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Poly(vinyl pyrrolidone) Conjugated Lipid System for the Hydrophobic Drug Delivery

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Abstract: Water soluble polymer, poly(vinyl pyrrolidone) was chosen to conjugate with 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-*N*-(succinyl) (*N*-succinyl DPPE) to make a new drug delivery system. PVP with an amine group (amino-PVP) was polymerized by free radical polymerization. The amine group of amino-PVP was conjugated with the carboxylic group of *N*-succinyl DPPE. The resultant conjugate could form nanoparticles in the aqueous solution; these nanoparticles were termed a lipid-polymer system. The critical aggregation concentration was measured with pyrene to give a value of 1×10^{-3} g/L. The particle size of the lipid-polymer system, as measured by DLS, AFM and TEM, was about 70 nm. Lipophilic component in the inner part of the lipid-polymer system could derive the physical interaction with hydrophobic drugs. Griseofulvin was used as a model drug in this study. The loading efficiency and release profile of the drug were measured by HPLC. The loading efficiency was about 54%. The release behavior was sustained for a prolonged time of 12 days. The proposed lipid-polymer system with biodegradable and biocompatible properties has promising potential as a passive-targeting drug delivery carrier because of its small particle size.

Keywords: PVP, *N*-succinyl DPPE, lipid nanoparticle, drug delivery.

Introduction

Drug delivery carriers using lipid in the pharmaceutical technology have actively been studied to use them for the delivery of hydrophobic drugs and genes.[1-3] For the cancer therapy, the polymeric nanoparticles were designed as carriers of specific water-insoluble drugs.[4,5] The polymeric nanoparticles usually have the hydrophobic segments and hydrophilic segments. It is the spherical nano-particle made by self-assembled amphiphilic polymers in the aqueous solution.[6-8] In the aqueous solution, hydrophilic parts cover the particle's corona, while hydrophobic fragments form the core of nanoparticle. The lipid nanoparticles serve as a cargo space for the pharmaceuticals which have the poor water solubility. Hydrophobic drugs can be loaded in the core of nanoparticle by the hydrophobic interaction.[3,4,9] In the cancer therapy, the lipid nanoparticles can be localized in the tumor site due to the enhanced permeability and retention effect (EPR effect). The EPR effect is that the small particle can be easily permeated and accumulated inside the tumor due to the leakiness of vascular endothelial cell of tumor tissues.[10] The nanoparticles which have proper sizes (50-500 nm) can be accumulated at the solid tumor. The hydrophilic segments can give the long blood-circulation property by preventing the capture by the reticulo-endothelial system (RES) and opsonization.[4,19]

Phospholipid has two long hydrophobic tails and a small hydrophilic head group. It has the biocompatible and biodegradable property. It consists main ingredients of the cellular membrane.[11] There is a long history in the pharmaceutical application to use poly(vinyl pyrrolidone) (PVP) as the hydrophilic polymer.[12-14] VP monomers are toxic but they are synthesized to non-toxic polymer by the radical polymerization. PVP has non-toxic, water-soluble and biocompatible property similar to PEG.[15-18] The plasma half-life of PVP was longer than PEG after the systemic administration.[16]

In various studies, PEG coated lipids are used as the long blood-circulating system *in vivo*.[19] In this study, we made lipid-polymer system which was made by the conjugation between lipid and PVP. Lipid-polymer system forms the lipid nanoparticle in the aqueous solution such as blood. It inhibits the biodegradation of lipid and extends their blood circulation life like PEG.[12] Lipid-polymer system are the nanoparticles with several tens or hundreds nanometers. Hydrophobic drugs like paclitaxel and doxorubicin can be loaded in the lipid parts of the micelle.

In this study, we used griseofulvin as the model drug to investigate the loading possibility of hydrophobic drugs. Griseofulvin has been used in the treatment of dermatophyte infections.[20] The size and shape of lipid-polymer system was studied. Fluorescent spectroscopy and HPLC were used to check the drug loading and the release profiles. The lipid-

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polymer system with hydrophobic or water-insoluble drug is possible to use as a passive targeting carrier by the EPR effect.

Experimental

Materials. 1-Vinyl-2-pyrrolidone (VP) and 1,3-dicyclohexylcarbodiimide (DCC) were purchased from Aldrich. Azobis(isobutyronitrile)(AIBN) was supplied from JUNSEI and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-*N*-(succinyl) (*N*-succinyl DPPE, Sodium Salt) was obtained from Avanti Polar Lipids. 2-Mercaptoethylamine and griseofulvin were purchased from Sigma. Dialysis membranes were purchased from Spectra/Por®.

