Preparation and Functional Evaluation of Agarose Derivatives

Jai Prakash Chaudhary,[1] Stalin Kondaveeti,[2] Vishal Gupta,[2] Kamalesh Prasad,[2,3] Ramavatar Meena[1,3]

[1]Scale-Up and Process Engineering Unit, CSIR-Central Salt and Marine Chemicals Research Institute, G. B. Marg, Bhavnagar 364002, Gujarat, India

[2]Marine Biotechnology and Ecology Discipline, CSIR-Central Salt and Marine Chemicals Research Institute, G. B. Marg, Bhavnagar 364002, Gujarat, India

[3]Academy of Scientific and Innovative Research, CSIR-Central Salt & Marine Chemicals Research Institute, G. B Marg, Bhavnagar 364002, Gujarat, India

Correspondence to: R. Meena (E-mail: rmeena@csmcri.org)

ABSTRACT: A facile microwave-assisted one-pot synthesis of sodium carboxymethylagarose and calcium carboxymethylagarose from *Gracilaria dura* agarose (Ag) has been described. The process is user friendly, and the highest degree of substitution was obtained within 15 min compared with the conventional method, which requires more than 3 h. Solubility and gelling behavior of the modified Ag products were found to be dependent on degree of substitution of the products. The characterizations were done by using Fourier transform infrared spectroscopy, ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy, thermogravimetric analysis, scanning electron microscopy, Inductively coupled plasma spectrophotometry (ICP), rheology, conductometer analysis, and DNA gel electrophoresis. These agarose derivatives were easily soluble in water and exhibited low thermal hysteresis, improved conductivity, and improved the DNA resolution ability of the parent *G. dura* Ag hydrogels. These hydrogels may have potential applications in the areas including electrochemical devices, microbiology, biomedical, and pharmaceuticals fields. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40630.

INTRODUCTION

Low-temperature ($\leq 70^\circ$C) water-soluble polysaccharides, including agar or agarose (Ag), are particularly useful in foodstuffs when incorporation takes place at the pasteurization phase. Because of the high viscosity and nontoxicity, carboxymethylated biopolymers are frequently used in food science as a thickener, stabilizer, and as a constituent of other products, such as toothpaste, laxatives, diet pills, water-based paints, detergents, textile sizing, and various paper products. In the recent years, unmodified and modified biopolymer materials are becoming more promising than the synthetic polymer materials for food, pharmaceutical, and cosmetic industries because of their polyelectrolyte nature.[1–4] Carboxymethylated derivatives of various naturally occurring polysaccharides such as gellan, dextran, starch, chitosan, guar gum, cellulose, and xylan have been reported in the literature, using conventional heating for more than 3 h.[5–12] The multistep carboxymethylation of polysaccharide has also been reported in the literature to obtain the polysaccharide derivatives of desired properties for specific applications.[13–15] Anionic polymers are widely used to add coagulation, curtailing water produc-

tion in oil wells, release of chemicals, and water purification.[16–18] Gelation of Ag depends on several factors such as concentration, thermal hysteresis, presence of heavy metals, ions, and ionic strength of the solution.[19]

Ag, the red seaweed polysaccharide, is widely used in biomedical and bioengineering applications. The basic disaccharide repeating units of Ag consists of (1,3) linked β-D-galactose and (1,4) linked α-L$-$3,6-anhydrogalactose as shown in Supporting Information Figure S1(a). Polymer-based conducting hydrogel materials represent an important class of materials that synergize the advantageous features of hydrogels and organic conductors to be used for applications such as bioelectronics and energy storage devices.[20,21] In the year 2000, the chemistry Nobel Prize was awarded to Alan J. Heeger, Alan G. MacDiarmid, and Hideki Shirakawa for the discovery and development of conductive polymers, as well as the ability of salts to prevent the convulsive effects during administration of certain anesthetics; this led to the increase in interest in this field.[22,23] In modern technology, there exists a strong demand for materials that show conductivity, good transparency, and environmental friendly characters.[24] Griess et al.[25] have reported

conventional method for the preparation of only carboxymethyla-garose in the literature. They have also reported the effect of ionic strength in presence of NaCl and MgCl$_2$ as well as gel electrophoresis in the water cast derivatized Ag hydrogels. No rapid synthesis, physi-cochemical characterization, conductivity, and rheology of such Ag derivatives are accessible in the literature. Further, no comparative study of sodium carboxymethylagarose with its superior derivative calcium carboxymethylagarose was present in the literature. Numer-ous microwave-assisted functional modifications of polysaccharides have been reported in the literature. The technique is rapid and envi-ronmentally friendly compared with the conventional methods.[26–28]

Therefore, we report herein a facile microwave-assisted one-pot synthesis of water-soluble gelling and conducting sodium carboxy methylagarose (Na-CMA) and calcium carboxymethylagarose (Ca-CMA) products for enhancing DNA resolution capacity of the parent *Gracilaria dura* Ag hydrogels. The primary reason of this work was to modify *G. dura* Ag in an ongoing program of our laboratory on the functional modification of seaweed polysaccha-rides for newer applications. Na-CMA and Ca-CMA formed vary-ing strength hydrogels (rheology) in water, which can be used in soft matter electrochemical devices, such as soft organic diodes, polymeric batteries, as well as in biomedical applications.[21] Car-boxymethylagarose may also provide an efficient alternative approach for the oral delivery of hydrophilic macromolecules.

EXPERIMENTAL

Materials and Instruments

Ag was extracted from the red seaweed *G. dura*, which was col-lected from the south east coast of India (09°16.311′N, 78°59.921′E) following the method reported,[29] and the gel strength, sulfate, and ash contents of the Ag were 950 g cm^{-2} (0.5% gel), ≤0.25%, and 0.9%, respectively. Monochloroacetic acid (MCA) was purchased from M/s. Spectrochem Chemicals Ltd., Mumbai, India. Other chemicals used in this study were of analytical reagent grade except for isopropyl alcohol (IPA; labora-tory reagent grade), which were purchased from M/s S. D. Fine Chemicals, Mumbai, India. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin-Elmer FT-IR machine (Spec-trum GX; PerkinElmer Inc., Waltham, MA) on a KBr disc (2 mg sample in 600 mg KBr). ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance-II 500 (Ultra shield) Spectrometer with D$_2$O as solvent. Spectra were recorded at 70°C, using d_6-DMSO as the internal standard.[30] ^{13}C-NMR spectra of Na-CMA and Ca-CMA products with high degree of substitution (DS) were recorded at room temperature. ^{1}H-NMR spectra of MCA, Na-CMA, and Ca-CMA were also recorded (10 mg mL^{-1} of D$_2$O) under ambient conditions. Gel strength was measured in 1% (w/v) gel of modified Ag products and 1% (w/v) gel of the parent Ag at 20°C on a Nikkansui-type gel tester (Kiya Seisakusho Ltd., Japan). Gelling and melting points were recorded following the reported method.[31] Apparent viscosity was measured (in 1% w/v sample) on a Brookfield viscometer (DV-II +Pro), using SC4–18 spindle at 60 rpm at 80°C. Thermogravimetric analysis (TGA) was done on a Mettler Toledo Thermal Analyzer, model TGA/SDTA 851e. Presence of sodium and calcium contents in modified Ag products was confirmed by Inductively coupled plasma spectrophotometry (ICP) analysis.

