An Amphiphilic Nanocarrier Based on Guar Gum-*graft*-Poly(ε-caprolactone) for Potential Drug-Delivery Applications

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Abstract

Amphiphilic guar gum grafted with poly(ε-caprolactone) (GG-*g*-PCL) was fabricated as a drug-delivery carrier using microwave irradiation. The structure of the GG-*g*-PCL co-polymer was characterized by ^{1}H-NMR spectroscopy. By microwave irradiation, GG-*g*-PCL with high grafting percentage (>200%) was obtained in a short reaction time. The GG-*g*-PCL co-polymer is capable of self-assembling into nanosized spherical micelles in aqueous solution with the diameter of around 75–135 nm and 60–100 nm, as determined by DLS and TEM, respectively. The critical micelle concentration (CMC) of GG-*g*-PCL was found to be approx. 0.56 mg/l in a phosphate buffer solution. The drug-release profile showed that the GG-*g*-PCL micelles provided an initial burst release followed by a sustained release of the entrapped hydrophobic model drug, ketoprofen, over a period of 10–68 h. Under physiological conditions, the GG-*g*-PCL co-polymer hydrolytically degraded into lower-molecular-weight fragments within a 7-week period. These results suggest that the GG-*g*-PCL micelles could be used as a nanocarrier for *in vitro* controlled drug delivery.

Keywords

Guar gum, poly(ε-caprolactone), nanocarrier, drug delivery, biodegradation

1. Introduction

Polymeric micelles are supramolecular core–shell structures formed by self-aggregation of individual amphiphilic macromolecules comprised of hydrophobic and hydrophilic domains [1]. Micelles formed from amphiphilic block co-polymers have found widespread use in the field of controlled drug delivery because they

can solubilize various hydrophobic drugs, increase bioavailability and stay unrecognized during blood circulation [2]. Polymeric micelles used for drug delivery in intravenous administration must be non-toxic to the human body. In this context, amphiphilic systems based on polysaccharides and their derivatives as drug-delivery carriers have recently gained much interest due to their biocompatibility, biodegradability, ability to form hydrophobic clusters, drug loading and controlled release properties [3–5]. For example, polymeric micelles with diameters $<$100 nm for drug-delivery applications were prepared from N-phthaloylchitosan-*g*-polyvinylpyrrolidone co-polymer [6]. Moreover, micelles based on dextran-*g*-methoxypolyethylene glycol/poly(ε-caprolactone) co-polymers with diameters of approx. 100–200 nm were reported as drug-delivery carriers [7]. Guar gum (GG) is an edible carbohydrate polymer found in the seeds of *Cyanaposis tetragonolbus*. It is a non-ionic, branched chain polymer, consisting of linear chain of mannose units joined by β-D-(1 $\rightarrow$ 4) linkages and a branched chain of α-D-galactopyranose units attached to linear chain by (1 $\rightarrow$ 6) linkages [8]. Due to its favorable properties such as biodegradability and non-toxicity, GG has wide application in the biomedical and pharmaceutical fields [9, 10]. Chemically-modified GG has varied utility in controlled release and targeting studies of almost all classes of bioactive molecules. Therefore, the interest in GG and its derivatives as excipients in drug delivery has been increased in recent years. Poly(vinyl alcohol)-GG interpenetrating network microspheres were prepared by cross-linking with glutaraldehyde for anti-hypertensive drug delivery [11]. A graft co-polymer of GG with acrylamide was prepared and cross-linked with glutaraldehyde to form hydrogel microspheres for anti-hypertensive drug delivery [12]. Recently, a pH-sensitive alginate-GG hydrogel cross-linked with glutaraldehyde was reported for the controlled delivery of protein drugs [13].

Developing an amphiphilic GG-based graft co-polymer, which is capable of self-assembly into nanosized polymeric micelles, seems to be a promising delivery system for hydrophobic drugs. Therefore, in this study, poly(ε-caprolactone) (PCL) was grafted onto GG backbone to produce an amphiphilic GG-*g*-PCL co-polymer using microwave irradiation method as efficient tool for the graft co-polymerization [14, 15]. PCL was used as hydrophobic segments because of its excellent biodegradability, biocompatibility and non-toxicity. GG-*g*-PCL can be self-assembled into micelles composed by hydrophobic PCL segments as the internal core and hydrophilic GG segments as a surrounding corona in aqueous solutions due to its amphiphilic character. The hydrophobic PCL core of micelles improves the drug loading and release ability of micelles. The non-ionic hydrophilic GG shell of micelles essentially prevent possible protein adsorption onto the micelle surface and the subsequent uptake of the micelles by macrophages presenting in the bloodstream, thereby prolonging the circulation duration of the corresponding micelles [16]. The structure of GG-*g*-PCL co-polymer was confirmed by ^{1}H-NMR. The critical micelle concentration (CMC) of the GG-*g*-PCL co-polymer was analyzed by fluorescence. The size and morphology of the GG-*g*-PCL micelles was analyzed

