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# Release of adriamycin from poly(γ-benzyl-L-glutamate)/poly(ethylene oxide) nanoparticles

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**Abstract**

Prolonged circulation of anticancer agent in blood is expected to decrease the host toxicity and enhance the anticancer activity. The purpose of this study is to develop and characterize the prolonged and sustained release formulation of anticancer agent using biodegradable poly(γ-benzyl-L-glutamate)/poly(ethylene oxide) (PBLG/PEO) polymer nanoparticles. PBLG/PEO polymer is a hydrophilic/hydrophobic block copolymer and forms a micelle-like structure in solution. Spherical nanoparticles incorporating adriamycin were prepared by a dialysis method. The fluorescence intensity of adriamycin in the nanoparticles was increased when sodium dodecylsulfate was added. It is one of the evidences of entrapment of adriamycin in the polymer nanoparticles. Only 20% of entrapped drug was released in 24 h at 37°C a and the release was dependent on the molecular weight of hydrophobic polymer. The endothermic peak of adriamycin at 197°C disappeared in the nanoparticles system, suggesting the inhibition of a crystallization of adriamycin by polymer adsorption during the precipitation process. The mean residence time of adriamycin from the nanoparticles was more than threefold that from a free adriamycin. These results suggest usefulness of PBLG/PEO nanoparticles as a sustained and prolonged release carrier for adriamycin. 

*Keywords:* Adriamycin; Nanoparticle; Poly(γ-benzyl-L-glutamate)/poly(ethylene oxide); Sustained release

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## 1. Introduction

In recent years, microspheres, liposomes, and biodegradable polymers have been used to develop the site-specific drug delivery systems. Especially, nanoparticles have been widely investigated as the drug carriers (Brannon-Peppas, 1995; Langer et al., 1997; Maruyama, et al., 1997; Paul et al., 1997). Biodegradable poly(D,L-lactide) (Coffin and McGinity, 1992; Le Ray et al., 1994), polybutylcyanoacrylate (Zhang et al., 1998) and poly(ε-caprolactone) (Masson et al., 1996) are being used to prepare nanoparticles. The advantages of the nanoparticles are the reduced drug toxicity, the improvement of biodistribution, and the increased therapeutic efficacy.

Diblock copolymers have been studied in the sustained release system as an alternative drug carrier (Gao and Eisenberg, 1993; Hruska et al., 1993) since they are known to form a micelle structure. Hydrophilic–hydrophobic diblock copolymers exhibit amphiphilic behavior and form micelles with core-shell architecture. These polymeric carriers have been used to solubilize hydrophobic drugs, to increase blood circulation time, to obtain favorable biodistribution and to lower interactions with reticuloendothelial system (Yokoyama et al., 1991; Kataoka et al., 1993; Kwon et al., 1994). In the present study, we report the preparation and characterization of the polymeric nanoparticles containing anticancer drug. The nanoparticles are obtained from the poly(γ-benzyl-L-glutamate)/poly(ethylene oxide) (PBLG/PEO) diblock copolymer, which form a hydrophobic inner core and a hydrophilic outer shell of micellar structure (Cho et al., 1997; Jeong et al., 1998). Diblock copolymer nanoparticles were prepared through a dialysis procedure in an aqueous milieu. Adriamycin was used as a model drug. It intercalates into DNA, interacts with plasma membranes and forms free radicals through bioreductive activation (Sinha et al., 1989; Kataoka et al., 1993). The in vitro and in vivo release profiles of adriamycin from polymeric nanoparticles were also examined to investigate the possibilities of achieving effective cancer chemotherapy.

## 2. Materials and methods

### 2.1. Materials

PBLG/PEO block copolymers were prepared by the previously reported method (Cho et al., 1990). For the synthesis of PBLG/PEO block copolymers we used the mono amine-terminated PEO with an average molecular weight of 12 000. We attempted to produce copolymer chains with different size of PBLG blocks by changing the mole ratio of benzyl-L-glutamate monomer to PEG. Adriamycin was obtained from Dong-A Pharm. Ind. Co. (Seoul, Korea). Daunorubicin was purchased from Sigma Chemical Co. (USA). All solvents were of HPLC grade and other reagents were of analytical grade.