Synthesis of Amine-Terminated PVP. The polymerization of VP was carried out under the dry argon atmosphere by the radical polymerization. VP monomer (27.85 mL, 0.25 mol) in the dioxane (100 mL) solution was polymerized in the presence of azobis (isobutyronitrile) (2.463 g, 0.015 mol) as the initiator for 24 h at 60 °C. To prepare amine-terminated PVP (amino-PVP), 2-mercaptoethylamine (1.543 g, 0.08 mol) was added to the system as the chain transfer agent. The reactants were dialyzed for 24 h using the dialysis membrane (molecular weight cut-off: 2,000) in the aqueous solution. Then, it was lyophilized using the freeze dryer. Characterization of amino-PVP was performed by GPC, FT-IR (MAGNA-IR 560 spectrometer, NICOLET) and ^{1}H-NMR (JNM-AL400, 400 MHz, JEOL Ltd.) in $CDCl_3$ (spectra δ = 7.3 ppm, 1 wt% TMS).

Conjugation of Amino-PVP and Lipid. *N*-succinyl DPPE (100 mg) was dissolved in chloroform (5 mL) and its carboxyl group was activated by DCC (23.8 mg, 0.115 mmol) for 4 h at room temperature. Amino-PVP (231 mg, 0.115 mmol) was dissolved in chloroform (5 mL). Then, it was added in the lipid solution. After stirring for 24 h at room temperature, the solvent was removed and the lipid-polymer conjugate was washed twice with cold acetone. The formation of lipid-polymer was confirmed by FT-IR and ^{1}H-NMR. The reaction mechanism of amino-PVP and lipid is shown in Figure 1.

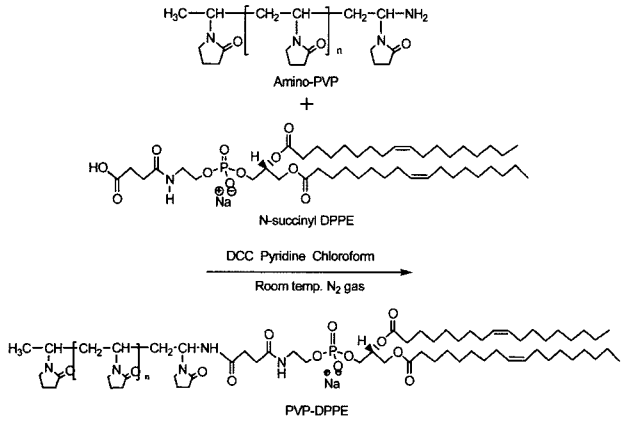

Figure 1. Reaction mechanism of amino-PVP and lipid.

Analysis of Lipid Nanoparticle. To prepare nanoparticles, lipid-polymer was dissolved in THF. The solution was dialyzed for 48 h using dialysis membrane (molecular weight cut-off: 2,000) against 2 L of distilled water (pH 7.4) at room temperature. The presence of nanoparticle and their size were determined using dynamic light scattering (Zeta-Plus, Brookhaven Instruments Corporation) at the fixed scattering angle of 90° and at the constant temperature of 25 °C. The size and shape of nanoparticle were also observed with atomic force microscope (AFM, Nano Scope IIIA, Digital Instruments) and transmission electron microscope (TEM, CM20, Philips).

To estimate critical aggregation concentration (CAC) values for lipid-polymer, the method was based on the solubilization of hydrophobic fluorescent dye, pyrene, in the micelle. After the addition of pyrene in micelles with various concentrations, the concentration of solubilized pyrene in the micellar phase was determined by the Spectrofluorophotometer (RF-5301 PC, Shimadzu) as wavelengths of excitation (λ_{ex}) 339 nm and emission (λ_{em}) 390 nm.

Drug Loading in Lipid Nanoparticles. To incorporate drugs in the micelle, griseofulvin (4 mg) as a hydrophobic drug was dissolved in acetonitrile, and then it was added in 8 mg/mL lipid-polymer solution (5 mL). The lipid-polymer solution with drugs was mixed by the ultrasonicator (1 min) and homogenizer (4 h). Mixtures were dialyzed for 24 h against PBS buffer solution (pH 7.4) at room temperature. The griseofulvin concentration was estimated by HPLC (LC-6A, Shimadzu) at wavelength of 293 nm. The shape of drug-loaded nanoparticles was observed with AFM.

