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# Carboxymethyl kappa carrageenan derivatives synthesized from kappa carrageenan of the red alga Kappaphycus striatus cultivated in Vietnam: Characterization, structure and biological activity

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**Short Report**

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Table 1 and 2 are available in the Supplementary Files section.

Scheme 1 is available in the Supplementary Files section.

# Carboxymethyl kappa carrageenan derivatives synthesized from kappa carrageenan of the red alga *Kappaphycus striatus* cultivated in Vietnam: Characterization, structure and biological activity

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**Abstract**

Carboxymethyl kappa carrageenans (CMKCs) have been synthesized from kappa carrageenan extracted from the red alga *Kappaphycus striatus* with different molar ratios of monochloroacetic acid (MCA) in the presence of sodium hydroxide. The structural and physicochemical characteristic of products wase evaluated by FT-IR, $^{13}C$ and $^{1}H$ NMR spectra, degree of substitution, molecular weight, zeta potential and biological activity. Degree of substitution of carboxymethyl group in kappa carrageenan ranged from 0.24 to 1.12. FT-IR and $^{13}C$ NMR spectra proved the substitution of carboxymethyl group in kappa carrageenan disaccharide unit. Molecular weight of CMKCs increased slightly while their zeta potential was lower compared to the parent kappa carrageenan. CMKCs exhibited a slight difference in the lowest hemagglutination concentrations on native human blood group types and showed an increase in antioxidant activity. The results indicate that carboxymethyl kappa carrageenan derivatives from kappa carrageenan of the red alga *K. striatus* cultivated could promise be a good source of materials for application as carriers.

**Keywords**: Biological activity; Degree of substitution; Carboxymethyl kappa carrageenan; *Kappaphycus striatus;* Physicochemical properties; Structures

## Introduction

Carrageenan is natural sulfated polysaccharides obtained from the harvested naturally or cultivated red algae (Rhodophyta) (Zia et al. 2017). Carrageenans are mainly composed of D-galactose residues linked alternately of 3-linked-β-D-galactopyranose and 4-linked-α-D-galactopyranose units, and are classified as three main types (kappa-, iota- and lamda carrageenan), depending on their degree of sulfation that occurs on free hydroxyl groups (Campo et al. 2009). Carrageenans have attracted attention as a new biomaterial source for broad range of applications, due to their ability to participate in biochemical processes and also used for tissue regeneration with therapeutic biomacromolecules and for drug delivery (Li et al. 2014; Yegappan et al. 2018).

In order to develop new biomaterials as to increase application of polysaccharides from higher plant and algal polymers, carboxymethylation of polysaccharides have also been carried out in various natural polymers (Xie et al. 2021). At present, some biomaterials from carboxymethylated kappa/iota carrageenan derivatives were successfully developed as carriers for delivery of bioactive molecules (Leong et al. 2011a, 2011b; Fan et al. 2011; Tranquilan-Aranilla et al. 2012; Sahoo et al. 2017; Madruga et al. 2020, 2021).

In Vietnam, the red alga *Kappaphycus striatus* was extensively cultivated since 2005 as a source not only of materials for carrageenan production, but also a source of income for coastal farming households (Hung et al. 2019). Previously, the physicochemical properties and structure of kappa carrageenan from the red alga *K. striatus* have also been reported (Hung et al. 2021). However, to improve their application, it is very important to conduct new products from the red alga *K. striatus*. This target led us to synthesize carboxymethyl derivatives from kappa carrageenan of this alga (Scheme 1), with the main objective to evaluate properties, structure and biological activity from their derivatives, towards developing them as a biomaterial source for application.

## Materials and methods

### Materials

The red algae *Kappaphycus striatus* "sacol" brown strain (F. Schmitz) Doty ex P. Silva were harvested over a cultivation period of 60 days at Vanphong Bay, Vietnam (120°29′ N, 109°10′ E) in March 2021. After collection, algae were brought to the laboratory, washed with tap water to eliminate sand, debris and epiphytes. Fresh algal materials were homogenized with ethanol for 4 h to remove pigments and then washed with absolute ethanol at room temperature. The residues were dried by sunlight and stored at -20 °C until used. Sodium hydroxide, monochloroacetic acid, ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl were supplied by Sigma-Aldrich Co. (St. Louis, MO, USA). Human A, B, AB and O blood cells were obtained from healthy donors at Khanh Hoa General Hospital, Vietnam. All chemicals used were analytical grades.

### Extraction of carrageenan

Carrageenan extraction was carried out according to method previously described (Hung et al. 2021). Briefly, dry algal powder was incubated in 6 % KOH solution at 80°C for 2 h. Then the alga was washed and extracted with distilled water at 90°C for 2 h. Thereafter, the mixture was filtrated and gellified with 0.2 % KCl, frozen overnight and then thawed, washed with fresh water and ethanol at least twice, dried at 60°C and milled into powder.

