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Synthesis and characterization of PEPO grafted carboxymethyl guar and carboxymethyl tamarind as new thermo-associating polymers

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ABSTRACT

New thermo associating polymers were designed and synthesized by grafting amino terminated poly(ethylene oxide-*co*-propylene oxide) (PEPO) onto carboxymethyl guar (CMG) and carboxymethyl tamarind (CMT). The grafting was performed by coupling reaction between —NH_2 groups of PEPO and —COOH groups of CMG and CMT using water-soluble EDC/NHS as coupling agents. The grafting efficiency and the temperature of thermo-association, T_{assoc} in the copolymer were studied by NMR spectroscopy. The graft copolymers, CMG-g-PEPO and CMT-g-PEPO exhibited interesting thermo-associating behavior which was evidenced by the detailed rheological and fluorescence measurements. The visco-elastic properties (storage modulus, *G'*; loss modulus, *G"*) of the copolymer solutions were investigated using oscillatory shear experiments. The influence of salt and surfactant on the T_{assoc} was also studied by rheology, where the phenomenon of "Salting out" and "Salting in" was observed for salt and surfactant, respectively, which can give an easy access to tunable properties of these copolymers. These thermo-associating polymers with biodegradable nature of CMG and CMT can have potential applications as smart injectables in controlled release technology and as thickeners in cosmetics and pharmaceutical formulations.

1. Introduction

Over the past decade, thermo-associating polymers have gained considerable attention and are being widely investigated due to their interesting properties in response to temperature as a stimulus (Liu & Urban, 2010; Meng & Hu, 2010). Contrary to the conventional water-soluble polymers, thermo-associating polymers in aqueous solution exhibit an opposite temperature dependence to the rheological properties in which the inter-chain interactions increase and gel with increase in temperature. In most cases, the thermo-associating behavior is characterized by the presence of a polymer exhibiting an interesting thermodynamic property of lower critical solution temperature (LCST) (Bokias, Mylonas, Staikos, Bumbu, & Vasile, 2001). Therefore, if a polyelectrolyte chain is grafted with small chains of polymer having an LCST, the grafts start to become incompatible/hydrophobic with water upon increasing the temperature and the resulting hydrophobic character induces an association, which manifests into transient network formation leading to increase in viscosity. The precipitation of the polymer however, is prevented by the high hydrophilic nature of the backbone polymer. Various combinations of synthetic water-soluble polymers and polymers with characteristic LCSTs have been shown to exhibit thermo-associating behavior. For example, poly(*N*-isopropylacrylamide) [PNIPAm], poly(acrylic acid)-g-poly(ethylene oxide) [PAA-g-PEO] or poly(acrylic acid)-g-poly(*N*-isopropylacrylamide) [PAA-g-PNIPAm] have been shown to display thermo-associating behavior (Durand & Hourdet, 1999; Kjoniksen, Iversen, Nystrom, Nakken, & Palmgren, 1998). The thermo-associating behavior of these polymers can be easily controlled by external parameters such as polymer concentration, nature and amount of added salt, pH and temperature. Nevertheless, the nature of both components (backbone and the polymer chains with LCST) is expected to play an important role in the macroscopic properties. Hourdet et al. (Durand & Hourdet, 1999) have developed a whole family of thermo-associating polymers by grafting side chains like PEO, PNIPAm, PEPO onto a hydrophilic polymer namely, sodium poly(acrylate) [PAA-Na]. An excellent review on thermo-responsive copolymers with the fundamental aspects and applications has been reported by Liu, Fraylich, and Saunders (2009).

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Besides totally synthetic polymers, polysaccharide based thermo-responsive polymers are attracting increasing attention recently in biomedical applications. Polysaccharides are available in a variety of structure with different properties. Since they contain reactive functional groups, they can be easily modified chemically. Furthermore, their high stability, non-toxicity and biodegradability with gel forming property lead their applications in food and pharmaceuticals. In the recent past, many studies have been devoted to hydrophobically modified polysaccharides. For example, hydrophobically modified chitosan polymers (Bokias et al., 2001; Kjoniksen et al., 1998; Thatte, 2004), thermo-responsive graft copolymers of carboxymethyl cellulose (CMC) have been reported in the literature. An interpenetrating networks (IPNs) based on guar gum and poly(*N*-isopropylacrylamide) PNIPAm, with a major focus on swelling kinetics has been reported (Li, Wu, & Liu, 2008). Recently, Zhang et al. (2009) have reported on the synthesis and characterization of thermo-sensitive graft copolymer of poly(*N*-isopropylacrylamide) and carboxymethyl chitosan. Similarly, Shi and Zhang (2007) and Zhang et al. (2009) studied the grafting of poly(*N*-isopropylacrylamide) onto carboxymethylhydroxypropyl guar (CMHPG). However, it was intriguing to note from their study that, they did not observe the formation of hydrogel in their system but obtained thermo-sensitive polymer solutions. The polymer solutions did not exhibit thermo-thickening behavior but resulted in macroscopic phase separation. Huh, Hashi, Ooya, and Yui (2000) reported on the synthesis and characterization of carboxymethyl dextran and poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide). Very recently, Pasale, Cerroni, Ghugare, and Paradossi (2014) have reported on the design and synthesis of multiresponsive hydrogels based on azide-derivative of hyaluronate and propargyl end functionalized telechelic PNIPAm using "click chemistry"approach.

Most of the thermo-associating polymers based on synthetic and natural polymers, exhibit either phase separation or decrease in viscosity upon heating. The true thermo-thickening (viscosity increase with temperature without undergoing macrophase separation) behavior is observed in a few cases which strongly depends on the balance of hydrophilic/hydrophobic components, concentrations and molecular weight of LCST side chains, pH and presence of salts in aqueous solutions.

