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Preparation and characterization of a poly(ethylene glycol) grafted carboxymethyl konjac glucomannan copolymer

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ABSTRACT

Carboxymethyl konjac glucomannan (CKGM) grafted methoxy poly(ethylene glycol) (mPEG) (CKGM-g-mPEG) copolymer was prepared by reacting the CKGM with methoxy poly(ethylene glycol) amine in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (EDC) and N-hydroxysuccimide (NHS). By varying the mPEG-OCOCH2NH2/CKGM feed ratio, the degree of substitution (DS) of mPEG could be adjusted. The degree of substitution (DS) of mPEG into CKGM was calculated from TGA data. The chemical structure and physical properties of the products were identified by means of FT-IR, ^{1}H NMR, DSC and TGA. Solubility and the viscosity tests showed that CKGM-g-mPEG has better solubility and lower viscosity at low shear frequency as compared to non-pegylated CKGM.

1. Introduction

Natural polymers have attracted significant attention by chemists and biochemists due to their outstanding properties such as biocompatibility, bioactivity and biodegradability. In the past decade polysaccharides, one of the natural polymers, have been shown to play an important role in the biomedical, controlled release and biochemical fields with several industrial applications (Chen, Jo, & Park, 1995; Liu, Jiao, Wang, Zhou, & Zhang, 2008). Konjac glucomannan (KGM), a high molecular weight and water soluble natural polysaccharide isolated from the tubers of the widespread plant *Amorphophallus konjac* in China and Japan, is composed of β-(1 → 4) linked β-D-glucose and β-D-mannose in the molar ratio 2:3–1:1.6 (Shimahara, Suzuki, Sugiyama, & Nishizawa, 1975). KGM has the ability to lower blood cholesterol and sugar level, helps with weight loss, promotes intestinal activity and reduces the risk of developing diabetes and heart disease (Chen, Cheng, Liu, Liu, & Wu, 2006; Li, Xia, Wang, & Xie, 2005). Moreover, KGM with its characteristics of low cost, good biocompatibility and biodegradability displays promising application in food and food additives, pharmaceuticals, biotechnology and the fine chemical industry (Zhang, Xie, & Gan, 2005). In recent years, the indepth development and applications of KGM and its derivatives have been widely reported (Du et al., 2004; Nakano, Takikawa, & Arita, 1979; Wang et al., 2008). Several chemical modification techniques have been applied to develop bio-related functional materials having properties unrepresentative to the initial KGM, e.g. palmitoylation (Tian, Dong, & Chen, 1998), graft (Xiao, Gao, Li, & Zhang, 1999), carboxymethylation (Kobayashi, Tsujihata, Hibi, & Tsukamoto, 2002), sulfation (Zhang, Gan, Xie, & Xiao, 2005) and quaternization (Yu, Huang, Ying, & Xiao, 2007). However, the synthesis and characterization of modified KGM with another polymer or oligomer has not been reported yet.

Poly(ethylene glycol) (PEG) is a neutral, water soluble and non-toxic polymer which has been employed for pharmaceutical and biomedical applications (Harris & Zalipsky, 1997). PEG is widely used by the FDA for internal consumption and injection in a variety of food, cosmetics, medicines and drug delivery systems (Cavalla, 2001). It was introduced into several biomaterials and drugs because of good immunogenicity and the ability of preserving the biological properties of proteins. PEGylation of chitosan (Ganji & Abdekhodaie, 2008; Jeong, Kim, Jang, & Nah, 2008; Sugimoto, Morimoto, Sashiwa, Saimoto, & Shigemasa, 1998), poly(lactic acid) (Huh & Bae, 1999) and alginate (Laurienzo, Malinconico, Motta, & Vicinanza, 2005) to improve their solubility, gelation ability or stability have been reported.

This study describes the synthesis and characterization of a carboxymethyl KGM grafted monomethoxy PEG (CKGM-g-mPEG)

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copolymer with grafted PEG oligomers on the high molecular weight KGM. This novel CKGM-g-mPEG graft polymer is expected to combine the beneficial properties of both KGM and PEG for advanced applications in several fields including biotechnology and pharmaceuticals.

