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Aqueous N,N-diethylnicotinamide (DENA) solution as a medium for accelerated release study of paclitaxel

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Abstract—N,N-Diethylnicotinamide (DENA) was identified as an excellent hydrotropic agent for paclitaxel (PTX) in our previous studies. The aqueous solubility of PTX was increased by several orders of magnitude in the presence of DENA. Because of such a high hydrotropic property, DENA was used as a release medium providing a sink condition for the release of PTX from poly(lactic-co-glycolic acid) (PLGA) matrices. The release profiles of PTX from PLGA matrices into DENA, serum and phosphate-buffered saline (PBS) were compared. The stability of PTX in DENA and the degradation of PLGA molecules in DENA were examined. The degradation rate constant of PTX in 2 M DENA was similar to those in other aqueous solutions. The use of 2 M DENA as a release medium allowed differentiation of the release profiles of PTX from PLGA matrices made of different PLGA compositions. The PTX release from PLGA matrices was much faster in DENA solution than in serum or PBS, and the concentration of DENA affected the PTX release rate. The presence of DENA in the release medium increased the hydrolysis rate of PLGA polymers. The faster release of PTX from PLGA matrices in DENA solution may be due to the high PTX solubility and faster degradation of PLGA polymers in the presence of DENA. Our study suggests that the aqueous DENA solution can be used for the accelerated release study of PTX from PLGA matrices.

Key words: Hydrotropic agent; paclitaxel; *in vitro* release; PLGA; accelerated release.

INTRODUCTION

Paclitaxel (PTX) has been used as an effective agent against ovarian, breast and lung cancers [1]. Recently, PTX was also shown to be effective in preventing restenosis after it is released from the drug-eluting stents [2–5]. In one study, PTX was loaded into a poly(lactic-co-glycolic acid) (PLGA) matrix that was subsequently released from the stents into the surrounding tissue [5]. While many studies have shown that

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the release of PTX from the stents is highly beneficial in preventing restenosis, the optimum release kinetics of PTX from the stents has not been examined thoroughly. While the most realistic PTX release profile from the stents can be obtained from *in vivo* animal studies, such *in vivo* studies require the use of numerous animals that may not be realistic for testing many samples. For practical reasons, most drug release kinetics are measured from *in vitro* studies.

The *in vitro* release or dissolution testing of poorly soluble drugs poses problems due to their very low aqueous solubility. Phosphate-buffered saline (PBS) [6], PBS with surfactants [7] and PBS with albumin [8, 9] were used for the *in vitro* release of PTX from microspheres or paste. The release media were replaced with fresh media frequently to maintain the sink condition. The assay methods usually require a concentration step by lyophilization and/or extraction with organic solvents to measure the amount of paclitaxel released. Although the residual amount of PTX in microparticles can be determined [10], such a method requires a lot of samples with large volumes for each *in vitro* release experiment.

Recently, it was shown by our group that the aqueous solubility of PTX was significantly increased by using a hydrotrope, N,N-diethylnicotinamide (DENA) [11]. The aqueous solubility of PTX in DENA was much higher than that in acetonitrile or ethanol [12]. The PTX solubility in 2 M DENA is about 1 mg/ml and, thus, even a very small volume, as low as 1 ml, can be used to provide a sink condition in the *in vitro* PTX release if the total amount of PTX released is 200 μg/ml or less. In addition, a small volume of a release sample can be directly injected into an HPLC system for the determination of PTX concentration without concentration and extraction steps. Thus, use of DENA aqueous solution as a release medium is attractive. In this study, the DENA solution was used as a medium for the *in vitro* release study of PTX, and the effect of the release medium on the PTX release from model PLGA/PTX films was studied.

MATERIALS AND METHODS

Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA) polymers with various co-polymer compositions were obtained from Birmingham Polymers (Birmingham, AL, USA). Calf serum bovine and thimerosal were obtained from Sigma (St. Louis, MO, USA).

HPLC assay

The concentration of PTX was determined by reversed-phase HPLC using the Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA). Each sample of 5–20 μl was injected into a Symmetry C_{18} 5 μm column (3.9 $\times$ 150 mm, Waters, Milford, MA, USA). A mixture of acetonitrile and water (45 : 55, v/v) was used as a mobile phase at a flow rate of 1.0 ml/min at 25°C. A diode array detector was set at 227 nm.

Stability of PTX in an aqueous DENA solution

PTX needs to be released for at least a few weeks from stents and, thus, PTX should be stable in the release medium for accurate determination of the amount of PTX released. In order to use an aqueous solution of DENA as a medium for PTX release, the stability of PTX in 2 M DENA (in distilled water) was studied.

A PTX solution of about 100 μg/ml concentration was prepared in 2 M DENA and dispensed into small glass vials. Sample vials were incubated at 37°C. At predetermined time intervals, duplicate samples were stored frozen until analyzed by the HPLC method.

Effect of PLGA co-polymer composition on PTX release from films

PLGA/PTX films were prepared using PLGA(50/50) (intrinsic viscosity 0.63 dl/g, weight-average molecular weight (M_w) 43×10^3, polydispersity 1.8), PLGA(85/15) (intrinsic viscosity 0.72 dl/g, M_w 105.6×10^3) and poly(D,L-lactic acid) (PLA, intrinsic viscosity 0.66 dl/g, M_w 106×10^3) to examine the effect of co-polymer compositions on the release of PTX in 2 M DENA. Solutions of 10% (w/v) PLGA/1% (w/v) PTX in methylene chloride were prepared, and 81 μl of the solution was placed on a glass slide (0.9 cm $\times$ 0.9 cm) to make films about 100 μm in thickness. After overnight air-drying, PLGA/PTX film samples were vacuum-dried at room temperature for 1.5 days.

