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Polymeric nanoparticle composed of fatty acids and poly(ethylene glycol) as a drug carrier

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Abstract

Diamine-terminated poly(ethylene glycol) (ATPEG) was hydrophobically modified with long-chain fatty acids (FAs) through a coupling reaction using *N*,*N*′-dicyclohexyl carbodiimide (DCC). FA–PEG–FA conjugates have different physico-chemical properties according to the chain length of the fatty acid (FA). Synthesized FA–PEG–FA conjugate was confirmed by Fourier transform-infrared (FT-IR). Since FA–PEG–FA conjugates have the amphiphilic characteristics in aqueous solution, polymeric nanoparticles of FA–PEG–FA conjugates were prepared using a simple dialysis method in water. The results of ^{1}H nuclear magnetic resonance (NMR) spectroscopy and fluorescent spectroscopy suggest that the FA–PEG–FA conjugate has a typical core–shell type nanoparticle structure made by a self-assembling process. From the analysis of fluorescence excitation spectra, especially, the critical micelles concentration (CMC) of this conjugate was changed unpredictably, i.e. the critical association concentration (CAC) value was decreased below a FA carbon number of 16 but, above increased a FA carbon number of 16. Transmission electron micrograph readings showed the spherical morphologies of the polymeric nanoparticles. The particle size was continuously decreased until below a FA carbon number of 20, but it was increased above a FA carbon number of 20. Clonazepam (CNZ), as a model drug, was easy to entrap into polymeric nanoparticles of the FA–PEG–FA conjugates. The drug release behavior was changed according to the FA chain length and was mainly diffusion controlled from the core portion.

Keywords: Poly(ethylene glycol); Fatty acid; Core–shell type; Polymeric nanoparticles

1. Introduction

Nanoparticles are colloidal particles ranging in size from 10 to 1000 nm, and they are extensively employed for targeted drug delivery systems.

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Nanoparticles have several advantages over conventional drug carriers; small particle size, ease of administration, drug targeting to the specific body site, solubilization of hydrophobic drug, nanoparticles avoid the reticuloendothelical system (RES), and reduced side effects of anticancer drugs. (Yokoyama et al., 1990; Kreuter, 1991; Kwon et al., 1995).

Recently, block copolymers or polymeric conjugates were synthesized to make core–shell type nanoparticles and polymeric micelle. In previous studies, we have synthesized poly(L-lactic acid)/poly(*N*-isopropylacrylamide) block copolymers and the core–shell type nanoparticles were prepared by a dialysis method. In this report, we showed that reversible size changes were observed as a result of a lower critical solution temperature (LCST) characteristic of poly (*N*-isopropylacrylamide (PNIPAAm)) (Kim et al., 2000a). In another report, we showed that triblock copolymers of poly(ε-caprolactone)/poly(ethylene glycol)/poly(ε-caprolactone) were synthesized to make core–shell nanoparticles and the drug release mechanism of hydrophobic drug was governed by a diffusion process rather than by a degradation of polymers(Ryu et al., 2000). Poly(ethylene glycol) (PEG) is popularly used as a hydrophilic polymer in such core–shell structure systems due to their good water-solubility, non-toxic, non-immunogenic, and biocompatible characteristics. PEG consists of a hydrophilic outer shell in the core–shell structure, and it represents the protective behavior of the blood protein and reticuloendothelial system (RES) uptake by the hydrophilic nature (Lee et al., 1989).

In this study, we synthesized the amphiphilic polymeric conjugate composed of the diamine-terminated PEG (ATPEG) and various fatty acid (FA) chain-length to use as a noble drug carrier. ATPEG has two amine groups at both terminals, so it can be easily modified by the carboxylic groups of FA. FAs are believed to be non-toxic since they exist fluently in the body. It can be expected that FAs form hydrophobic inner cores due to their water-insoluble characters. We have used several for conjugation such as decanoic acid (C_{10}), lauric acid (C_{12}), myristic acid (C_{14}), palmitic acid (C_{16}), stearic acid (SA, C_{18}), arachidic acid (C_{20}), lignoceric acid (C_{24}), and octacosanoic acid (C_{28}). We examined the effects of various FAs on the formation of polymeric nanoparticles, physicochemical properties, and drug release behaviors.