by dynamic light scattering (DLS) and transmission electron microscopy (TEM) method, respectively. The feasibility of using these micelles as a delivery carrier for the transfection of a hydrophobic model drug, ketoprofen, was investigated. Moreover, the *in vitro* biodegradation of GG-*g*-PCL co-polymer at pH 7.4 was analyzed by weight loss determination.

2. Materials and Methods

2.1. Materials

GG (approx. 445 kDa) was purchased from Merck (Germany) and purified by the barium complexation method as reported in the literature [17]. From this material, a 25.6-kDa GG was prepared by acid hydrolysis [18]. Stannous octanoate ($Sn(Oct)_2$) and ε-caprolactone (CL) were obtained from Sigma (USA). Barium hydroxide (Merck) was used without further purification. The model drug ketoprofen was supplied by Aldrich (USA) and used as supplied. All other chemicals used were of analytical reagent grade.

*2.2. Preparation of GG-*g*-PCL Co-polymer*

Microwave graft co-polymerization of CL onto GG was carried out using a 2.45 GHz microwave oven (GE, WES1130DMWW). First, 1 g GG (25.6 kDa) was placed in a dried glass vessel and a solution of CL (2 g) and stannous octoate (i.e., 1 mol% to CL) was added. The vessel was sealed under N_2 gas and stored overnight. Thereafter, the vessel was irradiated under microwave oven at 550 W microwave power for different time periods, as indicated in Table 1. The obtained product (GG-*g*-PCL) was repeatedly washed with water and Soxhlet extracted with methanol for 48 h to remove the homo-polymer of PCL. The percent grafting of PCL onto GG was calculated using the equation $(W_1 - W_0)/W_0 \times 100$, where W_1 is weight of GG-*g*-PCL and W_0 is weight of GG.

Table 1.
Synthesis of GG-*g*-PCL under 550 W microwave irradiation

Sample	Exposure time (min)	GG-*g*-PCL	
		Yield (g)	Grafting % (wt%)
1	1	1.45	110
2	3	1.74	145
3	6	2.13	180
4	9	2.30	285
5	12	2.32	284
6	15	2.31	280

Feed amount of GG = 1 g and of CL = 2 g.

2.3. *Preparation of Drug-Loaded GG-g-PCL Micelles*

Drug-loaded GG-*g*-PCL micelles were prepared using the membrane dialysis method. Briefly, the co-polymer (50 mg) and ketoprofen (25 mg) were dissolved in 5 ml DMF under stirring. With this mixture, 15 ml of deionized water was added drop-wise. Thereafter, the mixture was dialyzed against deionized water using dialysis tubing (molecular mass cut-off 2 kDa) for 48 h. Thereafter, the drug-loaded micelles were freeze-dried. A similar procedure was followed to prepare the free GG-*g*-PCL micelles without using drug. In order to determine the drug loading level, a weighed quantity (25 mg) of drug-loaded micelles was extracted with ethanol at room temperature for 48 h under uniform stirring. After centrifugation, the ketoprofen in the supernatant was assayed by UV-Vis spectrophotometry at a wavelength of 263 nm. All the experiments were carried out in triplicate.