PLGA/PTX films were transparent but were tightly bound to the glass surface; thus, films attached to the glass surface were used in the release study. Triplicate samples of each PLGA/PTX (10 : 1, w/w) film were immersed in 3 ml of 2 M DENA solution at 37°C. At predetermined time intervals, 0.3-ml samples were taken and stored frozen. The same volume of fresh medium was added back to the release medium. The amount of PTX in the release medium was determined by HPLC.

In vitro *PTX release in different media*

Polyethylene (PE) discs (1.12 cm diameter) were firmly attached onto the glass surface using double-sided tape. PLGA(50/50) (intrinsic viscosity 0.63 dl/g) and PTX were dissolved in acetonitrile or methylene chloride to make 10% PLGA(50/50), 10% PLGA(50/50)/1% PTX, or 10% PLGA(50/50)/3% PTX solutions. The PLGA or PLGA/PTX solution (100 μl) was placed on the PE disc. After overnight air-drying, films were vacuum-dried for 2.5 days and stored in a desiccator until use. PLGA or PLGA/PTX films could be peeled off easily from the PE disc after drying.

Different concentrations of DENA were used as release media. In the release experiments using DENA solution, the total volume of the release medium was 10 and 30 ml for film samples in 2 M and 1.5 M DENA, respectively. Volumes of all DENA solutions, including 1.2 M and 1 M DENA, were enough to maintain the total amount of the released PTX to less than 15% of the PTX solubility in the DENA solution, which ensures the sink condition. Release medium was incubated at 37°C. The samples in DENA solution were used for HPLC analysis after filtration.

For the release study in PBS, 500 ml of PBS was completely replaced with a fresh solution at every sampling point. Of those samples 10 ml was lyophilized and reconstituted in 1 ml of a acetonitrile/water mixture (25 : 75, v/v).

Film samples were also incubated in 5 ml of calf serum containing 0.02% (w/v) thimerosal as a preservative. In addition, in order to prevent the contamination in serum, glass vials or tips were autoclaved before use, and all the samples using serum were handled under aseptic conditions. Serum was replaced every 4 days. 0.3 ml of release sample was stored frozen.

After the release study, the residual PTX amounts in the films from PBS and serum were determined as follows. Film samples were washed with distilled water (DW), vacuum-dried and dissolved in 5 ml of acetonitrile. After transferring 0.5 ml of solution to a new tube, 0.5 ml of DW was added to precipitate any large MW PLGA polymers. Since the PTX solubility in 50% acetonitrile is 4.3 mg/ml, it was likely that most of the PTX remained dissolved. The mixture was sonicated for 30 s and filtered. The concentration of PTX was determined by the HPLC method. The extraction efficiency of PTX from serum was determined as shown in the next section and was used for the quantification of PTX released into serum.

To examine whether PTX crystal forms on the PLGA/PTX films upon incubation in PBS, films with different PLGA(50/50)/PTX ratios (10 : 0, 10 : 1 and 10 : 3, w/w) were incubated in two different volumes, 5 ml or 80 ml, of PBS at 37°C for 1 day. According to *in vitro* PTX release data from the PLGA(50/50)/PTX (10 : 1, w/w) films, the amount of PTX released in PBS during the first day was about 3.3 μg. Since PTX solubility in PBS is 0.45 μg/ml, 5 ml of PBS represents the non-sink environment, where the PTX concentration will exceed the PTX solubility. A larger volume (80 ml) of PBS represents the sink condition for PLGA(50/50)/PTX (10 : 1, w/w) films. After freeze-drying, microscopic images were taken for those samples using a light microscope (Labophot-2, Nikon, Japan).

Extraction of PTX from serum

For the extraction of PTX from tissue samples, organic solvent or solid phase extraction techniques were mainly used [13]. In this study, a modified method of Koshkina *et al.* [14] was used for the extraction of PTX from serum samples using ethyl acetate.

10 μl PTX working stock solution in acetonitrile was added to 0.99 ml of serum containing 0.02% (w/v) thimerosal in an Eppendorf tube to prepare solutions of 0.1, 0.2, 0.5, 1, 2, 5 and 10 μg/ml PTX in serum. Thimerosal was used as a preservative. Triplicate samples containing serum with PTX were incubated at 37°C for 12 h, in order to obtain equilibrium binding of PTX to serum proteins.

300 μl serum, containing PTX, was transferred to a new Eppendorf tube, and 0.9 ml of ethyl acetate was added. After vortex-mixing for 30 s, samples were centrifuged ($10^4 \times g$, 5 min). The ethyl acetate layer was transferred to a new glass vial. The extraction step was repeated, and the ethyl acetate supernatant layer was pooled together. Ethyl acetate from the pooled supernatant was removed by

vacuum-evaporation in about 1 h. Liquid nitrogen was used in the trap to collect evaporated ethyl acetate.

Dried serum extract was dissolved in 0.2 ml of acetonitrile/water (1 : 1, v/v) by vortexing for 30 s. After filtering through a 0.25 μm nylon filter, 20 μl of each filtrate was used for HPLC analysis. The HPLC analysis was implemented as described previously, but with a different mobile phase: 43% of acetonitrile in DW was used until 28 min to separate a PTX peak from a serum-originating peak, and then the acetonitrile concentration was gradually increased to 100% to remove other peaks originating from the serum.

After extraction and HPLC analysis, a regression line of AUC *versus* PTX concentration was prepared, and the slope of the regression line was compared with that of PTX standard solutions in acetonitrile (data not shown). PTX extracted from serum had a smaller slope than that of the PTX standard. Extraction efficiency was calculated as: ((slope of regression line of PTX extracted)/(slope of regression line of PTX standard)) $\times$ 100% = 73.4%. This value was used for the adjustment of the released PTX amount into serum.