2. Experimental section

2.1. Materials

KGM was purchased from Chengdu Root Industry Co., Ltd. (Chengdu, China). Methoxy poly(ethylene glycol) (mPEG) (M_n = 2000), *N*-*tert*-butoxycarbonylglycine (*N*-*t*-Boc-glycine), 4-(dimethylamino) pyridine (DMAP), 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (EDC), dicyclohexylcarbodiimide (DCC), morpholinoethane sulfonic acid (MES), *N*-hydroxysuccinimide (NHS) and trifluoroacetic acid (TFA) were procured from Sigma Aldrich (Shanghai, China). All other reagents were of analytical grade and were used directly without further purification. All solvents and water were redistilled freshly.

CKGM was prepared as described by Shinsaku, Shigetomo, Naruhiro, and Yoshinori (2002). The degree of substitution was found to be 63.7% measured according to methods described in the literature (Smith, 1967).

2.2. Preparation of CKGM-g-mPEG

The synthesis procedure of CKGM-g-mPEG is shown in Scheme 1.

2.2.1. Synthesis of mPEG-$OCOCH_2NH_2$

mPEG-$OCOCH_2$NH-Boc was synthesized according to the method of Eiselt, Lee, and Mooney (1999). Briefly, mPEG was dried under high vacuum at 50 °C for 24 h before use. mPEG (2.5 mmol), N-*t*-Boc-glycine (5.5 mmol) and DMAP (catalysis amount) were dissolved in 30 mL CH_2Cl_2. Subsequently, 6 mmol DCC was added, followed by stirring for 24 h at 0 °C. After removing dicyclohexylurea (DCU) by filtration, the filtrate was concentrated using a rotavapor at 30 °C. The resultant solid was dissolved in minimal amount of acetone and cooled overnight at 4 °C. The precipitated DCU was subsequently filtered off. The solvent was evaporated, and the obtained product was then put in a vacuum oven at room temperature for further drying. mPEG-$OCOCH_2NH_2$ was prepared by removing the *tert*-butoxycarbonyl (Boc) group of mPEG-$OCOCH_2$NH-Boc with TFA. mPEG-$OCOCH_2$NH-Boc was dissolved in a mixture of CH_2Cl_2/TFA (1/1, v/v). The solution was stirred for 2 h at 0 °C and then evaporated at room temperature to remove CH_2Cl_2 and part of the TFA. The residual mixture was dissolved in a 15% saline solution, and the pH was carefully adjusted to 5.0 using NaOH (1.0 M) solution and TFA. After removing the precipitate by filtration, the filtrate was extracted at least three times with chloroform. The organic phases were then combined and dried over Na_2SO_4. The drying agent was filtered and the solvent evaporated. The oil residue was dried under vacuum over P_2O_5 at room temperature for 24 h.

2.2.2. Synthesis of CKGM-g-mPEG

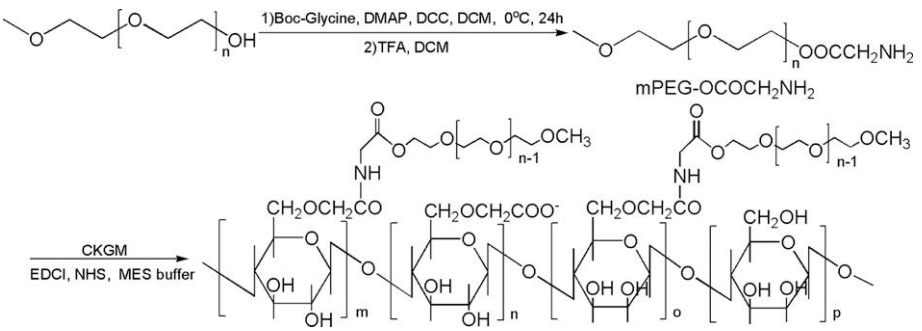
The CKGM solution was prepared in a buffer solution of 0.1 M MES and 0.5 M NaCl at a concentration of about 2.5% (w/v), and the pH was adjusted to 6.0. Quantitative EDC and NHS (molar ratio of EDC:NHS:COO^- = 1:0.5:1) were then added to the CKGM solution to activate the carboxylic acid groups of the polysaccharide backbone. The solution was agitated and was followed by adding the mPEG-$OCOCH_2NH_2$ solution (also prepared in the MES buffer solution at a concentration of 6.0% w/v). The precise weight of NHS, EDC and mPEG-$OCOCH_2NH_2$ differed according to the different graft ratios of CKGM-g-mPEG. The reaction was undertaken at room temperature for 10 h. The resulting mixture was dialyzed against pure water for 36 h and evaporated to dryness. The dried product was washed in acetone and chloroform to remove the residual mPEG and the organic solvent was removed by vacuum drying. Graft copolymers with various ratios of mPEG/CKGM units were prepared. The notations CP20, CP40, CP80 and CP100 indicate that the molar ratio of mPEG/CKGM unit feed ratio is 0.2, 0.4, 0.8 and 1.0, respectively.

