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Synthesis and characterization of amphiphilic poly(ethylene glycol) graft copolymers and their potential application as drug carriers

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Amphiphilic graft copolymers comprising monomeric units of stearyl methacrylate, methyl acrylate, acrylic acid and poly(ethylene glycol) acrylate were synthesized and their properties in aqueous systems characterized. The structures of these copolymers were analysed by Fourier transform infra-red and nuclear magnetic resonance spectroscopies while their molecular weights were estimated by static light scattering. The study of critical micelle concentrations and micellar sizes indicated that the formation of micelles is primarily determined by the hydrophobic/hydrophilic properties of these copolymers. Encapsulation of pyrene (as a drug model) into the micelles was found to be dependent on their stearyl methacrylate content. These copolymers also exhibited a sustained release pattern for pyrene in aqueous solutions and might indicate their future applications as potential drug delivery systems. © 1997 Elsevier Science Ltd. All rights reserved.

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INTRODUCTION

Amphiphilic graft copolymers of poly(ethylene glycol) (PEG) have been the subject of increasing attention from the viewpoints both of their preparation strategies and physicochemical properties. In addition, interest in their applications, especially in the design of drug-delivery systems and for modification of biomedical polymer surfaces, has been rapidly growing owing to PEG's unique aqueous properties, i.e. its low protein adsorption and low cell adhesion in aqueous systems.

The preparation of amphiphilic PEG-grafted copolymers by either copolymerization of vinyl monomers with PEG macromonomers or synthesis of polymeric precursors followed by PEG conjugation via polymeric analogous reactions, has been extensively reported[1–14]. For example, Bo *et al.* successfully prepared various comb-shaped copolymers from PEG monomethacrylate by free-radical polymerization[2]. However, a high concentration of PEG macromonomer should be excluded during polymerization to avoid gel formation. Lee *et al.* also prepared copolymers of alkyl methacrylates with methoxyPEG (mPEG) methacrylates for use as surface coatings to reduce protein adsorption[3]. Copolymerizations of the macromonomer, mPEG methacrylate, with methyl methacrylate, hexyl methacrylate and lauryl methacrylate, respectively, were carried out by free-radical polymerization.

The procedure for preparing comb-shaped PEG-grafted copolymers proposed by Wesslen and Wesslen, on the other hand, employed the method of polymeric precursors[4]. For example, polymerization of 2-ethylhexyl acrylate with glycidyl methacrylate was performed, followed by transesterification of mPEG in the presence of potassium methoxide. Twaik *et al.* and Thierry and Skoulis also grafted PEG onto poly(methyl methacrylate) in similar manners[5,6]. Derand and Wesslen reported that conjugation of mPEG onto the copolymers of styrene with maleic anhydride and/or methyl methacrylate resulted in anionic, PEG-containing, comb-shaped copolymers[7]. In addition, a more complex process for preparing PEG-grafted copolymers was documented by Jannasch *et al.*[8]. They showed that ionization of the copolymers styrene-*co*-acrylamide or styrene-*co*-methacrylamide by potassium t-butoxide or potassium naphthalene initiated grafting polymerization of ethylene oxide onto the backbone polymers[8].

The biomedical applications of PEG-grafted copolymers have been of great interest owing to their highly effective exclusion properties (including high protein repulsion and low cell adhesion) in aqueous solution[15,16]. PEG-containing polymers have also attracted increasing attention in the design of drug-delivery systems[17–19]. In addition to the promising results of PEG–protein conjugates with regard to their therapeutic effects, amphiphilic PEG-grafted copolymers, which are capable of forming micelles in aqueous solutions, have been studied in attempts to achieve drug sustained release[17,20–22].

Among microspheric drug-delivery systems, the formation of polymeric micelles in aqueous solutions from amphiphilic macromolecules undoubtedly offers an appropriate vehicle for drug delivery[23]. Hydrophobic therapeutic agents can be effectively encapsulated into the cores of micelles to achieve sustained release and/or potential for

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targeting of drugs. However, the extensive cellular uptake of these micelles by phagocytes is one of the major difficulties in prolonging their circulation half lives. To circumvent this problem, PEG chemically incorporated into the copolymer will aid the formation of micelles in aqueous solution and prohibit protein interaction, and therefore reduce their cellular recognition[17,21,24].

In this study, copolymers comprising monomeric units of stearyl methacrylate, methyl acrylate, acrylic acid and acrylate derivative of PEG have been synthesized and their structural characteristics evaluated. This type of copolymer consists of hydrophobic (primarily stearyl groups) and hydrophilic (PEG part) side chains as well as anionic carboxylic groups and becomes capable of forming polymeric micelles in water. The critical micelle concentrations (*CMC*s) of these copolymers were determined by static laser light scattering measurements. Changes in the size of micelles formed by various PEG-containing copolymers with time was recorded by dynamic light scattering measurements. In addition, measurements of the encapsulation capacity and release rate of a hydrophobic compound (using pyrene as a drug model) from micelles were also performed.