Molecular weight determination of PLGA polymers

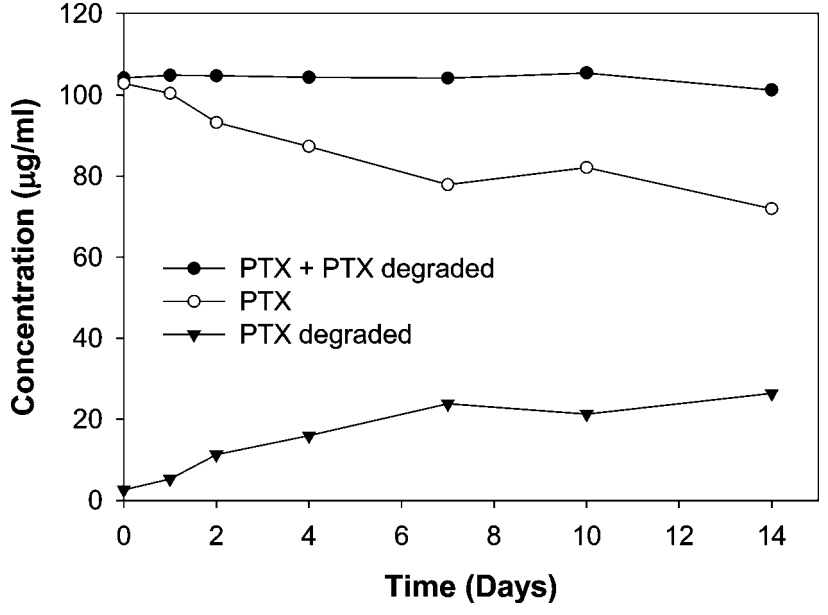
PLGA(50/50) or PLGA(50/50)/PTX (10 : 1, w/w) films were placed individually in glass vials containing 5 ml of 2 M DENA (in DW), 5 ml of 2 M DENA (in PBS), 15 ml of PBS, or 15 ml of calf serum containing 0.02% (w/v) thimerosal. The incubation temperature was 37°C and the medium was replaced every 5 days. At predetermined time intervals, film samples were washed with distilled water, lyophilized to remove water, and then stored frozen until analysis.

The molecular weight of PLGA polymer was determined by gel-permeation chromatography (GPC) using the Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) with a refractive index detector. The molecular weight calibration curve was established using polystyrene standards (Polymer Laboratories, Amherst, MA, USA) covering a molecular weight range of 580 to 283.3 $\times$ 10^3. For GPC analysis, lyophilized polymer samples were dissolved in 0.5 ml of chloroform stabilized with 1% ethanol (Mallinckrodt Baker, Paris, KY, USA). After filtering through a 0.45 μm Teflon syringe filter (Pall Gelman Laboratory, Ann Arbor, MI, USA), 50 μl of sample was injected into a PLgel 5 μm mixed-D column (Polymer Laboratories). Chloroform was used as a mobile phase at 1 ml/min and 35°C.

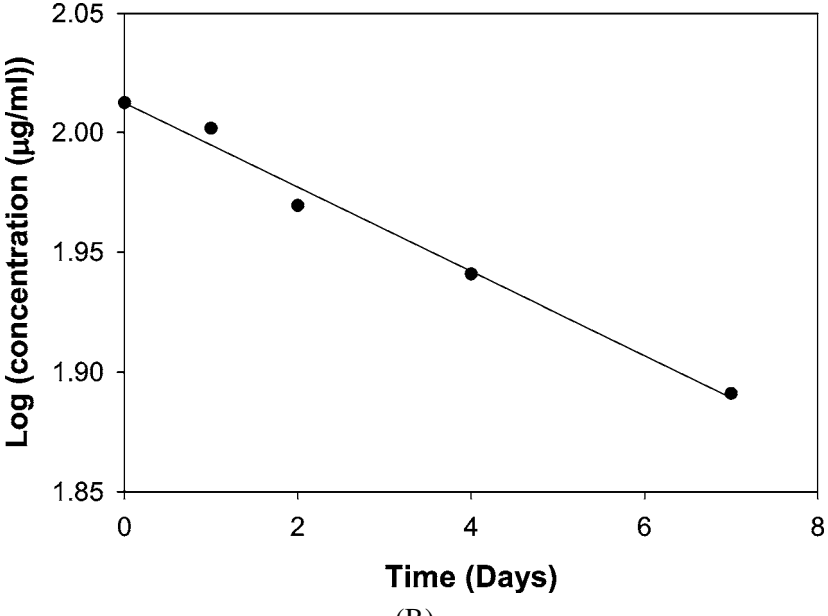
RESULTS

Stability of PTX in aqueous DENA solution

Before an aqueous DENA solution was used as a release medium for PTX release, the stability of PTX in the release medium was first examined for accurate determination of the amount of the released PTX.



(A)



(B)

Figure 1. Stability of PTX in 2 M DENA at 37°C. The retention times in the HPLC chromatogram were 11.4 min for PTX and 21.9 min for degraded PTX. The mobile phase was a mixture of acetonitrile and water (45 : 55, v/v). Duplicate samples were used at each time point. Changes of PTX concentration in linear scale (A) and in log scale (B) as a function of time.