Some of the polysaccharides used in the design and synthesis of thermo-associating polymers include Chitosan (Chung, Bae, Park, Lee, & Park, 2005; Kim, Cho, Lee, & Kim, 2000; Seetapan, Mai-ngam, Plucktaveesak, & Sirivat, 2006; Zhang et al., 2009), Hyaluronic acid (Yang, Kataoka, & Winnik, 2005b), carboxymethyl cellulose, alginates (Karakasyan, Lack, Brunel, Maingault, & Hourdet, 2008; Karakasyan et al., 2010), Pullulan (Belbekhouche, Ali, Dulong, Picton, & Le Cerf, 2011; Deguchi, Akiyoshi, & Sunamoto, 2003; Dulong, Mocanu, Picton, & Le Cerf, 2012; Mocanu, Mihai, Dulong, Picton, & Lecerf, 2011) and dextran (Huh et al., 2000; Karakasyan et al., 2008; Kurisawa & Yui, 1998). Bokias et al. (2001), have reported on the synthesis and aqueous solution properties of thermo-associating graft copolymers based on carboxymethyl cellulose [CMC] bearing PNIPAm side chains, CMC-g-PNIPAm. Tizzotti et al. (2010) have reported on thermo-associative guar-based grafted copolymers by "grafting onto" method through the CuAAC coupling showing reversible physical gelation. However, polysaccharides namely, guar gum (GG) and tamarind kernel powder (TKP) are not fully explored in synthesizing thermo-associating polymers based on them. Guar gum is a water-soluble nonionic polysaccharide extracted from the seeds of Cyanopsis tetragonoloba, constituted by a β-1, 4- mannose backbone with randomly distributed α-1,6-galactose side chains. The mannose/galactose ratio is generally 2:1 (Staros & Swingle, 1986). Tamarind kernel powder (TKP) is derived from the seeds of the tree *Tamarindus indica*. TKP is composed of (1→4)-β-D-glucan backbone substituted with side chains of α,D-xylopyranose and β-D- galactopyranosyl. It is a non-ionic, neutral, branched polysaccharide in which glucose, xylose and galactose units are present in the ratio of ~3:2:1 (Staros, Wright & Swingle, 1986) because of which it is widely known as Xyloglucan.

Since, GG and TKP are non-ionic in nature their solubility in water is poor and hence the commercial grades of carboxymethyl derivatives (carboxymethyl guar, CMG and carboxy methyl tamarind, CMT) (see Fig. 1) of these polysaccharides are more useful and improve the aqueous solubility significantly.

In this paper, we report on the synthesis and thermo-associating properties of two graft copolymers namely (i) carboxymethyl guar-g-PEPO [PEPO = poly(ethylene oxide-*co*-propylene oxide)], CMG-g-PEPO and (ii) carboxymethyl tamarind-g-PEPO, CMT-g-PEPO. The grafting was performed by coupling reaction between amino terminated thermo-responsive PEPO with CMG and CMT using water-soluble EDC as a coupling agent. The aim of the work was to examine a structure-property relationship in terms of thermo-associating and rheological behavior and to investigate the influence of external parameters on the process of association. The structural characteristics of all the graft copolymers were performed using FT-IR and NMR spectroscopy. The thermo-associating behavior of graft copolymers was studied using fluorescence spectroscopy and rheometry. Since these polymers are non-toxic, bio-compatible and thermo-thickening at tunable temperature close to physiological conditions, they show promising applications as smart injectables in controlled drug delivery.

2. Experimental

2.1. Materials

Carboxymethyl guar (CMG) and carboxymethyl tamarind (CMT) were commercial products and were kindly provided by Dabur India Ltd., India; Jeffamine M-2005 (Pluronics), an amino terminated poly(ethylene oxide-*co*-propylene oxide) (PEPO) with 84 mol% of propylene oxide, was purchased from Huntsman, Belgium. The coupling agent, 1-(3-(dimethylamino) propyl)-3-ethyl carbodiimide hydrochloride (EDC) was obtained from Fluka, USA; *N*-hydroxysuccinimide (NHS) was purchased from Aldrich Chemical Company, USA; Other solvents used were of analytical grade and obtained from S.D Fine Chemicals, India. Except CMG and CMT, all the other chemicals were used as received. Water used for the experiments was purified using a Millipore laboratory unit (*Q*-Millipore, 18.2 MΩ).

2.2. Purification of polysaccharides (CMG and CMT)

The commercial CMG and CMT samples were purified using the following procedure:

Ten gram of CMG/CMT was taken in a 1 L conical flask and 500 mL acetone was added to remove any organic soluble moieties. The entire mass was stirred with a magnetic needle on a magnetic stirrer for 12 h at room temperature (25 °C). Then it was filtered and dried. The dried CMG/CMT was dissolved in 1 L deionized water and centrifuged at 8000 rpm for 40 min. The supernatant liquid was dialyzed against water using a dialysis membrane with MWCO of 12 kDa. The dialysis was carried out for 24 h by frequently changing the external water. The dialyzed solution was then freeze-dried to obtain white cotton like material. The purified CMG/CMT were used for all the experiments.

2.3. Characterization of CMG, CMT and PEPO

The average molecular weight and polydispersity of CMG and CMT were measured by size exclusion chromatography (SEC) using OHpak SB-804HQ and Pullulan Standards from Shodex. The solvent,

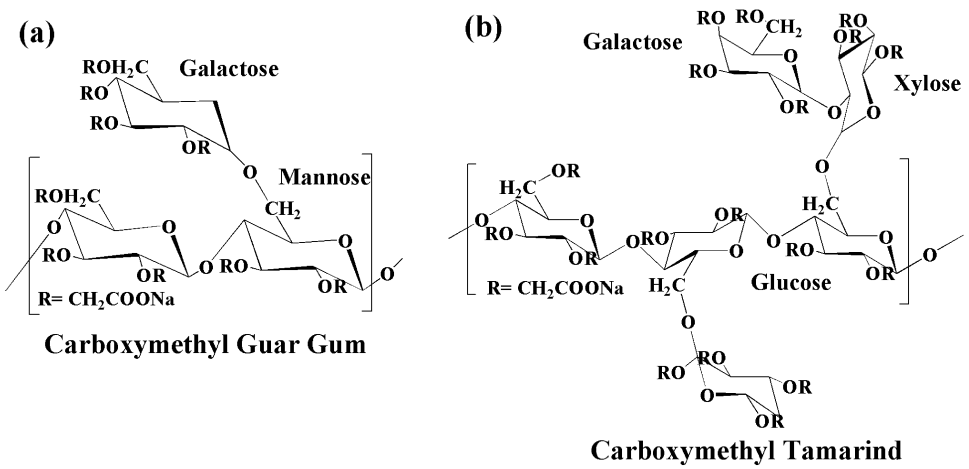


Fig. 1. Chemical structures of (a) CMG and (b) CMT.