EXPERIMENTAL

Materials

Stearyl methacrylate, obtained from Mitsubishi Rayon Company, Japan, was recrystallized from dry methanol twice before use. Acryloyl chloride (96%), obtained from Aldrich, was purified by distillation and dried over magnesium sulfate. Monomethoxy poly(ethylene glycol) (mPEG) with molecular weights of 2000 and 5000 g mol^{-1} (Aldrich) was dried *in vacuo* for 24 h before use. The reagents 2,2′-azobisisobutyronitrile (AIBN), pyrene and pyridine were purchased from Aldrich, Milwaukee, WI. AIBN was recrystallized from methanol before use.

Syntheses

The polymeric precursor, poly(stearyl methacrylate-*co*-acryloyl chloride), was prepared by free-radical copolymerization of acryloyl chloride (AC) and stearyl methacrylate (SMA). To a solution containing acryloyl chloride (**A**: 35 mmol or **B**: 24 mmol) and stearyl methacrylate (**A**: 35 mmol or **B**: 16 mmol) in benzene (**A**: 400 ml or **B**: 200 ml), AIBN (**A**: 2.17 g l^{-1} or **B**: 2.48 g l^{-1}) was added to initiate polymerization. The reaction was carried out at 80°C with stirring under nitrogen for 24 h. The resultant polymeric precursors (**A** and **B**) were subsequently reacted with mPEG (M_w = 2000 and 5000 g mol^{-1}, respectively) in benzene/pyridine (10:1 vol/vol) solution at 85°C for 24 h under refluxing. Methanol was subsequently added to the reaction solution at 85°C for another 24 h. After the reaction was stopped, the volume of the polymer solution was reduced to *ca.* 30 ml in a rotary evaporator under reduced pressure. Polymers were precipitated from n-hexane and the precipitates collected and dried *in vacuo*. The resultant polymers were resuspended in water and subjected to centrifugation (Beckman, J2-21) at 5000 rev min^{-1} for 10 min and the supernatants collected. The supernatant aqueous solutions of these polymers were subsequently filtered to remove large undissolved particles. The filtrates were further purified by ultrafiltration (Microcarbosep, MWCO 50 000) until no PEG peaks were shown by gel permeation chromatography (g.p.c.) and lyophilysed. *Table 1* shows the reaction conditions used for preparing the copolymers selected for this study.

Characterizations

The structure of the polymeric precursors was characterized mainly by Fourier transfrom infra-red spectroscopy (*FT*i.r.) (Jasco, model 700). Along with the disappearance of peaks attributed to C=C at *ca.* 1610–1635 cm^{-1}, the typical peaks for acryloyl chloride and stearyl methacrylate (e.g. 2908, 2844 cm^{-1} for –C–C– and 1719 cm^{-1} for C=O of stearyl methacrylate; 1785 cm^{-1} for C=O and 765 cm^{-1} for chloride of acryloyl chloride) shown on the spectra were also in agreement with the syntheses of these polymeric precursors.

The purity of PEG-grafted copolymers was checked by means of g.p.c. (Sephadex G-50). Removal of unreacted mPEG was confirmed by g.p.c. spectra after thorough dialysis. The compositions of PEG-derived amphiphilic copolymers were evaluated by 200 MHz ^1H nuclear magnetic resonance (n.m.r.) spectroscopy. Samples were run in $CDCl_3$ at ambient temperature. The analysis is somewhat complicated because of the four monomeric units in the copolymers. The mole content of each monomeric unit was obtained by comparing their integrated signals. The signals from the methoxy part of mPEG (δ = 3.3 ppm) were used to calculate the methyl group of methyl acrylate at δ = 3.6 ppm, which overlaps with $-CH_2CH_2O-$ of mPEG. The content of stearyl methacrylate was obtained from the integrated signal of its methyl group at 0.9 ppm while the percentage of acrylic acid content was determined from the n.m.r. integrated signals of acryloyl chloride in the polymeric precursors.

Molecular weights of the amphiphilic copolymers were characterized by static laser light scattering (Otsuka, DLS-7000) equipped with a 10 mV He–Ne laser. The primary beam is vertically polarized with λ = 632.8 nm in the range 30–150°. All the measurements were performed in tetrahydrofuran (THF) at 25°C. Molecular weights of the copolymers were calculated by measuring the excess Rayleigh ratio (R_θ) from the following equation[25]:

$$KC/R_\theta = 1/M_w + (1/M_w)(16\,\pi^2/3\lambda^2)S^2 \sin^2(\theta/2) + 2A_2C \quad (1)$$

where K is the optical constant with the incorporated refractive index, S^2 the z-average radius, C the polymer

Table 1 Molar ratios of monomers and conditions for synthesis of the amphiphilic copolymers

Copolymer no.	AC (g)	SMA (g)	AC/SMA (mole ratio)	AIBN (g)	mPEG[a] (g)	Methanol (g)
I	3.20	11.96	1/1	0.869	31.8	8.48
II	3.20	11.96	1/1	0.869	22.9	8.48
III	3.20	11.96	1/1	0.869	79.5	8.48
IV	2.19	5.47	1.5/1	0.496	43.6	11.62

[a]The molecular weight of mPEG in copolymers **I**, **II** and **IV** is 2000 g mol^{-1}. The molecular weight of mPEG in copolymer **III** is 5000 g mol

concentration in g ml^{-1} and A_2 the second virial coefficient from a Zimm plot which extrapolates θ and C to the limits. The refractive index increment (dn/dc) was determined by an Otsuka Electronics differential refractometer (DRM 1201) with copolymers dissolved in THF at 633 nm (iodine lamp) at 25°C.