2.3. Characterization of CKGM-g-mPEG

FT-IRs were recorded at room temperature on a Perkin–Elmer Spectrum One FT-IR spectrometer (USA) in the range 400–4000 cm^{-1}. The ^1H NMR spectra were recorded with an Avance Bruker-600 spectrometer (Switzerland). Differential scanning calorimetry (DSC) studies on the CKGM-g-mPEG samples were performed under a nitrogen atmosphere with a flow capacity of 25 mL/min on DSC 204-F1 (NETZSCH, Germany) from 20 to 350 °C at a heating rate of 10 °C/min. Samples were dried under a vacuum drier for several days before tests. The thermal gravimetric analysis (TGA) for the samples were measured using a TA Q500 thermo analyser (TA instruments, Delaware, USA) at the heating rate of 10 °C/min under N_2 atmosphere in the temperature range of 25–500 °C.

2.4. Solubility determination

The solubility test of CKGM-g-mPEG was performed by adding 500 mg (every sample in excess) CKGM-g-mPEG into 2.0 mL deionized water followed by stirring for 2 h at reflux temperature. The paste was then cooled, centrifuged at 10,000 rpm and the 0.50 mL supernatant was lyophilized. The amount of the solute was recorded and the solubility accordingly calculated.



Scheme 1. Synthesis procedure of the CKGM-g-mPEG.

2.5. Dynamic viscosity determination

CKGM and CKGM-g-mPEG powders (CP100 was taken for example) were dispersed in water (2.0 wt.%) and stirred for 15 min by a mixer until the samples were dissolved totally. Dynamic viscosity measurements were undertaken using an advanced rheometer (Bohlin Gemini 200, Malvern Instruments Ltd., UK) in the parallel plate mode with a diameter of 40 mm at 30 °C. The parallel plate gap was 500 μm. Frequency dependence of the viscosity for CKGM-g-mPEG and CKGM were recorded. The scanning scope of frequency was 0.1–100 Hz.

3. Results and discussion

3.1. Synthesis of mPEG-$OCOCH_2NH_2$

For chemical coupling of mPEG onto CKGM, the terminal group of mPEG should be converted from the hydroxyl to a primary amino group. mPEG-$OCOCH_2NH_2$ was prepared via two steps: (1) synthesis of mPEG-$OCOCH_2$NH-Boc by esterifying mPEG with N-*t*-Boc-glycine, and (2) removal of the Boc group in mPEG-$OCOCH_2$NH-Boc. The formation of mPEG-$OCOCH_2$NH-Boc was evidenced by ^{1}H NMR (Fig. 1A). Signal g (1.45 ppm) corresponding to methyl protons of the Boc group indicated the formation of mPEG-$OCOCH_2$NH-Boc. The chemical shift of the terminal methyl of mPEG is 3.32. The peaks between 3.67 and 3.41 are assigned to the main-chain proton of CH_2 for mPEG. The chemical shift of the terminated CH_2 linked to the glycine segment of mPEG is 4.32. The peak at 3.91 is assigned to CH_2 of the glycine segment. The chemical shift of the proton of the NH is at 5.05. The Boc group was easily removed via hydrolysis in an acidic environment. Fig. 1B shows the ^{1}H NMR spectra of mPEG-$OCOCH_2NH_2$ and it is very similar to that of mPEG-$OCOCH_2$NH-Boc except for the absence of the signal of methyl for Boc and of NH for the acylamide. It indicates the disappearance of the Boc group in mPEG-$OCOCH_2NH_2$ because they were completely removed in the de-protecting procedure by TFA. It should be noted that pH control is important for removal of the Boc protecting group. The pH of the residual mixture should be carefully adjusted to 5.0, otherwise the intermediate mPEG-$OCOCH_2NH_2$ could be hydrolyzed to mPEG in a very short time. If the pH value of the system is higher than 7.0 in the de-protecting procedure, then the mPEG-$OCOCH_2$NH-Boc would be hydrolyzed to mPEG totally.

