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Graft Copolymerization of Methyl Methacrylate onto Silk Sericin Initiated by Ceric Ammonium Nitrate

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A novel redox system, ceric ammonium nitrate (CAN)-silk sericin (SS) was applied to initiate graft copolymerization of methyl methacrylate (MMA) onto silk sericin in an acidic aqueous solution medium. The effects of monomer concentration, initiator concentration, reaction temperature, and time on grafting parameters had been investigated in detail. The results showed that the graft copolymerization occurred even at as a low temperature as 35°C, and high grafting efficiency and grafting percentage could be achieved at the same time. Taking grafting percentage and grafting efficiency as functions, the optimum reaction conditions were obtained as follows: [SS] = 10 g L^{-1}*, [MMA] = 0.3 mol* L^{-1}*, [CAN] =* 5.7×10^{-3} *mol* L^{-1}*, temperature = 35°C, time = 60 min. The structure and morphology of SS and SS-g-PMMA were characterized by fourier transform infrared (FT-IR),* ^{1}H*-nuclear magnetic resonance (*^{1}H*-NMR), scanning electron microscope (SEM) and gel permeation chromatography (GPC). A possible mechanism was proposed to explain the formation of radicals and the initiation.*

Keywords silk sericin, methyl methacrylate, ceric ammonium nitrate, graft copolymerization

Introduction

Graft copolymerization is a useful technique for modifying the properties of synthetic and natural polymers, and graft copolymers have been used as a stabilizer (1) of emulsion and dispersion, sorption agents (2) and biomedical materials (3). Silk sericin (SS) is a natural macromolecular protein derived from the silkworm Bombyx mori, and is characterized by unusually high sericine content (ca. 35%) (4–6). Recently, SS has been widely used in enzyme immobilization (7), biomaterials (8), cosmetic, membrane material, and functional fibers (9, 10). Although silk sericin has many good properties, it has some drawbacks such as instability in water, poor solubility in organic solvents, and low strength against microbial attack. Chemical modification of SS is a useful method to improve these drawbacks, and meanwhile allows the acquisition of additional properties of modified SS.

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The graft copolymerization of various monomers onto SS has been reported, such as vinyl acetate (11), acrylic acid (12), acrylamide (12), and styrene (2, 13). In these reaction systems, potassium persulfate or its redox system is commonly used as an initiator. Wei et al. (2) studied the graft copolymerization of styrene onto SS initiated by potassium persufate-sodium bisulfite redox system, and the results showed that maxium grafting percentage was achieved at 70°C. Yao et al. (11) synthesized graft copolymers of SS and vinyl acetate using potassium persulfate as the initiator in alkaline aqueous medium beyond 70°C. However, low grafting efficiency and high initiation temperature limit their application in chemical modification of protein. Ceric ammonium nitrate (CAN) has preferential use as an initiator over other free radical initiation in grafting vinyl monomers onto different polyols. It is based on the simple reaction mechanism of single electron transfer, in which free radicals are directly produced on the backbone of the polymer itself, and initiates graft copolymerization at ambient temperature in aqueous medium (14–16). To our knowledge, there are no reports on the graft copolymerization of vinyl monomer onto silk sericin initiated by ceric ammonium nitrate (CAN). In the present paper, graft copolymerization of methyl methacrylate onto SS has been carried out using Ce (IV)-SS redox system in an acidic medium. The effects of variables on the grafting parameters have been investigated in detail, and the optimum grafting conditions were obtained. The structure and morphology of SS-g-PMMA were characterized by FT-IR, ^{1}H-NMR, SEM and GPC. Moreover, a possible mechanism of initiation is proposed.

Experimental

Materials

Silk sericin (SS) was purchased from Huzhou Atesi Biochemical Co., Ltd (China) and used without further purification. Analytical grade methyl methacrylate (MMA), obtained from Chengdu Kelong Chemical Industry (China), was inhibitor free by washing successively with a 10% (w/w) aqueous sodium hydroxide solution and distilled water, dried over anhydrous calcium chloride, and finally distilled under reduced pressure before use. Analytical grade ceric ammonium nitrate (CAN) was purchased from Tianjing Kemio Chemical Agent Develop Center (China), and used after being dried for 6 h at 110°C, for its being hydroscopic in nature (17). Deionized water was used for all experiments. All other chemicals and solvents used were of analytical reagent grade.