### Synthesis of carboxymethyl kappa carrageenan derivatives

Kappa carrageenan powder (10 g) obtained above was suspended in 200 mL of 80 % (w/v) 2-propanol solution and stirred at room temperature. Then, 20 ml of 10 N NaOH solution was slowly added dropwise over a period of 15 min and stirred at 40°C for 1 h. Next, monochloroacetic acid dissolved in a small volume of 80 % 2- propanol was added dropwise into reaction mixture and kept for 4 h at 55°C with vigorously stirring. After that, the mixture was adjusted the pH to 7.0 by acetic acid. The carboxymethyl kappa carrageenan product was collected by vacuum filtration and washed 3 times with 80 % 2-propanol followed by pure 2-propanol and dried at 50°C.

Kappa carrageenan was synthesized at same conditions without monochloroacetic acid (MCA) used as a blank and coded as KC 0s. The various molar ratios of MCA to kappa carrageenan were used in this study and their derivatives were coded as CMKC 1s, CMKC 2s, CMKC 3s, CMKC 4s and CMKC 5s.

Prior to evaluate the properties and structures, all samples were repurified by dialysis with Mw cut-off 10,000 Da membrane against Milli-Q water overnight. After dialysis, samples were filtrated under filter (0.45 μm) and recovered by precipitation with 2-propanol, dried at 60°C and milled into powder.

**Determine 3,6-Anhydrogalactose and sulfate contents**

3,6-anhydro galactose content in kappa carrageenan and their derivatives was determined according to the method of Yaphe and Arsenault (1965) using D-fructose as a standard. Sulfate content was determined according to the method of Terho and Hartiala (1972) using $Na_2SO_4$ as a standard. Prior to determining sulfate content, all samples were hydrolyzed in 0.1 N HCL for 6 h.

**Determine molecular weight**

The molecular weight (Mw) of all samples was determined by Gel Permeation Chromatography (Shimadzu LC-20AD, Japan), using a refractive index detector and GPC/SEC Suprema Ultrahigh column (8.0 mm × 300 mm, Germany). A 20 µl aliquot of 0.2 % solution of each sample was injected on the column and eluted with 0.1 M $LiNO_3$ with flow rate of 1.0 mL $min^{-1}$ at 40°C. Dextran with different molecular weights was used as standards.

**FT-IR spectroscopy**

Samples of kappa carrageenan and their derivatives were recorded in the range of 4000 to 500 $cm^{-1}$ on a Fourier transform infrared spectrophotometer SHIMADZU, Japan.

**$^{1}$H and $^{13}$C nuclear magnetic resonance (NMR) spectroscopies**

Kappa carrageenan or their derivatives was dissolved in 1 mL of $D_2O$ containing 10 mM of 2,2-dimethyl-2-silapentane-3,3,4,4,5,5-$d_6$-5-sulfonate (DSS as an internal standard). The $^{1}$H NMR spectrum was recorded at 70°C and operated at 600.4 MHz. Sixty-four scans were accumulated at a pulse angle of 90° with an interpulse delay of 12.8 s and an acquisition time of 1.70 s. The $^{13}$C NMR spectrum was measured at 70°C and operated at 150.97 MHz with an interpulse delay of 6.50 s and an acquisition time of 0.85 s. All samples were measured on a Bruker Avance Neo 600 MHz spectrometer, Switzerland. Chemical shifts were corrected relative to the internal standard of DSS ($\delta$ = 0.000 ppm for both $^{1}$H and $^{13}$C NMR) (Van de Velde et al. 2004).

**Determine zeta potential**

Zeta potential of kappa carrageenan and their derivatives was measured on Model SZ-100Z Horiba equipment (Japan). A 0.2 % solution of each sample in buffer (pH 7.0) was prepared before measurements.

**Determine the degree of substitution**

Degree of substitution of carboxymethyl groups in carboxymethyl kappa carrageenan (CMKC) was calculated from the $^{1}H$ NMR spectrum using the chemical shift from 3.4 ppm to 5.2 ppm as described (Leong et al. 2011a; Madruga et al. 2020) according to Eq. 1.

$$x_i = \frac{A(\text{proton(s) of CMKC at } O_i}{A[\text{proton(s) of KC at } O_i] + A[\text{proton(s) of CMKC at } O_i]} \quad \text{(Eq.1)}$$

$$\text{Degree of substitution} = \sum x_i$$

A represents the peak area; O is the oxygen atom at position i (i= positions 2 and 6 of the β-D-galactopyranose-4-sulfate unit (G4S) and position 2 of the 3,6-anhydro-D-galactopyranose moiety (DA)); $x_i$ is the partial degree of substitution.