When PTX was incubated in 2 M DENA solution (in distilled water, pH 6.8), which has a PTX solubility of 1 mg/ml, the PTX concentration in the medium decreased gradually (Fig. 1A). A semi-log plot of the concentration *vs.* time is linear (Fig. 1B), which shows first-order degradation kinetics of PTX in 2 M DENA solution. From the slope of Figure 1B, the degradation rate constant of 1.69×10^{-3} h^{-1} was obtained. The reported degradation rate constants of PTX in phosphate buffer (pH 6) and citrate buffer (pH 6) were 1.25×10^{-2} h^{-1} and 2.97×10^{-3} h^{-1}, respectively [15]. Degradation of PTX in 2 M DENA is about the same as that in other aqueous buffer solutions.

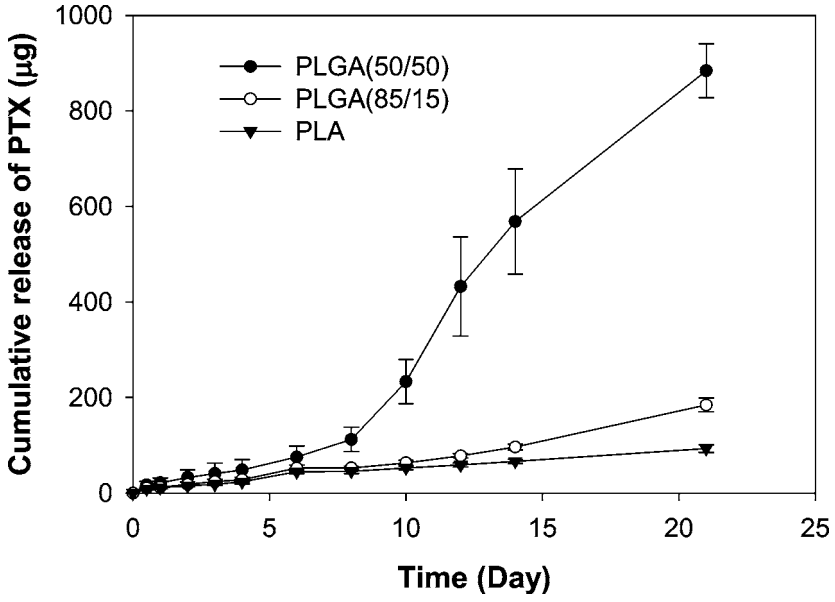
As the PTX concentration decreased upon incubation in 2 M DENA solution, a new peak appeared with a larger retention time (21.9 min) than that of the PTX peak (11.4 min). UV spectra of those two peaks, obtained from HPLC chromatograms using a diode array detector, showed that they were similar (data not shown). Thus, it is suggested that PTX degraded in 2 M DENA to produce another product with a 21.9 min retention time. Paclitaxel converts to 7-*epi*-taxol, which is a paclitaxel isomer, in many solvents, such as DMSO, isobutyl alcohol and methanol, and 7-*epi*-taxol also has a higher retention time than PTX [16]. Considering the retention time in an HPLC chromatogram and a UV spectrum, the PTX-deg peak is expected to be 7-*epi*-taxol.

The degradation product (PTX-deg) can be counted for calculating the total amount of drug released. When the decrease in the AUC of PTX ($-AUC_{PTX}$) in 2 M DENA was compared with the increase in the AUC of PTX-deg ($AUC_{PTX\text{-}d}$), there was a linear correlation between those variables (data not shown), and the ratio of $(-AUC_{PTX})/(AUC_{PTX\text{-}d})$ was 1.158. Thus, by converting AUC of PTX-deg to AUC of PTX, it was possible to calculate the total amount of PTX in the medium, which included the amount of PTX degraded. The total concentration of PTX, which is the sum of the intact PTX concentration and the degraded PTX concentration, remained almost constant until 14 days in 2 M DENA at 37°C (Fig. 1A). Thus, the amount of PTX released into 2 M DENA could be accurately measured by combining the PTX-deg peak with the native PTX peak together.

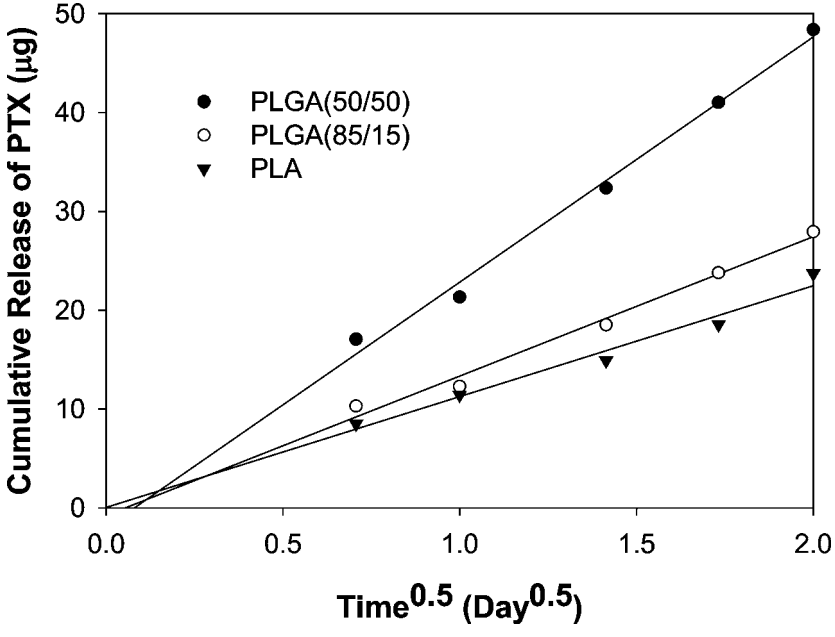
Effect of PLGA co-polymer composition on PTX release

PLGA/PTX (10 : 1, w/w) films attached on the glass surface were used for PTX release in the 2 M DENA aqueous solution. The release profiles of PTX from PLGA/PTX films are shown in Fig. 2A. The release rate of PTX was in the order PLGA(50/50) > PLGA(85/15) > PLA. In addition, PLGA(50/50)/PTX films showed an abrupt release of PTX between 10 and 20 days, while PTX was released slowly in other films composed of PLGA(85/15) or PLA.