Critical micelle concentration measurements

The *CMC* measurements were performed by using static light scattering according to the method of Ito *et al.*[26]. In brief, copolymers of various concentrations were prepared in redistilled water. The samples were thermostated in a refractive index matching liquid (toluene) at 37°C. The ratios of the intensity of excess light scattered from polymer solutions at 90° to the intensity of the incident light at the same angle as the Rayleigh ratios (R_{90}) were recorded. The values of *CMC* were determined from the plot of R_{90} *versus* polymer concentration; the concentration (*CMC*) at which a clear change in the slope was observed.

Measurements of molar masses of micelles

The molar masses of micelles were determined in a similar manner to the measurements of the weight-average molecular weights of the copolymers. Aqueous solutions of the copolymers with various concentrations in distilled water were filtered through a 1.2 μm membrane and allowed to stand at room temperature overnight for equilibration before the measurements. The excess Rayleigh ratio (R_θ) was determined by static light scattering (Otsuka, DLS-7000). The molar masses of micelles were estimated according to the following modified equation[27]:

$$K(C - CMC)/R_\theta = 1/M_w + (1/M_w)(16\pi^2/3\lambda^2)S^2 \sin^2(\theta/2) + 2A_2(C - CMC) \quad (2)$$

Micelle stability measurements

The stability of micelles in aqueous solutions was evaluated in terms of the variation of micellar size with time. The average polymeric micelle size data were obtained from dynamic light scattering (Otsuka, Photal LPA-3000/3100). The polymeric micelle sample was prepared by dissolving 0.04 g copolymer in 25 ml of pH 7.4 phosphate buffer saline solution. The concentration of NaCl was maintained at 0.09% (w/v) in this study. The resultant mixture was filtered through a 1.2 μm membrane to remove the insoluble species (if any) and then homogenized (W-385, Heat System-Ultrasonic, Inc., output power = 10%) for 20 min. Before micellar size measurements, the sample was passed through a 0.8 μm filter. The number of photons counted per second (cps) was adjusted to the range of 8000–12 000. The number of accumulation times was set at 50 throughout this work. The micellar size data reported in this work represent an average of at least three measurements.

Measurements of release rates of pyrene from micelles

Pyrene in 20 ml THF solution (8.4×10^{-3} mg ml^{-1}) was added dropwise to the micelle solution in phosphate saline buffer, pH 7.4 (vol: 40 ml; concentration: 500 times the *CMC* for each corresponding copolymer). After 1 h stirring, the solution became semi-transparent and the volume of aqueous solution was partially reduced to 28 ml by rotary evaporation. The loading amounts of pyrene in micelles were measured spectrometrically (λ_{max} = 338.2 nm, $\varepsilon = 3.32 \times 10^5$ M^{-1} cm^{-1} in benzene) from redissolution of the dried micellar copolymers containing pyrene in benzene. The weight-average partition coefficients (K_w) of pyrene between micelles and aqueous solutions were then calculated[21]. The solubility of pyrene in micelle solution was evaluated as well.

Release rates of pyrene from micelles were determined by the membrane dialysis technique. Micelles encapsulated with pyrene were dialysed (Spectrapor, MWCO 3,500) against phosphate saline buffer (pH 7.4) at room temperature and 2 ml of micelle solution was withdrawn at certain time intervals. The phosphate saline buffer (1 l) was switched to a fresh one every 24 h to maintain a sink condition during dialysis. About 85% of the pyrene in the originally saturated aqueous solution in the absence of polymeric micelles was removed from the dialysis membrane tube within 35 min. This result confirms that diffusion of pyrene from micelles into the aqueous phase is the rate-limiting step. The aliquots withdrawn were dried thoroughly and redissolved in benzene. The content of pyrene mixed with micellar copolymer in benzene was subsequently determined spectrometrically.

RESULTS AND DISCUSSION

Syntheses of PEG-derived amphiphilic copolymers have been reported elsewhere[1–14]. Lee *et al.* and Konak *et al.*, for example, prepared graft copolymers of alkyl methacrylates with mPEG methacrylate macromonomers by free-radical polymerization[3,28]. In this study, the preparation of amphiphilic copolymers was performed first by syntheses of the polymeric precursors by radical polymerization of stearyl methacrylate (SMA) with acryloyl chloride (AC), followed by esterification of mPEG as well as methanol with the resultant polymeric precursors (*Figure 1*). In preparation of these polymeric precursors, two initial monomer feed ratios were used (**A**: AC/SMA = 1.0/1.0 and **B**: AC/SMA = 1.5/1.0). Characterization of these polymeric precursors by *FT*i.r. was in agreement with their structures. These two precursors were reacted with mPEG (M_w = 2000 and 5000 g mol^{-1}, respectively) under various conditions to give four graft copolymers. Excess of methanol was added to the reaction solution after 24 h of reaction time to convert the residual acid chloride to its methyl ester. The reaction mixtures were subsequently subjected to centrifugation and filtration to remove large precipitates in aqueous solutions. Ultrafiltration was performed to remove unreacted mPEG from the graft copolymers. The purity of these graft copolymers was confirmed by the absence of a PEG peak on g.p.c. spectra[29]. From the *FT*i.r. spectra, along with the disappearance of the chloride peak at 765 cm^{-1}, the observation of a peak at 1109 cm^{-1} (C–O–C of PEG) was noticed.