**Determine antioxidant activity**

The antioxidant activity of kappa carrageenan and carboxymethyl kappa carrageenan derivatives was assayed according to the method described (Shimada et al. 1992), using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, 1 mL of 0.1 mM solution of DPPH in MeOH-deionized water (1:1) was mixed with 3 mL of each sample solution containing 400 μg $mL^{-1}$ in MeOH–deionized water (1:1). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free-radical scavenging activity. Ascorbic acid at various concentrations in

MeOH-deionized water (1:1) was used as a positive control and MeOH–deionized water (1:1) as a negative and the experiments were carried out as above described. The DPPH radical scavenging activity (%) (RSA %) was calculated by the equation Eq.2:

$$RSA\ (\%) = (1 - A_1/A_0) \times 100 \quad \text{(Eq.2)}$$

where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of samples.

**Hemagglutination assay**

The hemagglutination assay was carried out by a microtiter method using a 96-well microtiter V-plate (Hung et al. 2009). Each human blood cell (A, B, AB and O group) was washed five times with 0.15 M NaCl solution. After washing, a 2% erythrocyte suspension (v/v) was prepared in 0.15 M NaCl solution.

Kappa carrageenan or carboxymethyl kappa carrageenan derivatives were prepared in 0.15 M NaCl solution at an initial concentration of 10 mg $mL^{-1}$. First, 25 µL each of serially two-fold dilutions of each kappa carrageenan or carboxymethyl kappa carrageenan solution was prepared in saline on a microtiter V-plate. To each well, 25 µL of a 2 % erythrocyte suspension was added and the mixtures were gently shaken and incubated at room temperature for 2 h. Control consisted of 25 µL of each erythrocyte suspension and 25 µL of 0.15 M NaCl solution,. Hemagglutination was observed as positive in the case that more than 50% of the erythrocytes in the well were agglutinated. The reciprocal of the highest two-fold dilution exhibiting positive hemagglutination was calculated as the lowest concentrations of kappa carrageenan or carboxymethyl kappa carrageenan derivatives which agglutinated erythrocytes.

**Results and Discussion**

**Chemical property of kappa carrageenan and carboxymethyl kappa carrageenan derivatives**

The physicochemical properties of kappa carrageenan and carboxymethyl kappa carrageenan derivatives were presented in Table 1. Content of 3,6-anhydro-D-galactose (DA) in KC 0s synthesized without MCA increased slightly while the sulphate contents decreased compared to the parent kappa carrageenan, indicating that alkaline treatment results in removal of sulphate group at C-6 of α-D-galactopyranose to a free hydroxyl group and converted into 3,6-anhydro-D-galactose as reported (Aguilan et al. 2003; Tranquilan-Aranilla et al. 2012).

It should be noted that our investigation only examines the influence of various molar ratios of monochloroacetic acid (MCA) to kappa carrageenan (nMCA/KC 0s) on the degree of substitution (DS). As a result, increasing in molar ratio of nMCA/KC 0s in carboxymethylated process of samples caused reduction effect, leading hydrolysis of $SO_3$ - in C-4 of the β-D-galactose and could break 3,6 anhydro bridge of 3,6 anhydro-α-D-galactose as reported (Tranquilan-Aranilla et al. 2012). The molecular weight of KC 0s was decreased as compared to that of KC, in which KC 0s was synthesized in the alkaline treated conditions without monochloroacetic acid. The depolymerization occurred in strong alkaline treated process as evidenced by the decrease in molecular weight reported for kappa carrageenan and other polysaccharides (Ren et al. 2008; Xu et al. 2009; Tranquilan-Aranilla et al. 2012). However, in the next step for carboxymethylation of KC, molecular weight of carboxymethyl kappa carrageenan derivatives increased following increasing of degree of substitution compared to that of KC 0s (Table 1). Similar result was also recorded for increasing molecular weight of carboxymethyl kappa carrageenan derivatives synthesized from kappa carrageenan (Tranquilan-Aranilla et al. 2012).

**FT-IR analysis of polysaccharide and derivatives**

Fig. 1 showed the typical adsorption bands reported for kappa carrageenan, including bands at 925 $cm^{-1}$ for C–O–C of 3,6- anhydro-D-galactose, 848 $cm^{-1}$ for C4–O–S stretching and 1225 $cm^{-1}$ for O=S=O symmetric vibrations of β-D-galactose-4-sulphate (Mendoza et al. 2002; Aguilan et al. 2003; Arman and Qader 2012; Souza et al. 2018; Hung et al. 2021).

In FT-IR spectra of CMKC samples (Fig. 1c, d, e, f, g), the appearance of the new bands at 1589 $cm^{-1}$ and 1415 $cm^{-1}$ were assigned to the asymmetrical and symmetrical stretching vibrations of carboxylate anions (- $COO^-$) (Fan et al. 2011; Tranquilan-Aranilla et al. 2012; Madruga et al. 2020) and the band at 1325 $cm^{-1}$ was reported for the -$CH_2$- scissoring in the carboxymethyl group (Fan et al. 2011; Madruga et al. 2020). The presence of three new bands confirms the successful carboxymethylation of kappa carrageenan.