The release of PTX from a biodegradable PLGA matrix was governed by both diffusion and biodegradation of the polymer matrix, as was the case for mifepristone release from PLGA films [17]. The PTX release in the initial period is governed by diffusion. As shown in Fig. 2B, the amount of PTX release during the initial 4 days is linearly proportional to the square root of time, which



(A)



(B)

Figure 2. *In vitro* cumulative release of PTX from PLGA/PTX films of various lactide/glycolide ratios in PLGA in 2 M DENA at 37°C as a function of time (A) and $(\text{time})^{1/2}$ (B). $n = 3$.

suggests that the mechanism of PTX in this period is diffusion, according to Higuchi's model. During the initial period, the PLGA polymer matrix swells in the medium, and the polymer chains degrade slowly into smaller molecular weight chains. Although polymer degradation occurs in this period, the polymer chains are still compact enough to restrict the diffusion of drug molecules. The difference in the initial release rate between PLGA films is due to a difference in swelling of the PLGA/PTX films, since hydrophilicity of the films is in the order PLGA(50/50) > PLGA(85/15) > PLA.

After the initial period of slow release, the PLGA(50/50)/PTX film released a large amount of PTX abruptly, while the other two films still showed the slow release of PTX. This can be explained by the different susceptibility of PLGA polymers to degradation. Higher glycolide composition in PLGA leads to faster polymer degradation, because those chains are more susceptible to hydrolysis by water molecules. Thus, the degradation rate of PLGA is in the order of PLGA(50/50) > PLGA(85/15) > PLA. Both PLGA(85/15) and PLA chains degrade much slower than PLGA(50/50), while PLGA(85/15) degrades faster than PLA. Once polymer chains become very small, they cannot maintain their film structure any more, and the drug molecules are liberated into the release medium. Thus, the abrupt release of PTX from PLGA(50/50) can be explained by significant degradation of PLGA(50/50) chains. Since PLGA(85/15) and PLGA polymers are more resistant to hydrolysis than PLGA(50/50), slower PTX release was still observed until 22 days without showing an abrupt PTX release.

The increase in the surface area also played a certain role in the faster PTX release from the PLGA(50/50) film. PLGA/PTX films attached onto the glass surface were used for the release study. PLGA(50/50)/PTX films were detached from the glass surface during the release period, while PLGA(85/15) or PLA films remained attached to the glass surface. Thus, the surface area of the PLGA(50/50)/PTX film was doubled during the release period, which should result in faster release of PTX from PLGA(50/50)/PTX. Thus, for further release studies, free PLGA/PTX films were used to avoid the change in the surface area during the release study. This result suggests that aqueous solution of DENA can successfully differentiate the release of PTX from PLGA films having different co-polymer composition ratios.

Effect of release media on the PTX release

Although the 2 M DENA aqueous solution allowed differentiation of the PTX release from films with different co-polymer compositions, it was necessary to compare it with different release media that are commonly used, such as PBS, serum and lower concentrations of DENA. Figure 3 shows the release profiles of PTX from PLGA(50/50)/PTX (10 : 1, w/w) films in various media. PTX solubility was very low when the concentration of DENA was below 1 M, thus DENA concentrations between 1 M and 2 M were used in this experiment. In DENA solutions, abrupt PTX release due to polymer degradation occurred between 10 and 30 days in the order 2 M DENA in PBS (pH 7.5) > 2 M DENA in DW (pH 6.8) > 1.5 M

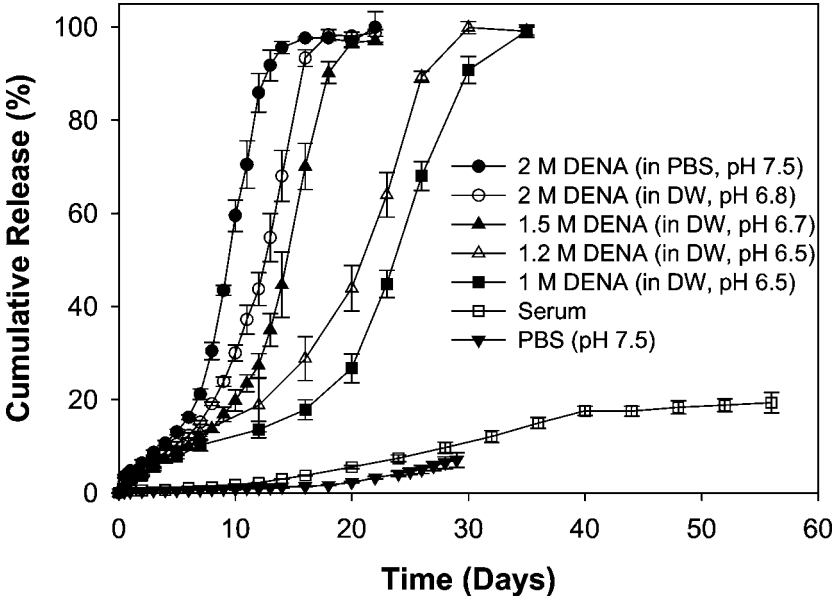


Figure 3. *In vitro* release of PTX from PLGA(50/50)/PTX (10 : 1, w/w) films in various media. $n = 3$.