0.1 N $NaNO_3$ was used as an eluent at a flow rate of 0.3 mL/min. The molecular weights, PDI and degree of substitution of CMG and CMT are shown in Table 1.

The amino terminated poly(ethylene oxide-*co*-propylene oxide) [PEPO, Jeffamine M-2005, from Huntsman, Belgium] was characterized by ^{1}H NMR spectroscopy (see Supplementary Fig. 1).

The terminal $-CH_3$ protons appear at 3.38 ppm and the $-CH_3$ protons of propylene oxide appear at 1.15 ppm while all the remaining protons are observed in the range of 3.45–3.75 ppm. The molar ratio of EO:PO calculated from the NMR spectrum accounted to 16:84. This corresponds for a PEPO chain, to 6 EO units and 29 PO units with a corresponding molar mass of 2000 g/mol in full agreement with the data reported by the supplier.

2.4. Synthesis of graft copolymers: CMG-g-PEPO and CMT-g-PEPO

The coupling reaction between carboxyl groups of polysaccharides and terminal amine of PEPO was carried out in cold water using EDC/NHS as coupling reagents (Scheme 1). The stoichiometry of reactions is given in Table 2. A typical experimental procedure can be described as follows: In a reaction vessel equipped with a magnetic stirrer, 2 g of polysaccharide was dissolved under stirring in 100 mL of water for at least 24 h at room temperature. A total of 2 g of PEPO-NH_2 (1 mmol of NH_2) was separately dissolved in 50 mL of cold water to get a homogeneous solution and the pH was adjusted around 5-6 with HCL (1 mol/L). After cooling the polysaccharide solution to $T = 6\,^\circ$C, the solution of PEPO-NH_2 was slowly added, and the pH was adjusted to 5–6. After 1 h of mixing, 0.092 g of NHS (0.07 mmol) and 0.488 g of EDC (3.1 mmol) were separately dissolved in 2 mL of water, slowly added into the reaction vessel, and the reaction was allowed to proceed further over a period of 16 h at 8 °C. Then the copolymer was progressively precipitated under vigorous stirring in ethanol at room temperature. The precipitate was washed several times with ethanol to remove unreacted reagents and carbodiimide byproduct, filtered off, and finally dried under vacuum. By using various molar ratios of CMG/CMT and PEPO, the graft copolymers were synthesized and the grafting efficiency was determined by NMR spectroscopy (see Table 2). Since the reaction was conducted in water and at a temperature lower than 18 °C (below the LCST of PEPO), the phase separation of PEPO chains was avoided.

Most of the characterizations were performed on samples CMG-g-PEPO 1:1 and CMT-g-PEPO 1:1 which have been prepared with the same feed weight ratio (50/50). These two samples will be denoted as CMG-g-PEPO and CMT-g-PEPO; otherwise the initial feed weight ratio will be indicated as given in Table 2.

Table 1
Characteristics of CMG and CMT.

Polymer	M_n (kg/mol)	M_w (kg/mol)	PDI	Degree of substitution
CMG	860	1600	2.0	0.46
CMT	800	1500	1.9	0.54

Table 2
Composition of CMG-g-PEPO from NMR spectroscopy.

Feed composition(wt)		Final composition CMG-g-PEPO (wt)*	
CMG/PEPO	CMG	PEPO	Y(%)
1.0/1.0	1.0	0.5	50
1.0/0.5	1.0	0.4	80
1.0/0.25	1.0	0.1	40

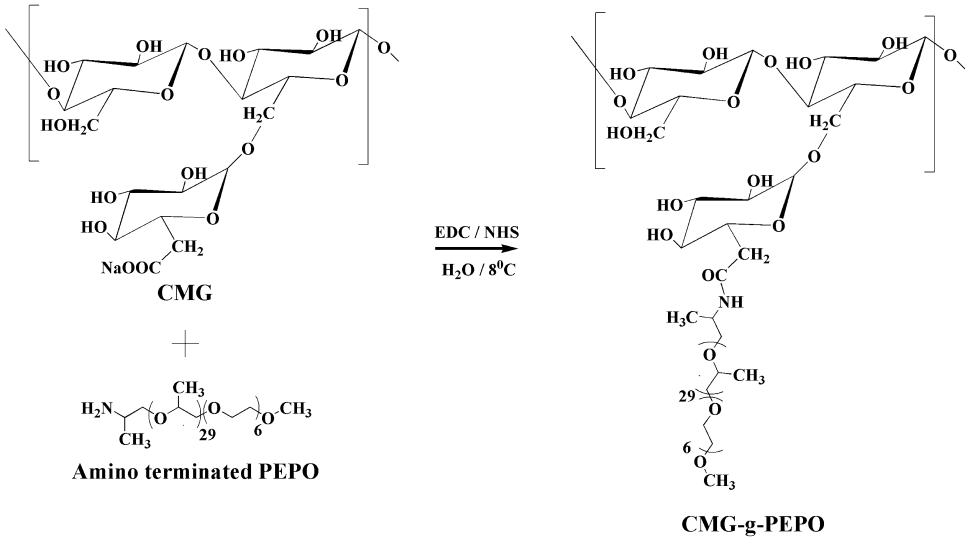
* Since the anomeric proton of CMG could not be resolved due to overlap/broadening, we used other sugar protons (in the range of 3.0–4.5 ppm) to analyse the composition of the copolymers. The total area coming from this region corresponds to 18 protons of the CMG repeat unit. Whereas, the peak at 1.18 ppm correspond to 3 protons of propylene oxide moiety in PEPO. The contribution of protons of PEPO units to the region of 3.0–4.5 ppm has been subtracted to get the final composition of the CMG (the ratio of PPO to PEO estimated by NMR in the PEPO was used for this purpose).