**$^1$H and $^{13}$C NMR spectroscopies and the degree of substitution (DS)**

Chemical shifts in structures of kappa carrageenan, KC 0s and their derivatives from $^1$H and $^{13}$C NMR spectra were shown in Fig. 2 and 3. Fig. 2a and b from $^1$H NMR and Fig. 3a and b from $^{13}$C NMR spectra showed the same shift signals of kappa carrageenan and KC 0s of the twelve hydrogen atoms and proton of -$OCH_3$, and the twelve carbon atoms and carbon of -$OCH_3$ group at C-6 of the β-D-galactose-4-sulphate in the disaccharide repeating unit, respectively. The chemical shifts are given in Table 2 and agree closely with reported values (Van de Velde et al. 2004; Hung et al. 2021).

Fig. 2 shows the comparison of chemical shifts between KC, KC 0s (Fig. 2a, b) and CMKCs (Fig. 2c, d, e, f, g) from $^1$H NMR spectra. Among of $^1$H NMR spectra of CMKCs, CMKC 3s spectrum was selected to exhibit new proton peaks (Fig. 2e), due to the highest degree of substitution. CMKC 3s spectrum revealed the appearance of new proton peaks at δ = 3.77, 4.08 and 4.24 ppm, attributed to the methylene protons in the carboxymethyl substituents at

positions C-2 and C-6 of the β-D-galactopyranose-4 sulphate unit (G4S-units) and position C-2 of the 3,6-anhydro-α-D-galactopyranose unit (DA-units), respectively, and these peaks are not present in KC 0s spectrum, indicating that hydroxyl groups of KC 0s are carboxymethylated. The partial degree of substitution of carboxymethyl group in each kappa carrageenan derivative from the $^{1}H$ NMR spectra was also determined according to method reported (Leong et al. 2011a; Madruga et al. 2020) and was shown in Table 1. The degree of substitution (DS) ranged from 0.24 to 1.12. The maximum substitution was achieved at a molar ratio of nMCA/KC 0s (3:1), higher molar ratios decreased DS. The decrease in DS at high MCA/KC molar ratios was recorded due to a side reaction, leading the formation of sodium glycolate from sodium monochloroacetate and NaOH (Volkert et al. 2004; Leong et al. 2011a; Fan et al. 2011; Madruga et al. 2020).

Fig. 3 shows the comparison of chemical shifts between KC, KC 0s (Fig. 3a, b) and CMKCs (Fig. 3c, d, e, f, g) from $^{13}C$ NMR spectra. Similar to $^{1}H$ NMR spectra of Fig. 2e, $^{13}C$ NMR spectrum of CMKC 3s was selected to exhibit new carbon peaks (Fig. 3e). The prominent peaks appear at δ= 179.95 and 179.10 ppm were assigned to the carbon of the carboxylate group ($COO^{-}$) and may be attributed to different possible positions of carboxymethyl substituents on the KC 0s. The appearance of signals from 177.7 - 182.6 ppm has also been reported for carboxymethyl substituents in kappa carrageenan (Leong et al. 2011a; Fan et al. 2011; Tranquilan-Aranilla et al. 2012; Madruga et al. 2020). The methylene (–CH2) carbon atom of the carboxymethyl substituents was attributed with peaks at δ= 72.77 and 73.60 ppm as recorded (Xu et al. 2009; Tranquilan-Aranilla et al. 2012; Madruga et al. 2020). In downfield shift, carboxymethyl substitution ($-CH_2COO^{-}$) at δ = 72.14 ppm was assigned to C-6 of the G4S and at δ = 76.72 and 79.91 ppm for C-2 of G4S and DA residues, respectively. The similar shifts of carboxymethyl substitution in a downfield shift have also been reported for carboxymethylation (Lawal et al. 2008a, 2008b; Tranquilan-Aranilla et al. 2012). Based on the ratio of peak intensity of $^{13}C$ NMR and values of DS from $^{1}H$ NMR indicates that the hydroxyl groups in kappa

carrageenan (KS 0s) were carboxymethylated successfully and the reactivity of OH groups was arranged to be in order of O-$C_2$G4S > O-$C_6$G4S > O-$C_2$DA and these results are agreed with published reports (Tranquilan-Aranilla et al. 2012; Madruga et al. 2020).

As recorded, there have no the change in signal intensities of proton and carbon of -$OCH_3$ at C-6 of the β-D-galactose-4-sulfate (G4S) in all $^{1}H$ and $^{13}C$ NMR spectra (Fig. 2 and 3), indicating that the alkali-treated and carboxymethylated samples with increase in molar ratio of nMCA/KC 0s have not caused effect of -$OCH_3$ group in the disaccharide repeating unit.