3.2. Synthesis of CKGM-g-mPEG

Due to the presence of a carboxylic acid group, aqueous carbodiimide chemistry is a choice for the selective functionalization of CKGM. This approach uses a water soluble carbodiimide, EDC, which allows the formation of amide linkages by the nucleophilic attack of primary amines of PEG onto an *O*-acylisourea as an activated form of the carboxylic group. The co-reagent NHS, developed for peptide synthesis, is expected to limit rearrangement to the stable *N*-acylurea by the formation of an active ester intermediate

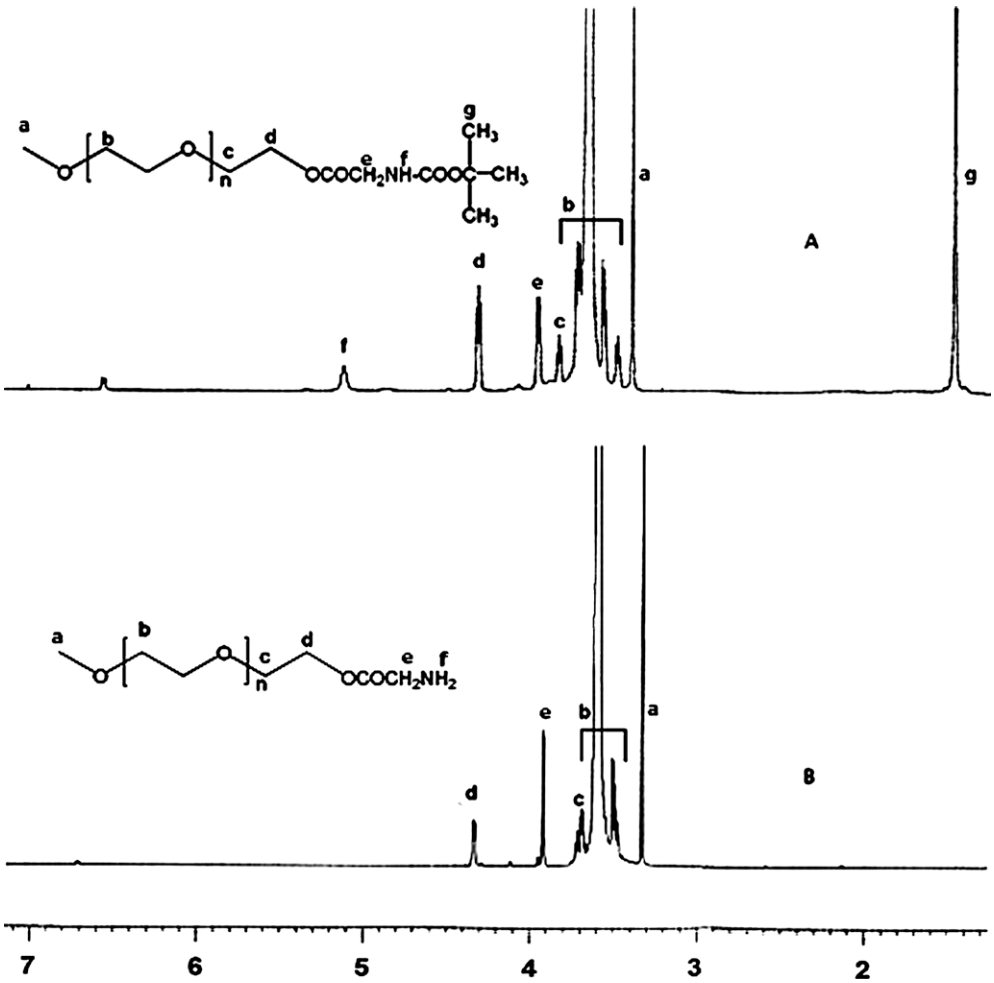


Fig. 1. The ^{1}H NMR spectra for (A) mPEG-$OCOCH_2$NH-Boc and (B) mPEG-$OCOCH_2NH_2$ in $CDCl_3$.

which is more stable than the *O*-acylisourea (Gomez, Chambat, Heyraud, Villar, & Auzély-Velty, 2006; Yang, Goto, Ise, Cho, & Akaike, 2002; Yoshioka, Tsuru, Hayakawa, & Osaka, 2003).