Rheological measurements were done on an Anton Paar Physica MCR 301 rheometer, USA, using parallel plate PP50/P-PTD200 geometry (50 mm diameter; 0.1 mm gap). The temperature was maintained at 25°C ± 1°C by a Viscotherm VT2 circulating water bath. Dynamic viscosities at varying shear rates were determined at 40°C. The rheological test of the elasticity and true gel charac-ters of the gel samples of parent Ag and modified Ag was meas-ured by performing frequency sweep. The frequency dependence of storage moduli (G') was carried out at 25°C, using frequency from 0.1 to 20 Hz. All rheological data presented were means of three replicate measurements.[29] Ionic conductivity was carried out using a digital conductivity meter (Systronics, Conductivity TDS Meter 308) in a manner similar to that described by Singh et al.[21] In brief, during the experiments sample and electrode were sealed in a glass cell and placed in a constant temperature water bath. The repeatability and expected uncertainty of the experiments were ca. ±1% and ±3%, respectively. The surface morphology of the parent Ag and modified Ag products was ana-lyzed by scanning electron microscopy (SEM), applying 20-kV accelerating voltage and ×2000 magnification on a Carl-Zeiss Leo VP 1430 instrument (Oxford INCA).

DS of the modified Ag products was estimated by using a method described in the literature.[32] Herein, 0.1 g dried samples were taken in a conical flask and dissolved in 25 mL of distilled water, followed by addition of 25 mL of 0.1M NaOH solution under stirring for 10–15 min. The resulting mixture was back titrated with 0.1M HCl, using phenolphthalein as the indicator. The amount of acid consumed was recorded, and the DS was calculated according to the below-given equation,

$$DS = 0.306X/(1-0.059X)$$

where X is the acid consumed per gram of the sample; "306" is the grams molecular mass of repeating unit of Ag, and "59" is the net increase in molecular mass of repeating unit of Ag for each carboxymethyl (CM) group substituted.

For DNA gel electrophoresis, 1.2% (w/v) Ag gel was prepared in 25 mL 1X Tris-acetate–EDTA buffer (pH 8.0). Similarly, 1.2% (w/v) Ag gels were prepared by adding 0.2% (w/v) solid powder of modified Ag products (Na-CMA and Ca-CMA) of DS 0.27 to 1.11. 1.2 kb DNA ladder (Invitrogen, Carlsbad, CA) was run over the above men-tioned gels at a constant voltage of 75 V in electrophoresis unit (Bio-Rad, Berkeley, CA) for 2 h. After electrophoresis, gels were observed under UV (UV tube 8W, 312 nm; Bangalore Genei, Bengaluru, India) light to monitor the effect of modified Ag products on DNA migration and resolution of bands. Further, to evaluate the effect of modified Ag products on resolution and migration, amplified frag-ment length polymorphism (AFLP) experiment was conducted. For this, DNA sample was extracted from the green seaweed *Utricularia reticulata* using the protocol described in the literature.[33] The extracted DNA was subjected to AFLP analysis using the commercial kit (Invitrogen) following the instructions in user manual. The AFLP bands were size fractionated over the above-mentioned gel samples. Gels were run and visualized under UV light as mentioned above.

Synthesis of Na-CMA/Ca-CMA

In a typical batch, Ag (306 mg or 1 mmol repeating unit) was dispersed in 17.5–22.5 mL IPA with constant stirring for 5–10

Table I. Physicochemical Properties of Parent Agarose and Modified Agarose Products

Sample and molar ratios	DS	Yield (%)	Gel strength (g cm^{-2})	G_T (°C)	M_T (°C)	Viscosity (cP) at 80°C
Agarose	NA	NA	2600 ± 50	35 ± 1	98 ± 1	7.0 ± 0.5
Na-CMA (1 : 0.5)	0.27	96 ± 0.5	900 ± 50	30 ± 1	60 ± 1	7.3 ± 0.5
Na-CMA (1 : 1)	0.46	95 ± 0.5	500 ± 50	27± 1	48 ± 1	7.5 ± 0.5
Na-CMA (1 : 2)	0.69	94 ± 0.5	<100	≤7	38 ± 1	8.6 ± 0.5
Na-CMA (1 : 3)	1.11	92 ± 0.5	NA	NA	NA	9.2 ± 0.5
Na-CMA (1 : 4)	1.11	90 ± 0.5	NA	NA	NA	10 ± 0.5
Ca-CMA (1 : 0.5)	0.27	96 ± 0.5	1000 ± 50	37 ± 1	63 ± 1	8.5 ± 0.5
Ca-CMA (1 : 1)	0.46	95 ± 0.5	600 ± 50	32 ± 1	51 ± 1	10.5 ± 0.5
Ca-CMA (1 : 2)	0.69	94 ± 0.5	<100	≤7	40 ± 1	12.2 ± 0.5
Ca-CMA (1 : 3)	1.11	92 ± 0.5	<100	≤7	35 ± 1	13 ± 0.5
Ca-CMA (1 : 4)	1.11	90 ± 0.5	<100	≤7	35 ± 1	14 ± 0.5

DS, degree of substitution; G_T, gelling temperature; M_T, melting temperature; NA, not applicable.

min at room temperature. To the above dispersion, 2.5–7.5 mL (e.g., 0.5 to 1.5 mol NaOH, with respect to total reaction mixture) of 20% aqueous NaOH solution was added slowly, followed by the addition of MCA (0.5–4 mmol) under stirring at room temperature. The reaction was carried out under microwave irradiation from 55°C to 70°C for 10–20 min applying 400 W power. The reaction time (Supporting Information Table SII) and temperature (Supporting Information Table SIII) were optimized on the basis of highest DS. The resulting reaction mixtures were cooled, and 70% CH$_3$OH was added in the mixture followed by neutralization with 90% acetic acid. Na-CMA was isolated by precipitation in 1 : 2 v/v IPA. Finally, Na-CMA was washed with fresh IPA under stirring, followed by vacuum drying at 50°C. For the synthesis of Ca-CMA, the obtained Na-CMA was treated with 3%–6% CaCl$_2$ solutions under microwave irradiation for 2 min at 65°C. The reaction mixtures were purified by dialysis method, followed by IPA precipitation and drying as described above to obtain the dried Ca-CMA. The same sets of the experiments were carried out using the conventional method, as described in the literature for carboxymethylation of various biopolymers.[5–12] The highest DS and ionic conductivity of the resulting Na-CMA and Ca-CMA were observed for 1 : 3 molar ratio of Ag : MCA under microwave (15 min at 65°C) irradiation, whereas the conventional method produced similar products with 1 : 3 molar ratio of Ag: MCA after heating at 60°C for 240 min (Supporting Information Table SII).

DNA Gel Electrophoresis

Migration of DNA bands was faster in 1.2% parent Ag gel; however, resolution of bands was better in Ag gel incurred with 0.2 wt % sodium carboxymethylagarose of DS 0.46 and calcium carboxymethylagarose of all DS.

RESULT AND DISCUSSION

Physicochemical Properties

Optimization studies revealed that microwave irradiation for 15 min at 65°C led to the formation of ester bonds between carboxyl group of MCA and hydroxyl groups of Ag. The highest DS

