N-Phthaloylchitosan-g-mPEG design for all-trans retinoic acid-loaded polymeric micelles

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\begin{abstract}
The amphiphilic grafted copolymer N-phonyaloylchitosan-grafted poly(ethylene glycol) methyl ether (PLC-g-mPEG) was synthesized using chitosan with four different degrees of deacetylations (DD) (80, 85, 90 and 95%). All-trans retinoic acid (ATRA) was incorporated into PLC-g-mPEG by dialysis method in an attempt to optimize carriers for ATRA delivery. Morphological investigation by transmission electron microscopy (TEM) showed that the particles had round and uniform shapes. The particle sizes of ATRA incorporated into micelles were about 80–160 nm depending on the initial drug-loaded and %DD of chitosan. Physicochemical properties of ATRA-loaded polymeric micelles were also investigated. %DD of chitosan, which corresponded to the N-phonyaloyl groups in the inner core of the micelles, was a key factor in controlling the incorporation efficiency, stability of the drug-loaded micelles and drug release behavior. As the %DD increased, the incorporation efficiency and ATRA-loaded micelles stability increased. The sustained release profiles were also obtained at high %DD (90 and 95%). When compared to the unprotected ATRA, ATRA loaded in PLC-g-mPEG micelles was efficiently protected from photodegradation. This result suggested that loading of ATRA in micelles improved the chemical stability of ATRA.
\end{abstract}

\section{Introduction}

All-trans retinoic acid (ATRA), an active metabolite of retinol, has been shown to exert anti-cancer activities against a number of cancer cells and tissues (Otsuki et al., 2003; Arce et al., 2005). Recently, it has been extensively used for the treatment of acute promyelocytic leukemia (APL) (Lengfelder et al., 2005). However, a gradual decrease in the ATRA concentration in the blood circulation after prolonged treatment (Muindi et al., 1992) and highly variable bioavailability after oral administration were observed (Ozpolat and Lopez-Berestein, 2002). Therefore, a parenteral formulation may provide a reliable approach for ATRA administration. In recent years, considerable emphasis has been placed on the development of new formulations of ATRA that are suitable for intravenous administration. However, the poor aqueous solubility of ATRA hampers its administration in solution form. Attempts have been made to develop parenteral formulations of ATRA by loading it in lipophilic carriers to overcome its solubility limitation. ATRA dispersal systems such as liposomes (Estey et al.,...
2005; Kawakami et al., 2006), solid lipid nanoparticles (Lim et al., 2004), polymeric micelles (Zuccari et al., 2005; Kawakami et al., 2005) and phospholipid-based microemulsions (Hwang et al., 2004) have been developed for parenteral administration purposes. Among these approaches, liposomes have been a promising parenteral delivery system for ATRA because of their superior ability to maintain plasma concentrations of ATRA over oral administration. In particular, clinical trials have demonstrated that parenteral liposomal ATRA offers potential pharmacological advantages over oral administration for APL therapy (Ozpolat and Lopez-Berestein, 2002). However, the limited solubility of ATRA within the liposomal membrane allows only large vesicle multilamellar-type liposomes to provide efficient ATRA loading. In this regard, other nano-sized carriers that effectively load ATRA will provide an alternative for ATRA parenteral delivery.

Amphiphilic block or graft copolymers have recently emerged as a class of materials with a wide range of pharmaceutical application. They can form nanoscopic core–shell structures above the critical micellar concentration (CMC) (Tuzar and Kratochvil, 1976). In the past 20 years, polymeric micelles have attracted great interest because of their potential as a drug carrier system. The hydrophobic core serves as a reservoir for hydrophobic drugs, while the hydrophilic part serves as an interface between the bulk aqueous phase and the hydrophobic domain. The highly hydrated outer shells of the polymeric micelles can inhibit intermicellar aggregation of their hydrophobic inner cores. Consequently, the polymeric micelles maintain a satisfactory aqueous stability irrespective of high contents of hydrophobic drug incorporated into the inner core of micelles. Furthermore, polymeric micelles in a size range <200 nm can escape from being scavenged by the non-selective reticuloendothelial system (RES) and shows enhanced permeability and retention effects (EPR effect) (Greish et al., 2003) at solid tumor sites for passive targeting.