The compositions of graft copolymers were calculated primarily from their ^{1}H n.m.r. spectra[30]. In addition to characterization of the graft copolymers by n.m.r., the structures of the two monomers (SMA and AC) and of mPEG at defined concentrations in $CDCl_3$ were also analysed as calibrations. All the n.m.r. measurements were performed under the same conditions. *Figure 2* shows an n.m.r. spectrum of copolymer **I** as an example. Owing to overlap of the integrated peaks at *ca.* 3.6 ppm from the backbone of mPEG and the methoxy group of methyl acrylate, the PEG contents of graft copolymers were obtained from the integrated peaks at 3.3 ppm

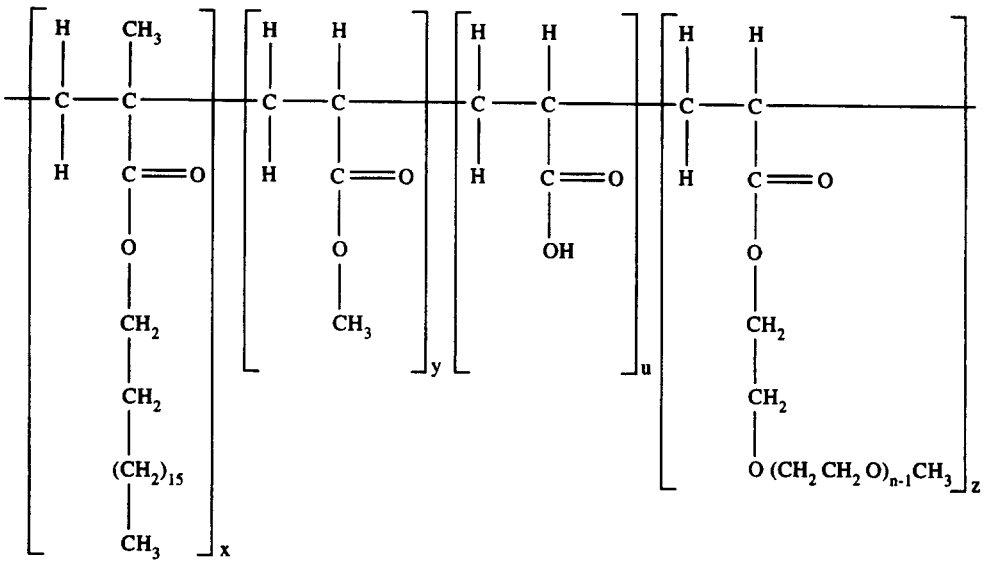

Figure 1 Molecular structure of the amphiphilic copolymers

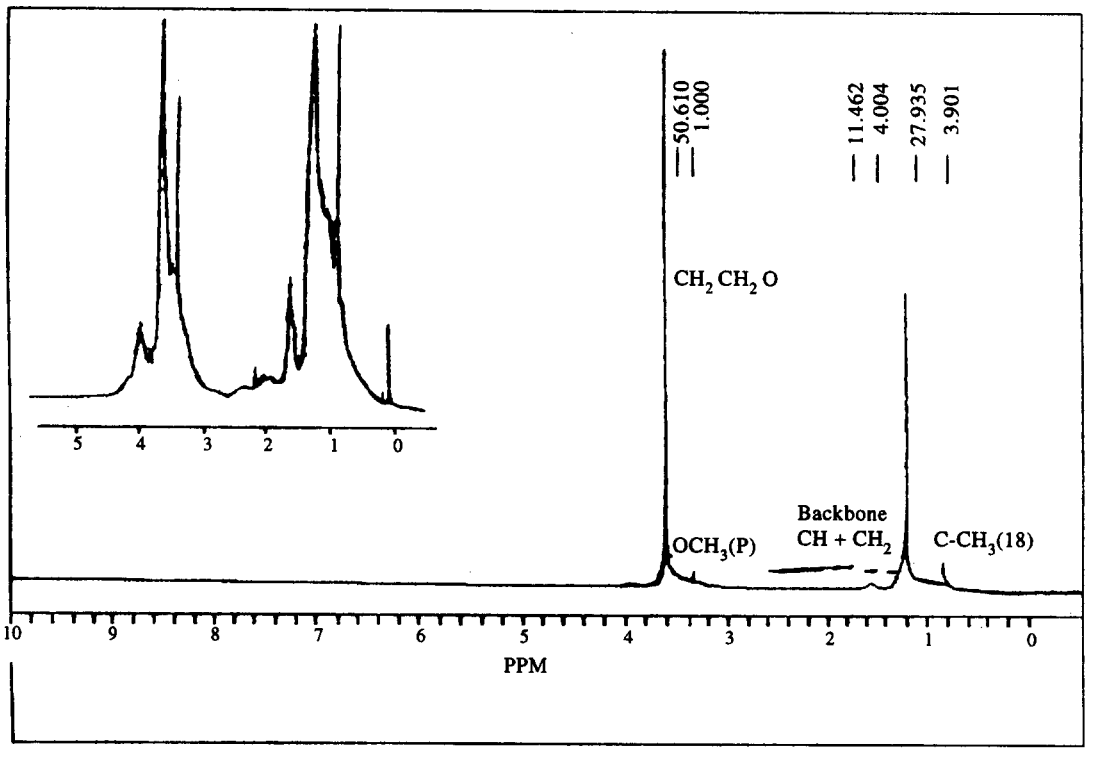

Figure 2 ^{1}H n.m.r. spectrum of copolymer **I** in $CDCl_3$

corresponding to the methyl end group of mPEG as compared with its n.m.r. calibration spectrum. Taking the contribution of mPEG at 3.6 ppm into account, the contents of methyl acrylate were easily determined. Compared with the calibration sample, the mole contents of stearyl methacrylate were obtained from their corresponding integrated methyl peaks at 0.9 ppm. The contents of acrylic acid, detected by *FT*i.r. spectra, were determined from the integrated peaks of acryloyl chloride of the polymeric precursors (at 6.2, 6.4 and 6.6 ppm, respectively). The contents of acrylic acid may thus be obtained by the reasonable assumption that all acryloyl chloride was completely converted to mPEG acrylate, methyl acrylate and acrylic acid. The composition data for the graft copolymers are summarized in *Table 2*.

The mole percentages of PEG conjugation range from *ca.* 4.3 to 12.1% for all the polymeric precursors, even for the precursor with a higher content of acryloyl chloride (*ca.* 12% conjugation only). The results are attributed to the steric hindrance between the polymeric precursors and PEG in benzene. Copolymer **III** has the lowest degree of conjugation obviously as a consequence of the higher molecular weight of mPEG (5000 g mol^{-1} as compared with 2000 g mol^{-1} for the rest of the copolymers). Copolymer **IV** has the highest degree of conjugation since its polymeric precursor contains a higher level of acryloyl chloride. The higher content of acryloyl chloride may also reduce the effect of steric hindrance and, thereby, result in a higher grafting efficiency of mPEG. As a consequence, copolymer **IV** contains the lowest contents of methyl

Table 2 Characterization of the PEG-derived amphiphilic copolymers

Copolymer no.	mPEG/MA/AA/SMA[a]	mPEG content (wt%)	Degree of grafting (grafts/100 monomers)	M_w (g mol^{-1}) (copolymer)[b]
I	0.20/0.79/1.27/1.00	45.2	6.1	130000
II	0.22/1.08/0.40/1.00	50.8	8.1	102000
III	0.13/1.52/0.39/1.00	57.0	4.3	132000
IV	0.21/0.52/0.00/1.00	53.0	12.1	64000

[a]Calculations based on n.m.r. spectroscopy
[b]Based on static light scattering measurements in THF