was obtained with the molar ratio 1 : 3 (Ag : MCA, *cf.* Table I). The synthetic process of Na-CMA/Ca-CMA from Ag is shown in Supporting Information Scheme S4. The yields of Ag derivatives decreased with increased DS of the products. The yields of the products were in the range of 90% ± 0.5% to 96% ± 0.5% (Table I). The maximum (96% ± 0.5%) and minimum (90% ± 0.5%) yields were obtained for the products of Ag having DS 0.27 and 1.11, respectively (Table I). Modification of *G. dura* Ag was mainly carried out to obtain the desired properties such as low temperature solubility, low thermal hysteresis hydrogels (gels having small difference between gelling and melting temperatures), soft and low gelling temperature hydrogels, improved DNA resolution, and conductivity for their potential applications. An increase in the molar ratio of MCA to Ag leads to a significant increase in DS of modified products except for a molar ratio of 1 : 4 (Ag : MCA) as shown in Table I. At a molar ratio of 1 : 0.5: 0.5 (Ag : MCA: NaOH), the DS 0.27 is significantly low compared with DS 1.11 at a molar ratio of 1 : 3: 1 (Ag : MCA: NaOH), and no increase in DS above this alkali concentration. Product having low DS (0.27) at molar ratio of 1: 0.5 (Ag: MCA) may be due to the insufficient amount of aqueous NaOH to activate the Ag polymer. This is in good agreement with the study reported for xylan.[12] The DS was 0.46 on applying a molar ratio of 1.0 mole MCA/mole Ag and 0.75 mole NaOH/mole Ag. The maximum DS 1.11 was obtained at a molar ratio of 3.0 moles of MCA/mole Ag, and the DS attained plateau beyond this molar ratio of MCA. Correspondingly, the DS increases up to 1.11 by further increase in NaOH concentration up to 1 mole NaOH/mole Ag. Further increase in the concentration of NaOH solution decreases the DS values to 0.96 with 1.25 mole NaOH/mole Ag and 0.73 with 1.5 mole NaOH/mole Ag compared with 1.11 for 1 mole NaOH/mole Ag at a molar ratio of 1 : 3 (Ag : MCA). It may be due to the fast hydrolysis of the MCA in the concentrated NaOH solution.[12]

Ag used in this work took 2 min to dissolve at 100°C, and solubility was increased significantly after carboxymethylation of Ag with MCA. All the modified Ag products with DS from 0.27 to 0.46 dissolved at low temperature and formed hydrogels at

room temperature. It may be due to the presence of low amount of CM functional groups in Ag backbone, whereas Na-CMA with DS 0.69 to 1.11 are readily soluble in water at room temperature, and Ca-CMA with DS 0.69 to 1.11 are soluble at $70°C \pm 1°C$ and formed viscous solutions at room temperature, but these solutions formed soft gel at very low temperature ($\leq 7°C$). This can be explained on the basis of high ionic strength/ionization in water because of high amount of CM groups as well as sodium and calcium ions. The sodium salt form of carboxymethylcellulose is water soluble. Carboxymethylation of polymers have been widely used to make water-soluble polymer from insoluble one for application in various fields to control the behavior of aqueous systems.[34]

The gel strength, gelling temperature, and melting temperature of 1% parent Ag decreased significantly after carboxymethylation with MCA (Table I). Consequently, the gel strength of Na-CMA and Ca-CMA decreased with the increase in DS from 0.27 to 1.11. Among all modified Ag products, Na-CMA and Ca-CMA with the lowest DS 0.27 exhibited the highest gel strength 900 ± 50 and 1000 ± 50 g cm^{-2}, respectively, whereas the respective products having DS 0.69 formed soft gel at low temperature ($\leq 7°C$). Na-CMA with DS 1.11 is nongelling even at low temperature ($\leq 7°C$), and Ca-CMA with DS 1.11 formed soft gel at low temperature ($\leq 7°C$) as shown in Table I. Na-CMA and Ca-CMA having DS 0.27 produced the stronger gel in water compared with their higher DS, which may be due to the limited presence of CM functional groups on the Ag backbone, thus assisting the formation of hydrogel networks through hydrogen bonding as well as because of the increase in number of "junction zones" in the gel network. The results are in good agreement with the literature reports of Ag derivatives.[35] Result of this study revealed that the modification technique used in this study produced quick soluble and low gelling and melting Ag-based hydrogels with low thermal hysteresis compared with parent Ag, which may be useful for some specific applications.[34,36] The change in properties may be due to the presence of agents that disrupt hydrogen bond formation and reduces the gelling and melting temperatures, and gel strength of Ag gels.[37] Therefore, the difference in the gelation properties of the modified and the parent Ag gels can be explained on the basis of insertion of CM functional groups in Ag backbone, which results in tailoring of gelation properties. These observations show that CM groups affect the gelation behavior of Ag by imparting steric hindrance to the regular chain of Ag and interfering with the conformational transition and aggregation of the polymer to form gel networks. Similar observation was reported for the study done with carrageenans.[38] Singh et al.[19] have reported that addition of sodium sulfate in the aqueous Ag solutions weakens the gel structure by weakening the Ag–water interactions and facilitates the easy release of water from gel network. Rees[39] and Arnott et al.[40] have also reported that agents that disrupt hydrogen bond formation will decrease the gel strength and gelation temperature of Ag gels or even inhibit the formation of the strong gel.

The viscosity values of the modified Ag products and parent Ag were measured at 80°C. The viscosity of the parent Ag polymer was found to be less when compared with modified Ag products (Table I). The viscosities of modified Ag (Na-CMA and Ca-CMA) increased on increasing the DS up to 1.11 (Table I). Ca-CMA with DS 0.27–1.11 exhibited higher viscosity values compared with Na-CMA with DS 0.27–1.11, respectively. The apparent viscosities of Na-CMA$_{DS\ 0.27}$, Ca-CMA$_{DS\ 0.27}$, Na-CMA$_{DS\ 1.11}$ and Ca-CMA$_{DS\ 1.11}$ were 7.30 cP, 8.50 cP, 10.00 cP and 14.00 cP respectively (Table I). The difference in the viscosity of Na-CMA and Ca-CMA may be due to the higher extent of intermolecular interactions in Ca-CMA.[41] It may be the result of cross-linking of Ca^{2+} with carboxyl groups or otherwise higher hydration power of the calcium ions having greater nuclear charge compared with sodium ion. Stygar et al.[42] have reported that viscosity increases with the decrease in the size of alkali or alkaline earth metal cation. Iglauer et al.[43] have reported that viscosity of carrageenan increased when concentration of calcium ion increased in the solution. They have also reported that an increase in ionic strength of solution led to higher viscosities if the cation (Ca^{2+}) and anion are present in constant ratios, whereas viscosity of solution decreased with an increase in ionic strength, if a brine mixture contains both Na^+ and Ca^{2+} ions.