**Zeta potential of kappa carrageenan and their derivatives**

Kappa carrageenan contains only one sulphate group (Campo et al. 2009) while carboxymethyl kappa carrageenans, in addition to the sulphate group, carry carboxymethyl groups. At neutral pH, KC, KC 0s and carboxymethyl kappa carrageenans (CMKCs) are complete dissociation of negative charges. However, due to only one sulphate group in the disaccharide repeating unit, zeta potential of KC and KC 0s attained a highly charged ionised state (Table 1). On the contrary, carboxymethyl kappa carrageenan derivatives with increase in degree of substitution of carboxymethyl groups leads to increase of the negatively charged densities. As a result, the intra- and intermolecular repulsion interactions increased, leads to a change in zeta potential of CMKCs and recorded with the lowest value at CMKC 3s (-48.5 mV). Furthermore, changes in the surface charge density of CMKCs s may affect the electrostatic repulsion between molecular chains, resulting in change of the zeta potential as reported for carboxymethyl cellulose derivative (Gu et al. 2005; Roy et al. 2018).

**Antioxidant activity**

The radical scavenging activity (RSA) of KC, KC 0s and CMKCs were expressed as percentage (Fig. 4a). The highest RSA was achieved at CMKC 3s, which has the highest DS (1.12), confirming that carboxymethylation increases the RSA of KC 0s. CMKCs were recorded for RSA ranged from 15.2 - 25.8 μg $mL^{-1}$ of ascorbic acid equivalent (Fig. 4b). RSA and ascorbic acid equivalent values of KC are similar to those of KC 0s, thus was not plotted on Fig. 4. Improving the antioxidant activity under addition of carboxymethyl groups into polysaccharide structures has also been reported (Yuan et al. 2005; Chen et al. 2014; Machova et al. 2014; Sun et al. 2015; Madruga et al. 2020).

**Hemagglutination activity**

KC 0s and their carboxymethylated derivatives showed a slight difference in hemagglutination activity on native human blood groups A, B, O and AB with the lowest hemagglutination concentrations ranged from 0.315 – 1.25 mg $mL^{-1}$ (Fig. 5). The hemagglutination activity of KC was similar to that of KC 0s, thus was not plotted on Fig. 5. The lowest hemagglutination concentrations of all samples were recorded on native B blood group (0.315 mg $mL^{-1}$). The difference in hemagglutination activities of samples on native human blood groups may be due to differences of specific sugars on the surface of each blood group cell (Takasaki et al. 1978). The results indicated that the addition of carboxymethyl groups into a sulphated polysaccharide promoted the hemagglutination activity. It is also suggested that the positive charges of glycoprotein on surface of the erythrocyte membrane could be attracted by the negative charges of kappa carrageenan or their carboxymethylated derivatives, result increases the ability of erythrocytes to adhere and form agglutination (Wang et al. 2021). The similar agglutination has also been recorded for carrageenan (Silva et al. 2010; McKim 2014; Campos-Sánchez et al. 2023).

Carboxymethyl kappa carrageenan derivatives were synthesized from kappa carrageenan of the red alga *K. striatus* showed the physicochemical properties, structure and biological activities similar well to those of carboxymethyl kappa carrageenans reported, including degree and sites of carboxymethyl group substitution in kappa carrageenan structure, molecular weights, antioxidant and hemagglutination activity. The results indicate that carboxymethyl kappa carrageenan derivatives in this study could promise be a good source of materials for application as carriers. However, to obtain a better understanding of relationship between their structure and biological activities, we need studies furthermore in details.

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**Authors' contributions**

HTTN, DTT and TTTT made the experiments. VTDT, PDT and TTTT analyzed the data. HTTN prepared the scheme, figures and tables and wrote the original draft of the manuscript. LDH administrated project, supervised all the assays and reviewed and edited the manuscript. All authors reviewed the manuscript and have read and agreed to the published version of the manuscript.

**Data availability**

The data supporting the findings of this study are available from the corresponding author, upon reasonable request.

**Ethics Declarations**

**Competing interests**

The authors declare no competing interests.

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**Legend to Figures**

**Scheme 1** The synthesis of carboxymethyl kappa carrageenan

**Fig. 1** FT-IR spectra of kappa carrageenan (KC), kappa carrageenan was synthesized at same conditions without monochloroacetic acid (KC 0s) and carboxymethyl kappa carrageenan derivatives (CMKC 1s – 5s)

**Fig. 2** $^{1}H$ NMR spectra of kappa carrageenan (KC), kappa carrageenan was synthesized at same conditions without monochloroacetic acid (KC 0s) and carboxymethyl kappa carrageenan derivatives (CMKC 1s – 5s). DA: 3,6-anhydro-α-D-galactose; G4S: β-D-galactose-4-sulphate

**Fig. 3** $^{13}C$ NMR spectra of kappa carrageenan (KC), kappa carrageenan was synthesized at same conditions without monochloroacetic acid (KC 0s) and carboxymethyl kappa carrageenan derivatives (CMKC 1s – 5s). DA: 3,6-anhydro-α-D-galactose; G4S: β-D-galactose-4-sulphate

**Fig. 4** Antioxidant activity of kappa carrageenan was synthesized at same conditions without monochloroacetic acid (KC 0s) and carboxymethyl kappa carrageenan derivatives (CMKC 1s – 5s). (a) Radical Scavenging Activity (RSA) of DPPH and (b) activity in equivalent of ascorbic acid