acrylate and acrylic acid. For copolymers **I**, **II** and **IV** with mPEG conjugation ranging from 6.1 to 12.1%, it is believed that, at this level of conjugation density and length of ethylene oxide, mPEG should have covered the micellar surface well since it has a high degree of mobility and flexibility in aqueous solutions. Copolymer **III** containing PEG of molecular weight of 5000 g mol^{-1} should also give sufficient masking on its corresponding micelles.

The weight-average molecular weights of the graft copolymers were evaluated by static light scattering and the results shown in *Table 2*. The graft copolymers were completely dissolved in THF as a consequence of their better solubilities in this solvent and, therefore, the absence of aggregates (or micelles) in the polymer solution. The weight-average molecular weights were determined by Zimm plots. Molecular weights determined this way might lead to different values than the measurements in water. Limited solubilities of amphiphilic copolymers in water have frequently resulted in a complex situation in the analyses of static light scattering data, as reported in the literature[35,36]. The weight-average molecular weights of copolymers **I**, **II** and **III** were in the range 100000 to 130000 g mol^{-1}, while copolymer **IV** gave a molecular weight of 64000 g mol^{-1}.

The molar mass of polymeric micelles in water was also estimated by static light scattering. Xu *et al.* assumed that unimers and aggregates of the amphiphilic copolymers were capable of reaching an equilibrium state in aqueous solution[27]. Therefore, the micellar solution was treated as the aggregates in the 'solvent' of the polymer solution. When the polymer concentration was lower than its *CMC*, the concentration of micelles became negligible. Equation (1) was, therefore, able to be transformed into equation (2) according to the method of Xu *et al.* and used to determine the molar mass of aggregates[27]. *Figure 3* shows a typical modified Zimm plot for measurement of the molar mass of micelles obtained from copolymer **I** in aqueous solutions. The results are shown in *Table 3*. The molar mass of the polymeric micelles is in the range 3.0×10^6 to 6.5×10^6 g mol^{-1}. Kwon *et al.* have prepared micelles based on block copolymers of mPEG and poly(β-benzyl L-aspartate) (PBLA) in aqueous solutions[33]. The molar mass of micelles measured by static light scattering was *ca.* 2.8×10^6 g mol^{-1}, which is comparable to the molar mass of micelles in this study, although the molecular weights of the block copolymers of mPEG with PBLA was one order of magnitude lower (*ca.* 7000 g mol^{-1}). It is interesting to note that the micellar molar mass of copolymer **IV** is comparable to the other three although its polymer molecular weight is much lower (see *Tables 2 and 3*). Taking the weight-average molecular weights of the graft copolymers measured in THF as references, the average molecular numbers of micelles aggregated (N_{agg}) in aqueous solutions were calculated and the results are shown in *Table 3*. Copolymer **IV** has an