### 2.2. Preparation of adriamycin loaded nanoparticles

PBLG/PEO nanoparticles entrapped with adriamycin were prepared by a dialyzing method (Kwon et al., 1995). 25 mg of adriamycin HCl and 20 mg of PBLG/PEO were dissolved in 10 ml of dimethylformamide and triethylamine. The solution was stirred at room temperature until all the components are dissolved completely. To form core-shell type nanoparticles and to remove free drug, a dialysis bag containing drug solution was suspended in 1.0 *l* of 0.1 M acetate buffer (pH 5.5). Subsequently, $3 \times 1.0$ *l* of distilled water was used over 24 h to remove free adriamycin. The prepared nanoparticles were freeze-dried using a freeze dryer and were dried at 50°C for 6 h.

### 2.3. Determination of particle size and drug entrapment

The mean particle size and size distribution of nanoparticles were measured by dynamic light scattering particle size analyzer (Malvern, UK) with an argon laser beam at a wavelength of 488 nm. The value is expressed in number-averaged scales as unimode. The scattering angle of 90° was used. Nanoparticles were dispersed in distilled water for particle size measurement. The lyophilized sample was sonicated for 1 min in

Table 1
Average particle size and drug entrapment of nanoparticles depending on the PBLG chain length ($n = 3$)

| Sample | PBLG content (mole%) | Molecular weight | Drug entrapment (% w/w polymer) | Particle size (nm) |
|---|---|---|---|---|
| GE-1 | 60.5 | 103 700 | 12.6 | $362 \pm 28$ |
| GE-2 | 40.0 | 51 800 | 10.7 | $282 \pm 76$ |
| GE-3 | 12.4 | 20 400 | 7.3 | $250 \pm 34$ |

deionized water and the particle size was measured without filtering.

The size and shape of nanoparticles were observed by scanning electron microscopy (Jeol, Japan). A drop of the nanoparticle suspension was placed on a graphite surface. After freeze-drying, the sample was coated with gold/paladium using an ion sputter (Jeol, Japan). Observation was performed at 25 kV.

The amount of adriamycin in nanoparticles was determined by dissolving them in dimethylformamide. The concentration of adriamycin was measured by using UV spectrophotometer (Hewlett Packard, USA) at 484 nm.

### 2.4. Thermal and X-ray diffraction analysis

The thermal analysis of adriamycin, polymer and nanoparticles were performed on a TG/DTA thermal analyzer (Seiko, Japan). Each sample was scanned at a speed of 10°C/min under air purging. The temperature was increased from 20 to 350°C.

X-ray diffratometry was carried out using a diffractometer (Rigaku, Japan) with a nickel filter CuK$\alpha$ radiation operating at 40 kV. The scanning speed was 3°/min and sampling interval was 0.02°.

### 2.5. Fluorescence measurements

Fluorescence spectra of adriamycin were measured with a fluorescence spectrophotometer (Jasco FP-777, Japan). Emission spectra of adriamycin were recorded with excitation at 484 nm having 10 nm of bandwidths. The concentrations of adriamycin were adjusted to 10 μg/ml equivalents. Sodium dodecylsulfate solution was added in order to determine the effects of surfactant on the fluorescence of adriamycin entrapped in the nanoparticles. All of the fluorescence experiments were carried out at room temperature.

### 2.6. In vitro release of adriamycin

Dried sample (10 mg) was transferred to a dialysis tube and dialyzed against 10 ml of phosphate buffered saline solution at 37°C. The dialysis solution was agitated by a magnetic stirrer at 150 rpm. At various intervals, dialysis solution was taken and the same volume of the buffer solution was replaced. The concentration of drug was determined by measuring the fluorescence emission at 590 nm.