The formation of CKGM-g-mPEG is evidenced by ^{1}H NMR (CP80 is shown as an example in Fig. 2B). The strong –CH_2– peaks of mPEG are present between 3.50 and 3.80 ppm which cannot be seen in the spectra of CKGM (Fig. 2A); the methyl group of mPEG appears at about 3.42 ppm. The peaks specific to CKGM appear between 2.80 and 4.80 ppm. The assignment for the peaks of ^{1}H NMR of the polysaccharide and its derivates are difficult. Therefore, the assignment is based on the work of Jeong et al. (2008) as they studied methoxy poly(ethylene glycol) grafted water soluble chitosan, which is similar to CKGM-g-mPEG. A series of CKGM-g-mPEG copolymers with varying content of the mPEG side chain were synthesized. The degree of mPEG substitution (DS) to CKGM is listed in Table 1.

3.3. FT-IR analysis

The FT-IR spectra for CKGM, mPEG, and CKGM-g-mPEG copolymers in the wave number range of 450–4000 cm^{-1} are shown in Fig. 3. CKGM has characteristic absorptions at 3436, 2925, 1616 and 1070 cm^{-1} corresponding to the stretching of –OH groups, C–H of methyl, and C–O of associate hydroxyl groups.

The FT-IR spectrum of mPEG shows –OH group stretching vibration at 3435 cm^{-1} and symmetric stretching of CH_2–O at 2889 cm^{-1}. Peaks at 1280, 1343 and 1468 cm^{-1} illustrate the presence of CH_2 and CH_3 groups. The highest peak at 1111 cm^{-1} is assigned to the stretching vibration of ether bonds in mPEG.

In the cases of CKGM-g-mPEG copolymers, peaks at 1108, 1281, 1343 and 1467 cm^{-1} indicate the presence of mPEG in the CKGM backbone. The weak peak at 2925 cm^{-1} that is assigned to C–H in the CKGM has changed to a strong peak at 2887 cm^{-1}, and the peak at 1070 cm^{-1} that is assigned to C–O in CKGM has changed to 1108 cm^{-1}. This further confirms the connection of mPEG and CKGM. It is clear that the peak height of mPEG peaks increased with an increase in the molar ratio feed of the mPEG/CKGM unit.

3.4. DSC analysis

The thermal behavior of CKGM and CKGM-g-mPEG copolymers were investigated by means of DSC analyses and the results are