2.4. *Characterization*

The ^{1}H-NMR spectrum of the samples was recorded on a Bruker DRX-300 spectrometer using tetramethylsilane as an internal standard and D_2O as a solvent at 25°C. The absorbance measurements were carried out in an Ocean Optics HR4000 UV-Vis spectrophotometer. The calibration curve of absorbance against different concentrations of ketoprofen was made at 263 nm. To determine the critical micelle concentration (CMC), aliquots of pyrene solutions (15 μM in acetone, 400 μl) were added to 10 ml volumetric flasks, and the acetone was allowed to evaporate. GG-*g*-PCL solutions at concentrations ranging from 0.005 to 1.0 g/l were prepared in PBS solution from the stock polymer solution. Ten ml of the aqueous polymer solutions was then added to the volumetric flasks containing the pyrene residue. All the sample solutions contained excess pyrene content at the same concentration of 0.616 μM. The solutions were allowed to equilibrate for 24 h at room temperature. Thereafter, fluorescence spectra of the GG-*g*-PCL solutions were recorded on a fluorescence spectrometer (Photon Technology) at room temperature. The excitation spectra were recorded from 280 to 360 nm with an emission wavelength of 390 nm [19]. For TEM studies, a drop of GG-*g*-PCL micelles (0.1 mg/ml) containing 0.01 wt% phosphotungstic acid was deposited onto a 200 mesh copper grid coated with carbon and dried at 37°C. The shape and size of the micelles were observed at 75 kV with FEI Morgagni 268D TEM. The micellar size of GG-*g*-PCL was determined by DLS using a Beckman Coulter PCS submicrometer particle size analyzer at 90°. The concentration of the polymer solution was 0.5 mg/ml.

2.5. In Vitro *Drug Release*

The *in vitro* drug-release study was performed in an apparatus with 100 ml phosphate-buffered saline (PBS, pH 7.4) at 37°C. GG-*g*-PCL micelles loaded with a known amount (50 mg) of ketoprofen were dispersed in 5 ml PBS and placed in a dialysis bag (molecular mass cut-off of 3 kDa). The dialysis bag was then immersed in 95 ml of the release medium and kept in a horizontal laboratory shaker at 37°C and 100 rpm. To measure the drug-release content, samples (1 ml) were

periodically taken out and an equivalent volume PBS was used as replacement. The amount of released ketoprofen was analyzed with a spectrophotometer at 263 nm. The experiment was performed in triplicate for each of the samples.

2.6. In Vitro *Enzymatic Degradation*

The degradation behavior of the GG-*g*-PCL co-polymer was studied in PBS (pH 7.4) at 37°C with 5% CO_2. The GG-*g*-PCL co-polymer (100 mg) was incubated in a 96-well plate with 15 ml PBS containing endo-1,4-β-mannanase solution (70 U/ml) and esterase (30 U/ml) at 37°C. The weight loss of the polymer was determined quantitatively at predefined time periods.

3. Results and Discussion

*3.1. Synthesis and Characterization of GG-*g*-PCL*

GG-*g*-PCL was prepared under microwave heating according to the reaction scheme shown in Fig. 1. Microwave heating, which is totally different from conventional heating, was utilized here to favor a more rapid and efficient reaction.

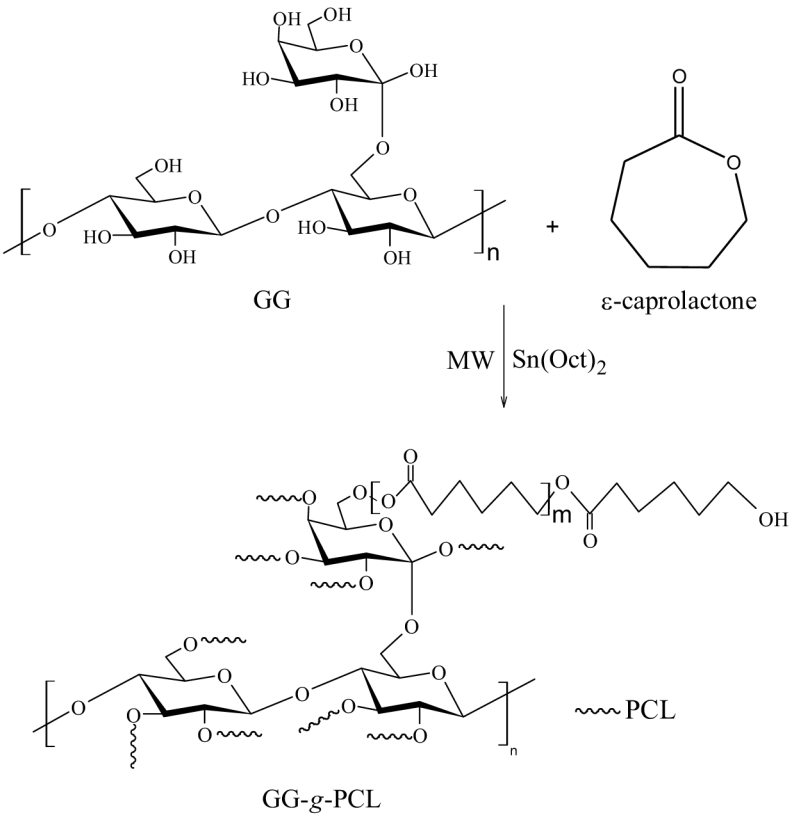

Figure 1. Reaction scheme for the synthesis of GG-*g*-PCL.