2. Materials and methods

2.1. Materials

ATPEG with a number-average molecular weight of 2000 was supplied by the Texaco Chem. Co. (Ballaire, TX). FAs used in this study were decanoic acid and octacosanoic acid obtained from the Aldrich Chemical Co. (Milwaukee, USA), myristic acid and SA obtained from the Wako Pure Chemical Industries, Ltd. (Tokyo, Japan), palmitic acid, arachidic acid, and lauric acid purchased from the Sigma Chemical Co. (St. Louis, USA), lignoceric acid obtained from the Tokyo Kasei (Tokyo, Japan). The coupling agent *N*,*N*′-dicyclohexyl carbodiimide (DCC) was obtained from the Aldrich Chemical Company (Milwaukee, USA). *N*-hydroxysuccinimide (NHS) was purchased from the Sigma Chemical Co. (St. Louis, USA). The dialysis membranes with a molecular weight cutoff (MWCO) of 2000 g/mol were purchased from Spectra/Por™ Membranes. Tetrahydrofuran (THF), dimethyl sulfoxide (DMSO) and other chemicals were of reagent grade and used without further purification.

2.2. Synthesis of FA–PEG–FA conjugate

The FA–PEG–FA conjugate was prepared by conjugating carboxylic acid of FA and amine groups of ATPEG using DCC as a coupling agent (Fig. 1). FA (1 mmol), DCC (1.2 mmol), NHS (1.2 mmol) and ATPEG (0.5 mmol) were dissolved separately in DMSO. The DCC/DMSO solution was added to the FA/DMSO solution, and stirred for 30 min to activate the FA carboxyl group. The NHS/DMSO solution was added to an activated FA solution, and the reaction was conducted at room temperature for 12 h. In the reaction mixture, dicyclohexylurea (DCU) was formed, which was then filtered to remove the DCU. The ATPEG/DMSO solution was added to the reac-

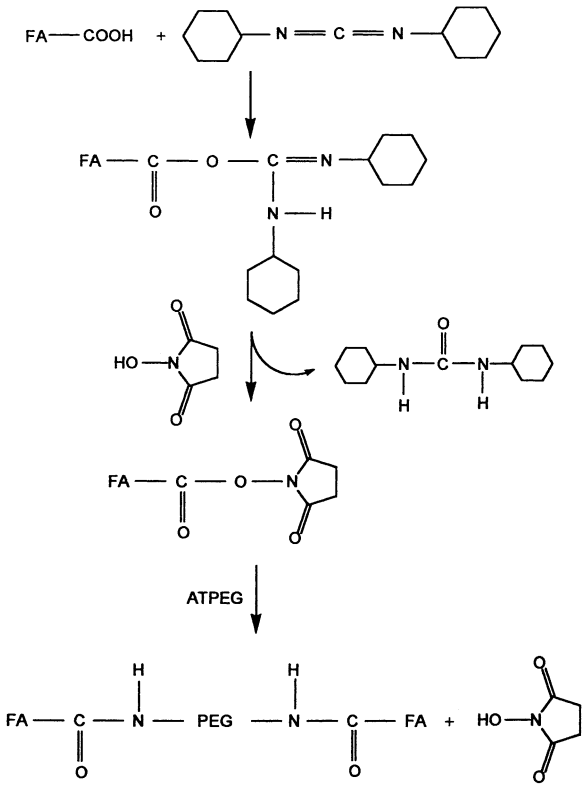

Fig. 1. Synthetic scheme of FA–PEG–FA conjugate.

tion mixture, and stirred for 30 min to complete the conjugation of activated FA and ATPEG. The resulting solution was placed in a dialysis membrane and dialyzed against distilled water for 7 days. The dialyzed solution was freeze-dried, and purified by repeated dissolving and re-dialyzing processes in THF and distilled water. The freeze-dried FA–PEG–FA conjugate was stored in a refrigerator at 4 °C until use.

2.3. Fourier transform-infrared (FT-IR) spectroscopy

Fourier transform-infrared (FT-IR) spectroscopy measurement (FT-IR Magna IR 550, Nicolet) was used to confirm the synthesis of FA–PEG–FA conjugate.

2.4. Preparation of FA–PEG–FA conjugate polymeric nanoparticles

The formation of FA–PEG–FA polymeric nanoparticles was carried out by the dialysis method (Kim and Kim, 2001). Twenty milligrams of FA–PEG–FA conjugate was dissolved in 5 ml of THF. To form polymeric nanoparticles, the solution was dialyzed against distilled water for 24 h using MWCO 2000 g/mol dialysis membrane. The dialyzed solution was then analyzed or freeze-dried.

In order to determine the core–shell structure of the FA–PEG–FA conjugate, the ^{1}H nuclear magnetic resonance (NMR) spectra was measured in $CDCl_3$ and D_2O using a 300 MHz spectrometer (Jeol, Japan).

2.5. Differential scanning calorimetry (DSC) measurement

The melting temperatures (T_m) of FA–PEG–FA polymeric nanoparticles were measured with a Universal V2.4F (TA instruments) differential scanning colorimeter. The measurements were carried out at temperatures from −80 to 250 °C under nitrogen at a scanning rate of 10 °C/min.