Chitosan is soluble in aqueous acidic solutions but cannot form micelles in water. Recently, the production of chitosan spheres for drug delivery system was achieved by some specific processing techniques, such as suspension cross-linking, spray-drying coagulation and emulsification/solvent evaporation. Modifications on the chitosan structure can be carried out in order to optimize it for the intended application by changing hydrosolubility, adhesivity, haemocompatibility and others. The modification of polymeric materials by graft copolymerization has been intensively studied by several authors (Sashiwa and Aiba, 2004) because it can provide materials with desired properties through the appropriate choice of the molecular characteristics of the side chain to be grafted. Some modified chitosans, e.g. N-lauryl-carboxymethyl-chitosan (Miwa et al., 1998) and N-octyl-O-sulfate chitosan (Zhang et al., 2004), have been reported for the preparation of polymeric micelles for paclitaxel. In our previous report, we have concentrated on the modification of chitosan based on the balancing of polarity on the chain in order to obtain novel derivatives. We synthesized N-phthaloylchitosan-grafted poly(ethylene glycol) methyl ether (PLC-g-mPEG), which produced self-assembling, nano-level sphere-like particles (50–100 nm) depending on the chain length of mPEG, as observed by scanning electron microscope (SEM) and transmission electron microscope (TEM) (Yoksan et al., 2004). The previous study showed that the high camptothecin-loaded micelles have been successfully prepared from the PLC-g-mPEG with 95% DD using dialysis method (Opanasopit et al., 2006).

The purpose of this study was to investigate PLC-g-mPEG micelles as a drug delivery system for ATRA. The incorporation efficiency, stability of ATRA-loaded micelles and in vitro release behavior were investigated. Moreover, photodegradation of ATRA in ATRA-loaded micelles and in vitro release behavior were investigated. Moreover, photodegradation of ATRA in ATRA-loaded micelles was evaluated and compared with ATRA in ethanol solution.

2. Materials and methods

2.1. Materials

All-trans retinoic acid was purchased from Sigma-Chemical Company (St. Louis, MO, USA). Chitosans with 80, 85, 90 and 95% deacetylation (Mw 5.78 × 105 Da) were provided by Seafreach Chitosan (Lab), Co., Thailand. Phthalic anhydride and succinic anhydride were purchased from Fluka Chemika, Switzerland. Poly(ethylene glycol) methyl ethers (mPEG, Mn 2000 Da) were obtained from Aldrich Chemical Company, USA. 1-Ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide (EDC) was purchased from TCI, Japan. 1-Hydroxy-1H-benzotriazole, monohydrate (HOBt) was obtained from BDH Laboratory Supplies, UK. All other chemicals were of analytical grade.

2.2. Synthesis of N-phthaloylchitosan-grafted mPEG

N-Phthaloylchitosan was prepared as previously reported (Yoksan et al., 2004). Briefly, chitosans (DD = 80, 85, 90 and 95%, Mw 5.78 × 105 Da) were reacted with phthalic anhydride