DENA in DW (pH 6.7) > 1.2 M DENA in DW (pH 6.5) > 1 M DENA in DW (pH 6.5), which shows the DENA concentration-dependent profile when DENA aqueous solution was prepared in DW. The abrupt PTX release was not observed in PBS and serum until 29 and 56 days, respectively. The residual amounts of PTX were 83% and 65% for samples incubated in PBS and serum, respectively. The released amount calculated from the residual amount is much larger than that measured from the release medium. The difference in the released amount from two measurements might be due to the degradation of PTX in the serum and a very low concentration of PTX in the medium. PTX adsorption to the container surface might also have played a certain role [10].

Even though we consider the higher amounts of PTX released, which is the difference between the initial amount and the residual amount, it is evident that there is a difference in the rate of PTX release between DENA solutions and serum or PBS. In addition, a higher concentration of DENA showed a faster release of PTX. There was a possibility that PTX crystal might have formed in PLGA/PTX films when they were incubated in PBS or serum, thereby dissolution of PTX from crystals becoming the rate-limiting step in the PTX release.

Figure 4 shows the microscopic images of the films incubated in PBS. When the films were incubated in the non-sink condition, PTX crystals formed on the surface of the PLGA(50/50)/PTX films. More PTX crystals were observed in films with a higher PTX concentration (10 : 3, w/w, PLGA/PTX). However, upon incubation in 80 ml of PBS, which is a sink condition for PLGA(50/50)/PTX 10 : 1 (w/w) films, PTX crystal was not observed in PLGA(50/50)/PTX films (10 : 1 or 10 : 3, w/w).

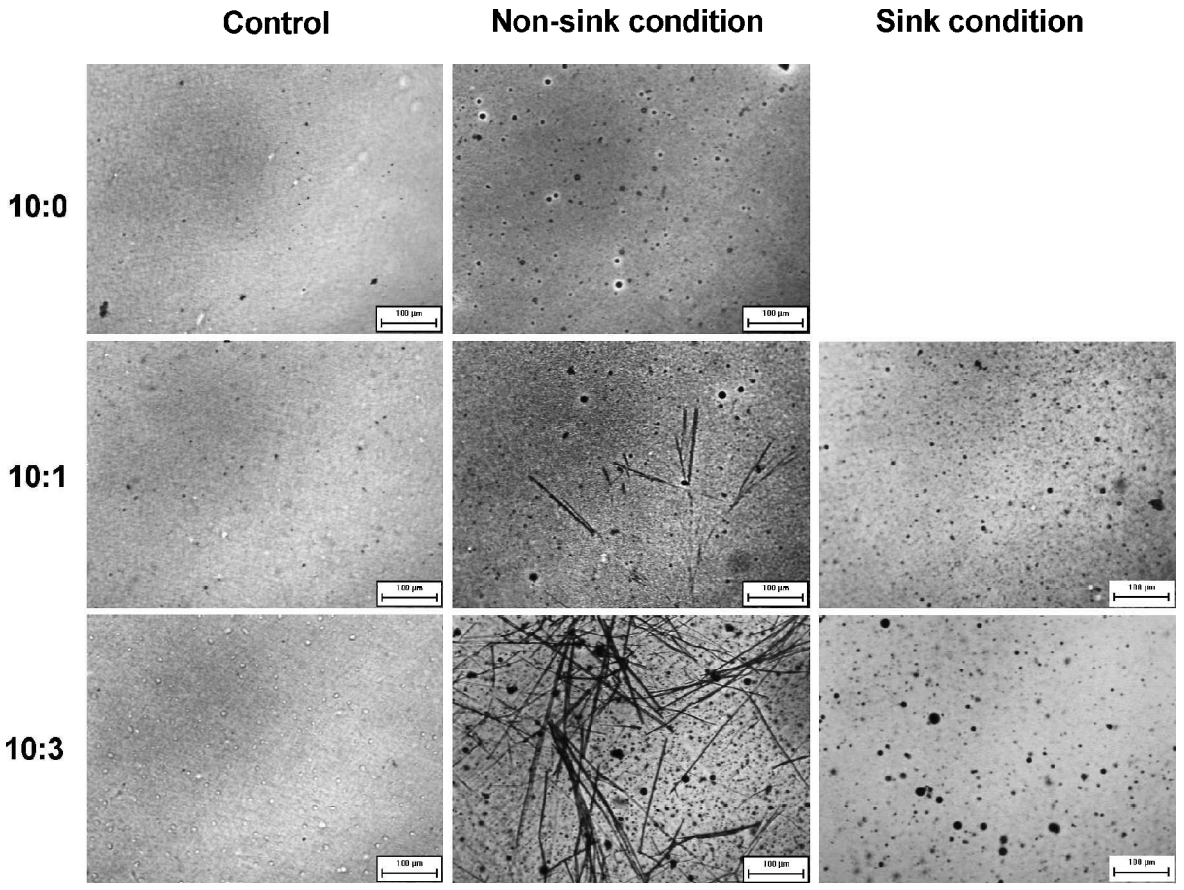
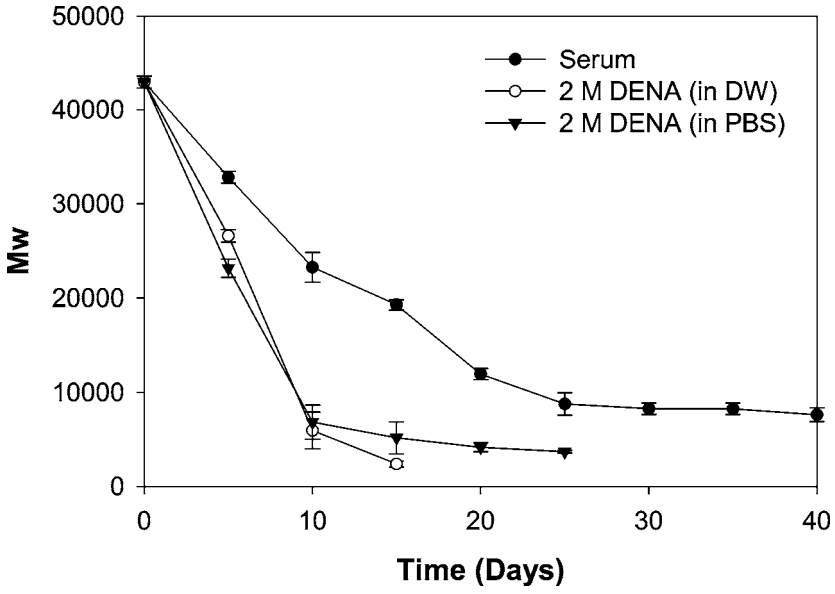
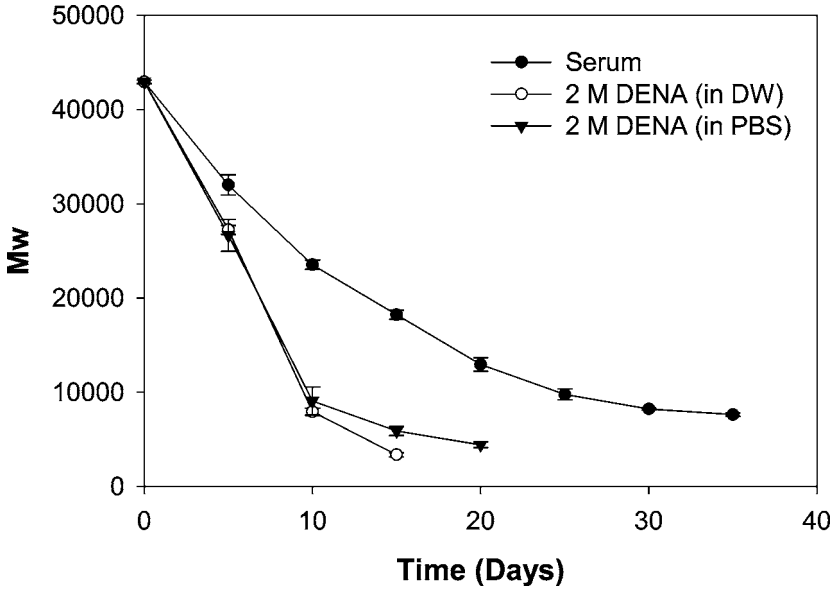