### 2.7. In vivo release

Male Sprague–Dawley rats weighing 130–150 g were obtained from Taehan Experimental Animal Center (Seoul, Korea). Water and food were freely supplied and they were stabilized for more than 2 weeks in a temperature-controlled environment (20–25°C). Under light ether anesthesia, the femoral vein and artery of rat weighing 200–250 g were cannulated with polyethylene tubing for drug administration and blood sampling, respectively. Bolus injection of sample equivalent to 4 mg/kg of adriamycin was made intravenously to each rat. Blood samples were withdrawn from the femoral artery at appropriate time intervals. After centrifugation at 12 000 rpm for 3 min, plasma samples were frozen at $-20$°C until they are analyzed. Adriamycin in rat plasma was analyzed using a method described by Rolland (1988) with some modification. Daunorubicin solution was used as an internal standard. The samples were extracted with 4 ml of ethyl acetate. After mixing organic phase with 100 μl of 0.1 N sulfuric acid

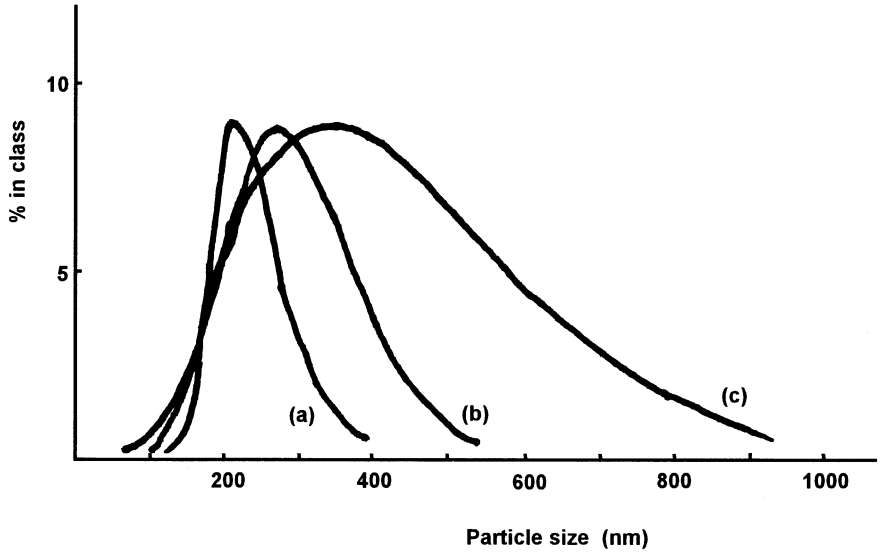


Fig. 1. Particle size distribution of adriamycin loaded polymer nanoparticles. (a) GE-3; (b) GE-2; (c) GE-1.

and centrifugation for 5 min, the aqueous lower phase was quickly transferred into a tube containing 100 μl of a 0.2 M sodium acetate/methanol solution. Intra-day and inter-day precision was tested using blank rat plasma samples spiked with adriamycin and daunorubicin.

### 2.8. Statistics and pharmacokinetic analysis

We used moment analysis for determination of pharmacokinetic parameters. All calculated values were expressed as mean $\pm$SE. Statistical differences were assumed to be significant when

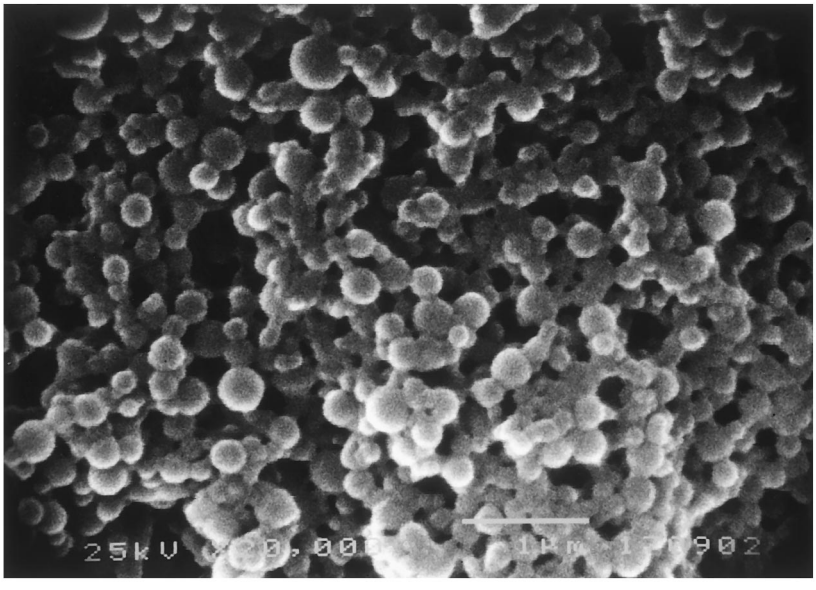


Fig. 2. Scanning electron micrograph of polymer nanoparticles (GE-3).