***In vitro* Drug Release from Lipid Nanoparticles.** The release of griseofulvin from the nanoparticles in the dialysis membrane (molecular weight cut-off: 1,000) was examined in 95 mL of PBS buffer solution (pH 7.4) at 37 °C with 70 rpm strokes. At scheduled time intervals, the released media was collected and estimated by HPLC. After the sampling, the entire media was replaced with the fresh PBS buffer solution to maintain the sink condition.

Results and Discussion

Characterization of Amino-PVP. Amino-PVP was prepared by the free radical polymerization of VP in dioxane with 2-mercaptoethylamine as the chain transfer agent. The molecular structure of the amino-PVP was identified by ^{1}H-NMR and FT-IR. The spectra are shown in Figure 2. In NMR spectra, we confirmed the presence of amino group in PVP because of the peak at 2.07 ppm. It was also confirmed at 1250 cm^{-1} (C-N) in FT-IR spectra. A strong peak at 3500 cm^{-1} is -OH peak. This is due to the hydrophilic property of PVP. The molecular weight of amino-PVP was about 2,000 estimated by GPC.

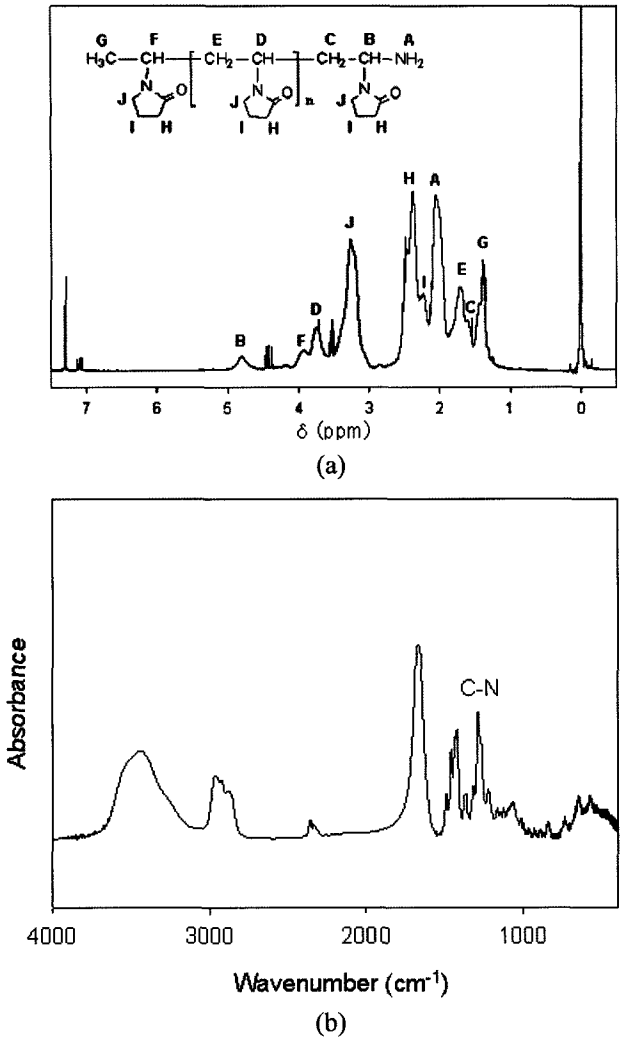

Figure 2. Spectra of amino-PVP. (a) ^{1}H-NMR spectra in $CDCl_3$ and (b) FT-IR spectra.