<table>
<thead>
<tr>
<th>Table 1 – Molecular weight and chemical composition of N-phthaloylchitosan-grafted mPEG</th>
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<tr>
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<tr>
<td>---</td>
</tr>
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<tr>
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<tr>
<td>PLC (90)-g-PEG</td>
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<tr>
<td>PLC (95)-g-PEG</td>
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* Calculated from 1H NMR.
(5 mol equivalent to pyranose rings in dimethylformamide (DMF)) at 100 °C under vacuum for 6 h. The temperature was reduced to 60 °C, and the reactions were left overnight. The product was reprecipitated in cold water. The precipitate was collected, washed with ethanol, and vacuum dried to give a pale yellow compound. The compound (0.40 mol equivalent to MPEG) was stirred with MPEG in DMF solution. 1-Hydroxy-1-benzotriazole, monohydrate (3 mol equivalent to MPEG) was added as catalyst and stirred at room temperature until the solution became clear. 1-Ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide, hydrochloride (3 mol equivalent to MPEG) was reacted overnight. The mixture was diazyl in water and washed with ethanol to obtain white particles of PLC-g-MPEG. Table 1 shows the molecular weight and chemical composition of PLC-g-MPEG.

2.3. Critical micelle concentration determination

The CMC of PLC-g-MPEG polymer was determined using a spectrofluorophotometer (RF-1501, Shimadzu, Japan) with pyrene as a fluorescent probe. Experiments were set up with excitation and emission wavelengths of 352 and 383 nm, respectively. The concentration of pyrene was 6.0 × 10^{-7} M. The emission and excitation spectra of pyrene were recorded with a micelle concentration that ranged from 0.125 to 256 μg/ml. In each experiment, a 5 μl pyrene in acetone solution was added to a 4 ml polymeric micelle solution and stirred for 24 h until the acetone was completely evaporated prior to measurement. For the pyrene excitation spectrum, the ratio of fluorescence intensity at 334 and 337 nm (I_{334}/I_{337}) was calculated and plotted against the logarithm of the concentration of micelles.

2.4. Incorporation of ATRA into polymeric micelles

The incorporation of ATRA into polymeric micelles was carried out by dialysis method (Yokoyama et al., 1999). Briefly, 5 mg of PLC-g-MPEG polymer and ATRA (5–40% of polymer) were dissolved in 2 ml of DMSO in a glass tube. The mixture was stirred at room temperature until completely dissolved. The mixture was then placed in a dialysis bag (membrane: Spectra/Por® 12,000–14,000 MWCO, Spectrum Laboratories, USA) and dialyzed against distilled water overnight. The mean particle diameters were determined by dynamic laser light scattering (DLS) using the Zetasizer® 3000 (Malvern Instruments, Southborough, MA, USA). ATRA-loaded micelles were dissolved in a mixture of DMSO:HzO (9:1). The amount of ATRA incorporated into polymeric micelles was determined by UV absorption at 360 nm. The incorporation efficiency was calculated as the percentage of the initial drug used in the preparation that incorporated into the micelles.

2.5. Gel permeation chromatography (GPC)

The stability of drug-loaded micelles was determined by GPC as described previously (Opanasopit et al., 2004). High performance liquid chromatography (HPLC) was carried out using an Agilent HPLC system (Agilent 1100 series, USA) equipped with a Tosoh TSKgel G3000PWXL column at 40 °C. Samples (50 μl) were injected into the column and eluted with distilled water at a flow rate of 1.0 ml/min. The detection was performed by measuring absorption at 360 nm using a UV detector and a refractive index (RI) detector.

2.6. In vitro release

Release of ATRA from ATRA-loaded micelles was measured using a dialysis bag (membrane: Spectra/FoT® 12,000–14,000 MWCO, Spectrum Laboratories) as described previously (Opanasopit et al., 2004). One hundred milliliters of release medium (ethanol, polysorbate-80 and distilled water at 10, 15 and 75% (v/v) respectively; the pH of solution was adjusted to pH 8.5 with 1N NaOH) was added in a double jacket beaker with an external constant temperature circulation water bath under constant stirring at 37 ± 0.5 °C. One milliliter of ATRA-loaded micelles was placed in a dialysis bag and immersed in the medium. At the time intervals of 1, 2, 4, 8, 12, 24, 48, 72 and 96 h, 3 ml aliquots of the medium were withdrawn, and the same volume of fresh medium was added. The sample solution was analyzed by HPLC. All experiments were done in triplicate.