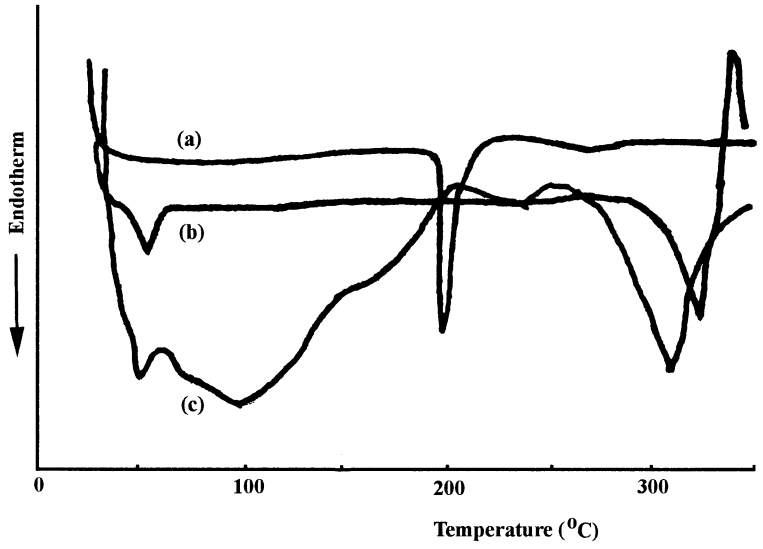


Fig. 3. DTA thermogram of (a) adriamycin, (b) PBLG/PEO block copolymer and (c) nanoparticles (GE-3).

$P < 0.05$ (Student's $t$-test). The total area under the plasma drug level versus time curve ($AUC_{0-Tn}$) was obtained by summation of each individual area between two consecutive time intervals using the trapezoidal rule.

## 3. Results and discussion

### 3.1. Preparation of polymeric nanoparticles

PBLG/PEO block copolymers having different size of PBLG blocks while constant size of PEO blocks were synthesized. The composition and number average molecular weight of the copolymers were shown in Table 1 and these estimation methods by NMR were described in other article (Jeong et al., 1998). The weight ratio of adriamycin entrapped into polymeric nanoparticles and the size of nanoparticles were shown in Table 1. GE-1 is the most hydrophobic polymer system tested due to the highest content of PBLG. Because free base of adriamycin is slightly soluble in water and the PBLG block is hydrophobic, GE-1 system had the highest loading content of adriamycin.

The particle size distribution of nanoparticles measured by dynamic light scattering was in the range of 0.1–1 μm as shown in Fig. 1. The size distribution of GE-3 system was the narrowest among tested. Generally, critical micelle concentration decreases and the micellar size increases, as the number of hydrophobic groups within a surfactant molecule increases (Florence and Attwood, 1988). In our study and the other study, block copolymers also exhibited similar tendency (Jeong et al., 1998). Number average size of adriamycin-loaded nanoparticles is presented in Table 1. The average size of the resulting nanoparticles was dependent on the size of hydrophobic block. The increase in the proportion of PBLG chain length resulted in an increase in the particle size. A scanning electron micrograph of adriamycin-loaded nanoparticles (GE-3 system) is presented in Fig. 2. Nanoparticles were found discrete and spherical with smooth surface. Most of the nanoparticles (GE-3 system) were in the size range of 50–500 nm. Although some nanospheres were fused together as shown in Fig. 2, the particles were dispersed easily in water by simple agitation.

### 3.2. Thermal and X-ray diffraction analysis

The thermal property of the adriamycin in the nanoparticle was investigated by DTA analyzer. The thermograms of free drug, polymer and