Synthesis and Purification of Graft Copolymers

Graft copolymerization was carried out in a four-neck round bottom flask equipped with a reflux condenser, nitrogen gas inlet, thermometer and magnetic stirrer. To control the reaction temperature, the flask was placed in a hydrothermal bath. For a total 100 mL of solution, 1.0 g SS was first dissolved in hot water, and then cooled to room temperature. The required amount of MMA was added to the sericin solution and purged with nitrogen for 30 min. Subsequently, the mixture was heated to the specified temperature before adding the initiator CAN, which was dissolved in 0.1 mol L^{-1} nitric acid. The graft copolymerization was carried out at different MMA concentrations, CAN concentrations, temperatures and reaction periods. Up to a specified reaction time, the inhibitor was added to terminate the reaction. The product was precipitated using methanol, filtered, and was washed repeatedly with deionized water. The product was then dried at 60°C in a vacuum oven to a constant weight. Poly(methyl methacrylate) (PMMA), which was probably formed during the graft copolymerization reaction, was separated from the grafted polymer by Soxhlet extraction (18, 19).

Acid Hydrolysis of SS-g-PMMA by Hydrochloric Acid Solution

Destruction of the silk sericin component in SS-g-PMMA was carried out by acid hydrolysis. This was done by, heating under reflux, a certain amount of copolymer in a required amount of 6M hydrochloric acid at 110°C for 20 h (20). The residue was then filtered to obtain the precipitate. The precipitate was dissolved in acetone, subsequently filtered, and the filtrate was then precipitated in water. This process was repeated three times, and finally the PMMA grafts from deionized water were dried in a vacuum oven at 60°C to a constant weight.

Measurements

FT-IR spectra of SS and grafted SS were recorded on a NICOLET 200SXV Fourier transform infrared (FT-IR) spectrophotometer using a potassium bromide disc technique, and the scanning was carried out from $4500\,\mathrm{cm}^{-1}$ to $400\,\mathrm{cm}^{-1}$. ^{1}H-NMR spectra were recorded for the solution of a side-chain polymer in $CDCl_3$ employing a Bruker AVANCE (300 MHz) instrument operated at room temperature. Chemical shifts were quoted in ppm with tetramethylsilane (TMS) as the referrer. Gel permeation chromatography (GPC) was performed on a GPC-StyrageIHR4E. Tetrahydrofuran was used as the eluent at a flow rate of 0.35 mL/min. The GPC system was calibrated with polystyrene standard samples. The surface morphology of SS and grafred SS were studied by SEM (KYKY-AMRAY-1000B), and the stub was coated with gold using a Bel-ted Scd 50 sputter coater.

Results and Discussion

The grafting parameters, including the percentage of grafting (GP) and grafting efficiency (GE) were calculated according to Athawale, V. D. et al. (21) as follows:

$$\mathrm{GP}\ (\%) = 100 \times (W_2 - W_1)/W_1$$
$$\mathrm{GE}\ (\%) = 100 \times (W_2 - W_1)/W_3$$

where W_1 is the weight of silk sericin, W_2 is the weight of PMMA grafted silk sericin, and W_3 is the weight of the monomer.

Effect of MMA Concentration

Figure 1 shows the effect of MMA concentration on grafting parameters. It can be seen that GP increased significantly with the increase of MMA concentration, and GE has a similar trend. The increase of GP and GE could be due to the greater availability of MMA to the grafting sites. It is not observed under the present reaction conditions that GP decreases with increasing the concentration of MMA, which is generally seen in other reaction systems (22, 23). This proves the high efficiency of CAN used as initiator in the reaction system, which results in the formation of little homopolymer (24).

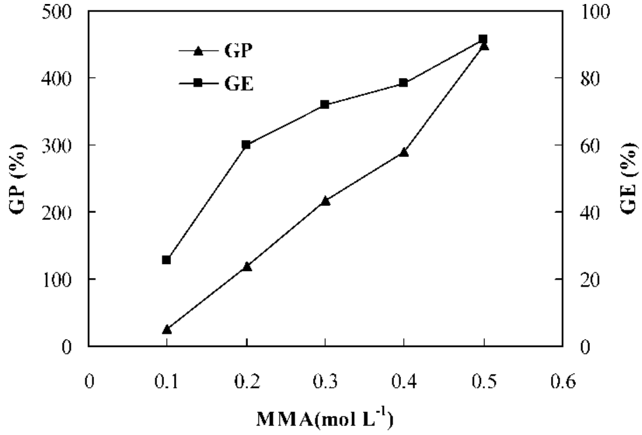


Figure 1. Effect of methyl methacrylate concentration on the graft copolymerization. [SS]:10 g L^{-1}; [CAN]: 7.6 × 10^{-3} mol L^{-1}; [HNO$_3$]: 2.0 × 10^{-2} mol L^{-1}; Time: 120 min; Temperature: 35°C.