2.5. NMR spectroscopy

The ^{1}H and ^{13}C NMR spectra of CMG, CMT, PEPO and the graft copolymers were recorded using a 5 mm QNP probe in a Bruker DRX-500 spectrometer operating at a proton frequency of 500.13 MHz. The samples were made in D_2O and TMS was used as a reference for chemical shifts (0 ppm). For high temperature measurements, the tubes were left to stabilize for 15 min at a desired temperature before recording the spectrum.

2.6. Fluorescence spectroscopy

Steady state fluorescence spectra were recorded on a Fluorolog spectrofluorometer (JY Horiba Inc.) equipped with a peltier plate to control the temperature of the measuring cell. Pyrene solution in acetone (1×10^{-3} M) was used as an external probe at a final concentration of 6×10^{-7} M. The excitation wavelength was 339 nm. The change in the intensity ratio (I_1/I_3) of the first and third vibronic bands at 373 nm and 384 nm for I_1 and I_3, respectively, in the emission spectra, were used to detect the hydrophobic micro domains.



Scheme 1. Synthesis of thermo-sensitive CMG-g-PEPO.

2.7. Steady shear and oscillatory rheological measurements

The visco-elastic properties of aqueous solutions of CMG-g-PEPO and CMT-g-PEPO copolymer solutions were performed on an Anton Paar MCR-301, controlled stress rheometer by using a cone-and-plate geometry (diameter = 50 mm, angle = 1°) for highly viscous gel samples. For low viscosity samples, couette (coaxial cylinder) geometry was used to measure viscosity, operating in flow or dynamic mode, respectively. The dynamic data was obtained as a function of strain amplitude first at a fixed frequency of 1 Hz to ensure that the measurements were performed in the linear visco-elastic regime. The frequency sweep experiments were then performed in the linear regime to determine storage (G') and loss (G'') moduli in the frequency range of 0.01–100 Hz (rad/s).

A particular care was taken to prevent water evaporation during measurements at high temperature using a homemade cover combined with the deposition of a film of silicone oil on the edges of the sample.

2.8. Sample preparation

All the solutions were prepared using pure de-ionized water. Very gentle stirring was employed to get homogeneity of the sample before taking them for experiments.

3. Results and discussion

3.1. Synthesis of graft copolymers, CMG-g-PEPO and CMT-g-PEPO

Use of carbodiimide chemistry has been widely reported in the literature and practiced in peptide synthesis for the coupling reaction between amino groups and carboxyl groups (Kast & Bernkop-Schnürch, 2001). In the case of water-soluble polymers, like polysaccharides, similar reaction can be carried out in aqueous medium using water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDC) as coupling agent. EDC alone is limited by low coupling yields therefore, addition of NHS to such reactions can greatly enhance the coupling efficiency (Staros & Swingle, 1986). In our case, the PEPO was selected according to its relative hydrophobicity and molecular weight (M_n = 2000 g/mol). The hydrophobicity of PEPO is controlled by the ratio of propylene oxide to ethylene oxide units (29/6 mol ratio) in the oligomer.

With both CMG and CMT precursors, the grafting reaction given in Scheme 1 was carried out with various initial weight ratio of CMG: PEPO (or CMT:PEPO) equal to 1:0.25, 1:0.5 and 1:1. At the end of the reaction, the qualitative grafting of PEPO on to polysaccharides can be rapidly revealed by putting the reaction vessel under hot water to see the thermo-thickening behavior.

3.2. ^1H NMR spectroscopy

The composition of graft copolymers were quantitatively determined by ^1H NMR spectroscopy and their composition are given in Table 2. The ^1H NMR spectra of CMG, PEPO and the graft copolymer, CMG-g-PEPO are shown in Supplementary Fig. 2(a). The graft copolymer, showed the characteristic peaks of both CMG and PEPO protons. The $-CH_3$ proton of PEPO and the graft copolymer appeared at 1.18 ppm which is completely absent in the homopolymer of CMG. All the other protons belonging to the polysaccharides or methylene and methyne groups of PEPO appear between 3.2 and 3.7 ppm, except for the anomeric proton of the glucose unit. The observation of characteristic proton peaks arising from both PEPO and CMG/CMT in the graft copolymers confirmed their structure. Similar observations are made in CMT-g-PEPO copolymer (Supplementary Fig. 2(b)).

As shown in Fig. 2, a quantitative analysis of the copolymer composition was carried out by taking into account the isolated $-CH_3$ proton of PO units assigned at about 1.18 ppm, and the other protons of CMG and PEPO observed in the range 3.0–4.5 ppm.

For all the copolymers, we can see that the yield of grafting varies between 40 and 100%, indicating that the grafting reaction does not undergo complete conversion. This can be due to a rather low content of carboxylate groups (<25–30%) which are moreover non-cyclizable and not able to form anhydride intermediates (Yang, Kataoka, & Winnik, 2005a). A possibility would be to use larger amounts of EDC/NHS to improve the activation of carboxylic acid units but unfortunately such alternative has been shown to promote ester bonds between hydroxyl and carboxyl groups of polysaccharide backbones giving rise to covalent cross-linking (Zhang, 2005). As previously discussed, the grafting reaction was performed at low temperature, typically below 10 °C, in order to work in a homogeneous condition and to facilitate the random distribution of the LCST grafts. But working at low temperature is also very important if one wants to favor the formation of amide bonds in comparison with ester cross-linkages.

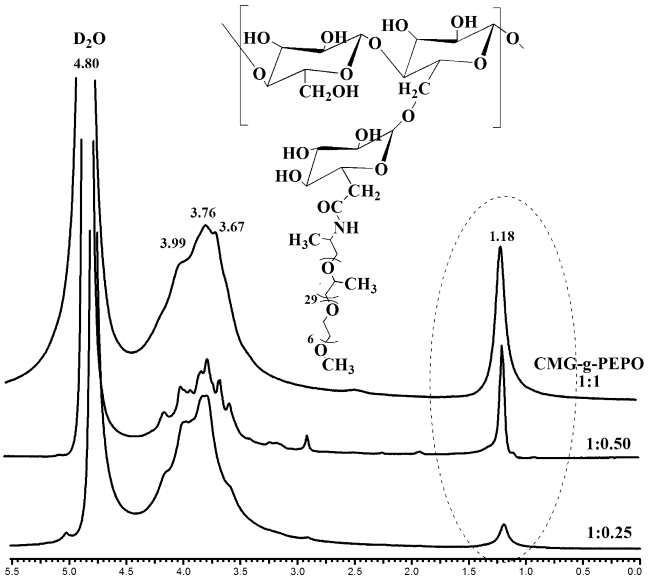


Fig. 2. ^{1}H NMR spectra of CMG-g-PEPO with different PEPO content.