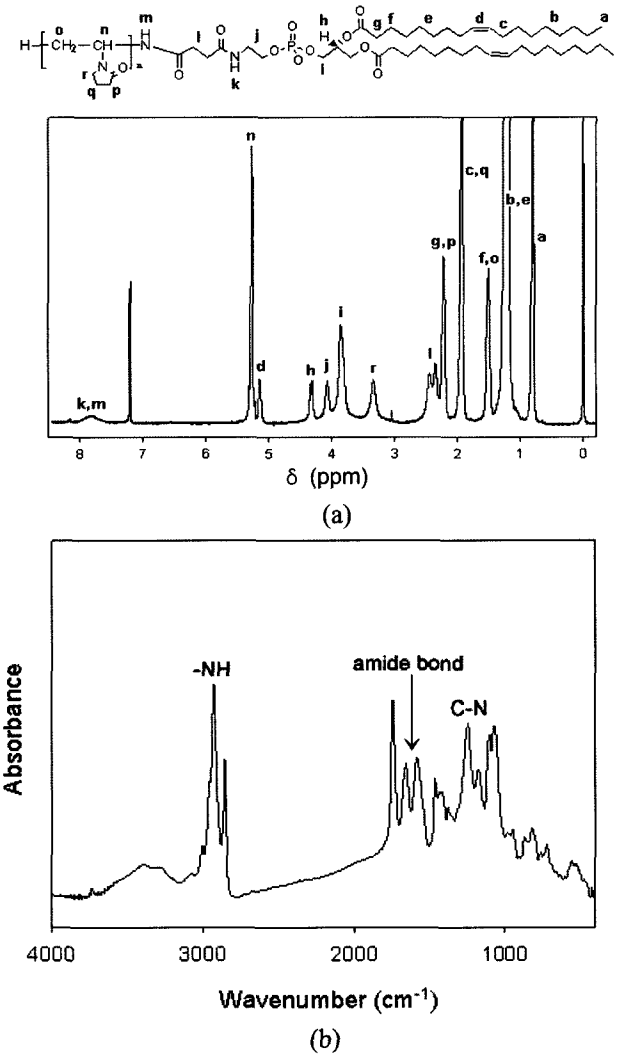

Figure 3. Spectra of the lipid-polymer system. (a) ^{1}H-NMR spectra in $CDCl_3$ and (b) FT-IR spectra.

Characterization of Lipid-Polymer. For the reaction between lipid and polymer, the carboxyl group of *N*-succinyl DPPE was activated by DCC. The activated carboxyl group was conjugated with amino-PVP to introduce the amide bond. The structure of lipid-polymer was identified by ^{1}H-NMR and FT-IR. It is shown in Figure 3. In NMR spectra, we confirmed the presence of amide bond because of the peak at 7.79 ppm. The peaks of 1.22 and 5.26 ppm showed the conjugation reaction between amino-PVP and *N*-succinyl DPPE. The peak at 1.22 ppm is repeating methin group of *N*-succinyl DPPE and the peak at 5.26 ppm is repeating methylene group of amino-PVP. In FT-IR spectra, a new peak was appeared at 1650 cm^{-1} compared with amino-PVP. The conjugation of amino-PVP and lipid was also confirmed by the peak at 1650 cm^{-1} (amide bond), 1250 cm^{-1} (C-N) and 3000 cm^{-1} (-NH). The lipid and PVP was successfully conjugated with our reaction condition.

Determination of CAC. The formation of nanoparticles from lipid-polymer was verified by the fluorescence spectroscopy using pyrene as a probe. The intensity ratio of I_{342}/I_{338} from pyrene excitation spectra are plotted against the concentration of lipid-polymer system. At low concentration, the low pyrene intensity was observed but it was increased beyond a specific concentration. Thus, the graph was formed with two linear segments having different slopes. The intersection point of these two segments gave CAC value, as shown in Figure 4. The CAC of lipid-polymer was 0.001 mg/mL.

Measurement of Particle Size and Shape. The lipid-polymer can be self-assembled in the aqueous solution. Dynamic light scattering method was used to measure the hydrodynamic diameter of lipid-polymer particle in PBS buffer solution. As shown in Figure 5, the mean diameter of lipid-polymer particle was 72.7 nm. The morphology of