The convenience of ring-opening polymerization of CL by microwave radiation offers the possibility of an extension of the method to grafting biodegradable esters on GG, whose hydroxyl groups are excellent candidates for initiating sites [5]. In this work, GG with a molecular mass of 25.6 kDa was used for the grafting reaction with CL. During the microwave-assisted ring-opening polymerization, the hydroxyl groups of GG can attack the carbonyl carbon of CL by nucleophilic addition reaction. The GG-*g*-PCL co-polymer prepared from the lower-molecular-mass GG is expected to provide the polymeric micelles with an adequate size for drug-delivery application. In order to optimize the reaction conditions, microwave-assisted graft co-polymerization was carried out using a fixed amount of GG and CL by varying the microwave exposure time as shown in Table 1. Both the yield of GG-*g*-PCL and the percentage grafting of PCL increased with the increase in treatment time from 1 to 9 min. After 9 min, these values did not improve much anymore. This observation indicates that the graft co-polymerization of CL onto GG was accelerated under microwave irradiation. The maximum grafting yield (285%) was obtained when 1 g GG was polymerized with 2 g CL at 550 W for 9 min.

The formation of GG-*g*-PCL co-polymer was confirmed by ^{1}H-NMR as shown in Fig. 2. The ^{1}H-NMR spectrum of the pure guar gum (Fig. 2A) showed the characteristic peaks of GG at 5 ppm due to anomeric protons, and at 3.5–3.9 ppm due to other sugar protons. In the ^{1}H-NMR spectrum of GG-*g*-PCL (Fig. 2B), in addition to the peaks from GG, the peaks at 2.3, 1.4 and 1.2 ppm were observed due to the methylene protons of the grafted PCL. These results clearly confirm the formation of the GG-*g*-PCL co-polymer. The grafting percentage of PCL was calculated by the relative intensity of the peaks at 1.4 and 1.2 ppm, which corresponds to 6 protons of b- and c-methylene groups of the PCL side-chain, and the peak at 5 ppm, which corresponds to the anomeric protons of GG backbone.

3.2. Micellar Properties of GG-g-PCL

In aqueous solutions the GG-*g*-PCL co-polymer formed micelles due to its amphiphilic nature. The formation of these micelles was accomplished through the balance of two competing functions. The hydrophobic function of the insoluble segments (i.e., PCL) led the co-polymer to aggregate away from the water phase to form the micelle cores, while the hydrophilic function of the soluble segments (i.e., GG) dissolved in the water to form the micelle shells [19]. To determine the micellar properties of GG-*g*-PCL in PBS solution, the CMC of the polymeric micelles was monitored by fluorescence spectroscopy using pyrene as a hydrophobic probe. In the excitation spectra, a definite shift from 332 to 336 nm was observed as GG-*g*-PCL concentration increased, indicating the formation of self-assembly of GG-*g*-PCL. It is reported that pyrene was preferentially solubilized into the hydrophobic cores of the micelles composed of core–shell structure when introduced into the aqueous phase [20]. The intensity ratio (I_{336}/I_{332}) of the pyrene excitation spectra *versus* the logarithm of co-polymer concentration is shown in Fig. 3. The CMC was obtained from the intersection of the baseline and the tangent of the