2.6. Wide angle X-ray diffractometer (WAXD) measurement

X-ray diffractograms were obtained using a Rigaku D/Max-1200 (Rigaku) with Ni-filtered Cuka radiation (35 kV, 15 mA).

2.7. Fluorescence spectroscopy

To investigate the core–shell structure formation and the critical association concentration (CAC), the FA–PEG–FA conjugate suspension was prepared as follows: 20 mg of the FA–PEG–FA conjugate was dissolved in 5 ml of THF and dialyzed using a MWCO 2000 g/mol dialysis membrane against distilled water for 1 day. The resultant solution in the dialysis membrane was then adjusted to various concentrations of the FA–PEG–FA conjugates.

The CAC of the FA–PEG–FA conjugate was estimated to demonstrate the potential for nanoparticle formation by a spectrofluorophotometer (Shimadzu RF-5301 PC, Tokyo, Japan) using pyrene as a hydrophobic probe (Kalyanasundaram and Thomas, 1977; Wilhelm et al., 1991; Kim and Kim, 2001). The samples were prepared by adding a known amount of pyrene in acetone to a series of 20 ml vials, and the acetone was then evaporated. The pyrene concentration was then adjusted to give a final concentration of 6.0×10^{-7} M in 10 ml of various concentrations of the FA–PEG–FA conjugate solution. The resulting solution was heated for 3 h at 65 °C to equilibrate the pyrene and the polymeric nanoparticles, and then left to cool overnight at room temperature. The emission wavelength was 390 nm for excitation spectra. The excitation and emission bandwidths were 1.5 and 1.5 nm, respectively.

2.8. Transmission electron microscope (TEM)

The morphologies of FA–PEG–FA polymeric nanoparticles were observed using a TEM (Jeol, JEM-2000 FX II, Japan) at 80 kV. A drop of polymeric nanoparticles suspension was placed on a copper grid coated with carbon film, and dried at 20 °C. The sample was negatively stained with 0.1% phosphotungstic acid.

2.9. Photon correlation spectroscopy (PCS)

Particle size distributions were measured by PCS using Zetasizer 3000 (Malvern Instruments, UK) with He–Ne laser beam at a wavelength of 633 nm (scattering angle of 90°). Polymeric nanoparticle suspension (concentration: 1 g/l) was used for particle size measurement without filtering.

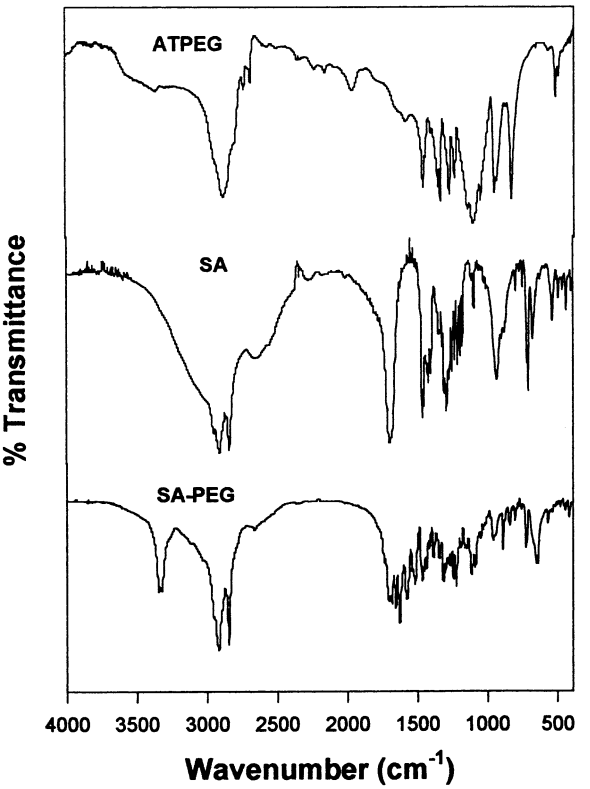

Fig. 2. FT-IR spectra of SA, ATPEG, SA–PEG–SA conjugate.

2.10. Drug loading and in vitro release studies

The clonazepam (CNZ)-loaded polymeric nanoparticles were prepared as follows: 20 mg of the

Table 1
Characteristics of Saturated FA

Common name	Number of carbon atoms	Molecular weight	Abbriviations
Capric acid	10	144.2	CA
Lauric acid	12	200.3	LA
Myristic acid	14	228.4	MA
Palmitic acid	16	256.4	PA
Stearic acid	18	306.5	SA
Arachidic acid	20	312.5	AA
Ligoceric acid	24	368.6	LiA
Ocatacosanoic acid	28	424.8	OA

FA–PEG–FA conjugate was dissolved in 4 ml of THF, and 20 mg of CNZ in 1 ml THF were added to this solution. To form the polymeric nanoparticles and remove the free drug, the solution was dialyzed against distilled water for 24 h using MWCO 2000 g/mol dialysis membrane. The medium was replaced every 1 h for the first 3 h and every 3 h for 21 h, and then freeze-dried.