2.7. Reverse-phase HPLC analysis of ATRA

Concentrations of ATRA were determined using a reverse-phase HPLC system (Sinico et al., 2005). The HPLC system consisted of a mobile phase delivery pump (P-1500, Thermo Separation Products, USA), UV detector (UV-1000, Thermo Separation Products, USA) and integrator (I-1000, Thermo Separation Products, USA). A C_{18} reverse-phase column (Water, USA), 5 μm, 150 mm × 0.5 mm was used. The mobile phase was 84.5:15.0:0.5 (v/v/v) mixture of acetonitrile:water:glacial acetic acid. The injection volume was 20 μl, and the flow rate was 1.5 ml/min. Detection was done at 360 nm.

2.8. Photodegradation of ATRA and ATRA-loaded polymeric micelles

To elucidate the effects of polymeric micelles on photodegradation protection over time in aqueous solution, ATRA-loaded polymeric micelles (10 μg/ml) were taken in a 1 cm quartz cuvette tightly stoppered and irradiated using a Xenon lamp. The cabinet temperature was 25 °C. Just after the preparation (t=0), a UV spectrum was recorded. Ten microliters of aliquots was withdrawn at time intervals (0.25, 0.5, 1, 2, 4, 6 h), followed by immediate dilution with ethanol. The amount of ATRA was determined by HPLC. For comparison, an ethanol solution of ATRA (10.0 μg/ml) was investigated by the same procedures as that for the micelles.

2.9. Statistical analysis

All measurements were collected in triplicate experiments. Values are expressed as mean ± standard deviation (S.D.). Statistical significance of differences in amount of ATRA was examined using one-way analysis of variance (ANOVA) followed by LSD post hoc test. The significance level was set at p<0.05.
3. Results and discussion

3.1. Characterization of PLC-g-mPEG

The PLC-g-mPEG polymers with four different DD (80, 85, 90 and 95%) of chitosan were synthesized, and the chemical structure was depicted in Fig. 1b. The structure of these graft copolymers was characterized by FTIR (Yoksan et al., 2004) and $^1$H NMR (Yoksan et al., 2003). $^1$H NMR exhibited peaks at 3.1–4.1 ppm belonging to methylene protons of mPEG, whereas the peaks at 3.1–4.2 and 7.6–7.9 represented pyranose ring and aromatic protons, respectively. The degree to which mPEG was substituted onto $N$-phthaloylchitosan was 16.50%, and the substitution of the phthalimido group was 66–69% as calculated by $^1$H NMR. Calculated results were summarized in Table 1. It was found that DD increased, ranging from 80 to 95%, with an increase in the phthalimido group on chitosan chain.

3.2. Characterization of PLC-g-mPEG polymeric micelles

Initially, suitable solvents for dissolving PLC-g-mPEG were investigated. The appearance of PLC-g-mPEG dispersed or dissolved in various solvents was shown in previous reports (Opanasopit et al., 2006). We found that the turbidity level largely depended on the type of solvents. PLC-g-mPEG was completely dissolved in dimethylsulfoxide (DMSO) and $N$-dimethylformamide. However, it formed a colloid (as seen from turbidity) in water, methanol, ethanol, 2-propanol and chloroform, and it was insoluble in toluene and $n$-hexane. The ability of DMSO and DMF to dissolve PLC-g-mPEG was probably due to a high dielectric constant and a high dipole moment of the solvents.

PLC-g-mPEG with 80 and 85% DD were slowly dissolved in DMSO (>3 h), whereas, PLC-g-mPEG with 90 and 95% DD immediately dissolved in DMSO (<5 min) after vigorously stirring using a magnetic stirrer. This might be the result of the decrease in chitin unit and an increase in chitosan and phthaloyl chitosan units (Table 1). In this study, DMSO was chosen as the solvent to dissolve PLC-g-mPEG. Nanosize (50–100 nm) micelles were formed when it was dialyzed against water. The tendency of these polymers to form self-assembling micelles might be based on the hydrophobic phthalimido groups on the chitosan chain and hydrophilic mPEG chain. The critical micelles concentration values of four PLC-g-mPEGs (DD = 80, 85, 90 and 95%), using pyrene as a fluorescent probe, were equal value of 28 μg/ml (Fig. 2). This result indicated that the increased $N$-phthaloylchitosan unit did not affect the CMC value.