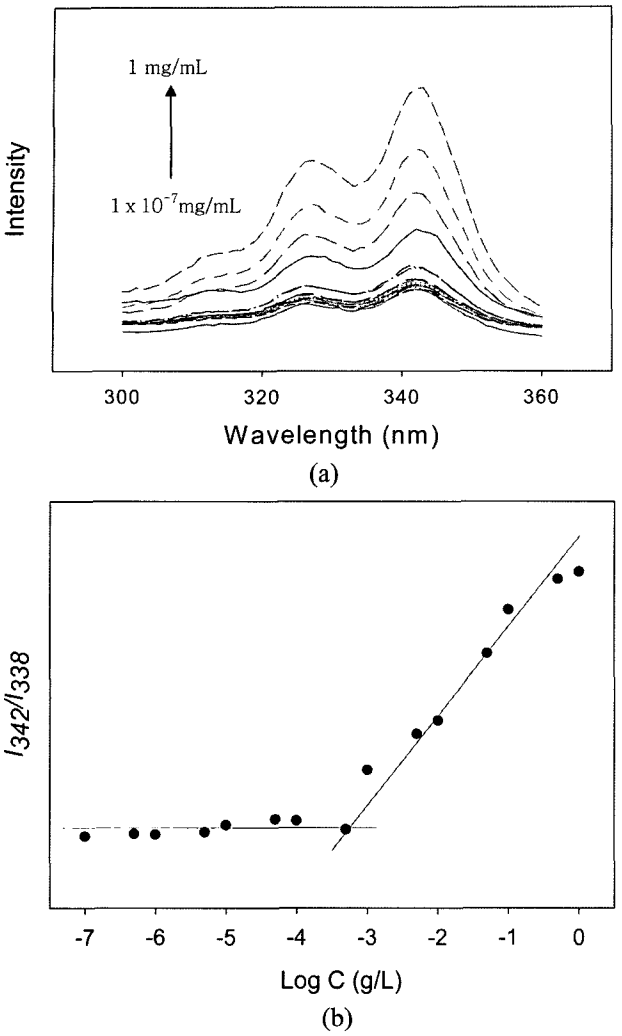

Figure 4. (a) Excitation spectra of pyrene with various lipid-polymer concentrations (1×10^{-7}~1 mg/mL) and (b) plots of fluorescence intensity of pyrene vs. logarithmic concentration of the lipid-polymer system.

lipid-polymer was confirmed by AFM and TEM as shown as Figures 6 and 7. The lipid nanoparticle had the spherical shape. The particle size from AFM was smaller than that determined by DLS. It was due to the collapse of particles during the sample preparation of AFM. The drug-loaded lipid-polymer had the larger size than lipid-polymer without the hydrophobic drug in the AFM images. Spherical nanoparticles were also confirmed in the TEM image.

Drug Loading and Release Test. The amount of drug in the solution was estimated by HPLC. The loading efficiency of drug in nanoparticles was calculated using the below equation.

$$\text{Drug loaded (\%)} = \frac{\text{the amount of drug loaded}}{\text{the amount of entire drug}} \times 100$$

The drug loading efficiency of nanoparticle was about

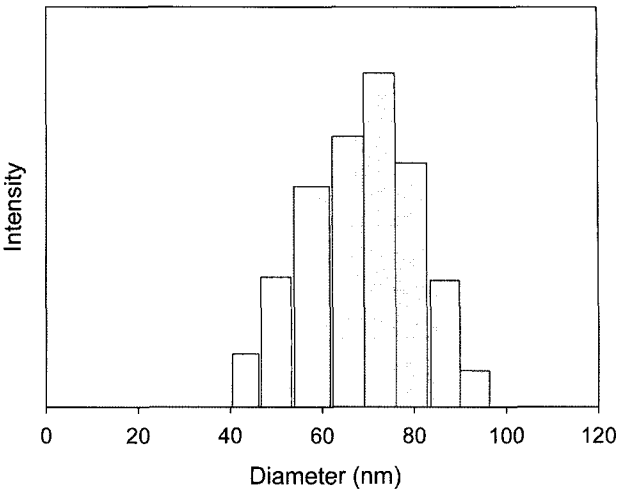

Figure 5. Particle size distribution of the lipid-polymer system by DLS.

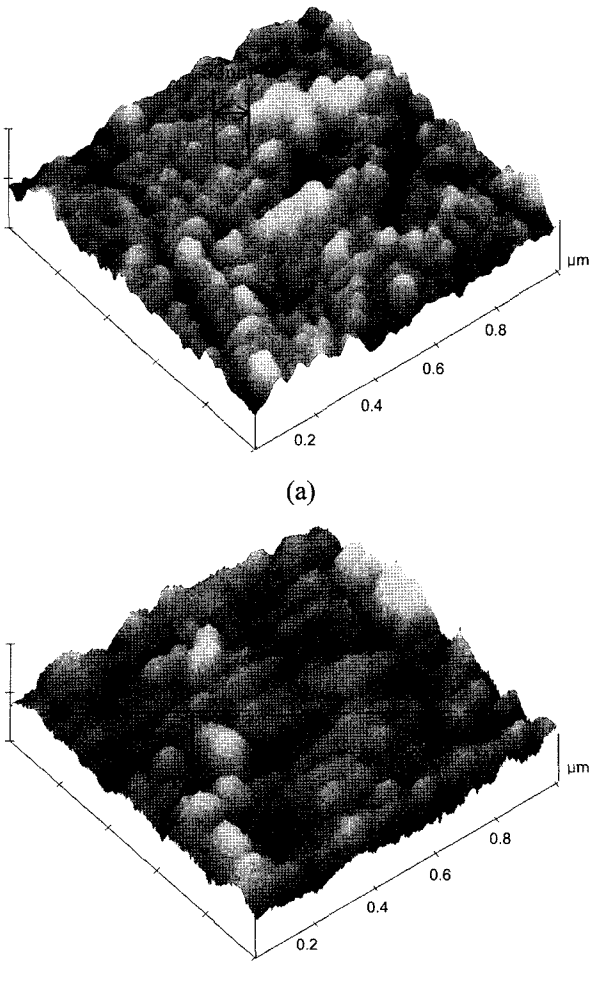

Figure 6. AFM image of the lipid-polymer system. (a) pure lipid-polymer system and (b) drug-loaded lipid-polymer system.