In vitro release studies, 5 mg of the CNZ-loaded FA–PEG–FA and 1 ml of PBS (0.1 M, pH 7.4) were placed into a dialysis membrane (MWCO 2000 g/mol), and the dialysis membrane was placed into a 20 ml vial with 10 ml of PBS. The medium was stirred at 100 rpm at 37 °C. At set time intervals, the entire medium was removed and replaced with the same amount of fresh PBS. The amount of the CNZ released from the polymeric nanoparticles was measured with a UV spectrophotometer (Shimadzu UV-1201, Japan) at 306 nm.

3. Results and discussion

Hydrophobic FAs with various chain lengths were conjugated to ATPEG to make FA–PEG–FA conjugates. Fig. 1 shows the synthetic scheme of SA–PEG–SA conjugate by amide bond formation from the carboxyl group of FA and the amine group of ATPEG using DCC as a coupling agent. The FT-IR spectra of SA, ATPEG, and SA–PEG–SA conjugate are shown in Fig. 2, and the assigned characteristic peaks are given in Table 1. Especially, two characteristic peaks on this spectra, i.e. amide stretch absorption at 3330 per cm,

Table 2
Characteristic peaks assigned on the FT-IR spectra of the FA, ATPEG, and FA–PEG–FA conjugate

Wavenumber (per cm)	Assignments
3330	$-NH_2$
2920	$-CH_3$
2850	$-CH_2-$
1750	$-C=O$
1570	$-NH_2$
1460, 1380	$-CH_3$
1150–1050	C–O

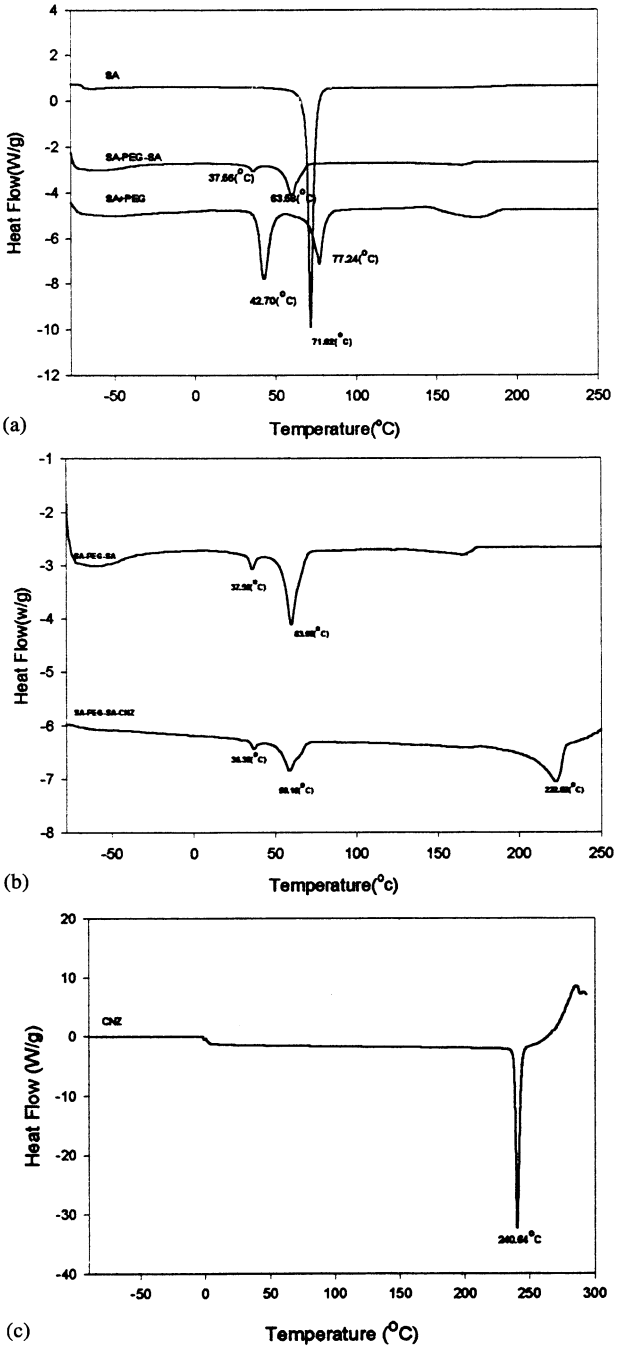

Fig. 3. DSC thermograms of SA, SA–PEG–SA polymeric nanoparticles, (a); SA, physical mixture of SA and PEG, and SA–PEG–SA (b); SA–PEG–SA and SA–PEG–SA–CNZ. (c); CNZ.

and amide bending at 1570 per cm, may be used to confirm the SA–PEG–SA conjugate (Table 2).