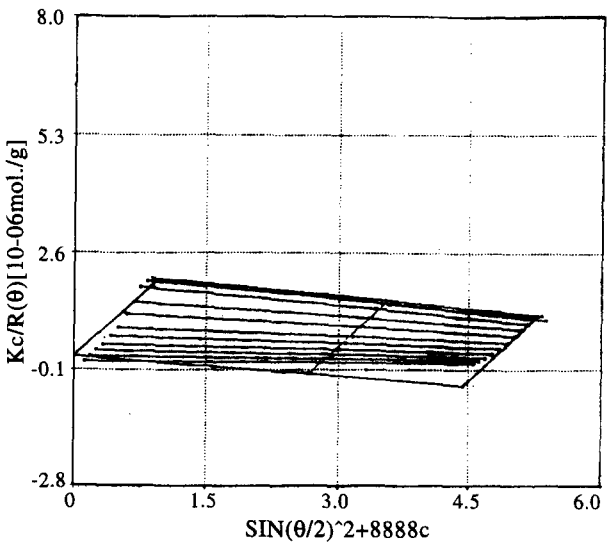

Figure 3 Modified Zimm plot for micelles of copolymer **I** in water

aggregation number of *ca.* 100, compared with no more than 40 for the other three copolymers. Copolymer **IV**, which possesses a higher mole content of stearyl methacrylate, exhibits a higher degree of association due to its enhanced hydrophobic interactions, whereas its lower content of hydrophilic comonomers reduces the extent of solvation with water. The lower molecular weight of this copolymer, compared with the other three, may also contribute to its higher degree of aggregation. Copolymer **III**, on the other hand, has the lowest aggregation number probably as a result of the relatively high molecular weight (5000 g mol^{-1}) of mPEG, although the PEG mole content of copolymer **III** is the lowest.

Critical micelle concentrations of these amphiphilic copolymers were determined by static light scattering. The ratios of excess light scattered intensity (R_{90}) are much lower in dilute solutions while the ratio rises as the micelle formation occurs. The value of *CMC* was, accordingly, determined by the distinct change in slope of the plot of R_{90} *versus* concentration of copolymers in aqueous solution. *Figure 4* shows a typical plot of R_{90} *versus* concentration for copolymer **I**. The *CMC* data are shown in *Table 3*. The values of *CMC* detected for copolymers **I**, **II** and **VI** are *ca.* 2 mg l^{-1}, while copolymer **III** gave a somewhat higher value of *ca.* 4 mg l^{-1}. The value of *CMC* is influenced by the hydrophobic/hydrophilic balance and molecular weight of the amphiphilic compounds. For example, a low-molecular-weight compound, sodium lauryl sulfate, forms micelles at a concentration of *ca.* 900 mg l^{-1} in aqueous solution while the *CMC* of the block copolymers of mPEG with PBLA was greatly reduced to *ca.* 5–10 mg l^{-1} [33]. Xu *et al.* also measured the *CMC* of PEG–poly(styrene) copolymers with

Table 3 Properties of micelles made from the amphiphilic copolymers in aqueous solution

Copolymer no.	M_w of micelles (g mol^{-1})[a]	N_{agg}	*CMC* (mg l^{-1})	Solubility of pyrene (M)[b]	K_w ($\times 10^{-3}$)[c]
I	4.28×10^6	33	1.90	1.0×10^{-7}	10.1
II	3.69×10^6	36	2.06	9.4×10^{-6}	9.4
III	2.89×10^6	22	4.02	1.1×10^{-5}	5.4
IV	6.45×10^6	101	1.71	9.7×10^{-5}	11.1

[a]Calculations based on static light scattering measurements in water
[b]Concentrations of copolymers in aqueous solution are 1.54 (copolymer **I**), 1.56 (copolymer **II**) and 1.36 (copolymer **IV**) mg ml^{-1}
[c]The weight-average partition coefficients of pyrene between micelles and aqueous solution

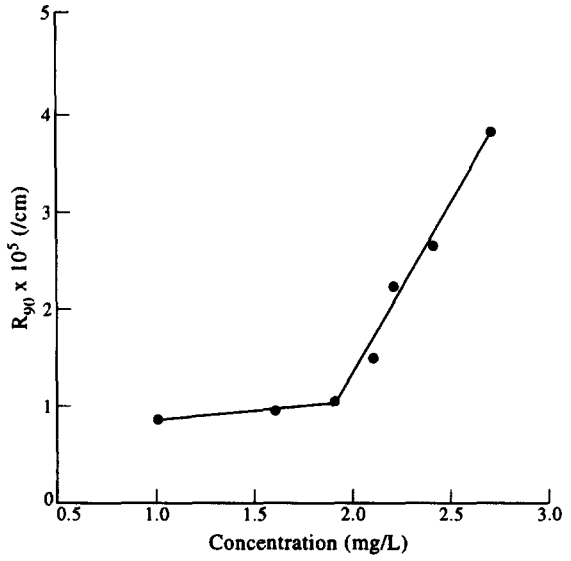

Figure 4 Measurement of R_{90} at various concentrations of copolymer **I** in aqueous solutions by static light scattering. Critical micelle concentration was determined at a concentration where a clear change of R_{90} value was observed