Effect of Reaction Time

The graft copolymerization had been recorded at different reaction times ranging from 10 to 150 min at constant concentration of the ceric ammonium nitrate (7.6 × 10^{-3} mol L^{-1}), methyl methacrylate (3.0 × 10^{-1} mol L^{-1}), and nitric acid (2.0 × 10^{-2} mol L^{-1}) at 35.0°C. With the increase of polymerization time, the GP increased rapidly up to 30 min, and beyond 30 min, the polymerization time had a small effect on the grafting reaction (Figure 2). GE showed a similar trend of changing to GP. The increase of GP and GE with time is attributed to the increase of the number of grafting sites on silk sericin backbone in the initial stage of reaction. A longer reaction time had little effect on GP and GE since the number of active sites remains almost stable. Thus, the optimum polymerization time is proposed as 60 min.

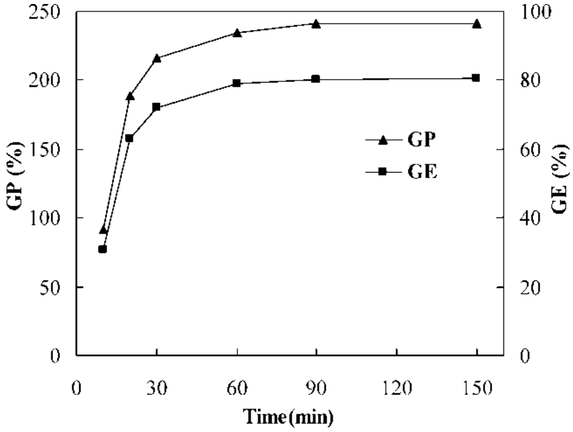


Figure 2. Effect of reaction time on the graft copolymerization. [SS]: 10 g L^{-1}; [MMA]: 0.3 mol L^{-1}; [HNO$_3$]: 2.0 × 10^{-2} mol L^{-1}; [CAN]: 7.6 × 10^{-3} mol L^{-1}; Temperature: 35°C.

Effect of Reaction Temperature

The effect of reaction temperature on grafting parameters is depicted in Figure 3. It can be seen that GP and GE showed an increasing trend up to 35°C. The increase of temperature can enhance the dynamic energy of monomer molecules, which leads to the increase of diffusion rate of monomer molecules from the reaction mixture to the active sites on silk sericin backbones, and ultimately results in the increase of GP and GE. With further raising the temperature, GP and GE decreased. This may be due to the increase of rate of chain transfer and chain termination reactions between monomer molecules and grafted chains.

Effect of CAN Concentration

The graft polymerization had been investigated at different CAN concentrations from 3.8×10^{-3} mol L^{-1} to 11.4×10^{-3} mol L^{-1} at constant concentration of methyl methacrylate (3.0×10^{-1} mol L^{-1}), and nitric acid (2.0×10^{-2} mol L^{-1}) at 35°C. It can be established from Figure 4 that GP increased with the increase of CAN concentration up to 5.7×10^{-3} mol L^{-1}, and GP showed a decreasing trend beyond 5.7×10^{-3} mol L^{-1}. Raising the CAN concentration will result in an increase of free-radical active sites on the silk sericin backbone, which may lead to the increase of GP. With the further increase of CAN concentration, it accelerates the dissociation rate of Ce (IV), which reduces the concentration of CAN participating in graft copolymerization. Meanwhile, a higher concentration of CAN makes Ce (IV) easier to be evolved in termination reaction. Therefore, GP decreases with a further increase of CAN concentration. GE has the same trend as GP on varying CAN concentration.