Fig. 3(a) shows the DSC thermograms SA–PEG–SA conjugate. As shown in Fig. 3, T_m of SA was observed at 71.62 °C whereas SA–PEG–SA has bimodal peaks, indicating that the lower melting peak, 37.56 °C are characterized to T_m of PEG and the higher melting peak, 63.58 °C was characterized to SA. These results were supported by the T_m of SA and PEG physical mixtures, i.e. SA and PEG showed the specific melting peak in the physical mixtures although each melting peak of SA and PEG of SA–PEG–SA conjugate were shifted to a lower temperature. To show the potential of SA–PEG–SA polymeric nanoparticles as a drug carrier, CZ-loaded polymeric nanoparticles were prepared and their thermo-properties were investigated. Fig. 3(b) shows the melting temperature of SA–PEG–SA and CZ-loaded polymeric micelles. Fig. 3(c) shows CZ itself melts at 240.6 °C whereas T_m of CZ for the CZ-loaded polymeric nanoparticles is 222.82 °C, indicating those parts of the entrapped drug exist in crystalline form. The melting point of CZ in CZ-loaded polymeric nanoparticles was decreased due to crystallization of CZ in the inner core of the polymeric nanoparticles (Jeong et al., 1998). These results indicated SA–PEG–SA polymeric nanoparticles have the potential as a drug carrier.

In aqueous media, it is expected that FA–PEG–FA conjugates can form the core–shell type polymeric nanoparticle structure through the self-assembling process since FA–PEG–FA conjugates have an amphiphilic characters. Due to the hydrophobic characters of FAs, the FA domain should be oriented towards the core of the polymeric nanoparticles while hydrophilic PEG is oriented in an outward direction as an outer shell of the polymeric nanoparticles.

The evidence of polymeric nanoparticles of FA–PEG–FA conjugate and limited mobility of the FA in the inner core of the polymeric nanoparticles were evidenced by ^{1}H NMR in $CDCl_3$ and D_2O as shown in Fig. 4. Since both of the SA and ATPEG are easily dissolved in $CDCl_3$ (Fig. 4(a)), the core–shell structure formation is not expected in an organic solvent. In $CDCl_3$, the characteristic peak of the methyl protons of the SA was shown

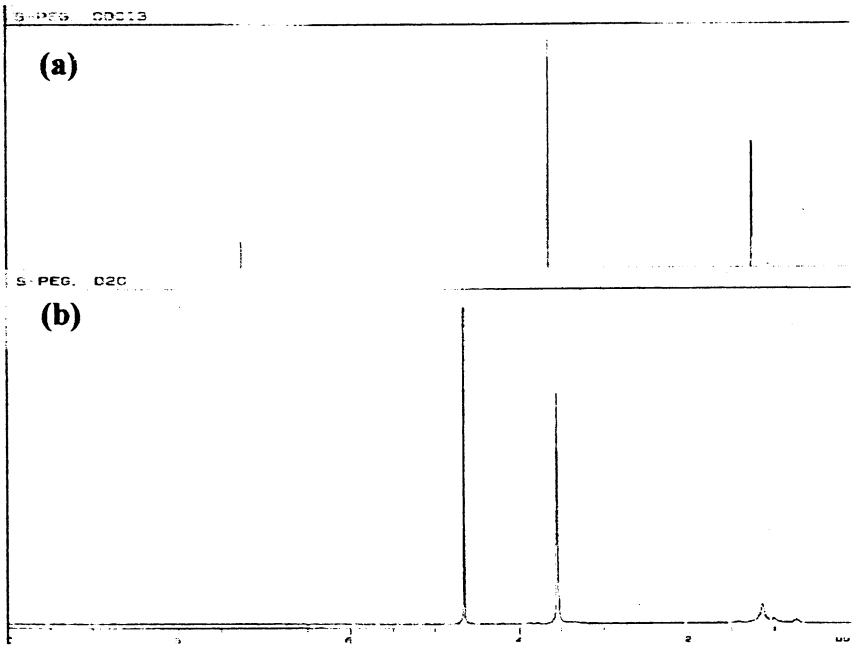

Fig. 4. ^{1}H NMR spectra of SA–PEG–SA conjugate polymeric nanoparticles dissolved in $CDCl_3$ (a) and redistributed in D_2O (b).