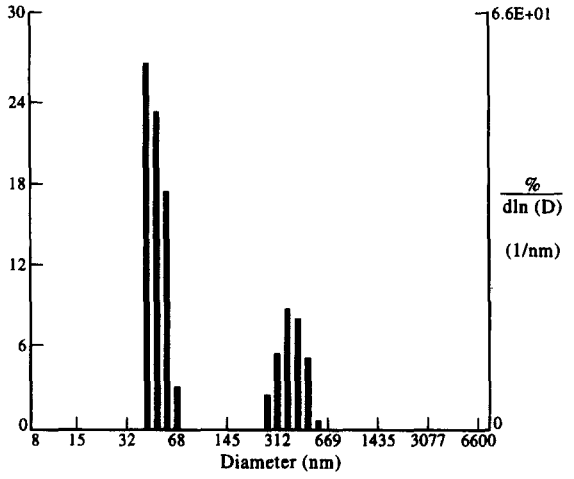

Figure 5 Particle size and distribution for the micelles of copolymer **III** as measured by dynamic light scattering

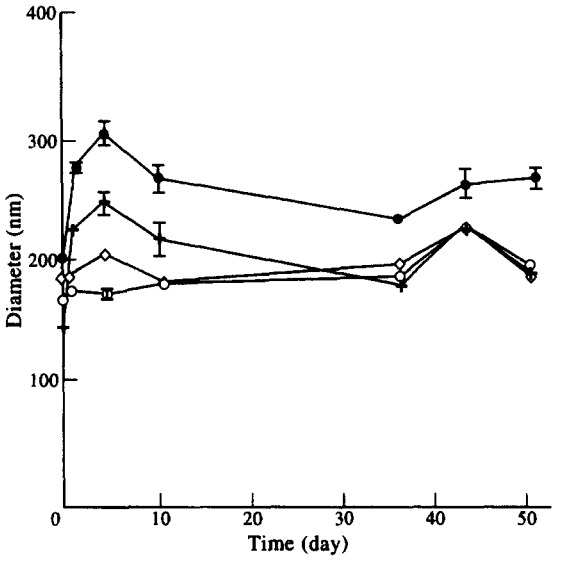

Figure 6 Variation of the diameters of the micelles from copolymer **I** (●), copolymer **II** (◇), copolymer **III** (○) and copolymer **IV** (+) in aqueous solutions with time as measured by dynamic light scattering

a molecular weight of 23 500 g mol^{-1} by fluorescence spectroscopy and found the value of *ca.* 4.0 mg l^{-1} [27]. In this study, these four PEG-derived copolymers exhibit low values of *CMC* resulting mainly from high hydrophobicity of the stearyl side chains. Copolymer **III**, conjugated with PEG of M_w = 5000 g mol^{-1}, is apparently more soluble in aqueous solution. In spite of its amphiphilic properties, PEG possesses good water solubility. The relatively low content of stearyl methacrylate in copolymer **III** (as shown in *Table 2*) may also contribute to its better solubility in aqueous solution. The relatively low *CMC* of copolymer **IV** was primarily a consequence of its high content of stearyl methacrylate.

The stability of the polymeric micelles in aqueous solution was evaluated by measuring the time evolution of the polymeric micellar diameter in aqueous solution with the dynamic laser light scattering technique. The results obtained from the Stoke–Einstein equation were processed by the histogram method. All the polymeric micellar solutions show a bimodal size distribution; for example, one population is centred around 50 nm and the other around 389 nm for copolymer **III** (see *Figure 5*). A similar observation was also reported by several authors in the study of similar systems[27,28,31,32,34,35]. It was reported by Xu *et al.* that a number of micelles derived from PEG-containing copolymers might aggregate into loose and large association complexes and reach thermodynamic equilibrium with micelles of smaller sizes[27]. Konak *et al.* also reported similar observations by the light scattering method for the aqueous solution of hydrophilic copolymers containing hydrophobic side chains[28]. They reported that large aggregates were produced by random association via attractive forces of hydrophobic side chains, whereas small micelles remained compact and dense by PEG to protect the hydrophobic cores from further association[28]. In spite of this, the results of the stability study shown in *Figure 6* obviously exhibit a pattern of small variations in their average sizes with time. The increase in micellar sizes

during the initial period is due to aggregation of the copolymers after intensive sonication to reduce the total interfacial area and, thereafter, the size remains roughly unchanged within 40 days. Little precipitate was observed at 50 days.

Encapsulation of pyrene (as a hydrophobic drug model in this study) in micelles was performed by evaporation of THF in THF/aqueous emulsion containing PEG-modified copolymers with pyrene. The loading amounts of pyrene in micelles were determined spectrometrically from the dried pyrene/micelle in benzene and the solubilities of pyrene in micelle solutions calculated. The results are shown in *Table 3*. In micellar solutions containing 1.4 to 3.2 mg ml^{-1} amphiphilic copolymers, the solubility of pyrene increases from 6×10^{-7} M (pyrene in water) to *ca.* 1×10^{-5} M. Kwon *et al.* reported similar observations on the increased solubility of pyrene in PEO–PBLA copolymer micelle solutions and established a linear relationship between pyrene solubility and micelle concentration[21]. The solubility of low-molecular-weight compounds in micellar solution is also strongly influenced by the structures of both solutes and polymeric micelles. Cao *et al.* demonstrated that the solubility of pyrene in the micellar solution of poly(methacrylic acid)–poly(styrene) copolymers was much higher due to the compatibility of pyrene with poly(styrene)[36]. In this study, copolymer **IV**, retaining a higher mole content of hydrophobic stearyl methacrylate, enhances the solubility of pyrene about tenfold compared with the other three micellar solutions. The weight-average partition coefficients of pyrene between micelles and their aqueous environments were also calculated, as shown in *Table 3*. The data obtained from this study were comparable with the systems developed by Kwon *et al.*, implying that the current system may have comparable loading capacities for hydrophobic compounds[21].