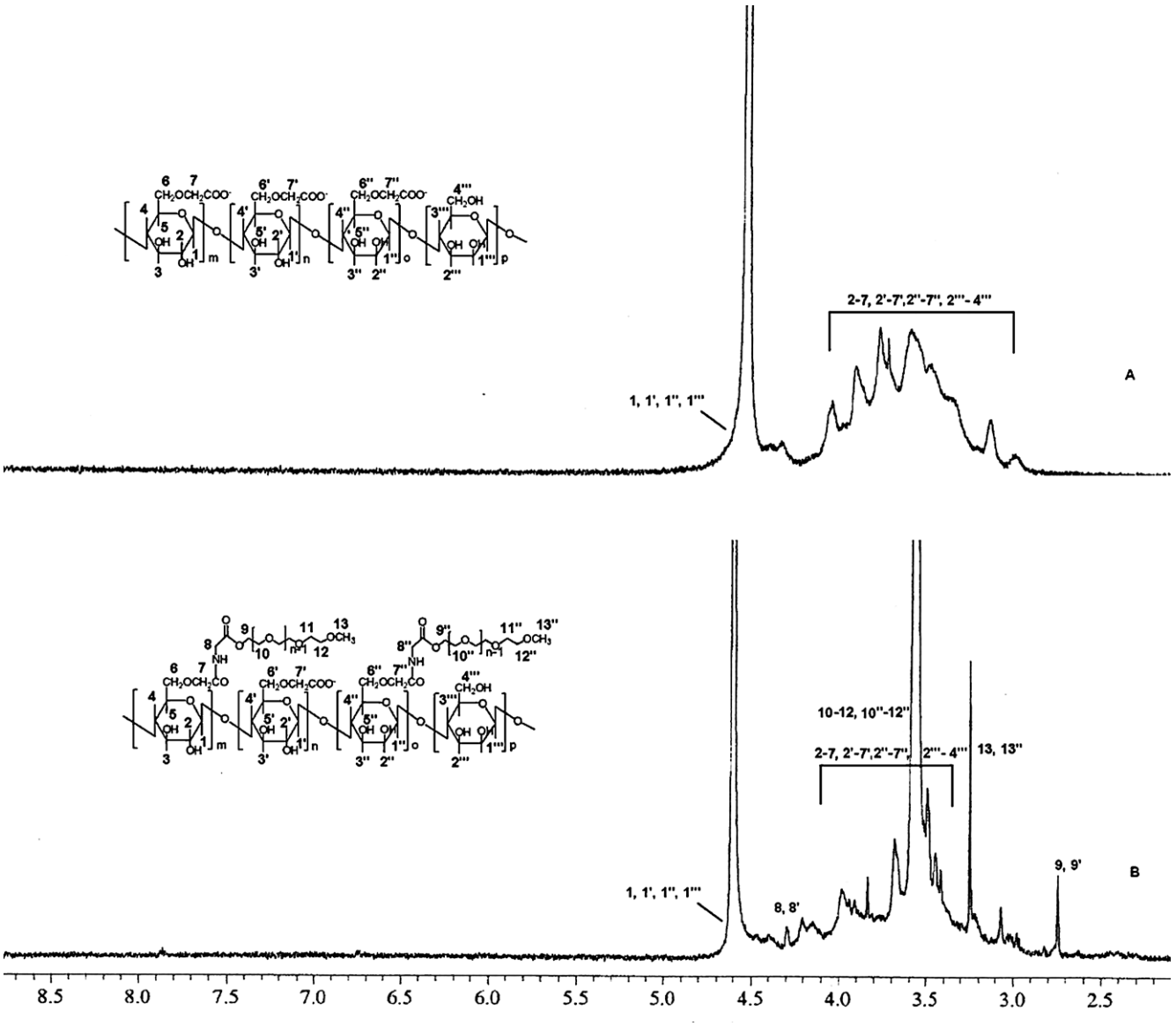


Fig. 2. The ^{1}H NMR spectra for (A) CKGM and (B) CP80 in D_2O.

Table 1
The degree of mPEG substitution (DS) of CKGM-g-mPEGs and the solubility of CKGM and CKGM-g-mPEG.

	mPEG:CKGM (feeding ratio, mol%)	DS of CKGM-g-mPEG (mol%)[a]	Solubility (g/100 mL H_2O)[b]
CKGM	–	–	11.70 ± 0.36
CP20	20	2.23	14.04 ± 0.17
CP40	40	4.38	14.36 ± 0.10
CP60	60	9.34	15.71 ± 0.29
CP80	80	11.68	16.53 ± 0.37
CP100	100	17.81	17.80 ± 0.30

[a] The DS of CKGM-g-mPEGs were calculated from TGA.
[b] The solubility of CKGM and CKGM-g-mPEGs were tested 3 times and average values were listed.

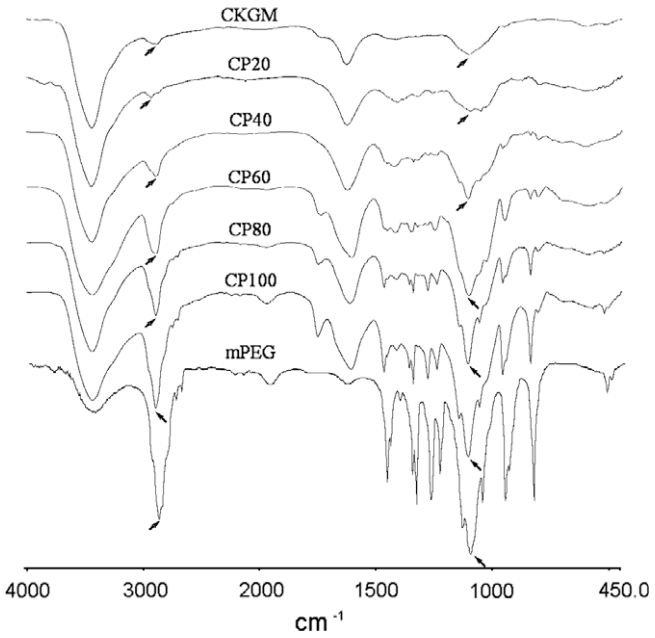


Fig. 3. FT-IR spectra of CKGM, CKGM-g-mPEG copolymers and mPEG.

shown in Fig. 4. The endothermic peaks around 40–60 °C can be seen in CP80 and CP100, corresponding to the melting peak of the mPEG constitution in the CKGM-g-PEG copolymers. The endothermic peak of CP20 and CP40 is too weak to be detected clearly, indicating the poor crystallinity of PEG units due to its lower content. The endothermic peak formed by loss of crystalline water during the drying process around 70 °C can be found in CKGM and CP20. The exothermic peaks around 260–290 °C, observed in CKGM and CKGM-g-mPEG copolymers, are attributed to the disintegration and thermal degradation of CKGM (Xu, Li, Kennedy, Xie, & Huang, 2007).