**Fig. 5** Lowest hemagglutination concentration (mg/mL) of kappa carrageenan was synthesized at same conditions without monochloroacetic acid (KC 0s) and carboxymethyl kappa carrageenan derivatives (CMKC 1s – 5s) on native human blood groups, A (▨), B (▩), AB (■) and O (目)

# Figures

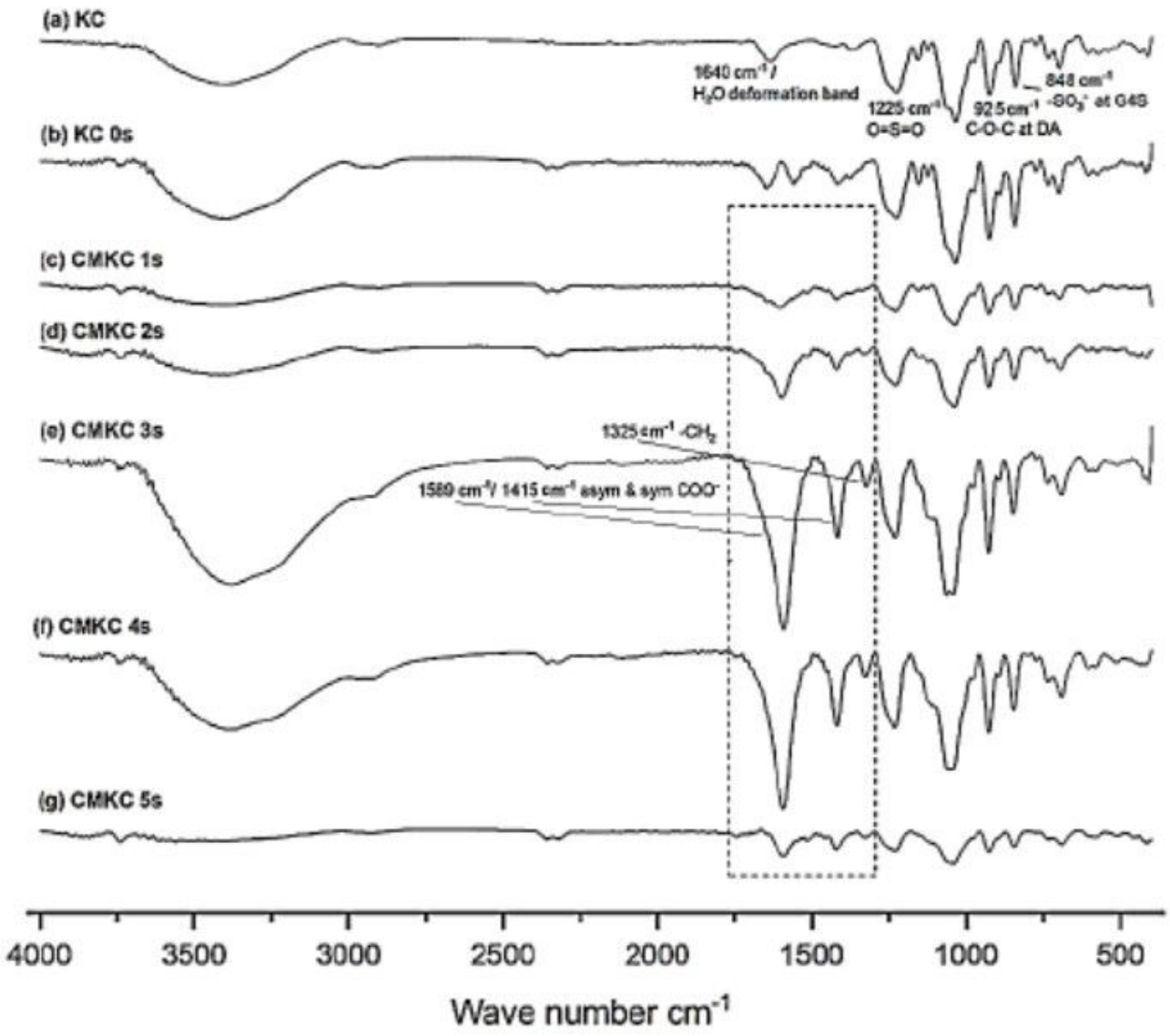



**Fig. 1**

**Figure 1**

FT-IR spectra of kappa carrageenan (KC), kappa carrageenan was synthesized at same conditions without monochloroacetic acid (KC 0s) and carboxymethyl kappa carrageenan derivatives (CMKC 1s – 5s)