3.3. Analysis of thermo-association by NMR spectroscopy

Thermo-association in these polymers occurs at a critical temperature, which is termed as temperature of association, T_{assoc}. At T_{assoc}, the LCST side chains start to self-assemble and give rise to an enhancement in viscosity of the solution. Interestingly, at T_{assoc}, one can observe a deviation of line width of certain peaks in NMR spectroscopy. This line width transition indeed mirrors the actual sol-gel transition occurring in a macroscopic way through the associated molecular motions. We show in Fig. 3(a), the stacked plots of static proton spectra of CMG-*g*-PEPO at different temperatures ranging from 10 to 75 °C. The line width of the CH_3 peak of the PEPO chain was measured at each temperature and plotted against the temperature in Fig. 3(b). It can be readily seen that, the ^{1}H static line width of the CH_3 peak of the PEPO chain showed a small gradual increase between 10 and 41 °C and exhibits an inflection point at about 41 °C. Above this temperature, the line width increases sharply indicating the formation of gel as a result of thermo-association.

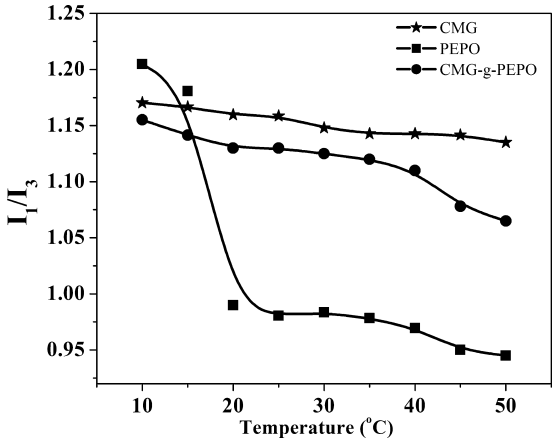


Fig. 4. I_1/I_3 vs. temperature for CMG, PEPO, CMG-g-PEPO 1:1 (C_p = 1.0 wt%).

The inflection point in the figure can be considered as T_{assoc}.

The T_{assoc} obtained from the NMR experiments corresponds very well with the observed associating behavior investigated by rheological measurements on the same samples (which are discussed in the later sections). Therefore, the NMR technique emerges as an important tool to determine T_{assoc} in the process of thermo-association. A similar exercise has been performed on the graft copolymer, CMT-g-PEPO and the T_{assoc} was found to be around 50–51 °C (data not shown).

3.4. Fluorescence spectroscopy

3.4.1. Fluorescence spectroscopy for CMG, PEPO and CMG-g-PEPO

Fluorescence spectroscopy has been extensively used to study the hydrophobic micro-environment of amphiphilic polymers in aqueous media with pyrene as an external probe. Two important properties of pyrene, a change in the intensity ratio of the first over third vibronic peak (I_1/I_3) in emission spectra and a (0, 0) band shift in excitation spectra upon the formation of hydrophobic micro-environment in aqueous media, are utilized to study the aggregation behavior of micelles. Fig. 4 shows the change in the intensity ratio (I_1/I_3) of pyrene excitation spectra as a function of temperature for CMG, PEPO and the copolymer, CMG-g-PEPO.

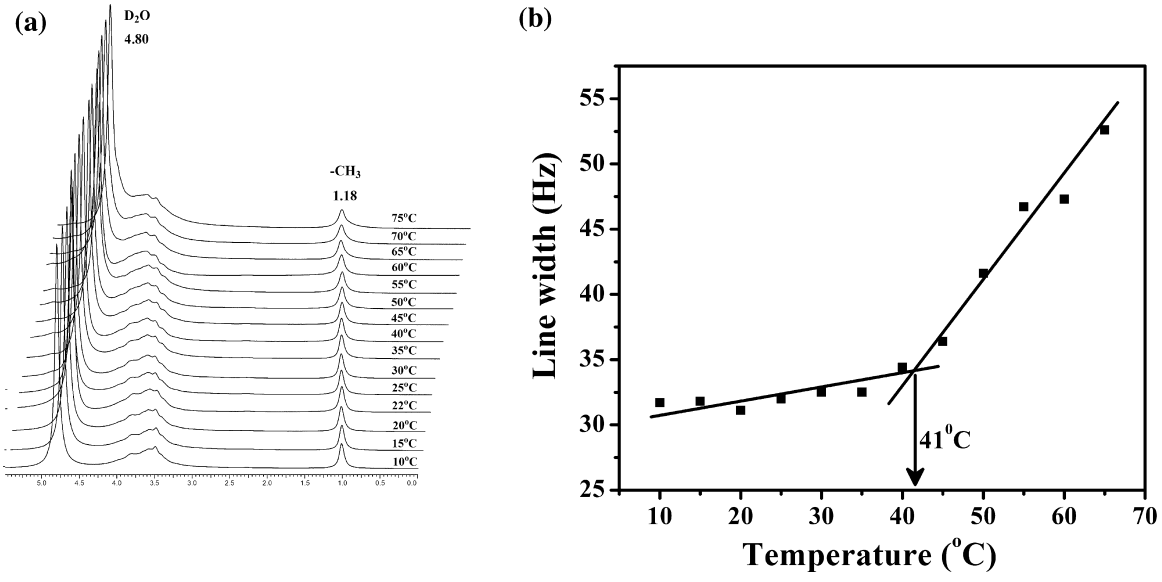


Fig. 3. (a) Stack plots of static proton spectra of CMG-g-PEPO at different temperatures (10–75 °C); (b) plot of proton line width of —CH_3 peak at different temperatures.