The diameter and distribution of the polymeric micelles were measured by dynamic laser light scattering and shown in Fig. 3. The mean particle sizes of the ATRA-loaded PLC-g-mPEG micelles ranged from 80 to 160 nm in diameter, which were larger than that of the bare micelles (50–100 nm). For both bare and ATRA-loaded PLC-g-mPEG micelles, the size distribution was narrow. The diameter increased with an increase in the weight ratio of ATRA to polymer (5, 10, 20 and 40% ATRA to polymer). Fig. 4 shows the TEM micrographs of ATRA-loaded polymeric micelles (5% ATRA to polymer). In all cases, the micelles were spherical in shape and nano-sized.

![Fig. 1 – Chemical structure of: (a) all-trans retinoic acid (ATRA) and (b) $N$-phthaloylchitosan-grafted mPEG polymer (PLC-g-mPEG).](image1)

![Fig. 2 – Effect of percent degree of deacetylation of chitosan: (●) 80%, (◊) 85%, (▲) 90% and (■) 95% measured as the $I_{337}/I_{334}$ band intensity ratio of pyrene as a function of the logarithm of the concentration of micelles forming from PLC-g-mPEG. Data are plotted in the mean ± S.D. of three measurements.](image2)

![Fig. 3 – The particle size of bare-polymeric micelles and ATRA-loaded polymeric micelles prepared from PLC-g-mPEG with 80, 85, 90 and 95% DD chitosan. (•) Bare-polymeric micelles; (□) 5%; (●) 10%; (▲) 20%; (■) 40% ATRA to polymer-loaded polymeric micelles. *p < 0.05 compared with 80% DD.](image3)
3.3. Incorporation of ATRA into PLC-g-mPEG micelles

As shown in previous studies, dialysis method was used for the physical incorporation of camptothecin into the PLC-g-mPEGs micelles (Opanasopit et al., 2006). In this study, ATRA was incorporated by dialysis method because both PLC-g-mPEG and ATRA dissolved in DMSO. This solvent could not be evaporated; therefore, evaporation and emulsion method could not be performed. Fig. 5a shows the effect of %DD and the initial drug on the efficiency of ATRA incorporation in PLC-g-mPEG micelles. The X-axis represents the initial drug used in preparation (percentage of ATRA), ranging from 5 to 40%, and the Y-axis represents the percentage of ATRA incorporated into the micelles (%yield). For all polymers tested, the incorporation efficiency decreased, ranging from 98 to 1.5% with an increase in the initial ATRA loading and the %DD. In all cases, 5% initial drug loading showed the highest drug incorporation. The important factor to control this incorporation process was mainly due to the hydrophobic interactions among hydrophobic N-phthaloylchitosan chain, ATRA and the solvent. Micelle formation and drug incorporation into micelles were expected to occur simultaneously. If ATRA interacted more favorably with the hydrophobic polymer chain than with solvent, incorporation efficiency should be high. If ATRA molecules interact with each other more strongly than with the hydrophobic polymer chain, ATRA will precipitate rather than be incorporated into the micelles. Increasing the initial drug concentration that was loaded results in an increased hydrophobic inner core, but incorporation efficiency was decreased. It was found that increasing the %DD dramatically increased the amount of ATRA incorporated into the micelles. PLC-g-mPEG micelles of 90 and 95% DD with 5% initial ATRA showed the highest incorporation efficiency (98% yield). As shown in Table 1, %DD was correlated to the hydrophobic N-phthaloylchitosan (PLC) units, and %DD ranked from 80 to 95, with the PLC units of 1829 and 2256, respectively. This suggests a large contribution of $\pi-\pi$ interaction between the aromatic groups of ATRA molecules (Fig. 1a) and the hydrophobic inner core (phthalimido group) of the PLC-g-mPEG polymers (Fig. 1b) rather than the effect of hydrophobic interaction. These results showed the advantages of this novel polymer since it can be employed for drug carrier systems in which the drug molecules need not to be chemically conjugated to the polymer. These results are likely to be consistent with previous results in which benzyl and methylnaphtyl PEG–poly(aspartate) block copolymer (phenyl/naphthyl group) at high esterification degree provided high camptothecin (CPT) incorporation stability. In contrast, n-butyl and lauryl PEG–poly(aspartate) block copolymer showed lower stability than benzyl and methylnaphtyl PEG–poly(aspartate) block copolymer (Opanasopit et al., 2004; Yokoyama et al., 2004).