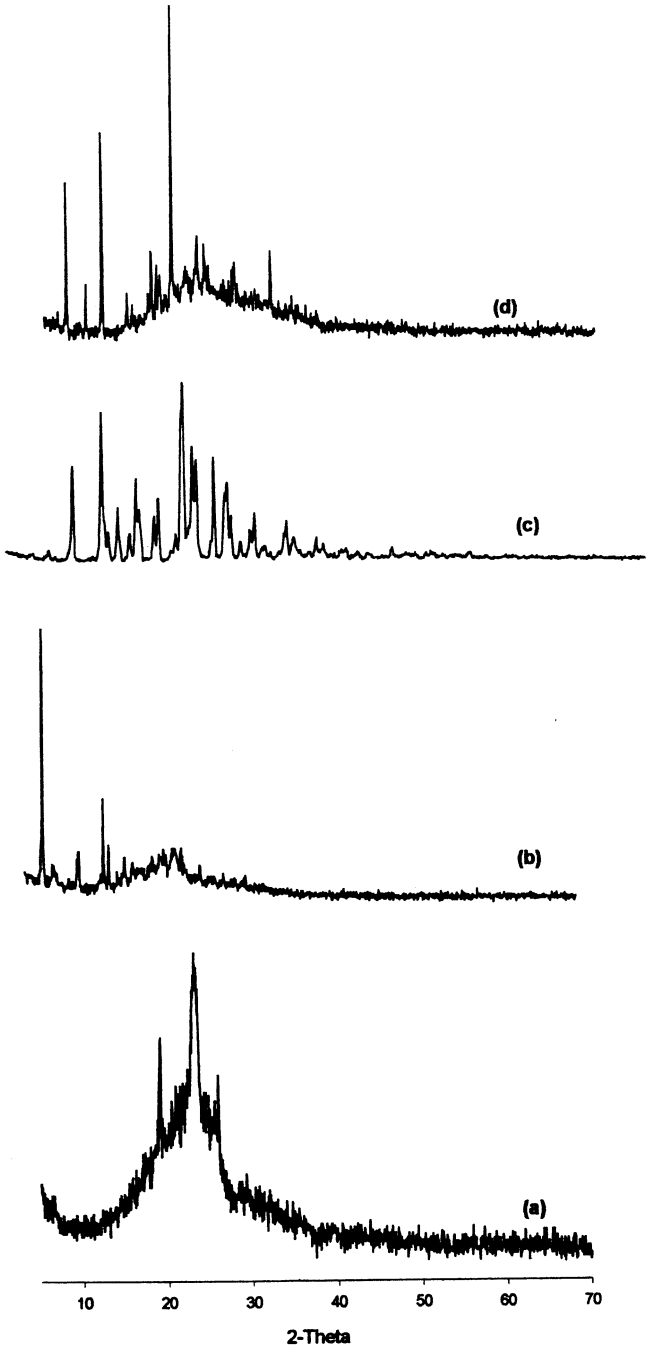

Fig. 5. WAXD patterns of FA–PEG–FA conjugates. NH_2–PEG–NH_2 (a); SA–PEG–SA (b); CNZ (c); CNZ-entrapped SA–PEG–SA nanoparticles (d).

about 0.9–2.4 ppm and protons of ethylene oxide of PEG segment were shown in 3.6 ppm. However, the characteristic peaks of SA were nonexistent in D_2O, whereas peculiar peaks of the ATPEG were remained wholly unchanged (Fig. 4 (b)). These results indicated that protons of SA display restricted motions within the inner core and are composed of a solid structure, whereas PEG domains are dissolved in the aqueous environment. This behavior of FA–PEG–FA core–shell type nanoparticles represents a similar tendency with poly(β-benzyl L-aspartate) (PBLA)/PEO diblock copolymer(Kwon et al., 1993). It was reported that the diblock copolymer has a rigid PBLA core.

To investigate the physico-chemical characteristics of CNZ-entrapped SA–PEG–SA core–shell type nanoparticles, X-ray powder diffraction was measured. Fig. 5 shows the X-ray powder diffraction scans of CNZ-loaded SA–PEG–SA core–shell type nanoparticles, SA–PEG–SA conjugates, and PEG. It can be observed that the X-ray diffraction patterns showed sharp peaks in CNZ drug crystals and in the SA–PEG–SA conjugates. When CNZ was entrapped into SA–PEG–SA core–shell type nanoparticles, the specific drug crystal peaks decreased rather than the CZ itself in the X-ray diffraction patterns. It was thought that drug crystallines showed sharply their specific crystal peak when it existed as a drug crystals but, that the drug existed as a molecular dispersion in the nanoparticles after drug entrapment into the nanoparticles (Gref et al., 1994). Also, these results showed that the CNZ was successfully entrapped into the nanoparticles as a molecular dispersion.