The formation of Na/Ca-CMA was confirmed by FT-IR and ^{13}C-NMR spectrometry (Figures 1 and 2). Formation of carboxylate anion (—COO—) bond of the CM group as a result of the reaction between hydroxyl group of Ag and MCA was confirmed by the appearance of the new bands at 1607 and 1422 cm^{-1},[44] which were assigned to the asymmetrical and symmetrical stretching vibrations of carboxylate anions (—COO—), whereas the band at 1328 cm^{-1} was a characteristic IR band of the —CH$_2$— scissoring vibration in the CM group as shown in Figure 1(a). This is in good agreement with the literature reports.[38] In addition, in the FT-IR spectrum of Na/Ca-CMA, the main characteristic absorption bands of Ag (1162, 1073, and 931 cm^{-1}) remained intact during modification reaction indicating that the backbone of Ag remains as such during chemical modification as shown in Figure 1(a,b).[35,45] The characteristic IR bands of MCA were obtained at 1733 cm^{-1} (sharp; —C=O, stretching vibration of carbonyl group of acid) and 1305 cm^{-1} (weak; —CH$_2$, scissoring in the CM group) as shown in Figure 1(c). ^{13}C-NMR of Na/Ca-CMA exhibited the prominent peak at 177.6 ppm (C$_a$), which was assigned to the carboxylate anion (—COO—) of the CM derivatives, whereas peaks at 76.3 ppm (C$_b$) were assigned to the methylene (—CH$_2$—) carbon atom of the CM substituent as shown in Figure 2, and are in good agreement with those of reported in the literature.[15,46] In addition, ^{13}C-NMR of Na/Ca-CMA showed other prominent peaks at 62.7 ppm (C-6′, linked with MCA) and 62.3 ppm (C6′* CH$_2$ linked with OH in unreacted Ag) (Figure 2). ^{13}C-NMR of the parent Ag exhibits the characteristic peaks of Ag polymer between 62.1 and 103.1 ppm.[30,35] Similarly, characteristic ^{13}C-NMR peaks of MCA were obtained at 172.3 ppm (—C=O/C$_a$) and 42.5 ppm (—CH$_2$—/C$_b$) as shown in Figure 2. The ^{1}H-NMR spectra of the Ag polymer show several signals between 3.33 and 5.12 ppm because of the protons linked to sugar carbon atoms [Supporting Information Figure S5(a)]. The ^{1}H-NMR Spectra of Na/Ca-CMA shows chemical shifting for
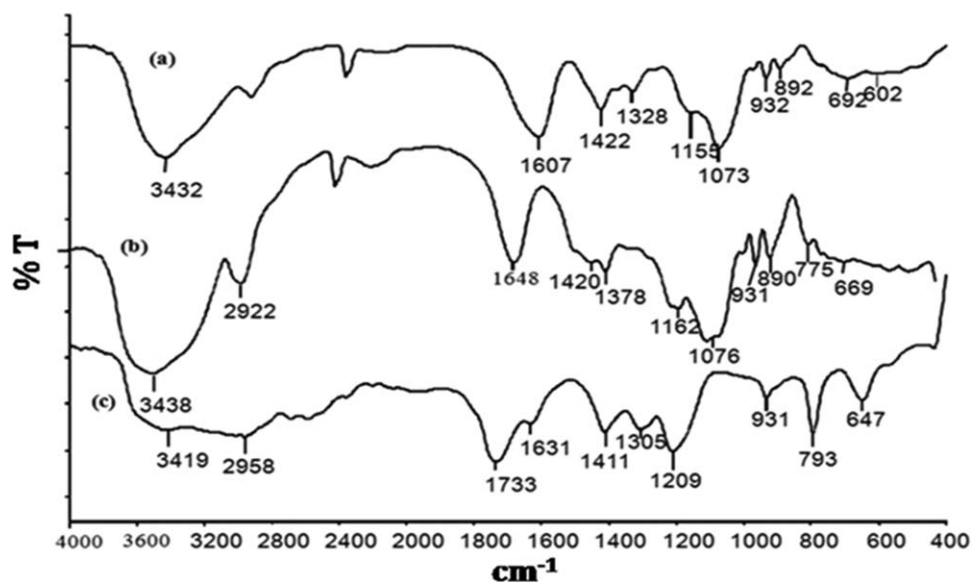
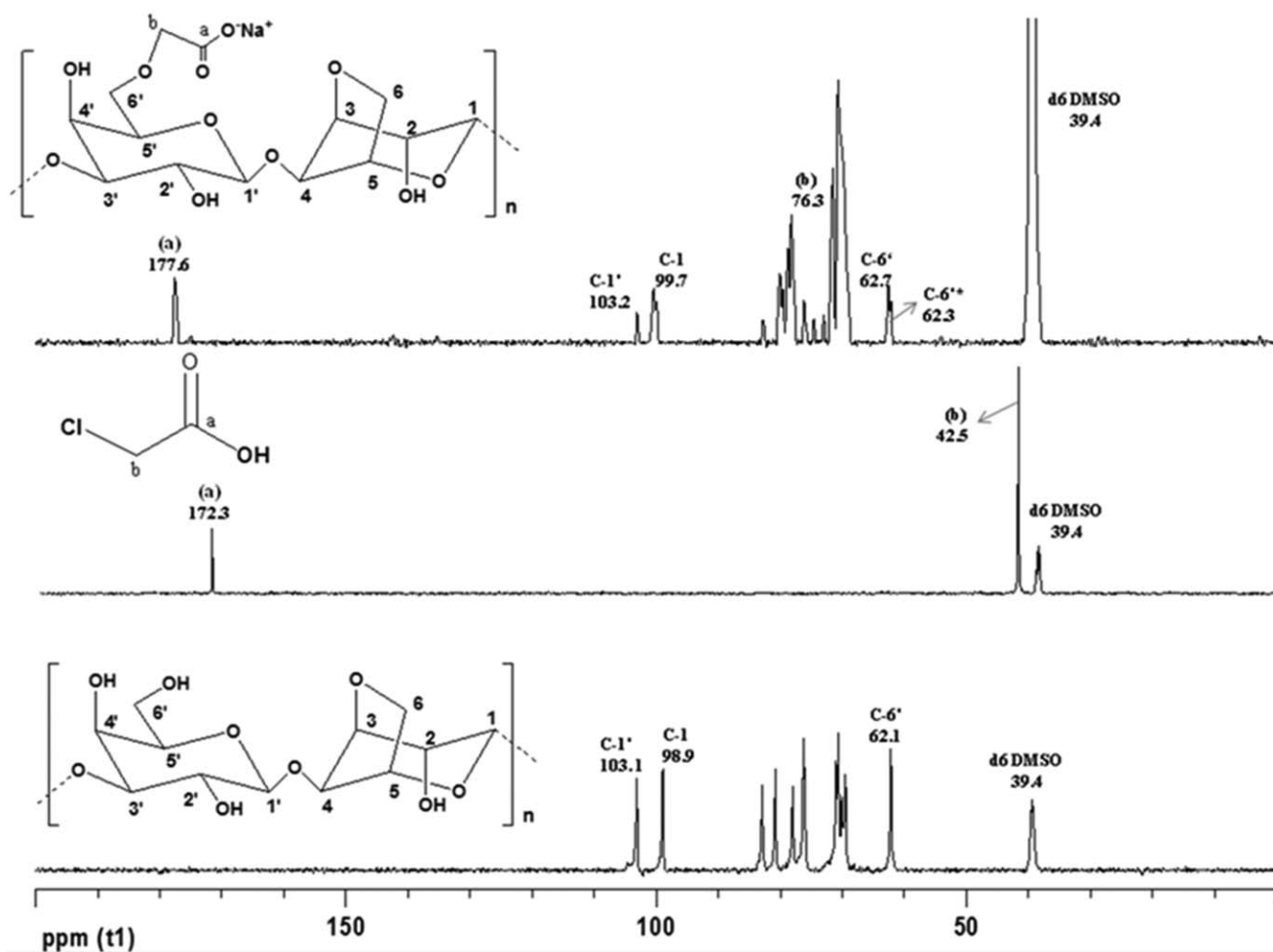**Figure 1.** FT-IR spectra of (a) Na/Ca-CMA, (b) parent agarose, and (c) MCA.

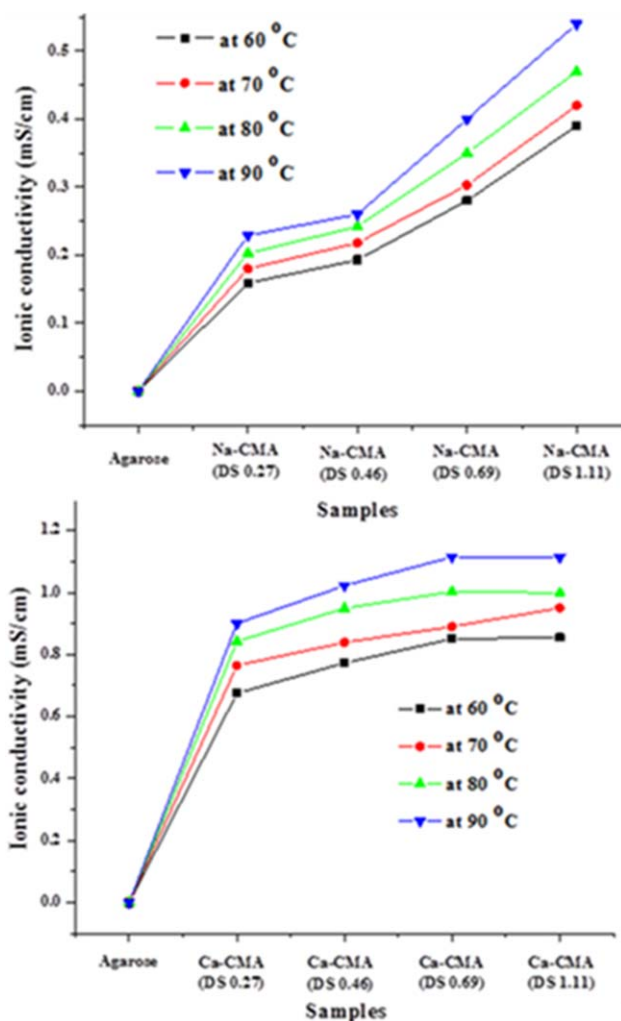**Figure 2.** ^{13}C-NMR spectra of parent agarose, MCA, and Na/Ca-CMA.