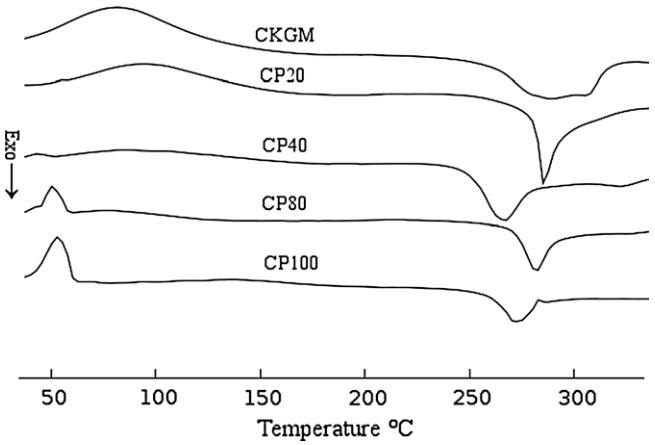


Fig. 4. DSC curves for CKGM, CP20, CP40, CP80 and CP100.

3.5. TG analysis

The TGA thermograms of CKGM, mPEG and CKGM-g-mPEG copolymers are shown in Fig. 5. The curves show that both CKGM and mPEG involve two steps of weight loss. The first slight weight loss at low temperature can be attributed to the dryness procedure when heating, and the subsequent second major weight loss occurring could be attributed to the decomposition of CKGM (266 °C) and mPEG (374 °C). In the cases of CKGM-g-mPEG copolymers, they involve three main stages of degradation. These include a moisture loss at a temperature around 100 °C, followed by a major weight loss of the CKGM component at about 270 °C and another subsequent weight loss of the mPEG component around 370 °C. The decomposition temperatures of the CKGM component and mPEG in graft copolymers are much higher than that of the pure CKGM and mPEG, which indicate the enhanced thermal stability of CKGM-g-mPEG copolymers.

As the decomposition temperature interval of the CKGM component and the mPEG component is significant, the degree of mPEG substitution (DS) can be calculated using the following Eqs. (1) and (2); and the results are shown in Table 1:

$$\text{Percentage of CKGM component in CP} = \frac{\text{CKGM component weight loss} + \text{sample residue}}{1 - \text{water weight loss}} \times 100\% \quad (1)$$

$$\text{Degree of mPEG substitution} = \frac{212.9 \times (1 - \text{percentage of CKGM component in CP})}{2000 \times \text{percentage of CKGM component in CP}} \times 100\% \quad (2)$$

where 2000 is the average molecular weight of mPEG, and 212.9 is the molar mass of a CKGM unit.

The degree of mPEG substitution (DS) to CKGM is shown in Table 1. The low DS results may be attributed to two reasons: (1) the large viscosity of the reaction system could entangle the reaction group ($-CH_2COO^-$ and NH_2) of the CKGM backbone and mPEG-$OCOCH_2NH_2$ and prevent reaction, and (2) the high molecular weight of the oligomer (mPEG, MW 2000) may cause steric hindrance to lower the reaction efficiency.

3.6. Solubility determination

Solubility tests were conducted to determine the solubility of CKGM-g-PEG. The solubility of the various samples (CKGM and CKGM-g-mPEG copolymers) are displayed in Table 1. As expected, introduction of the hydrophilic PEG to CKGM led to an increase in the solubility of CKGM-g-mPEG copolymers. The solubility of CKGM is 11.70 g/100 mL in water. The solubility of CKGM-g-PEGs increases with an increase in the PEG content. The highest solubility of all samples is CP100 which reaches 17.80 g/100 mL in water; which is more than a 50% increment in the solubility of CKGM.