Figure 4. Microscopic images showing PTX crystals formed on the surface of PLGA/PTX films after 1-day incubation in 5 ml (non-sink condition) or 80 ml (sink condition) of PBS at 37°C. The weight ratio of PLGA to PTX in the PLGA(50/50)/PTX films was 10 : 0, 10 : 1, or 10 : 3. Control is the film before incubation in PBS.



(A)



(B)

Figure 5. Changes in weight average molecular weight (M_w) of PLGA upon incubation of PLGA or PLGA/PTX films in different media. Each data point represents the mean ± SD of triplicate samples. (A), PLGA(50/50) film; (B) PLGA(50/50)/PTX (10 : 1, w/w) film.

A larger volume (80 ml) of PBS was enough to maintain the sink condition for both PLGA(50/50)/PTX films during the 1-day incubation. Thus, it is not likely that PTX crystallization and dissolution of crystal caused a slower PTX release in PBS or serum than in the DENA solutions, because a sink condition was maintained by replacing 500 ml of PBS frequently.

Effect of release media on PLGA degradation

There was also another possibility that the degradation rate of PLGA polymer was increased in DENA solutions, considering the DENA concentration-dependent PTX release. Thus, the molecular weight change of PLGA polymers upon incubation of films in different media was analyzed by GPC.

In both PLGA and PLGA/PTX films, the weight average molecular weight (M_w) of the PLGA polymer decreased much faster in 2 M DENA aqueous solutions than in serum (Fig. 5). Both 2 M DENA solutions in DW or PBS showed similar polymer hydrolysis profiles. The weight average molecular weight of PLGA from PLGA/PTX films was less than 6000 after 15 days in 2 M DENA solutions (Fig. 5), and most of the PTX was released between 10 and 16 days (Fig. 3). Thus, abrupt release of PTX from PLGA/PTX films in 2 M DENA would be related to rapid degradation of PLGA chains. In contrast, the molecular weight of PLGA in serum after 35 days was higher than 7000, which still may be high enough to hold PTX molecules in the film so as not to produce the abrupt release.

Since there was no difference in polymer molecular weight change between PLGA and PLGA/PTX films, the presence of PTX in PLGA films did not affect the degradation of PLGA polymer. This suggests that effective sink condition was maintained in both 2 M DENA and serum release media. The effective sink environment must have been helpful in removing PTX molecules from the PLGA films, thereby resulting in similar PLGA degradation profiles regardless of the presence of PTX in films.

From molecular weight analysis data, it is evident that DENA accelerated hydrolysis of PLGA chains. However, since the presence of PBS did not further facilitate the hydrolysis of PLGA in 2 M DENA solution, it is still not clear why PTX was released faster in 2 M DENA (in PBS) than in 2 M DENA (in DW).

DISCUSSION

An aqueous solution of DENA increased the PTX solubility significantly. Thus, the effect of using the DENA aqueous solution as a release medium on the PTX release from PLGA film matrices was studied. Release of PTX from film samples showed a difference in the PTX release profiles between different release media (Fig. 3). This may be explained by two factors: the solubility difference of PTX in different media and the accelerated degradation of PLGA polymers by DENA.