Figure 3. Temperature dependence of ionic conductivity of parent agarose and modified agarose products. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 4. Shear thinning behavior of parent agarose and modified agarose products. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

signals appeared between 3.31 and 5.78 ppm. In this interval, the new band at 4.49 ppm is seen, which may be due to the presence of methylene protons in the carboxymethylated Ag [Supporting Information Figure S5(b)], whereas MCA exhibited single peak at 4.20 ppm, which may be due to methylene protons [Supporting Information Figure S5(c)].

Ionic Conductivity

Figure 3 shows comparative ionic conductivity (κ) values of the parent Ag and modified Ag products at different temperatures from 60°C to 90°C. Ionic conductivity increased on increasing the temperature up to 90°C, which may be due to the higher mobility of ions at higher temperature. Ionic conductivity of the parent Ag polymer was negligible and increased significantly after carboxymethylation. The conductivity of Ca-CMA was significantly higher than Na-CMA, in each DS from 0.27 to 1.11 (Figure 3). An increase in conductivity of the modified Ag products with increase in DS up to 1.11 may be due to the increased concentration of charge carriers in the polymeric network. It may be the result of formation of charged ionic clusters
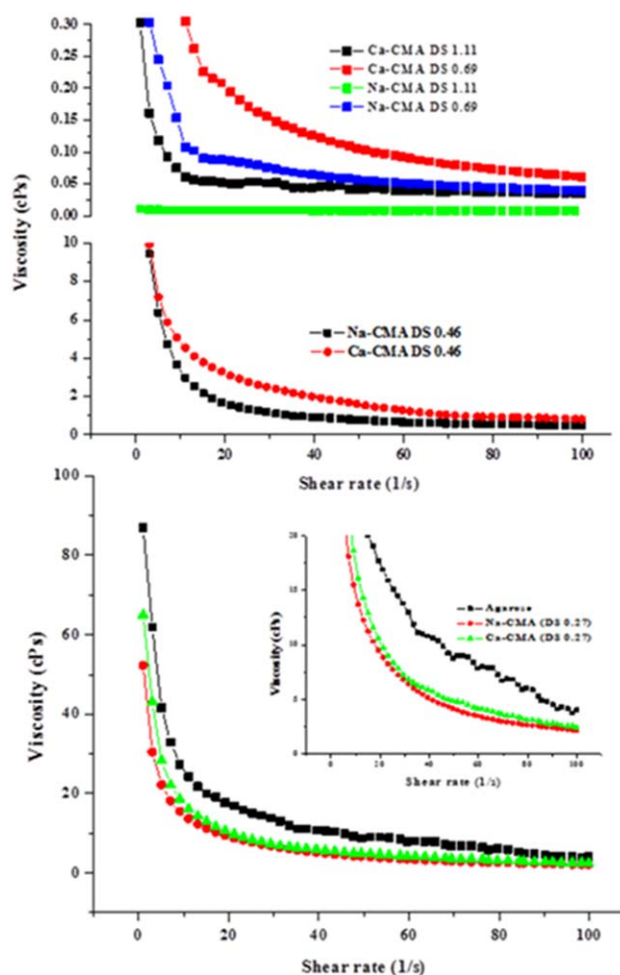
in the solution.[47] Induction of sodium and calcium in the modified Ag polymer structure probably changes the conductive mechanism or behavior of Ag matrix may be due to electronic transfer through ions. Similar observation has also been reported for conduction in poly(vinyl alcohol)–sodium polystyrene–sulfonate blends and glass materials.[41]

TGA Analysis

The thermal stability of the modified and unmodified Ag derivatives was investigated by using TGA technique. The TGA curves of the parent Ag and modified Ag (Na-CMA and Ca-CMA) are shown in Supporting Information Figure S6. The thermal stability of the parent Ag increased with increase in the DS of the products. The parent Ag exhibits lowest 27 wt % mass retention up to 600°C, whereas maximum mass retention of 45 and 50 wt % were obtained for Na-CMA$_{DS1.11}$ and Ca-CMA$_{DS1.11}$, respectively, synthesized with a molar ratio of 1 : 3 (Ag : MCA), revealing enhanced thermal stability of the modified Ag products. Further, Ca-CMA exhibits greater thermal stability compared with Na-CMA, as mass loss is lower for Ca-CMA (50%) than Na-CMA (55%) up to
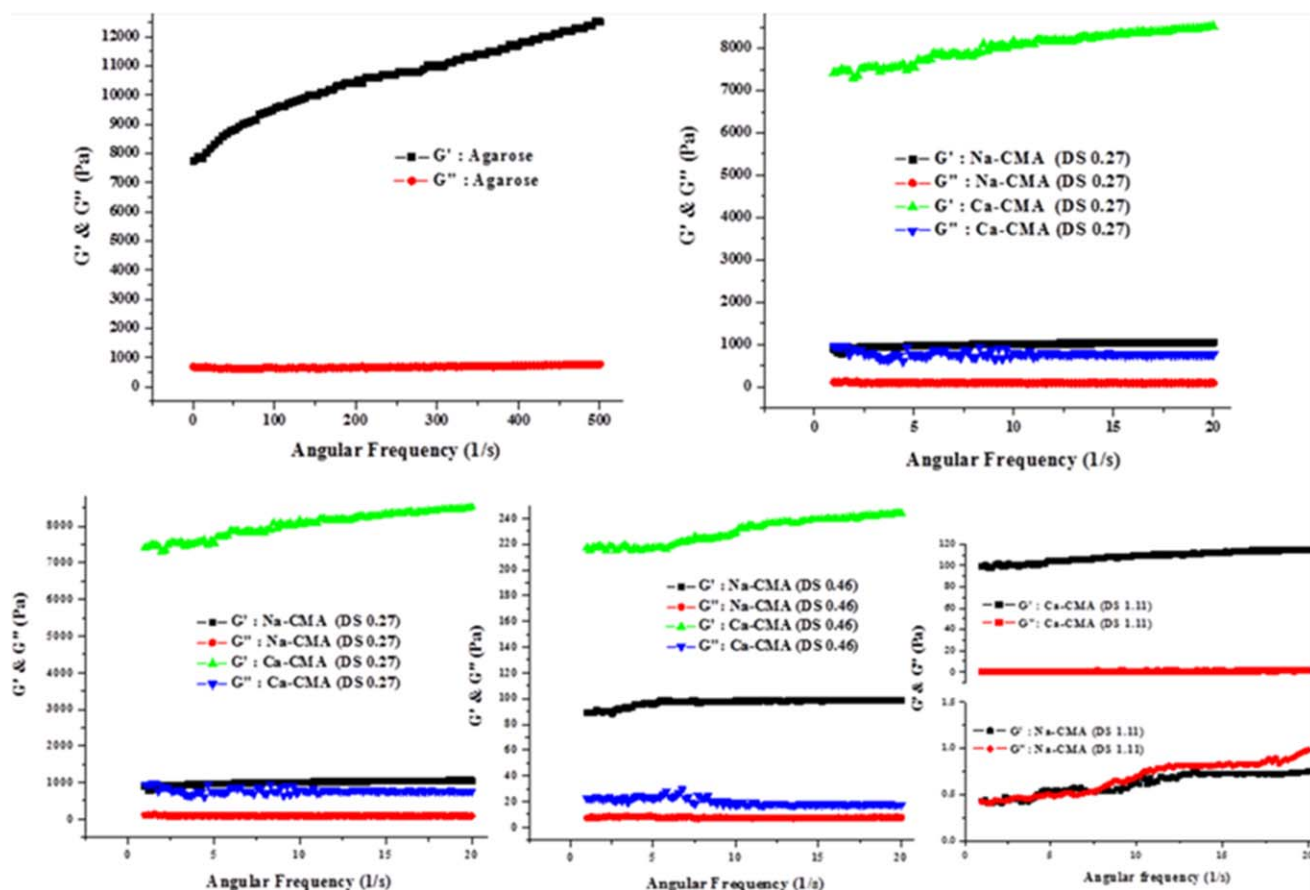
Figure 5. Dependence of angular frequency on storage modulus (G') and loss modulus (G'') of parent agarose and modified agarose products. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