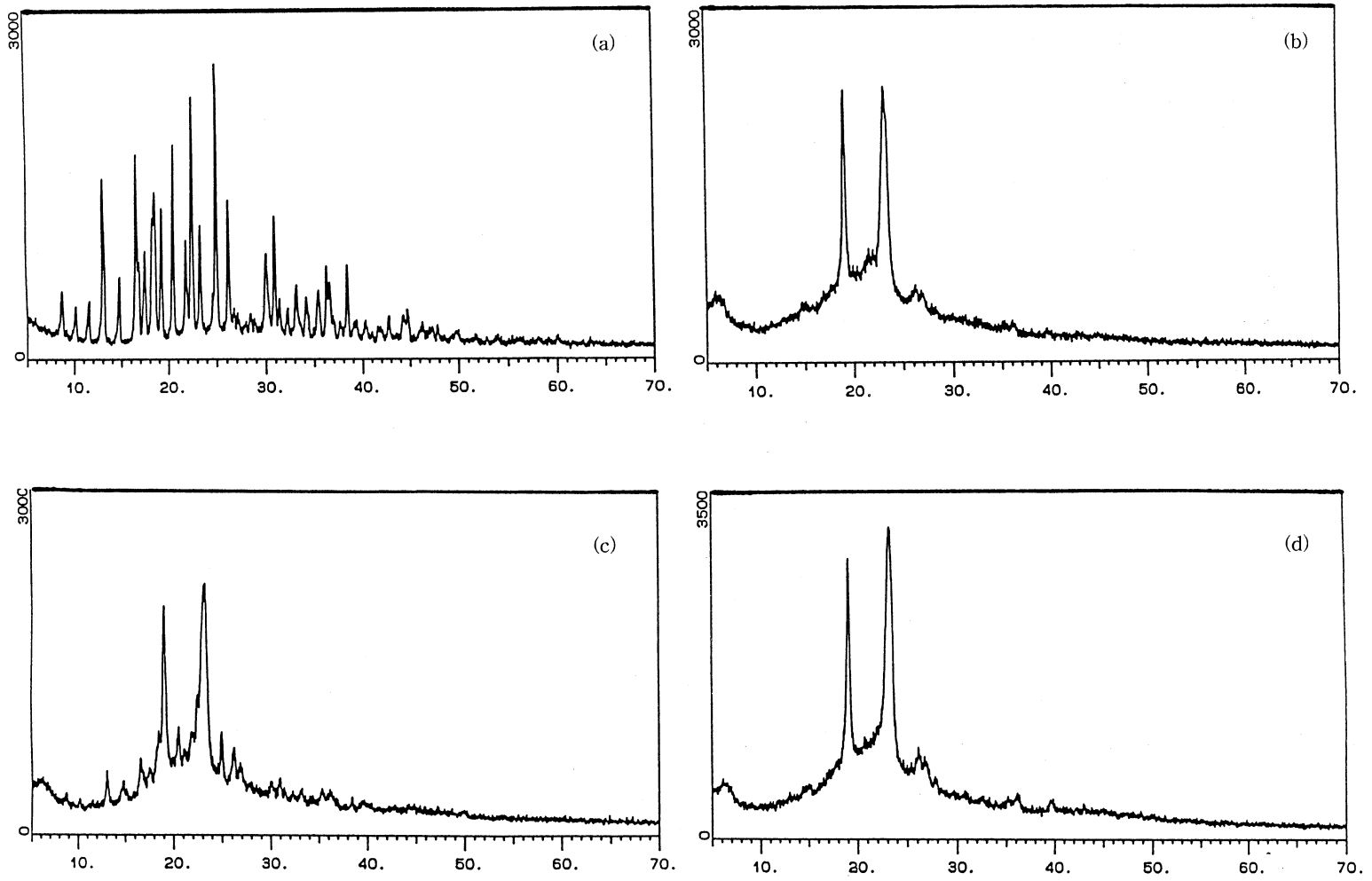


Fig. 4. Powder X-ray diffraction patterns of adriamycin and polymers. (a) adriamycin; (b) PBLG-PEO block copolymer; (c) physical mixture; (d) nanoparticles (GE-3).

nanoparticles are shown in Fig. 3. The adriamycin showed a single sharp endothermic peak at 197.31°C due to adriamycin melting. The polymer showed two peaks at 53.11 and 321.91°C. These peaks represent the melting point of PEO block and PBLG block, respectively. In the thermogram of the nanoparticles, the endothermic peak of adriamycin disappeared and those of polymer shifted to lower temperature. It is well known that polymers retard the crystal growth of drugs by adsorption at the surface of the crystal (Bhargava et al., 1996). The disappearance of adriamycin peak suggests that the crystallization of adriamycin was inhibited by the polymer during precipitation process. Adriamycin also interfered with the crystallization of polymer resulting in an imperfect crystal and a decreased melting temperature.