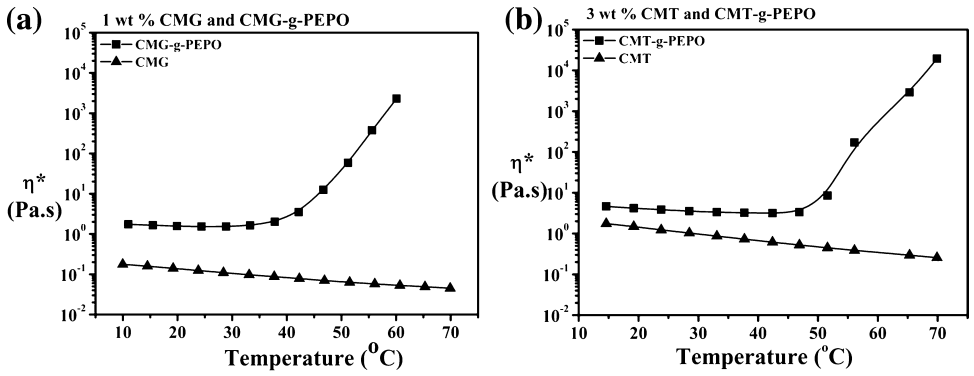


Fig. 5. Temperature dependence of complex viscosity of (a) 1 wt% CMG and CMG-g-PEPO, (b) 3 wt% CMT and CMT-g-PEPO.

For the homopolymer PEPO, a sharp decrease in the intensity ratio is observed between 15 and 20 °C, revealing the formation of hydrophobic micro-environment at this temperature. The high values of intensity ratio (I_1/I_3) indicate an aqueous polar environment whereas, lower values indicate the low polarity micro-environment, where pyrene molecules are preferentially solubilized (Ezzell & McCormick, 1991). In the case of CMG homopolymer, there is no rapid decrease in the intensity ratio (I_1/I_3) at any temperature in the whole temperature range studied. This indicates that there are no sharp transition in this range, even a rather low intensity ratio (I_1/I_3 = 1.15–1.2) suggests the existence of some hydrophobic contribution of the polysaccharide itself. The graft copolymer, CMG-g-PEPO on the other hand shows a noticeable decrease in the intensity ratio (I_1/I_3) at about 40 °C indicating the formation of hydrophobic microdomains. This can be attributed to the fact that, above the transition temperature of the grafted chains in the copolymer, a PEPO-rich phase can be formed, followed by an increase in the solubilization of pyrene in polymer-rich hydrophobic micro-environment. It is also seen that the sharpness and the amplitude of the transition, i.e. the hydrophobic environment is much weaker in graft copolymer as compared to the homopolymer, PEPO.

3.5. Visco-elastic properties of CMG and CMG-g-PEPO copolymer

We show in Fig. 5(a), the complex viscosities of 1.0 wt% CMG-g-PEPO 1:1 aqueous solution with respect to temperature and compared with the homopolymer, CMG. A slight decrease in viscosity with increasing temperature is observed for CMG solution. This is reasonable to expect since there is no formation of associations or aggregates in the CMG homopolymer solution alone. However, with the CMG-g-PEPO, the viscosities are higher than CMG homopolymer in the entire temperature range studied. This can be attributed to the presence of PEPO side chains which tend to associate and induce self-assembly. Furthermore, it can be readily seen from Fig. 5(a) that the influence of temperature is striking for CMG-g-PEPO wherein, there is a dramatic increase in the complex viscosity at about 41–42 °C exhibiting strong thermo-thickening behavior induced by the local microphase separation of PEPO side chains at this temperature (T_{assoc}) resulting in to a formation of transient network. The temperature at which these associations occur is further evidenced by the change in line width of PEPO chains in the NMR spectroscopy as discussed earlier. At lower temperatures i.e. below LCST the initial difference in the complex viscosities of CMG and CMG-g-PEPO could be due to the possibility of slight crosslinking of the polymeric chains during the coupling reaction which can increase the viscosity to some extent.

Similarly, the complex viscosities of 3 wt% CMT and CMT-g-PEPO aqueous solutions are reported in Fig. 5(b). The continuous drop in viscosity with increasing temperature is observed for CMT solution as expected since there are no associations or aggregate formations in CMT alone. At low temperatures, solutions of 3 wt% CMT-g-PEPO showed an increase in viscosity compared to the CMT solutions itself. The polymer solution is characterized by an initial decrease of viscosity in the low temperature range followed by a strong thickening induced by the local microscopic phase separation of PEPO side chains at about 50 °C.

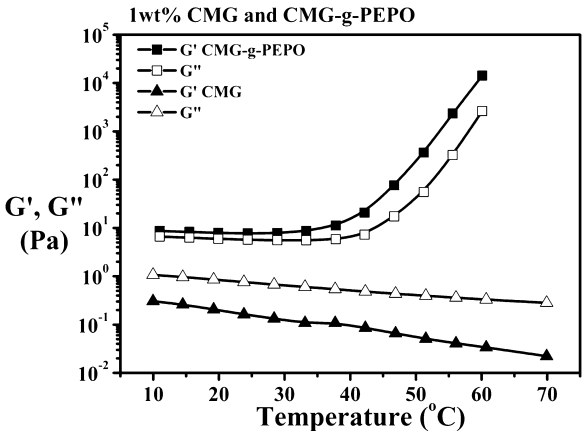


Fig. 6. Variation of dynamic moduli w.r.t. temperature for 1 wt% CMG and CMG-g-PEPO (f = 1 Hz).

We also show in Supplementary Fig. 3, the influence of PEPO content on the thermo-thickening behavior in the aqueous solutions. It can be seen that, the lower content of PEPO (CMG:PEPO ratio < 1:0.5) do not induce any thermo-thickening behavior whereas, samples with CMG:PEPO ratio 1.0:1.0 exhibit a very good thermo-thickening behavior at 41–42 °C. Therefore, it can be concluded that, in order to observe the thermo-thickening behavior in these polymers, there should be some minimum number of LCST chains with appropriate MW grafted onto the backbone water-soluble polymer. The variation in dynamic moduli (G' and G'') with respect to temperature (in the range of 10–70 °C) for CMG and CMG-g-PEPO is shown in Fig. 6.