Proof of Grafting Copolymerization

The proof of grafting was directly obtained from the FT-IR spectra (Figure 5) of pure SS and SS-g-PMMA after an exhaustive Soxhlet extraction with acetone. FT-IR spectrum of SS-g-PMMA (Figure 5b) shows a new absorption band at 1730 cm^{-1} corresponding to the

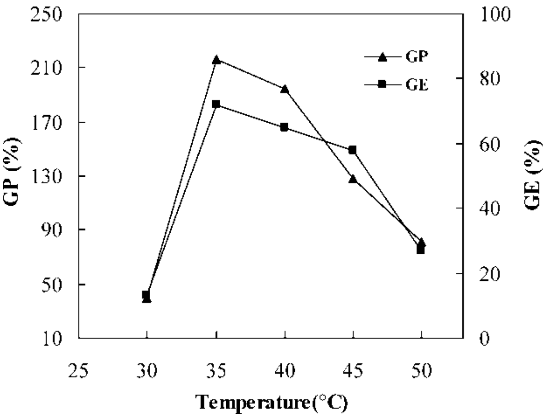


Figure 3. Effect of reaction temperature on the graft copolymerization. [SS]: 10 g L^{-1}; [MMA]: 0.3 mol L^{-1}; [HNO_3]: 2.0×10^{-2} mol L^{-1}; [CAN]: 7.6×10^{-3} mol L^{-1}; Reaction time: 60 min.

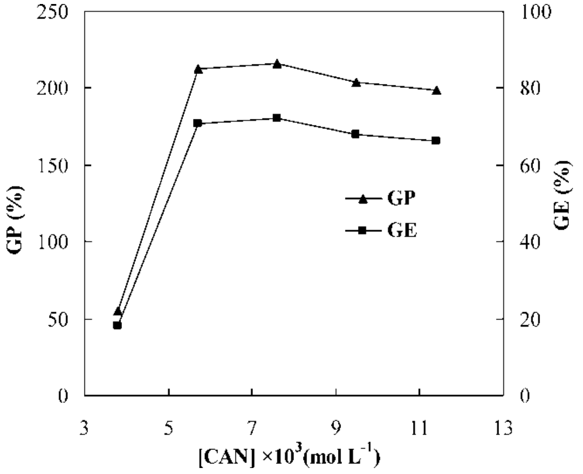


Figure 4. Effect of CAN concentration on the graft copolymerization. [SS]: 10 g L^{-1}; [MMA]: 0.3 mol L^{-1}; [HNO$_3$]: 2.0 × 10^{-2} mol L^{-1}; Temperature: 35°C; Reaction time: 60 min.

ester carbonyl group (C═O) of methyl methacrylate. In addition, the other two small peaks at 2998 cm^{-1} and 2951 cm^{-1} were assigned to the –OCH$_3$ and CH-aliphatic group of MMA, respectively (25). These peaks were obviously absent in the spectra of pure SS (Figure 5a) The appearance of the new absorption bands in the spectra of SS-g-PMMA gave evidence for the grafted product and suggested that MMA had been successfully grafted onto SS. Furthermore, the increase in weight of the product extracted using acetone for each control reaction, over that of the SS used initially, also confirms the evidence of grafting as well as the difference in the solubility characteristic of pure and grafted silk sericin.

Due to the lack of solubility of the graft copolymers in most common organic solvents, nuclear magnetic resonance (NMR) technique cannot be used directly to

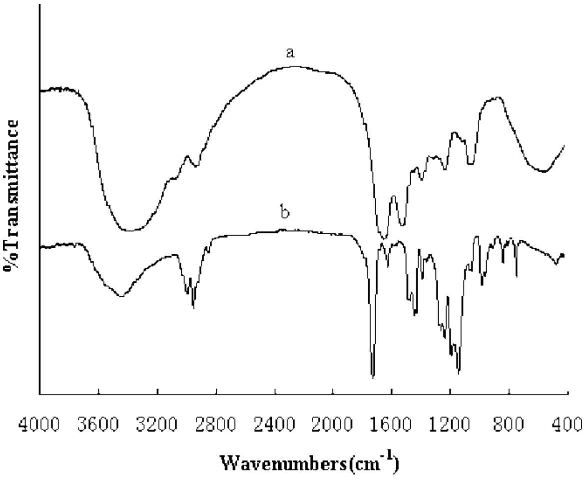


Figure 5. FT-IR spectra of (a) silk sericin and (b) SS-g-PMMA.

characterize the graft copolymers. However, the constitute of the graft copolymer can be obtained indirectly by acid hydrolysis technique, in conjunction with infrared spectroscopy (26).