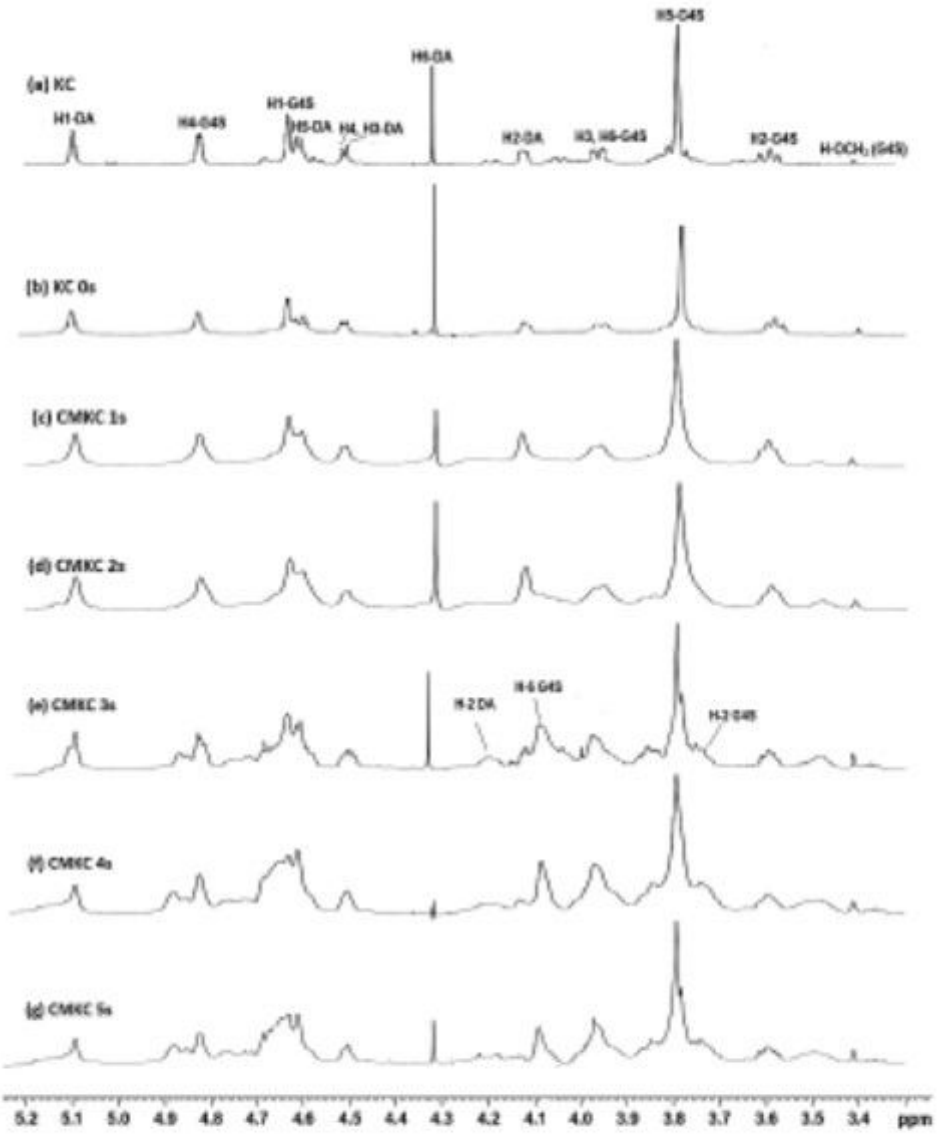
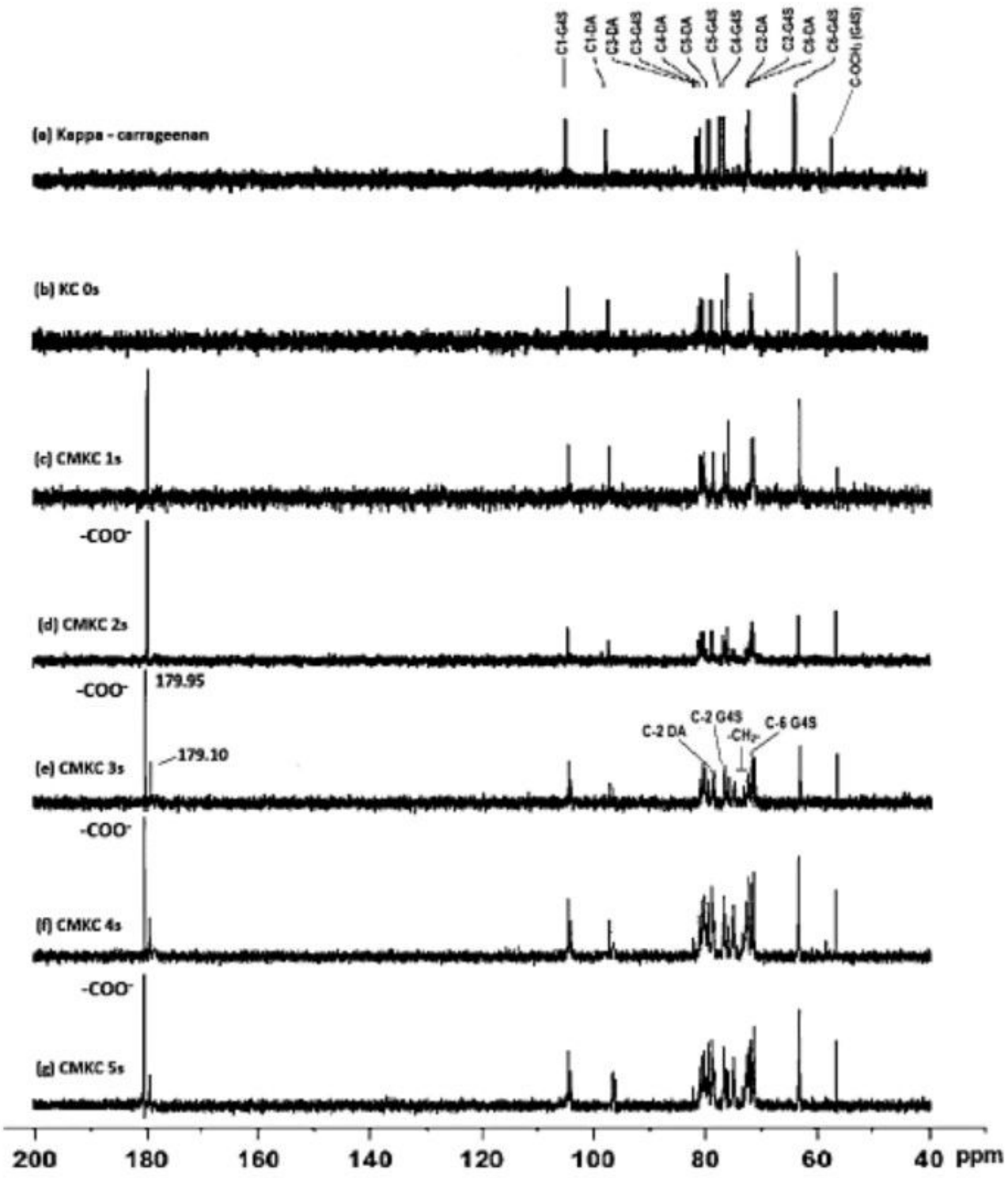