The backbone homopolymer, CMG showed slight decrease in the values of dynamic moduli (G' and G'') with increase in temperature. The $G'' > G'$ indicating the dominance of solution behavior in the temperature range studied. On the other hand, for the graft copolymer CMG-g-PEPO, the $G' > G''$ in all the temperature range and shows a rapid increase in both the moduli at 40–41 °C exhibiting a thermo-thickening behavior. This temperature at which the association takes place matches well with the earlier observation made with the complex viscosity (η^*) studies (see Fig. 5).

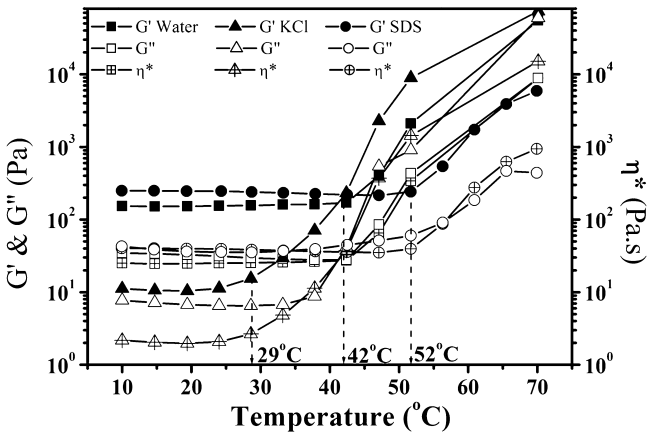


Fig. 7. Variation of complex viscosity and visco-elastic properties w.r.t. temperature for 1 wt% CMG-g-PEPO solutions in pure water, salt (0.6 M KCl) and surfactant (0.5 wt% SDS).

3.6. Influence of salt and the surfactant on the thermo-thickening behavior of CMG-g-PEPO and CMT-g-PEPO copolymers

In order to tune the thermo-thickening properties of the above copolymers without altering the chemical structure of the copolymers, the influence of salt and surfactant on the thermo-thickening behavior was investigated on CMG-g-PEPO and CMT-g-PEPO solutions. The results are shown in Fig. 7.

It can be readily seen that the addition of salt to the solution can be considered as an external trigger to easily tune the T_{assoc} of the copolymer since the initial critical temperature (~42 °C) notably decreases to ~29 °C with the addition of 0.6 M KCl. This phenomenon, well known as "salting-out" effect is attributed to the decrease of PEPO solubility in salt solutions, which promotes the PEPO dehydration and thus, the hydrophobic self-association. It further confirms that T_{assoc} of copolymers is governed by the thermodynamic properties of PEPO. Although, the thermo-thickening behavior is retained, the salt addition leads to a slight lowering of the thermo-thickening magnitude, which can be due to small precipitation of polymer coils in the presence of salt. On the other hand, in the presence of surfactant (0.5 wt% SDS), an interesting "salting-in" kind of effect was observed and the T_{assoc} shifted to 52.0 °C. Based on the above observations, it can be realized that the T_{assoc} of these copolymers can be shifted to low and high values using salt and surfactants respectively. Similar observations were made for CMT-g-PEPO polymers as well, where the copolymer exhibited T_{assoc} at 33 °C, 47 °C and 56 °C in 0.6 M KCL, water and 0.5 wt% SDS solutions, respectively (data not shown).

4. Conclusions

In this paper, we have designed and synthesized thermo-associating graft copolymers based on carboxymethyl guar (CMG) and carboxymethyl tamarind (CMT) with LCST grafted chains of poly (ethylene oxide-*co*-propylene oxide) (PEPO) by coupling reaction between them using water-soluble coupling agent namely, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC). The graft copolymers, CMG-g-PEPO and CMT-g-PEPO exhibited thermo-thickening behavior in water at 42 °C and 47 °C, respectively, which was evidenced by the detailed rheological and fluorescence measurements. At moderate concentrations (C_p = 10–30 g/L), the graft copolymer solutions showed soft gel-like nature and the visco-elastic properties (storage modulus, *G'* and loss modulus, *G"*) were studied using oscillatory shear experiments. It was observed that, CMG-g-PEPO showed more elastic like behavior where, *G'* > *G"* at all the frequencies and polymer concentrations studied. The temperature of association (T_{assoc}) where the thermo-thickening occurred was determined by microscopic line width measurements using NMR spectroscopy. The value of the T_{assoc} obtained matched very well with the macroscopic rheological measurements. The influence of salt and surfactant on the T_{assoc} was also studied by rheology where the phenomenon of "salting out" and "salting in" was observed for salt and surfactant respectively, which can give an easy access to the tunable properties for these copolymers. These thermo-associating polymers with the biodegradable nature of CMG and CMT can have potential applications as smart injectables in controlled release technology. They can also be used as thickeners in cosmetics and pharmaceutical formulations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.09.073.

References

Belbekhouche, S., Ali, G., Dulong, V., Picton, L., & Le Cerf, D. (2011). Synthesis and characterization of thermosensitive and pH-sensitive block copolymers based on polyetheramine and pullulan with different length. *Carbohydrate Polymers*, *86*(1), 304–312.

Bokias, G., Mylonas, Y., Staikos, G., Bumbu, G. G., & Vasile, C. (2001). Synthesis and aqueous solution properties of novel thermoresponsive graft copolymers based on a carboxymethylcellulose backbone. *Macromolecules*, *34*(14), 4958–4964.

Chung, H. J., Bae, J. W., Park, H. D., Lee, J. W., & Park, K. D. (2005). Thermosensitive chitosans as novel injectable biomaterials. *Macromolecular Symposia*, *224*(1), 275–286.

Deguchi, S., Akiyoshi, K., & Sunamoto, J. (2003). Solution property of hydrophobized pullulan conjugated with poly (ethylene oxide) poly (propylene oxide) poly (ethylene oxide) block copolymer. Formation of nanoparticles and their thermosensitivity. *Macromolecular Rapid Communications*, *15*(9), 705–711.

Dulong, V., Mocanu, G., Picton, L., & Le Cerf, D. (2012). Amphiphilic and thermosensitive copolymers based on pullulan and Jeffamine: Synthesis, characterization and physicochemical properties. *Carbohydrate Polymers*, *87*(2), 1522–1531.