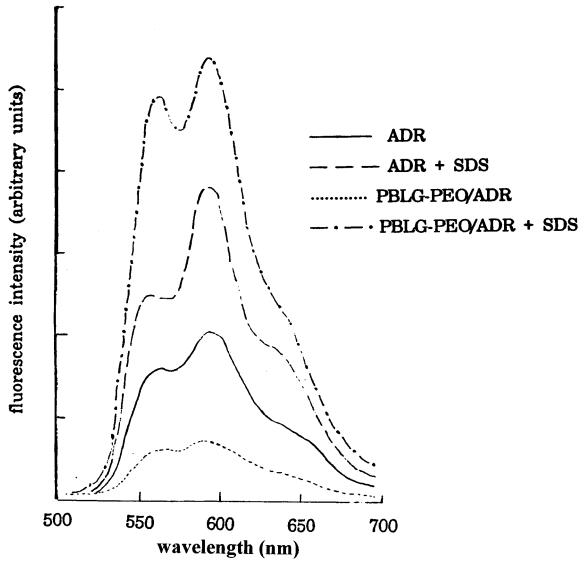


Fig. 5. Fluorescence emission spectra of adriamycin and PBLG-PEO/adriamycin nanoparticles (GE-3) in PBS (0.10 M, pH 7.4) with and without the addition of SDS (20 mg/ml). The concentration of adriamycin is equivalent to 10 μg/ml.

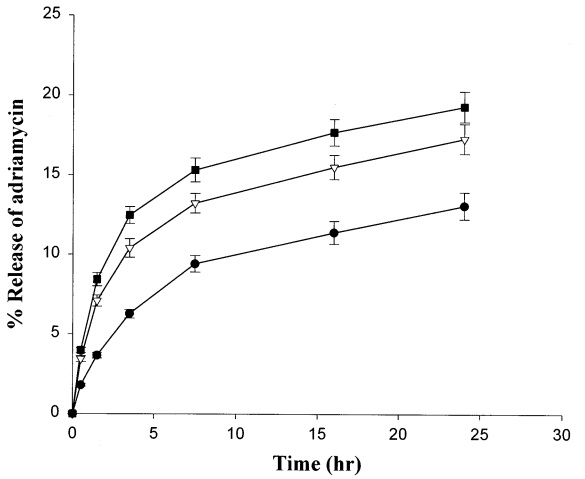


Fig. 6. Release of adriamycin from various PBLG/PEO block-copolymer nanoparticles at 37°C ($n = 3$). ●, GE-1; ▽, GE-2; ■, GE-3.

The absence of crystallinity of the drug from thermal analysis data may be due to the fact that drug was dissolved in the polymer during the DTA run. So we performed the powder X-ray diffraction study to investigate the crystallinity of adriamycin in the nanoparticles. Fig. 4 shows the powder X-ray diffraction patterns of adriamycin and nanoparticles. The diffraction pattern of the physical mixture was simply a superposition of those of the two components, while the nanoparticles showed the disappearance of characteristics of adriamycin. These results also indicate the production of the amorphous state of adriamycin in the nanoparticle system as thermal analysis.

### 3.3. Fluorescence studies

Fluorescence spectroscopy was used to examine the characteristics of core-shell type nanoparticles. When adriamycin-loaded nanoparticles were added to the PBS solution, a uniform colloidal dispersion was formed. Fig. 5 shows the fluorescence emission spectra of adriamycin. Fluorescence intensity of adriamycin in sodium dodecylsulfate micelle was higher than that in free state. The fluorescence intensity of adriamycin nanoparticle was also increased by the addition of sodium dodecylsulfate. The higher intensity results from the disruption of the PBLG/PEO nanoparticles containing adriamycin and the formation of micelle by sodium dodecylsulfate (Bahadur et al., 1988). A similar tendency was observed from the study with poly(ethylene oxide-co-β-benzyl-L-aspartate) nanoparticle system (Kwon et al., 1995). This result was the evidence of the entrapment of adriamycin in the core-shell type nanoparticles.