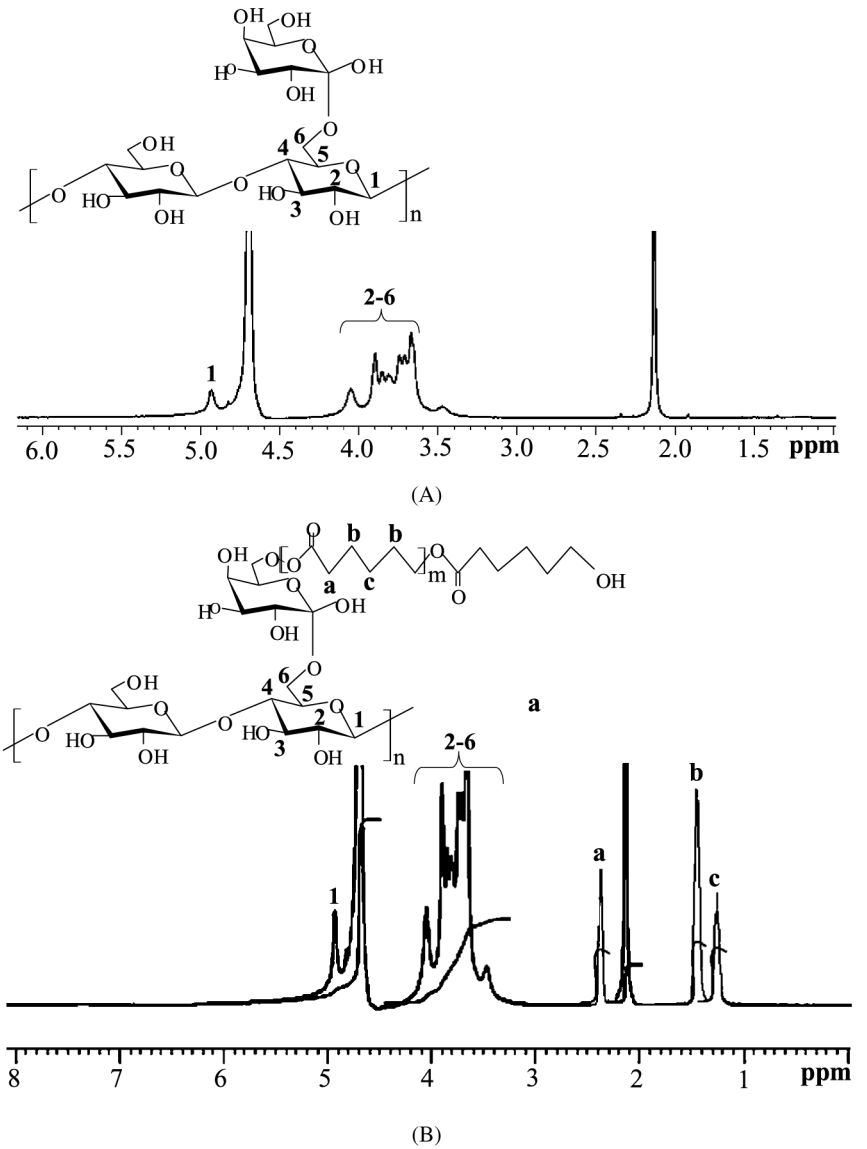

Figure 2. [1]H-NMR spectra of the (A) GG and (B) GG-*g*-PCL.

rapidly rising I_{336}/I_{332} curve in Fig. 3. The CMC of the GG-*g*-PCL co-polymer in PBS was determined as approx. 0.56 mg/l. Polymeric micelles with lower CMC will be suitable as drug-delivery carriers since they are stable in an aqueous environment and cannot easily dissociate on extremely diluted by blood in intravenous administration, and can prolong circulation in the bloodstream.

The size of the free and drug-loaded GG-*g*-PCL micelles was determined by DLS as shown in Fig. 4. Both the free and drug-loaded GG-*g*-PCL micelles exhibited a unimodal size distribution with a diameter range of 75–135 nm and

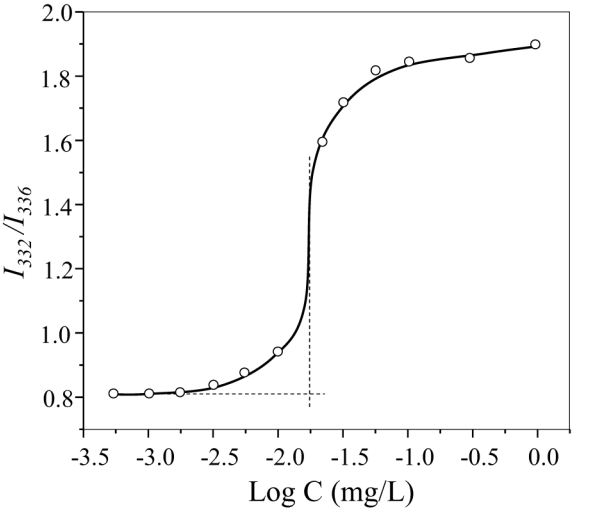

Figure 3. Change of the intensity ratio (I_{332}/I_{336}) *versus* the concentration of GG-*g*-PCL.