In addition to loading efficiency, the study of release rates of pyrene from micelles within 8 days was also performed (*Figure 7*). This system does exhibit a sustained release pattern within the time intervals measured. However, the authors believe that the release rates of the encapsulated compounds should increase significantly under more agitated conditions, such as in *in vivo* experiments when the system is applied for drug delivery. Copolymer **IV** shows the fastest liberation rate of pyrene probably owing to its highest average number of molecular aggregation (N_{agg} = 100) compared with copolymers **I** and **II**. For micellar particles with a bimodal size distribution, micelles with smaller sizes are more dense and stable than those with larger sizes. It is, therefore, expected that micelles made from copolymer **IV** display a more loose and flexible structure when they are formed from an average number of 100 macromolecules. In addition, the content of mPEG grafted onto the copolymers may influence the release rate to a certain extent. The extremely high mobility and flexibility of PEG in aqueous solution may exert a repulsion force against the compact structure of the micelles. Although copolymer **III** has the lowest aggregation number (N_{agg} = 22), the higher weight content of PEG in copolymer **III** results in a faster release rate of pyrene than for copolymer **I**. However, the dependence of the partition coefficient of pyrene between micelles and aqueous environment upon its release rate, as reported by Kwon *et al.*, was not observed in this study[21]. The greater the partition coefficient, the greater is loading amount of pyrene, which, in turn, may give rise to questions about its effect on the release of pyrene.

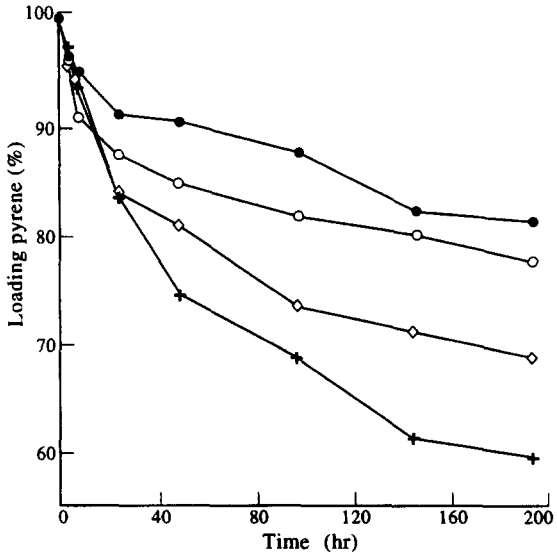

Figure 7 Release of pyrene from the micelles of copolymer **I** (●), copolymer **II** (◇), copolymer **III** (○) and copolymer **IV** (+) in aqueous solutions with time. Release rate was expressed in terms of the loading amounts of pyrene in the micelles with time

CONCLUSIONS

In this study, we have developed new amphiphilic graft copolymers comprising monomeric units of stearyl methacrylate, methyl acrylate, acrylic acid and PEG acrylate. The PEG-derived amphiphilic copolymers form polymeric micelles in aqueous solution and exhibit potential for controlled drug delivery. The structures and compositions of these copolymers were well characterized by *FT*i.r. and n.m.r. The structural analysis indicates that copolymers **I, II** and **IV** contain 6 to 12 grafts of mPEG (M_w = 2000 g mol^{-1}) per hundred monomeric units while copolymer **III** contains only four grafts of mPEG (M_w = 5000 g mol^{-1}) per hundred monomeric units.

In addition, these copolymers were subjected to static light scattering measurements and the molecular weights estimated in THF were taken as a reference for determination of the micelle properties. The molar mass of micelles in aqueous solution was also determined by the static light scattering method. Owing to the relatively high content of stearyl methacrylate, copolymer **IV** has the highest molar mass of micelles in spite of the lowest weight-average molecular weight of the copolymer. On the other hand, PEG of M_w = 5000 g mol^{-1} makes it more difficult for copolymer **III** to associate into large micelles and, therefore, reduces its aggregation number. Study of the critical micelle concentration of these copolymers in aqueous solution indicates that the critical micelle concentration was primarily determined by the hydrophobic/hydrophilic balance of the copolymers. Copolymer **III**, containing the highest (weight) content of mPEG and lowest amount of stearyl methacrylate, exhibits the best aqueous solubility and, therefore, the highest *CMC*. Micelles with a bimodal size distribution for aqueous solution of amphiphilic copolymers were also noticed by the dynamic light scattering method in this study. Little variation in the size of micelles with time was observed. Furthermore, measurements of both partition coefficients and release rates of pyrene within the micelles in aqueous solution show promising patterns and might indicate their potential

applications for sustained delivery of hydrophobic therapeutic agents. However, further investigations regarding their biological performance and biocompatibility, such as the toxicity of the materials, are necessary.

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