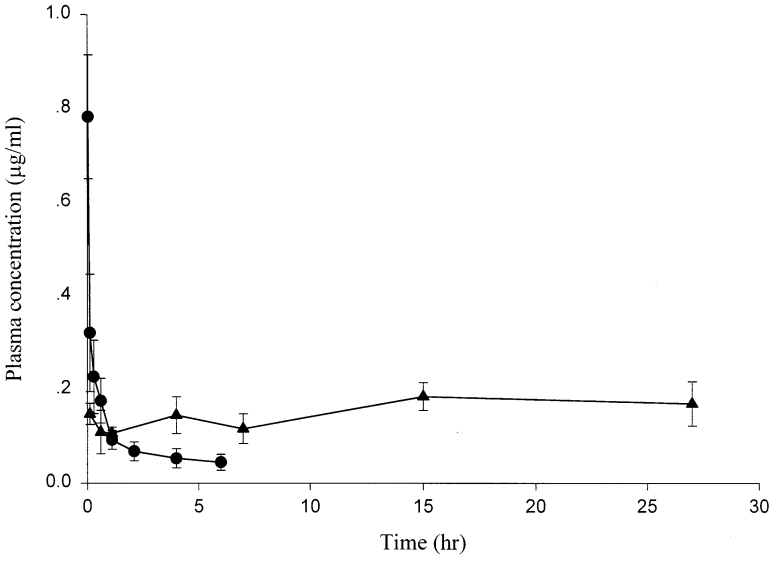


Fig. 7. Mean plasma concentration–time profiles of free adriamycin and PBLG-PEO/adriamycin nanoparticles ($n = 3$) by iv injection. Vertical bars represent the standard error of the mean. ●, free adriamycin; ▲, PBLG-PEO/adriamycin nanoparticles (GE-3).

### 3.4. In vitro and in vivo release

Fig. 6 shows the initial release profiles of adriamycin from PBLG/PEO nanoparticles as a function of time. In this release study, adriamycin-loaded nanoparticles showed markedly sustained release pattern. The release of adriamycin from the nanoparticles was dependent on the PBLG chain length. It was found that increase in PBLG chain length resulted in decrease in the release rate of adriamycin. This fact suggests that the more hydrophobic domain of polymer makes it possible to have stronger hydrophobic interaction.

Plasma concentration of adriamycin was determined by HPLC method. Coefficient of variation of concentration obtained on the same day and for several days was less than 5.9% for adriamycin and 4.5% for daunorubicin. The recovery rates of adriamycin and daunorubicin measured by the peak height ratios were 88.2 and 90.1%, respectively. Concentration profiles of adriamycin in blood after i.v. injection of polymeric nanoparticles (GE-3 system) and free adriamycin solution are shown in Fig. 7. Adriamycin-loaded nanoparticles showed significantly lower initial concentration and almost constant plasma level up to the end of the experiment when compared to free adriamycin. Some pharmacokinetic parameters were obtained from the concentration–time curves using the non-compartment model as shown in Table 2. Here, AUC, MRT and CL/F are the area under curve, the mean residence time and clearance, respectively. In comparison with free adriamycin, MRT of adriamycin in nanoparticles were significantly ($P < 0.05$) increased. The MRT of a drug provides a useful estimate of its persistence time in the body. This increase in MRT with nanoparticles may result from the slow release rate of adriamycin from nanoparticles.

In summary, adriamycin-loaded PBLG/PEO nanoparticles were prepared to develop a sustained release delivery of anticancer agent.

Table 2
Pharmacokinetic parameters of adriamycin

| Sample | AUC | MRT | CL/F |
|---|---|---|---|
| Free adriamycin | $0.844 \pm 0.075$ | $6.25 \pm 0.72$ | $4.74 \pm 0.94$ |
| Nanoparticles | $0.749 \pm 0.062$ | $23.78 \pm 3.54$ | $40.05 \pm 5.31$ |

Nanoparticles were prepared by dialysis method. Particle size, size distribution, thermal analysis, X-ray diffraction and fluorescence spectroscopy were carried out to characterize nanoparticles. Release of adriamycin from nanoparticles in vitro and in vivo was slow as expected. These results indicate that PBLG/PEO nanoparticles system is useful for the sustained release of adriamycin.

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