600°C. The varying thermal stability of Na-CMA and Ca-CMA compared with the parent Ag confirmed the formation of new materials.

ICP Analysis

The presence of sodium and calcium in the modified Ag was confirmed by ICP analysis (Supporting Information Table SVII). Sodium and calcium contents increased when the DS of the products increased and maximum were found in the products having DS 1.11.

Rheological Characterization

Figure 4 shows the plots of dynamic viscosities under varying shear rate 0–100 (1/s) for the modified Ag products and parent Ag. Shear rate and other rheological studies of the modified Ag products and parent Ag indicate a shear-thinning behavior over intermediate-to-high shear rate region. After carboxymethylation, shear thinning behavior of the parent Ag increased and varied as function of values of DS. Na-CMA and Ca-CMA
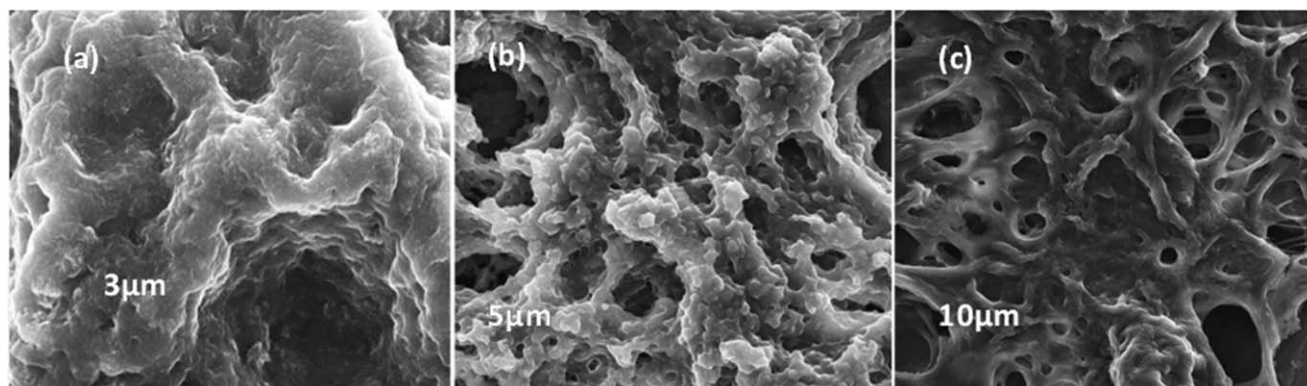

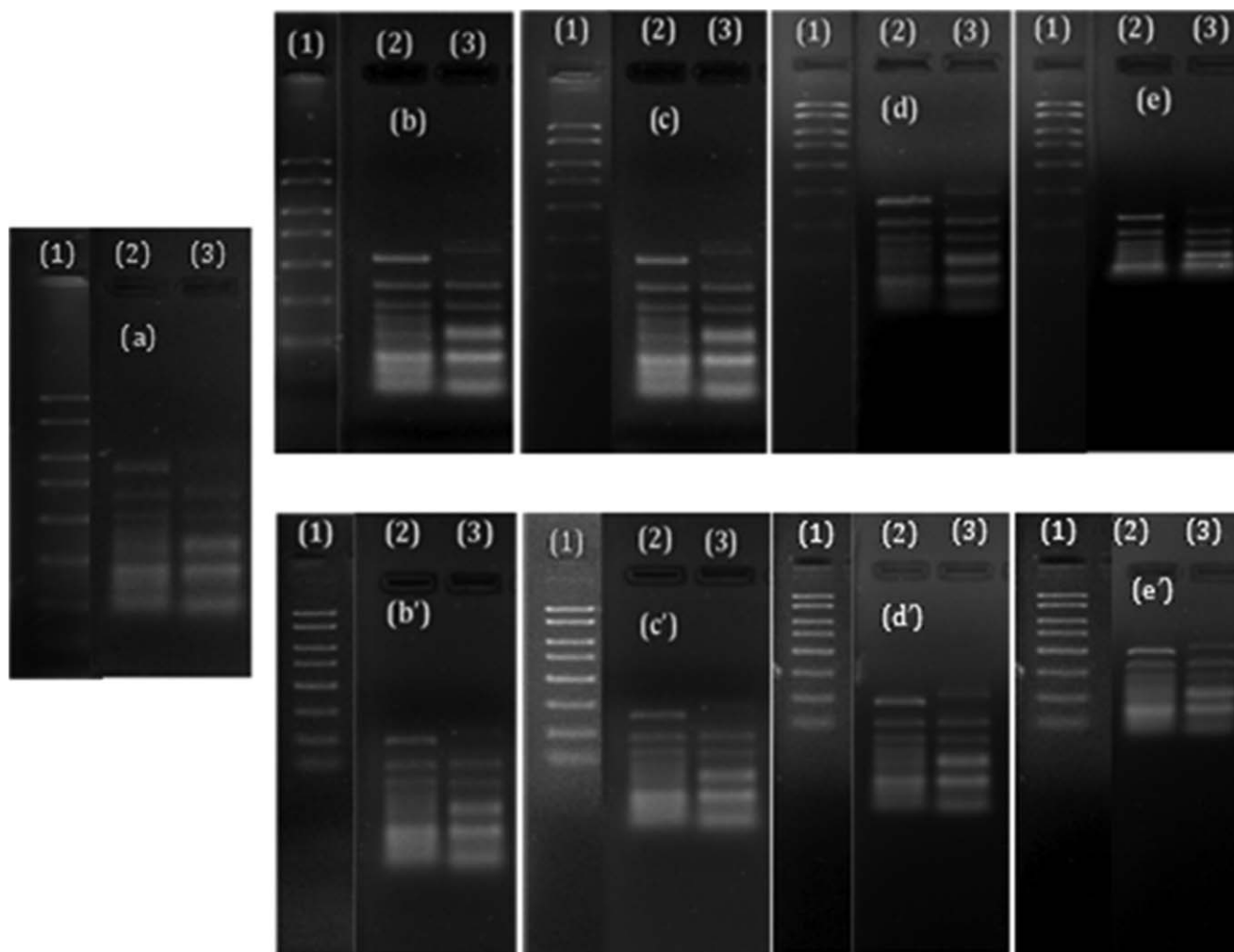**Figure 6.** SEM images of (a) agarose, (b) Na-CMA, and (c) Ca-CMA.