75–162 nm, respectively. The average hydrodynamic diameter of free and drug-loaded GG-*g*-PCL micelles was 105 and 113 nm, respectively, with a polydispersity index of 0.05. The marginal increase in the size of drug-loaded GG-*g*-PCL micelles might be due to the presence of drug inside the micelles. The size and morphology of GG-*g*-PCL micelles without drug was further evaluated by TEM. As shown in Fig. 5, the TEM images show that self-assembled GG-*g*-PCL micelles are well dispersed as individual micelle with regularly spherical shape, and the micelles are around 60–100 nm in diameter. The diameter of the micelles observed by TEM is smaller than its diameter obtained from the DLS experiment. The diameter of the micelles obtained from the DLS experiment reflects the hydrodynamic diameter of the micelles that are swollen in water, whereas the diameter of the micelles observed by TEM shows that of dried micelles. Therefore, an increase in the size of micelles obtained from DLS compared to that of TEM is assumed to be caused by the hydration of the shell portion of the micelles. The size range of the GG-*g*-PCL micelles (<150 nm) may be appropriate for drug targeting. The small GG-*g*-PCL micelles with a diameter less than 150 nm can avoid physical clearance by filtration in the lungs and in the spleen or excretion through the kidneys. As its unique core–shell structure and nanosize, it not only protects drugs from inactivation and prevents their sudden release in bloodstream in the physiological environment, but also can reduce the drug toxicity and make them suitable as long-circulating drug carriers.

3.3. Drug Release

Since GG-*g*-PCL micelles can solubilize hydrophobic compounds in aqueous solutions, we performed encapsulation and *in vitro* release studies under physiological conditions to evaluate the feasibility of using GG-*g*-PCL co-polymer as a hydrophobic drug-delivery carrier. The hydrophobic drug, ketoprofen, was used as

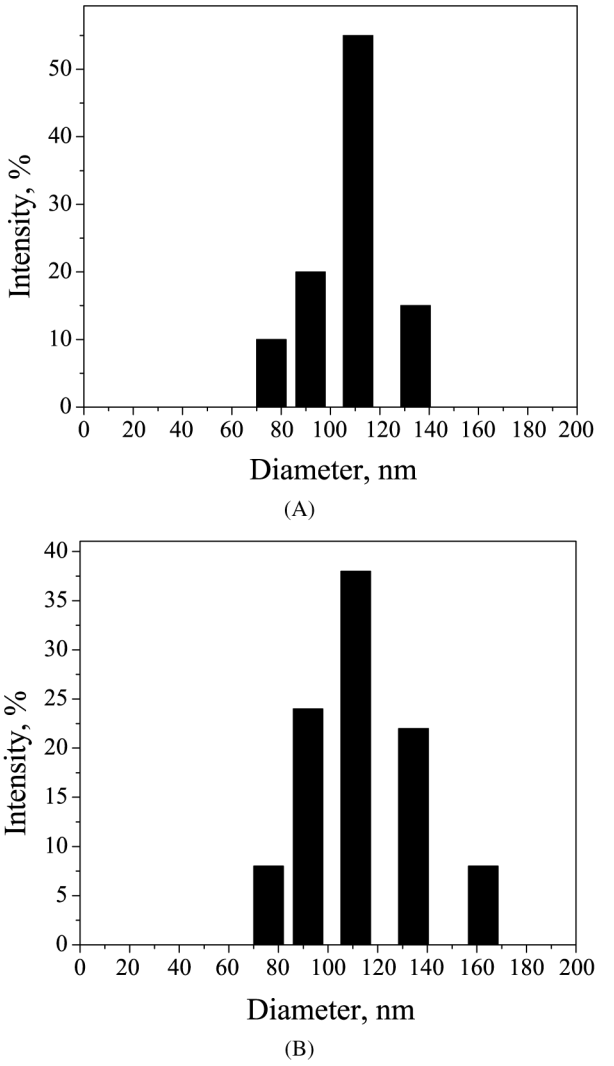

Figure 4. Size distribution of (A) GG-*g*-PCL micelles and (B) drug-loaded GG-*g*-PCL micelles.