3.4. Stability of ATRA-loaded polymeric micelles

The stability of ATRA-loaded micelles was characterized by GPC. It was observed that all samples formed polymeric micelle structures with micelle peaks near the gel-exclusion volume. Micelle peaks detected by the RI detector (for polymers) showed the same retention time (4.2 min) as detected by UV absorption at 360 nm for ATRA. GPC with UV detection allowed us to evaluate the nature of the polymeric micelles.
obtained and the degree of drug incorporation. Therefore, the stability of ATRA-loaded micelles was characterized by the peak area of the peak detected by UV absorption at 360 nm. This peak area represents the amount of ATRA loaded into the micelles. The small values of the peak area/ATRA concentration, [ATRA]/ATRA, means that most of the ATRA was adsorbed to the GPC column by hydrophobic interactions due to unstable packaging of ATRA in the micelles. The values of peak area/ATRA of the ATRA-loaded micelles decreased with an increase in the weight ratio of ATRA to polymer (5–40% ATRA to polymer), as shown in Fig. 5b. When 5% ATRA was initially loaded, we found that PLC (95)-g-mPEG showed the highest values of peak area/ATRA. These results indicate not only the drug content but also the quantity of the hydrophobic component (phthalimido group) was essential to form stable ATRA-loaded micelles. Particularly, greater than 85% DD was enough for stable micelle formation with high ATRA incorporation. These results were in agreement with the previous study that benzyl content of PEG–poly(benzyl-l-aspartate) block copolymer greater that 57% was enough for stable micelle formation. One hundred percent benzyl ester showed unstable CPT-loaded micelles (Opanasopit et al., 2004 ). Lacking a phthalimido group, which may provide the appropriate space required to insert ATRA molecules, resulted in good interactions with the adjacent phthalimido group by hydrophobic interaction.

3.5. Micelle drug release

Polymeric micelle drug delivery systems are advantageous for their wide applicability in delivering hydrophobic drugs. Micelle stability, long-circulation properties, and sustained drug release are critical factors for achieving highly selective delivery to tumor target sites. As previously reported, the camptothecin release rate was the slowest when it showed the highest GPC stability (Opanasopit et al., 2004, 2006). The weak interaction between drug and inner core of micelles partially contributed to the fast release of drug. Therefore, in this study, we investigated the release behavior of ATRA-loaded PLC-g-mPEG micelles. The ATRA released from PLC-g-mPEG micelles with 80, 85, 90 and 95% DD and 10% ATRA initially loaded is shown in Fig. 6. The results showed that ATRA release from PLC-g-mPEG micelles was sustained over 96 h, and %DD affected the release rate of ATRA from micelles. In PLC-g-mPEG with 80 and 85% DD, the ATRA release from micelles was rapid, and a high burst release to 70–80% was observed within 2 h. However, in PLC-g-mPEG with 90 and 95% DD, ATRA release was much slower than 80 and 85% DD, and the ATRA release reached about 50% at 96 h. These results might be due to PLC-g-mPEG with 80 and 85% DD having a lower interaction between the drug and hydrophobic inner core (phthalimido group) of the PLC-g-mPEG polymers as compared to PLC-g-mPEG with 90 and 95% DD. These release results were consistent with the GPC results. PLC (95)-g-mPEG showed the highest stability by GPC and also showed the slowest release rate. This implies that PLC-g-mPEG with 90 and 95% DD might be a good carrier for ATRA incorporation and sustained release for passive tumor targeting.