Figure 7. Gel electrophoresis images of (a) 1.2 % (w/v) parent agarose gel (Ag); (b) Ag gel with 0.2% (w/v) Na-CMADS0.27; (b′) Ag gel with 0.2% (w/v) Ca-CMADS0.27; (c) Ag gel with 0.2% (w/v) Na-CMADS0.46; (c′) Ag gel with 0.2% (w/v) Ca-CMADS0.46; (d) Ag gel with 0.2% (w/v) Na-CMADS0.69; (d′) Ag gel with 0.2% Ca-CMADS0.69; (e) Ag gel with 0.2% (w/v) Na-CMADS1.11; (e′) Ag gel with 0.2% (w/v) Ca-CMADS1.11. Lane 1: 1.2 kb DNA ladder; Lanes 2 and 3: samples after AFLP analysis with two different primers.

having the DS 0.27 exhibited highest shear thinning behavior compared with other Ag derivatives having the DS 0.46–1.11 (Figure 4).

Frequency dependence of storage modulus (G') and loss modulus (G'') of parent Ag and modified Ag products are shown in Figure 5. Parent Ag gel exhibits the highest G' and G'' values and the values decreased on carboxymethylation. Na-CMA$_{DS0.27}$ and Ca-CMA$_{DS0.27}$ synthesized at a molar ratio for 1 : 0.5 (Ag : MCA) exhibits highest G' and G'' value followed by Na-CMA and Ca-CMA of DS 0.46–1.11, synthesized with molar ratios of 1 : 1; 1 : 2; 1 : 3, and 1 : 4 (Ag : MCA). Na/Ca-CMA prepared at molar ratios of 1 : 3 and 1 : 4 (Ag : MCA) showed almost identical values of modulus, and the values of Na-CMA and Ca-CMA obtained at a molar ratio of 1 : 4 were not mentioned in Figure 5. The values of G' and G'' for Na-CMA and Ca-CMA products having DS 0.27 and 0.46 revealed the formation of strong or true hydrogel. This can be explained on the basis of limited existence of CM groups on the Ag backbone, which

may be due to the formation of hydrogel networks through hydrogen bonding as well as increased numbers of "junction zones" in the gel network.[28,32,34] The presence of more CM groups drastically reduced the gelation properties of the modified Ag products, resulting in lesser storage modulus and loss modulus values in the modified Ag products of highest DS (1.11). Further, the presence of CM groups in the modified Ag network may also disturb the double helix in Ag gel networks, resulting in the formation of lesser and weaker double helices in the modified Ag products of the higher DS. The G' and G'' values of Ca-CMA were found to be higher than Na-CMA for each DS (Figure 5). The less frequency dependence and higher G' values in Ca-CMA indicated a modified network structure of Ca-CMA. Comparing the effect of Na$^+$ and Ca^{2+} ions, it was found that Ag derivatives having Ca^{2+} are more effective in modifying the rheological behavior of Ag derivatives than Ag derivatives having Na$^+$ ion. The Ca^{2+} promoted interchain interactions and increased junction zones of the egg-box model and formed stronger gel for alginate, pectin, and gellan gum.[48]

The K$^+$ has been also reported to be more effective in enhancing the gelling ability of gum Arabic than Na$^+$ dose, which may be due to the formation of ion pairs favoring the interchain interactions and gel formation. However, in Ca-CMA, G' and G'' increased significantly, which revealed that Ca^{2+} might modify the network structure of Ca-CMA through cross-linking with carboxyl groups in addition to charge screening effects. This can also be explained on the basis of the stronger carboxylate–cation^{2+}–carboxylate interactions, rendering a higher capacity of adjacent helices cross-linking when divalent ions are involved.[49] Ca-CMA$_{DS1.11}$ showed significantly greater G' values than G'', which revealed that it formed gel-like structure, whereas higher G'' values of Na-CMA$_{DS1.11}$ revealed that Na-CMA$_{DS1.11}$ formed viscous solution (Figure 5).

SEM Studies

SEM images evidenced higher morphological differences between the modified Ag products and the parent Ag (Figure 6). Figure 6(a) presents SEM image for the parent Ag, which appears in globule-like morphology, whereas Figure 6(b,c) shows SEM micrographs obtained for sodium and calcium carboxymethylagarose. Upon carboxymethylation of Ag, the structure of parent Ag gets improved as shown in Figure 6(b,c), wherein the morphology of the globule-like structure of parent Ag was modified in such a way that because of the introduction of the hydrophilic groups onto Ag backbone, results improved solubility of modified Ag products may be the result of porous structure, which may permit rapid water penetration and large surface area for interaction. The morphology of the modified Ag products [Figure 6(b,c)] were compared with the parent Ag, it is clearly evident that the presence of CM groups along with sodium and calcium ions in modified Ag products have drastically changed the morphology of the parent Ag [Figure 6(a)]. It clearly indicates that carboxymethylation has been taken place. Na-CMA formed a more compact structure with less interconnected pores compared with the higher porous structure of Ca-CMA. On the other hand, the addition of Ca^{2+} ions determined the increase in gel porosity.[50] Furthermore, porous morphology of Ca-CMA may be the result of cross-linking of calcium ions with carboxylic ions present in the polymeric matrix in similar way as reported for alginate gel structure in the presence of calcium ions.[51]

DNA Gel Electrophoresis

The modified Ag products were investigated for improvement in functional properties of the parent Ag in terms of migration and resolution of DNA bands as shown in Figure 7. Results of this study revealed that there was retardation in DNA migration after addition of modified Ag products compared with parent Ag gel, and maximum was in Na-CMA with DS 1.11 [Figure 6(e)]. However, well-resolved and sharper DNA bands were observed with Na-CMA of DS 0.27–0.69 compared with the parent Ag gels, whereas no clear resolution of DNA bands was obtained in Ag gel having Na-CMA of DS 1.11. Consequently, fine resolution of DNA bands was obtained in Ag gels having Ca-CMA of DS 0.27–1.11. Difference might be the result of salt concentrations, binding affinity, interaction with ions, and salt type.[52,53] This gave an indication to evaluate the resolution among DNA bands having low molecular weight difference.

These results demonstrated a significant improvement of Ag properties after adding the modified Na-CMA and Ca-CMA of all DS, except Na-CMA of DS 1.11.

CONCLUSIONS

A facile rapid microwave-assisted environmentally friendly route for synthesis of water-soluble Na/Ca-CMA was developed from *G. dura,* Ag. All modified Ag products were forming low thermal hysteresis hydrogels except Na-CMA with highest DS. Compared with parent Ag, the maximum ionic conductivity of Na-CMA was found to be 1000 times more; however, the conductivity of Ca-CMA was around twofold higher than Na-CMA for same DS. Addition of Na-CMA and Ca-CMA in the parent Ag gels exhibited improved resolution and sharpness of DNA bands compared with parent Ag gel, except Na-CMA of highest DS. Thus, modified Ag products may have possible applications in the areas including electrochemical devices, controlled release, biomedical fields, molecular biology, and other biological applications.

REFERENCES

1. Silva, D. A.; de Paula, R. C. M.; Feitosa, J. P. A.; de Brito, A. C. F.; Maciel, J. S.; Paula, H. C. B. *Carbohydr. Polym.* **2004**, *58*, 163.