the model drug, and the drug-loaded micelles were prepared by the dialysis method as described in Materials and methods. The ketoprofen content entrapped into the GG-*g*-PCL micelles was found to be 18.3 wt%. Figure 6 shows the release profiles of ketoprofen from GG-*g*-PCL micelles in pH 7.4 buffer solutions and at 37°C as a function of time. These studies suggest that ketoprofen encapsulated with GG-*g*-PCL micelles present an initial burst (approx. 0–35% of the initial loading amount), followed by a sustained release (approx. 35–90% of the initial loading amount) and a non-release phase (beyond 90% of the initial loading amount). In addition, it was observed that the drug release equilibrium was reached only after 68 h. The initial burst release of ketoprofen from the micelles was attributed to ketoprofen molecules

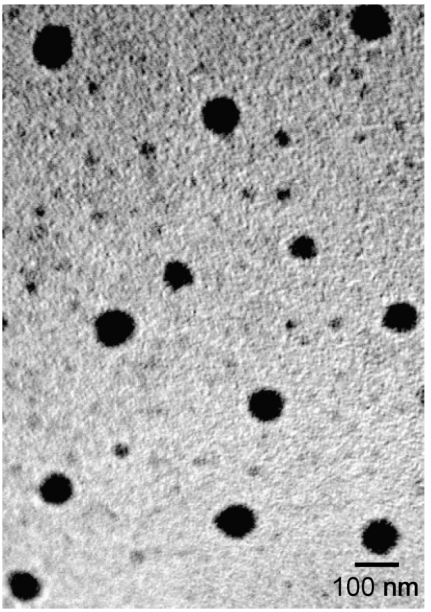

Figure 5. TEM image of GG-*g*-PCL micelles.

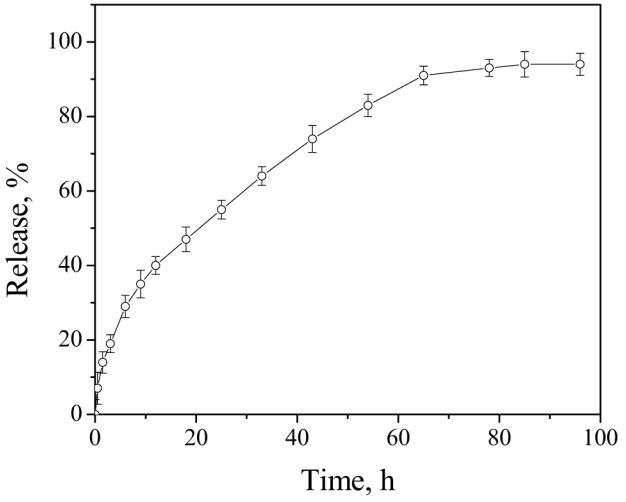

Figure 6. Ketoprofen release from GG-*g*-PCL micelles in pH 7.4 and at 37°C.

located within the shell or at the interface between the micelle core and shell [21]. The initial burst effect may also be attributed to the establishment of equilibrium between the drug inside micelles and the drug in solution present inside the tubing. The slow release of the drug from the GG-*g*-PCL micelles could be attributed to the hydrophobic–hydrophobic interactions between the drug molecules and the hydrophobic core of the GG-*g*-PCL micelles. Some of the drug molecules might

be adsorbed by tubing and solubilized into the hydrophobic core of the micelles, which may be the reasons for the observed non-release phase after 90% of drug release [22].

There are three primary mechanisms by which the release of drug molecules can be controlled: erosion, diffusion, and swelling followed by diffusion [23, 24]. The initial burst followed by a controlled drug release from the GG-*g*-PCL micelles showed that the release obeys a diffusion-controlled mechanism; however, the diffusion rates at each stage of the drug release differed considerably, suggesting that two different processes may be taking place. In the first 10 h, drug release occurs relatively faster. During this time, the drug present on the surface of shell or at the interface between the micelle core and shell may have good access to the surrounding aqueous environment through the micelle surface. However, diffusion after 10 h was dramatically slower, suggesting the drug had less access to the surrounding hydrophilic corona. This fraction of the drug loading could be entrapped in or even dissolved in dense solid regions of the hydrophobic core where polymer entanglement serves as a much greater impediment to drug transport [25].