Figure 6 shows the ^{1}H-NMR spectrum of the grafting polymer chains obtained from the acid hydrolysis of SS-g-PMMA. The single peak at 3.60 ppm is ascribed to the methyl protons of $-OCH_3$ in the side chain polymer (PMMA). The peak at 2.02 ppm is due to the methylene protons in the side chain polymer. The peaks from 0.99 ppm to 1.90 ppm are corresponding to the methyl protons of $-C-CH_3$. Therefore, it can be indirectly confirmed that methyl methacrylate has been successfully grafted onto silk sericin.

During the studies, the glass transition temperature (Tg) of PMMA segments cannot be obtained from differential scanning calorimetry (DSC) of SS-g-PMMA. The possible explanation of the result presented was that the molecular weight of grafting polymer chains was lower. It can be obtained from Figure 7 that the number-average molecular weight (M_n) of PMMA grafts was 75 kDa, which confirms the explanation described above and the evidence of grafting. Zhu et al. (27) showed that Tg of PMMA grafts could be seen from DSC of SS-g-PMMA in which the number-average molecular weight of PMMA grafts ranged from 280 kDa to 310 kDa.

Graft copolymerization considerably modifies silk sericin morphology. The surface morphology of pure SS and SS-g-PMMA was observed by scanning electron microscopy (SEM). It can be seen from Figure 8a that pure SS exhibits a smooth surface morphology, which may be due to the fact that SS is spherical protein, while SS-g-PMMA shows a rough surface morphology (Figure 8b), which may be attributed to the grafting polymer onto the SS.

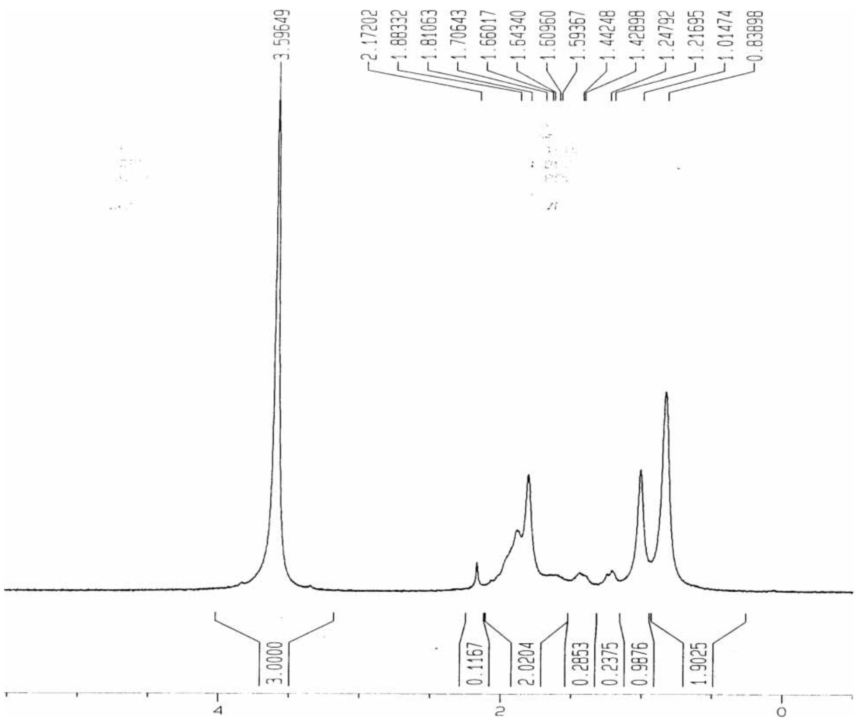


Figure 6. ^{1}H-NMR spectrum of PMMA grafts in $CDCl_3$.