Durand, A., & Hourdet, D. (1999). Synthesis and thermoassociative properties in aqueous solution of graft copolymers containing poly(*N*-isopropylacrylamide) side chains. *Polymer*, *40*(17), 4941–4951.

Ezzell, S. A., & McCormick, C. L. (1991). Synthesis and solution characterization of pyrene-labeled polyacrylamides. In S. W. Shalaby, C. L. McCormick, & G. B. Butler (Eds.), *Water-soluble polymers: synthesis, solution properties, and applications* (pp. 130–150). American Chemical Society.

Huh, K. M., Hashi, J., Ooya, T., & Yui, N. (2000). Synthesis and characterization of dextran grafted with poly (*N*-isopropylacrylamide-*co*-*N*, *N*-dimethylacrylamide). *Macromolecular Chemistry and Physics*, *201*(5), 613–619.

Karakasyan, C., Lack, S., Brunel, F., Maingault, P., & Hourdet, D. (2008). Synthesis and rheological properties of responsive thickeners based on polysaccharide architectures. *Biomacromolecules*, *9*(9), 2419–2429.

Karakasyan, C., Legros, M., Lack, S., Brunel, F., Maingault, P., Ducouret, G., et al. (2010). Cold gelation of alginates induced by monovalent cations. *Biomacromolecules*, *11*(11), 2966–2975.

Kast, C. E., & Bernkop-Schnürch, A. (2001). Thiolated polymers—thiomers: Development and in vitro evaluation of chitosan–thioglycolic acid conjugates. *Biomaterials*, *22*(17), 2345–2352.

Kim, S. Y., Cho, S. M., Lee, Y. M., & Kim, S. J. (2000). Thermo and pH responsive behaviors of graft copolymer and blend based on chitosan and *N*-isopropylacrylamide. *Journal of Applied Polymer Science*, *78*(7), 1381–1391.

Kjoniksen, A. L., Iversen, C., Nystrom, B., Nakken, T., & Palmgren, O. (1998). Light scattering study of semidilute aqueous systems of chitosan and hydrophobically modified chitosans. *Macromolecules*, *31*(23), 8142–8148.

Kurisawa, M., & Yui, N. (1998). Modulated degradation of dextran hydrogels grafted with poly (*N*-isopropylacrylamide-*co*-*N*, *N*-dimethylacrylamide) in response to temperature. *Macromolecular Chemistry and Physics*, *199*(11), 2613–2618.

Li, X., Wu, W., & Liu, W. (2008). Synthesis and properties of thermo-responsive guar gum/poly (*N*-isopropylacrylamide) interpenetrating polymer network hydrogels. *Carbohydrate Polymers*, *71*(3), 394–402.

Liu, F., & Urban, M. W. (2010). Recent advances and challenges in designing stimuli-responsive polymers. *Progress in Polymer Science*, *35*(1), 3–23.

Liu, R. X., Fraylich, M., & Saunders, B. R. (2009). Thermoresponsive copolymers: From fundamental studies to applications. *Colloid and Polymer Science*, *287*(6), 627–643.

Meng, H., & Hu, J. (2010). A brief review of stimulus-active polymers responsive to thermal, light, magnetic, electric, and water/solvent stimuli. *Journal of Intelligent Material Systems and Structures*, *21*(9), 859–885.

Mocanu, G., Mihai, D., Dulong, V., Picton, L., & Lecerf, D. (2011). New anionic amphiphilic thermosensitive pullulan derivatives. *Carbohydrate Polymers*, *84*(1), 276–281.

Pasale, S. K., Cerroni, B., Ghugare, S. V., & Paradossi, G. (2014). Multiresponsive hyaluronan-p(NiPAAm) click-linked hydrogels. *Macromolecular Bioscience*, *14*(7), 1025–1038.

Seetapan, N., Mai-ngam, K., Plucktaveesak, N., & Sirivat, A. (2006). Linear viscoelasticity of thermoassociative chitosan-g-poly (*N*-isopropylacrylamide) copolymer. *Rheologica Acta*, *45*(6), 1011–1018.

Shi, H. Y., & Zhang, L. M. (2007). New grafted polysaccharides based on *O*-carboxymethyl- *O*-hydroxypropyl guar gum and *N*-isopropylacrylamide: Synthesis and phase transition behavior in aqueous media. *Carbohydrate Polymers*, *67*(3), 337–342.

Staros, J. V., Wright, R. W., & Swingle, D. M. (1986). Enhancement by *N*-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions. *Analytical Biochemistry*, *156*(1), 220–222.

Thatte, M. R. (2004). *Synthesis and antibacterial assessment of water-soluble hydrophobic chitosan derivatives bearing quaternary ammonium functionality*. USA: Louisiana State University (Vol. Ph. D. dissertation)

Tizzotti, M., Creuzet, C., Labeau, M.-P., Hamaide, T., Boisson, F., Drockenmuller, E., et al. (2010). Synthesis of temperature responsive biohybrid guar-based grafted copolymers by click chemistry. *Macromolecules*, *43*(16), 6843–6852.

Yang, Y., Kataoka, K., & Winnik, F. M. (2005). Synthesis of diblock copolymers consisting of hyaluronan and poly(2-ethyl-2-oxazoline). *Macromolecules*, *38*(6), 2043–2046.

Yang, Y., Kataoka, K., & Winnik, F. o. M. (2005). Synthesis of diblock copolymers consisting of hyaluronan and poly(2-ethyl-2-oxazoline). *Macromolecules*, *38*(6), 2043–2046.

Zhang, H., Zhong, H., Zhang, L., Chen, S., Zhao, Y., & Zhu, Y. (2009). Synthesis and characterization of thermosensitive graft copolymer of *N*-isopropylacrylamide with biodegradable carboxymethylchitosan. *Carbohydrate Polymers*, *77*(4), 785–790.

Zhang, R. S. (2005). Synthesis, characterization and reversible transport of thermosensitive carboxyl methyl dextran/poly (*N*-isopropylacrylamide) hydrogel. *Polymer*, *46*(8), 2443–2451.