2. Thérien-Aubin, H.; Baille, W. E.; Zhu, X. X.; Marchessault, R. H. *Biomacromolecules.* **2005**, *6*, 3367.

3. Muzzarelli, R. A. A. *Carbohydr. Polym.* **2009**, *76*, 167.

4. Muzzarelli, R. A. A. *Carbohydr. Polym.* **2009**, *77*, 1.

5. Miyamoto, K.; Tsuji, K.; Nakamura, T.; Tokita, M.; Komai, T. *Carbohydr. Polym.* **1996**, *30*, 161.

6. Huynh, R.; Chaubet, F.; Jozefonvicz, J. *Die Angew. Makromol. Chem.* **1998**, *254*, 61.

7. Heinze, T.; Pfeiffer, K.; Liebert, T.; Heinze, U. *Starch/Stärke.* **1999**, *51*, 11.

8. Stojanović, Ž.; Jeremić, K.; Jovanović, S. *Starch/Stärke.* **2000**, *52*, 413.

9. Tijsen, C. J.; Kolk, H. J.; Stamhuis, E. J.; Beenackers, A. A. C. M. *Carbohydr. Polym.* **2001**, *45*, 219.

10. Abreu, F. R. D.; Campana-Filho, S. P. *Polímeros: Ciência e Tecnologia.* **2005**, *15*, 79.

11. Parvathy, K. S.; Susheelamma, N. S.; Tharanathan, R. N.; Gaonkar, A. K. *Carbohydr. Polym.* **2005**, *62*, 137.

12. Petzold, K.; Schwikal, K.; Heinze, T. *Carbohydr. Polym.* **2006**, *64*, 292.

13. Lazik, W.; Heinze, T.; Pfeiffer, K.; Albrecht, G.; Mischnick, P. *J. Appl. Polym. Sci.* **2002**, *86*, 743.

14. Heinze, T.; Koschella, A. *Macromol. Symp.* **2005**, *223*, 13.

15. Lawal, O. S.; Lechner, M. D.; Kulicke, W. M. *Int. J. Biol. Macromol.* **2008**, *42*, 429.

16. Aguilar, M. I.; Sáez, J.; Lloréns, M.; Soler, A.; Ortuño, J. F.; Meseguer, V.; Fuentes, A. *Chemosphere.* **2005**, *58*, 47.

17. Ogunberu, A. L.; Asghari, K. *J. Petrol. Sci. Eng.* **2006**, *50*, 176.

18. Haydar, S.; Aziz, J. A. *J. Hazard. Mater.* **2009**, *168*, 1035.

19. Singh, T.; Meena, R.; Kumar, A. *J. Phys. Chem. B.* **2009**, *113*, 2519.

20. Trivedi, T. J.; Srivastava, D. N.; Rogers, R. D.; Kumar, A. *Green. Chem.* **2012**, *14*, 2831.

21. Singh, T.; Trivedi, T. J.; Kumar, A. *Green. Chem.* **2010**, *12*, 1029.

22. Khalil, A.; Vijayalakshmi, V.; Olga, K.; Kamila, O.-S.; Catherine, A. *IEEE. Sens. J.* **2009**, *9*, 1942.

23. Arthur, J. S.; Unangst, M. R. *J. Chem. Phys.* **1941**, *9*, 195.

24. Colaneri, N. F.; Shacklette, L. W. *IEEE T. Instrum. Meas.* **1992**, *41*, 291.

25. Griess, G. A.; Guiseley, K. B.; Miller, M. M.; Harris, R. A.; Serwer, P. *J. Struct. Biol.* **1998**, *123*, 134.

26. Chhatbar, M. U.; Meena, R.; Prasad, K.; Chejara, D. R.; Siddhanta, A. K. *Carbohydr. Res.* **2011**, *346*, 527.

27. Peng, X.-W.; Ren, J.-L.; Zhong, L.-X.; Cao, X.-F.; Sun, R.-C. *J. Agric. Food. Chem.* **2010**, *59*, 570.

28. Sagar, A. D.; Pulle, J. S.; Reddy, S. M.; Yadav, M. V. *Int. J. Chem. Sci.* **2012**, *10*, 36.

29. Meena, R.; Siddhanta, A. K.; Prasad, K.; Ramavat, B. K.; Eswaran, K.; Thiruppathi, S.; Ganesan, M.; Mantri, V. A.; Subarao, P. V. *Carbohydr. Polym.* **2007**, *69*, 179.

30. Rees, D. A. *Adv. Carbohydr. Chem. Biochem.* **1969**, *24*, 267.

31. Mehta, G.; Meena, R.; Prasad, K.; Ganesan, M.; Siddhanta, A. K. *J. Appl. Phycol.* **2010**, *22*, 623.

32. Kweon, D. K.; Choi, J. K.; Kim, E. K.; Lim, S. T. *Carbohydr. Polym.* **2001**, *46*, 171.

33. Gupta, V.; Baghel, R. S.; Kumar, M.; Kumari, P.; Mantri, V. A.; Reddy, C. R. K.; Jha, B. *Aquaculture.* **2011**, *318*, 389.

34. Volkert, B.; Loth, F.; Lazik, W.; Engelhardt, J. *Starch/Stärke* **2004**, *56*, 307.

35. Mehta, G. K.; Kondaveeti, S.; Siddhanta, A. K. *Polym. Chem.* **2011**, *2*, 2334.

36. Bajpai, A. K.; Shukla, S. K.; Bhanu, S.; Kankane, S. *Prog. Polym. Sci.* **2008**, *33*, 1088.

37. Paepe, I. D.; Declercq, H.; Cornelissen, M.; Schacht, E. *Polym. Int.* **2002**, *51*, 867.

38. Tranquilan-Aranilla, C.; Nagasawa, N.; Bayquen, A.; Dela Rosa, A. *Carbohydr. Polym.* **2012**, *87*, 1810.

39. Rees, D. A. *Biochem. J.* **1972**, *126*, 257.

40. Arnott, S.; Fulmer, A.; Scott, W. E.; Dea, I. C. M.; Moorhouse, R.; Rees, D. A. *J. Mol. Biol.* **1974**, *90*, 269.

41. Enebro, J.; Momcilovic, D.; Siika-Aho, M.; Karlsson, S. *Biomacromolecules.* **2007**, *10*, 3253.

42. Stygar, J.; Biernat, A.; Kwiatkowska, A.; Lewandowski, P.; Rusiecka, A.; Zalewska, A.; Wieczorek, W. *J. Phys. Chem. B.* **2004**, *108*, 4263.

43. Iglauer, S.; Wu, Y.; Shuler, P.; Tang, Y.; Goddard, W. A. *Tenside Surfact. Det.* **2011**, *48*, 121.

44. Dodi, G.; Hritcu, D.; Popa, M. I. *Cellulose Chem.Technol.* **2011**, *45*, 171.

45. Bodard, M.; Christiaen, D. *Bot. Mar.* **1983**, *26*, 425.

46. Jing, X.; Wei, L.; Wenbing, Y.; Xiubing, P.; Dengke, Y.; Xiangdong, G. *Carbohydr. Polym.* **2009**, *78*, 227.

47. Yang, Y.; Zhang, J.; Zhou, C.; Wu, S.; Xu, S.; Liu, W.; Han, H.; Chen, B.; Zhao, X.-Z. *J. Phys. Chem. B* **2008**, *112*, 6594.

48. Hsiang-Yun, L.; Jui-Chu, T.; Lih-Shiuh, L. *Food Hydrocolloid.* **2009**, *23*, 2331.

49. Rodríguez-Hernández, A. I.; Durand, S.; Garnier, C.; Tecante, A.; Doublier, J. L. *Food Hydrocolloid.* **2003**, *17*, 621.

50. Patachia, S.; Florea, C.; Friedrich, C.; Thomann, Y. *Exp. Polym. Lett.* **2009**, *3*, 320.

51. Li, L.; Fang, Y.; Vreeker, R.; Appelqvist, I.; Mendes, E. *Biomacromolecules.* **2007**, *8*, 464.

52. Hargreaves, V. V.; Schleif, R. F. *Biochemistry.* **2008**, *47*, 4119.

53. Datta, K.; LiCata, V. J. *J. Biol. Chem.* **2003**, *278*, 5694.