Fig. 6 shows the TEM photograph of SA–PEG–SA polymeric nanoparticles. The polymeric micelles of SA–PEG–SA showed spherical shapes with the size range of about 10–25 nm.

To evaluate CAC of polymeric nanoparticles of the FA–PEG–FA conjugate, the fluorescence spectroscopy was performed and pyrene was used as a hydrophobic probe (Wilhelm et al., 1991). Fluorescence emission spectra showed the increased intensity along with the concentration of FA–PEG–FA as previously reported (Kim et al., 2000a,b; Ryu et al., 2000). In the excitation spectra, a red shift was also observed with an

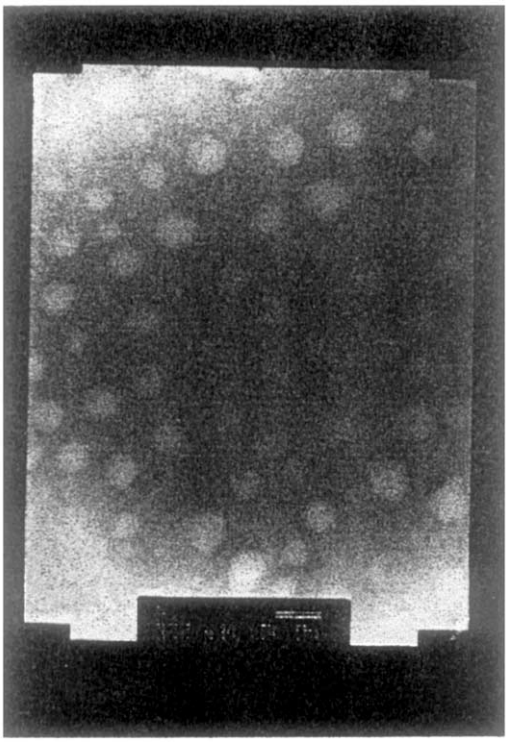

Fig. 6. TEM photograph of SA–PEG–SA polymeric nanoparticles.

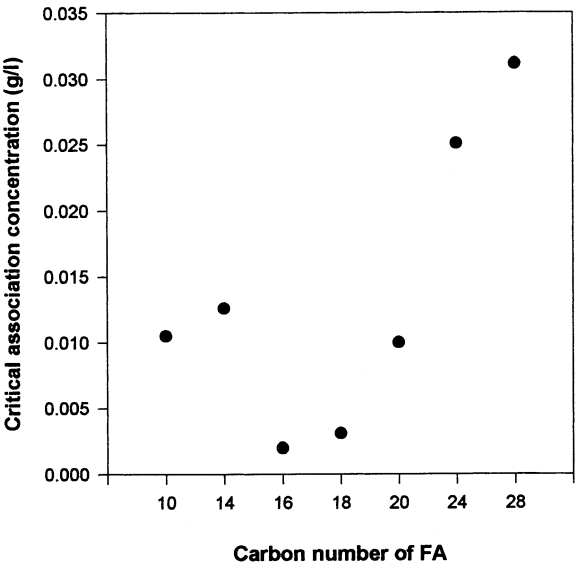

Fig. 7. CAC values against various FAs in the FA–PEG–FA conjugate.

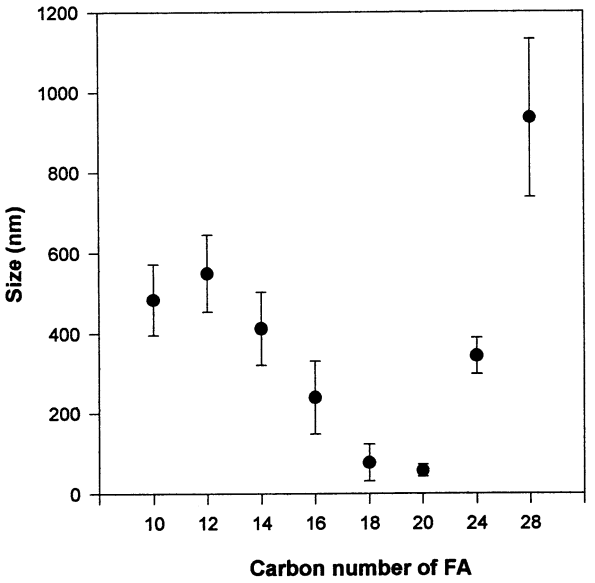

Fig. 8. Particle size distributions of FA–PEG–FA polymeric nanoparticles.

increasing concentration of FA–PEG–FA conjugate (not shown in figure). From the plot of I_{337}/I_{334} versus log C of the excitation spectra, the CAC values were taken from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low concentrations. The estimated CAC values of various FA–PEG–FA conjugate are shown in Fig. 7. The CAC values were decreased from C_{10} to C_{18}, but increased from C_{18} to C_{28}. In the short FA chains, as the carbon number decreases, the hydrophilicity of the conjugate increases. The FA–PEG–FA conjugate, therefore, aggregates to form the polymeric nanoparticles at high concentrations. In the longer FA chains, as the carbon number increases, the hydrophobicity of the conjugate increase. It can be expected that the CAC values increase by the hydrophobic/hydrophilic balance to form core–shell structure polymeric nanoparticles.