PTX solubility values in 2 M DENA, 1.5 M DENA, 1.2 M DENA, 1 M DENA and PBS are 980, 290, 101, 41 and 0.45 μg/ml, respectively. Effective solubility

of PTX in calf serum was 171 μg/ml [18], and it is attributed to PTX binding to protein components. Since PTX solubility is higher in 2 M DENA than in lower concentrations of DENA, this might have resulted in faster dissolution of PTX molecules inside the polymer film matrix in a higher concentration of DENA than in a lower concentration of DENA. Much slower release of PTX in PBS or serum than in the DENA solutions can also be explained by the difference in PTX solubility. Although proteins in serum might have diffused into the PLGA matrix and formed PTX–protein complexes, the complex itself will be diffused out more slowly than free PTX molecules, and this would be why serum with a higher PTX solubility does not show a much faster PTX release than PBS. However, the solubility difference between media does not explain why the presence of PBS in 2 M DENA facilitated the release of PTX further than in 2 M DENA (in DW), because it is not likely that the presence of PBS in 2 M DENA increases the PTX solubility significantly.

In addition to the PTX solubility, DENA itself could have facilitated the hydrolysis of polymer chains. The PTX release experiment from films showed that the higher concentration of DENA resulted in a faster degradation-induced PTX release than the lower concentration of DENA. This result suggested that DENA facilitated the degradation of PLGA.

Some basic drugs with tertiary amine have shown the ability to accelerate the degradation of polylactide or PLGA polymers [19–22]. Poly(L-lactic acid) (PLLA) microspheres loaded with basic drugs, such as meperidine, methadone, and promethazine, showed faster drug release and faster polymer chain degradation than microspheres loaded with naltrexone, which is also a basic drug [19]. Thus, although basic drugs can accelerate the hydrolysis of PLGA chains, there was variation in the extent of acceleration between drugs. An increase in the amount of basic drug loading resulted in faster degradation of PLLA or poly(D,L-lactide) chains [19, 22]. Maulding *et al.* also observed that polymer hydrolysis occurred even during the process of thioridiazine microencapsulation using poly(D,L-lactide), but microencapsulation at 4°C or the use of the pamoate salt slowed down the polymer hydrolysis [21]. Basic drug-induced degradation of a polyester, poly(β-hydroxybutyrate), was also observed even in the films stored in the air [20]. The films, which contained thioridazine, indenorol, or clonidine, stored in the air could have been exposed to moisture in the air. Also, water absorbed from the air might have acted as a substrate for polymer hydrolysis induced by a basic drug because storage of poly(D,L-lactide) microspheres loaded with methadone did not result in polymer hydrolysis upon 1-year storage under desiccated conditions [23].

DENA itself has a tertiary amine group, and the accelerated degradation of PLGA in 2 M DENA was confirmed (Fig. 5). Since DENA can be moved into the PLGA film together with water molecules while the PLGA film matrix swells, higher local concentrations of DENA can be maintained inside the film matrix when the concentration of DENA in the release medium is higher. Thus, PLGA degradation will be increased as the concentration of DENA increases.

The observation that PLGA degrades faster in a DENA solution than in serum or PBS can be utilized for simulation of the *in vivo* release. PLGA polymers are known to degrade much faster *in vivo* than *in vitro* [24], which can be attributed to the presence of lipids or other biological compounds that increase the mobility of polymer chains [25] or the autocatalytic effect of the accumulated acidic degradation products [26]. Enzymes such as esterase can also catalyze the degradation of PLGA [27]. The presence of serum albumin alone was also shown to increase the initial rate of PLGA degradation [28]. Since implanted stents will directly contact with the vessel wall and will be exposed to blood, PTX molecules available from the stent will be removed promptly from the stent surface by binding with blood proteins such as serum albumin, which have a high affinity to PTX [29], or by partitioning easily into membranes of the cells in the blood vessel due to their hydrophobic nature. Very effective sink conditions will be maintained *in vivo*, and there would be additional factors that facilitate PLGA polymer degradation. Thus, the *in vivo* release profile of PTX from stents may not be the same as the *in vitro* release in PBS or serum. By adjusting the release medium, such as the DENA concentration or the presence of PBS, a suitable *in vitro* release profile that would be close to the *in vivo* release profile can be obtained. *In vitro–in vivo* correlation can be studied by analyzing the amount of PTX released from an *in vitro* experiment and the residual PTX from an *in vivo* experiment using PLGA/PTX-coated stents. Once *in vivo* drug release data are obtained, the concentration of DENA in the *in vitro* release medium can be varied to find a condition that produces *in vitro* release profiles matching with the *in vivo* drug release profiles.

In addition, the *in vitro* release from the PLGA matrix can be accelerated in DENA solutions, which will significantly decrease the time for the testing of sustained release formulations. An accelerated release of peptide from the PLGA depot at an elevated temperature was reported [30], but high temperatures may not be suitable for compounds that are susceptible to heat. The degradation rate of PTX in 2 M DENA was not larger than that in PBS, suggesting that the use of DENA in a release medium did not increase the degradation rate of PTX. The 2 M DENA aqueous solution was successfully used as a release medium for *in vitro* PTX release from stents of various formulations [31]. The *in vitro* release test of controlled release parenterals is usually used for quality control purposes [32] and also for differentiating different formulations [30]. Thus, it is suggested that the accelerated *in vitro* test using DENA solutions as release media can increase the efficiency of comparing different formulations composed of PLGA polymers.

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