3.6. Photodegradation of ATRA and ATRA-loaded polymeric micelles

With a feature common to all compounds in the retinoids class, ATRA undergoes degradation when exposed to light (Brisaert and Plaizier-Vercammen, 2000). Therefore, the development of novel formulations characterized by high photoprotection towards the ATRA is important. The inclusion of ATRA in liposomes was reported to protect the drug against photodegradation, and a thorough characterization of ATRA–liposome (Brisaert et al., 2001) and niosome complexes (Manconi et al., 2002) has been described. Fig. 7 compares ATRA photodegradation profiles of ATRA and ATRA-loaded polymeric micelles at 25 ± 0.5 °C in the presence of

Fig. 6 – Release profile of ATRA from the micelles forming from N-phthaloylchitosan-grafted mPEG. (♦) 80% DD; (■) 85% DD; (▲) 90% DD; (●) 95% DD. Data are plotted as the mean ± S.D. of three measurements. *p < 0.05 compared with 80% DD at 96 h.

Fig. 7 – The rate of photodegradation of 10 μg/ml ATRA in ethanol solution (×) and ATRA-loaded polymeric micelles forming form N-phthaloylchitosan-grafted mPEG polymer as evaluated by reversed-phase HPLC. (♦) 80% DD; (■) 85% DD; (▲) 90% DD; (●) 95% DD (n = 3). *p < 0.05 compared with ATRA in ethanol solution.
UV light for 6 h. Photodegradation of ATRA followed first-order kinetics. The amount of ATRA versus time was fitted to Eq. (1).

\[ y = a \exp(-kt) \]  

(1)

\( a \) is equivalent to the total concentration of ATRA at \( t = 0 \), and \( K \) is the photodegradation rate constant.

The photodegradation rate constant (\( k \)) and the corresponding half-life (\( t_{1/2} = 0.693/k \)) of ATRA and ATRA-loaded polymeric micelles at 25 ± 0.5°C under the UV light for 6 h are summarized in Table 2. The photodegradation of ATRA in ethanol solution was very fast, whereas the micelle structure greatly contributed protection toward ATRA. After 1 h of UV light irradiation, only about 32% of the initial concentration of ATRA was still present, whereas ATRA-loaded polymeric micelles showed a slower photodegradation than ATRA in ethanol solution. Micelles protected the photodegradation of ATRA after 6 h with 74, 72, 59 and 80% of the initial concentration of ATRA still present, whereas ATRA-loaded polymeric micelles showed a slower photodegradation than ATRA in ethanol solution. Micelles protected the photodegradation of ATRA by GPC, and the release behavior of ATRA from micelles. Inclusion of the drug in the micelles notably hindered the photodegradation of ATRA. Therefore, this novel PLC-g-mPEG polymer has considerable potential in the development of ATRA delivery systems that are capable of improving its photostability.

<table>
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<tr>
<th>Compound</th>
<th>Hydrolysis rate (×10⁻³ h⁻¹)</th>
<th>Half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATRA in ethanol solution</td>
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</tr>
<tr>
<td>ATRA-loaded polymeric micelles</td>
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<tr>
<td>PLC (80)-g-PEG</td>
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<td>PLC (85)-g-PEG</td>
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<td>PLC (90)-g-PEG</td>
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<td>PLC (95)-g-PEG</td>
<td>0.35 ± 0.01</td>
<td>19.3</td>
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References