3.7. Dynamic viscosity determination

Fig. 6 shows the viscosity versus shear rate for CKGM and CP100 dispersion in water. It can be seen that grafting PEG onto CKGM decreases the viscosity of the solution at low shear rates. This is consistent with reports of PEGylation of polysaccharides such as PEG-chitosans (Jeong et al., 2008; Sugimoto et al., 1998). This result indicates that the introduction of hydrophilic PEG chains on the carboxyl groups destroyed the inherent structure

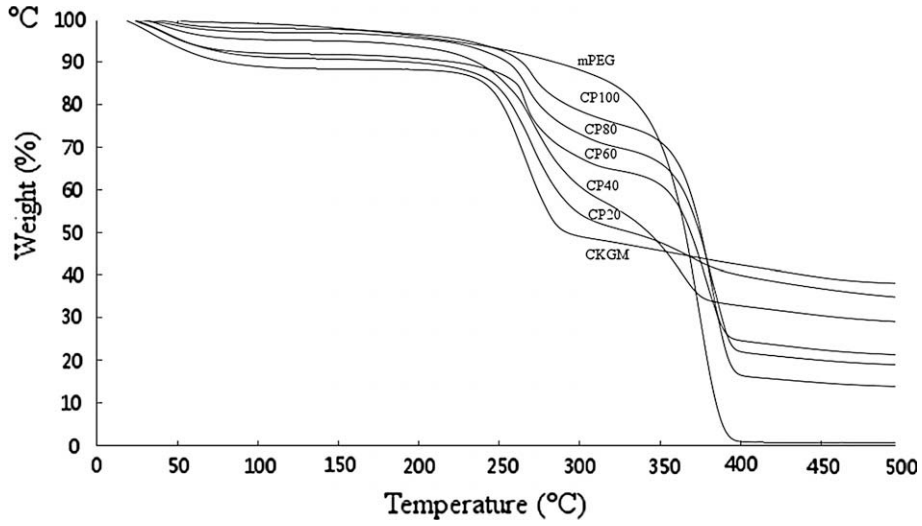


Fig. 5. TGA thermograms of CKGM, CKGM-g-mPEG copolymers and mPEG.

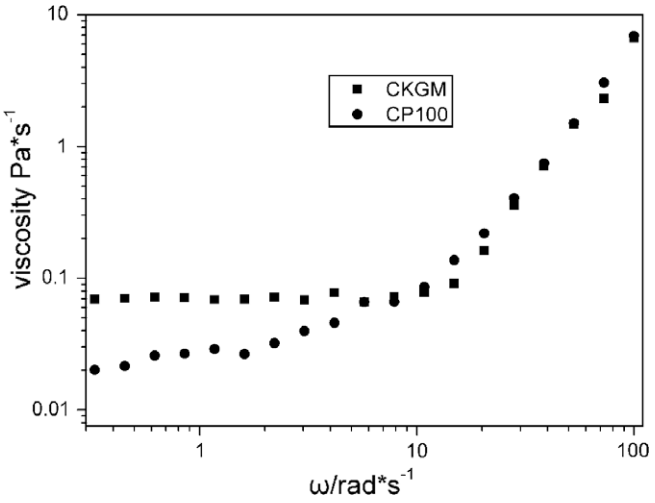


Fig. 6. Influence of frequency on the viscosity of CKGM (square) and CKGM-g-PEG CP100 (round) solutions at 30 °C.

of CKGM and disturbed the intra- and intermolecular hydrogen bonding in CKGM. It is interesting that both samples showed marked shear thickening behavior when the shear rate was increased above a critical shear rate. The mechanism for shear thickening is still a matter of discussion. Various explanations have been proposed to account for the shear thickening behavior of polymer solutions. One of the most accepted explanations is the "flow-induced formation of macromolecular associations" (Kishbaugh & McHugh, 1993a, 1993b).

4. Conclusion

A series of novel grafted polymers, the CKGM-g-mPEG copolymers with different DS were successfully synthesized and characterized. The DS of the products were controlled by varying the feeding amount of mPEG. Structure and properties of these products were characterized via ^{1}H NMR, FT-IR, DSC and TGA. Furthermore, it was confirmed that the CKGM-g-mPEG copolymers had higher solubility in water and lower viscosity at low shear frequency. Since PEG is highly biocompatible, the successful synthesis and characterization of the pegylated CKGM contributes to widening the available pool of advanced polymers for use in the biomedical, pharmaceutical, food and other related fields.

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