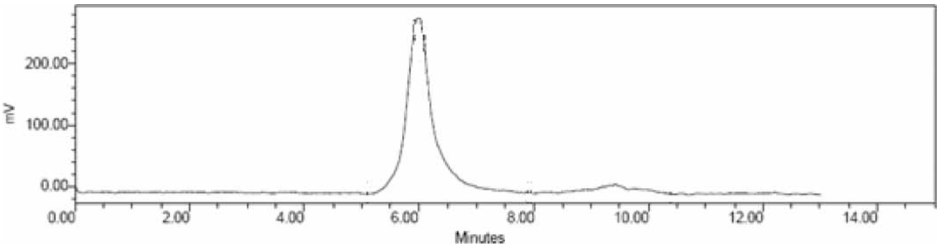
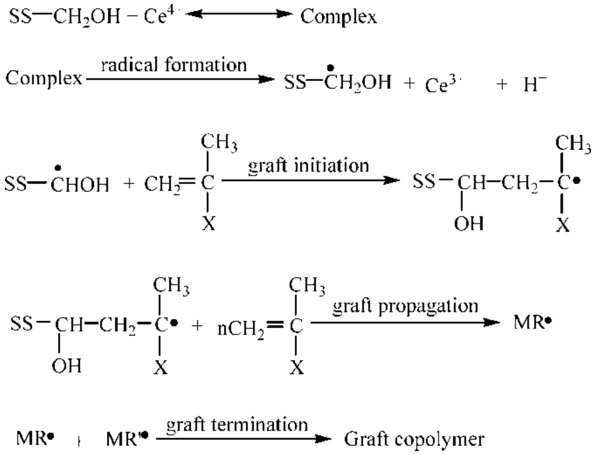


Figure 7. GPC of the grafting polymer chain after acid hydrolysis.

The Initiation Mechanism of Graft Copolymerization

Referring to KC Gupta (18) who studied the graft copolymerization of N-Isopropylacrylamide onto cellulose initiated by ceric ammonium nitrate, we propose a possible initiation mechanism of graft copolymerization as follows:



$$\mathrm{SS{-}CH_2OH - Ce^{4}} \longleftrightarrow \mathrm{Complex}$$

$$\mathrm{Complex} \xrightarrow{\text{radical formation}} \mathrm{SS{-}\dot{C}H_2OH + Ce^{3} + H^{-}}$$

$$\mathrm{SS{-}\dot{C}HOH + CH_2{=}\underset{X}{\overset{CH_3}{C}}} \xrightarrow{\text{graft initiation}} \mathrm{SS{-}\underset{OH}{CH}{-}CH_2{-}\underset{X}{\overset{CH_3}{C}}\bullet}$$

$$\mathrm{SS{-}\underset{OH}{CH}{-}CH_2{-}\underset{X}{\overset{CH_3}{C}}\bullet + nCH_2{=}\underset{X}{\overset{CH_3}{C}}} \xrightarrow{\text{graft propagation}} \mathrm{MR\bullet}$$

$$\mathrm{MR\bullet + MR'\bullet} \xrightarrow{\text{graft termination}} \text{Graft copolymer}$$

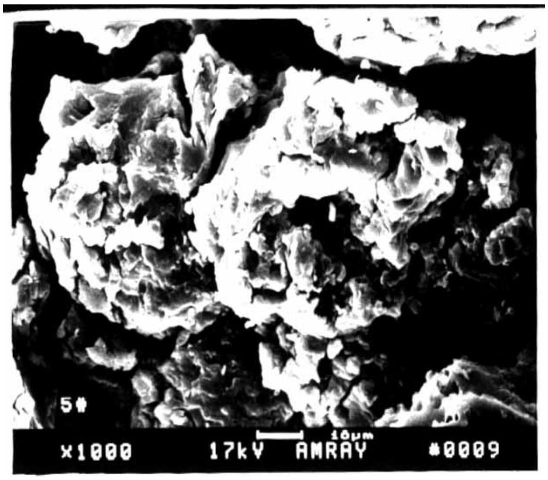


Figure 8. SEM of (a) SS and (b) SS-g-PMMA with grafting percentage 341%.

Where SS–CH_2OH is silk sericin with side –CH_2OH groups, X is –$COOCH_3$, and $MR^{\cdot}$ and $MR'^{\cdot}$ are macromolecular free radicals.

Conclusions

By using the novel redox system (CAN-SS), SS-g-PMMA was successfully synthesized in acidic aqueous medium. FT-IR, ^{1}HNMR and SEM techniques were used to give the evidence of graft copolymerization of MMA onto silk sericin. The Mn of PMMA grafts was obtained by GPC. Results showed that CAN-SS was an efficient redox system to synthesize SS-g-PMMA at ambient temperature, and variables such as concentrations of MMA and CAN, reaction temperature and reaction time had obvious effects on GP and GE. SS-g-PMMA have properties different from SS unmodified, and therefore further work will be necessary and significant to investigate the properties of SS-g-PMMA. Further studies in this direction are currently in progress.

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