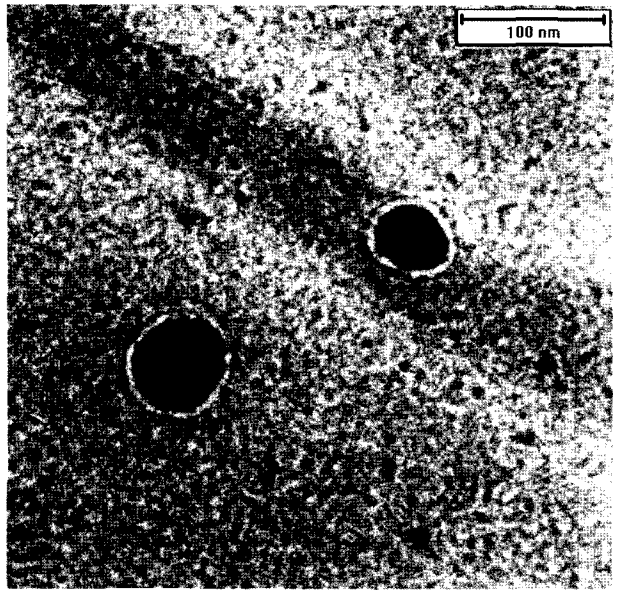

Figure 7. TEM micrograph of the lipid-polymer system after the freeze-drying.

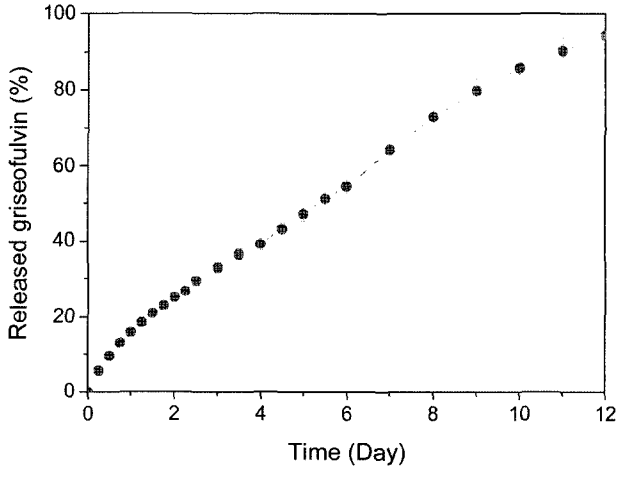

Figure 8. *In vitro* release profiles of griseofulvin from the lipid-polymer system.

54%. The drug-loaded nanoparticle was investigated to get the *in vitro* release profile. The release test was performed in the mimicking condition of biological systems. No initial burst effect was observed and the slope of release profiles was almost linear. It is possible to release the drug constantly for the prolonged time (12 days). Although drugs are used for the medical treatment, they might have the side effects. The side effects are occurred when the drug is medicated in the body with the excess amount of the dosage which is required for the treatment. Our lipid-polymer nanoparticle could release the drug steadily with the constant rate. Hence, it might easily control the amount of released drug within the therapeutic index. It could also sustain the efficacy of drug for 12 days after one injection. Our system can be used for the patient who needs the drug consistently.

Conclusions

Amino-PVP could decrease the cytotoxicity and increase the hydrophilicity of the lipid system. It is possible to use as a long circulating drug delivery carrier. In this study, lipid-polymer system was formed the self-aggregated lipid nanoparticle in the aqueous solution. The CAC values of lipid-polymer system were 1×10^{-3} g/L. The sizes and shapes of particles were determined by DLS, AFM and TEM. It was the spherical particle with about 70 nm size. The inner part of the nanoparticle can incorporate hydrophobic drugs due to the hydrophobic interactions. Griseofulvin was used as the model drug. The drug loading efficiency is about 54%. The release behavior was sustained for the prolonged time (12 days). We can use our lipid-polymer system with biodegradable and biocompatible properties as a passive-targeting drug delivery carrier because of its small particle size.

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