3.4. In Vitro *Enzymatic Degradation*

For the application of biodegradable polymers as drug-delivery carriers, it is very important to know the short-term degradation profiles (0–7 weeks). The short-term degradation profile is more important to predict the drug-release mechanism of common chemotherapeutics and their excretion process from the body after the drug release. Therefore, in this study, the *in vitro* enzymatic degradation of GG-*g*-PCL co-polymer in PBS in the presence of endo-1,4-β-mannanase solution and esterase at 37°C was examined and compared by weight loss as a function of time. Since both endo-1,4-β-mannanase and esterase are present in the body fluid, they may enhance the degradation of GG-*g*-PCL by the generation radicals [26]. Therefore, in this study, the concentrations of these enzymes were chosen to approximate the endogenous levels.

Initially, the GG-*g*-PCL co-polymer in the digestion media exhibited defined sharp edges. These edges changed to smooth, rounded edges after incubation in endo-1,4-β-mannanase/esterase digestion solution at 37°C for extended periods of time, indicating enzymatic degradation of GG-*g*-PCL co-polymer. As shown in Fig. 7, the weight loss of the degrading co-polymer in the medium increased as a function of incubation time. The weight loss of GG-*g*-PCL co-polymer after 7 weeks was 82%. Within 2 weeks of degradation, the weight loss of the co-polymer marginally increased, indicating that chain scission by enzymatic degradation did not actively occur during the first 2 weeks. The fast degradation of GG-*g*-PCL co-polymer after 2 weeks may be ascribed to the enzymatic hydrolysis of fast degradable GG and PCL units. GG degradation may occur predominantly *via* random chain scission by simple hydrolysis of the glycosidic linkage of GG back bone, resulting in water-soluble oligomers with hydroxyl groups at the chain end [27, 28], while PCL units degrade into water soluble products containing acid and

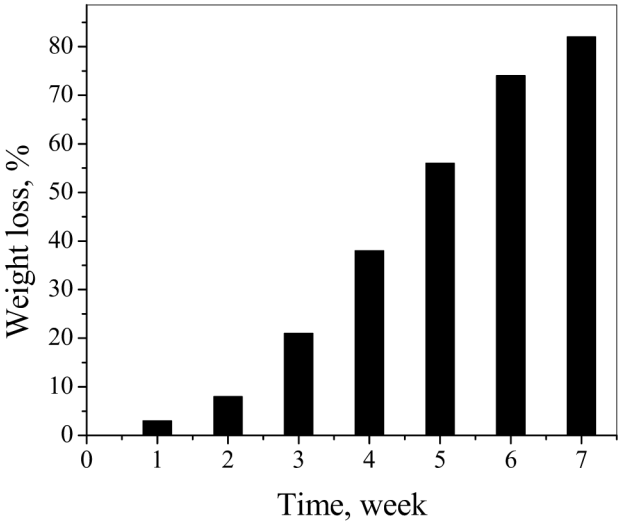

Figure 7. Weight loss of GG-*g*-PCL as a function of incubation time.

alcohol *via* enzymatic hydrolysis of the ester bond linkage [29]. From these results, it is clear that after 6 weeks of incubation at 37°C the GG-*g*-PCL co-polymer degraded into water-soluble lower-molecular-mass fragments, which could potentially be allowed excretion from the body *via* renal filtration in an *in vivo* application.

4. Conclusions

A novel type of non-toxic, biocompatible and biodegradable GG-*g*-PCL co-polymer was synthesized using microwave irradiation method. The grafting percentage of polycaprolactone was increased with increasing microwave treatment time. When the treatment time was about 9 min, the microwave graft polymerization of CL onto GG was greatly accelerated and improved. In aqueous solution, GG-*g*-PCL co-polymer self-assembled into spherical micelles due to its amphiphilic nature. DLS study revealed that the diameter of the GG-*g*-PCL micelles was in the range of 75–135 nm with a narrow distribution. The spherical shape micelles with the diameter range of 60–100 nm were observed by TEM. An anti-inflammatory drug, ketoprofen, was incorporated into the micelles with a loading capacity of about 18.3 wt%. The results of ketoprofen release experiments indicate that this system seems to be a very promising vehicle for the administration of hydrophobic drugs by controlled release. *In vitro* degradation results of the GG-*g*-PCL co-polymer show that degradation is slow in the first 2 weeks, which makes it an excellent drug carrier. After 2 weeks, an exhaustive degradation takes place, which provides the way for renal excretion. Overall, it is evident that the GG-*g*-PCL micelles could be used as drug carriers to achieve controlled drug release.

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