As shown in Fig. 8, the particle sizes decreased when increasing the carbon number, reached a minimum value for arachidic acid, and then significantly increased. According to these results, we proposed that the structure of polymeric nanoparticles were formed from FA–PEG–FA conjugates with different carbon chain of FA. At the short chain FAs, due to a decrease in the ratio of hydrophobic domains in the conjugate, the conjugate exhibited the increase in the hydrophilicity and then expected loosely packed polymeric nanoparticles as shown in Fig. 9. At the longer chain FAs, due to an increase in the ratio of hydrophobic domains in the conjugates, the conjugate exhibited the increase in the hydrophobicity and then expected larger and loosely packed polymeric nanoparticles (Piskin et al., 1995). By changing the ratio of hydrophilicity versus hydrophobicity, the conjugates produced a altered

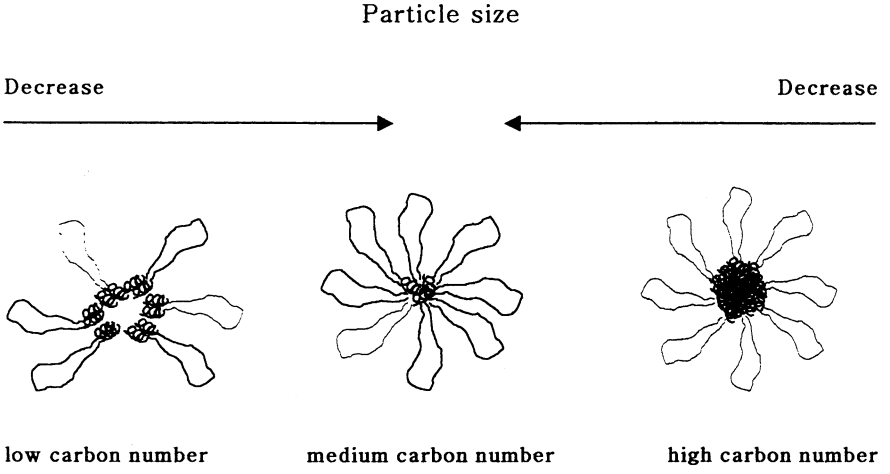

Fig. 9. Schematic illustrations of polymeric micelle formation of FA–PEG–FA conjugates according to the carbon-number.

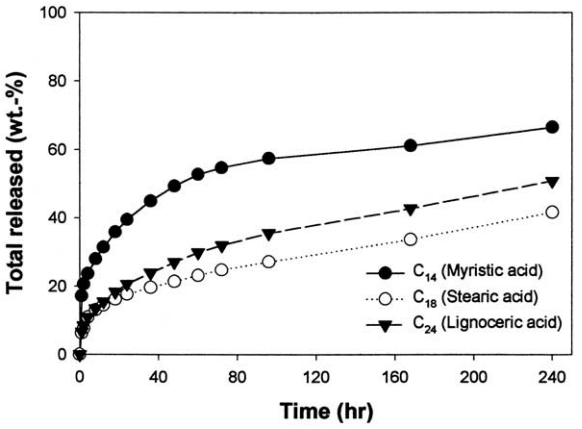

Fig. 10. CNZ release from the FA–PEG–FA polymeric nanoparticles in PBS (0.1 M, pH 7.4) at 37 °C($n = 3$). Drug loading content of C_{14}, C_{18}, and C_{24} were 16.2, 16.5, and 20.2%, respectively.

particle size, self-association behavior, and characteristics.

Fig. 10 shows the drug release from polymeric nanoparticles of various FA–PEG–FA conjugates. As shown in Fig. 10, polymeric nanoparticles with short carbon chain, myristic acid (C_{14})–PEG–myristic acid (C_{14}) conjugate, showed the significant initial burst effect during 40 h in drug release rate and more rapid drug release than polymeric nanoparticles with high carbon length, C_{18} (stearic acid) and C_{24} (lignoceric acid). These result supported by Fig. 9, indicating that particles, drug release and polymeric nanoparticles forming characteristics can be controlled by changing the carbon chain length of FA–PEG–FA conjugates.

Acknowledgements

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