Fig. 2

## Figure 2

$^{1}$H NMR spectra of kappa carrageenan (KC), kappa carrageenan was synthesized at same conditions without monochloroacetic acid (KC 0s) and carboxymethyl kappa carrageenan derivatives (CMKC 1s – 5s). DA: 3,6-anhydro-α-D-galactose; G4S: β-D-galactose-4-sulphate




## Figure 3

$^{13}C$ NMR spectra of kappa carrageenan (KC), kappa carrageenan was synthesized at same conditions without monochloroacetic acid (KC 0s) and carboxymethyl kappa carrageenan derivatives (CMKC 1s – 5s). DA: 3,6-anhydro-α-D-galactose; G4S: β-D-galactose-4-sulphate

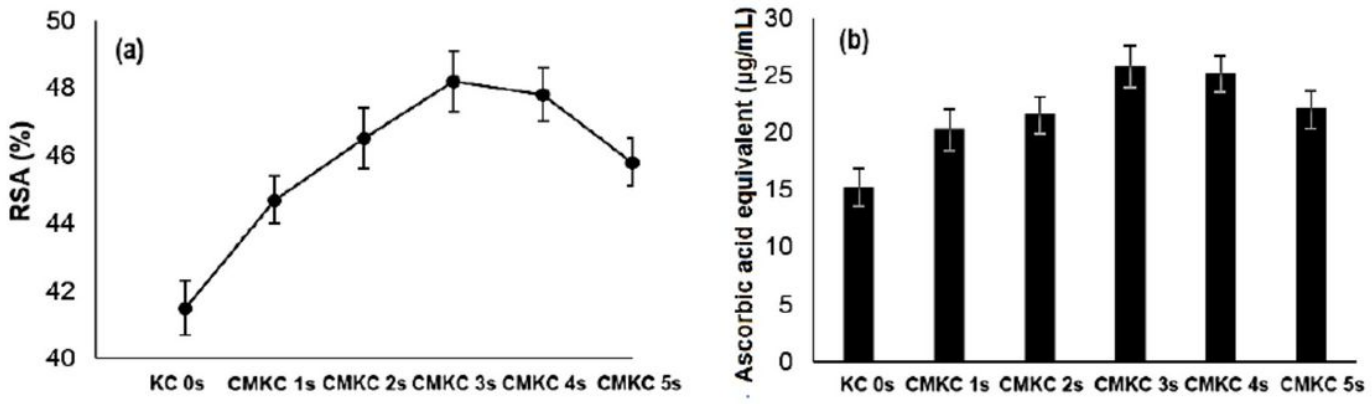



Fig. 4

## Figure 4

Antioxidant activity of kappa carrageenan was synthesized at same conditions without monochloroacetic acid (KC 0s) and carboxymethyl kappa carrageenan derivatives (CMKC 1s – 5s). (a) Radical Scavenging Activity (RSA) of DPPH and (b) activity in equivalent of ascorbic acid

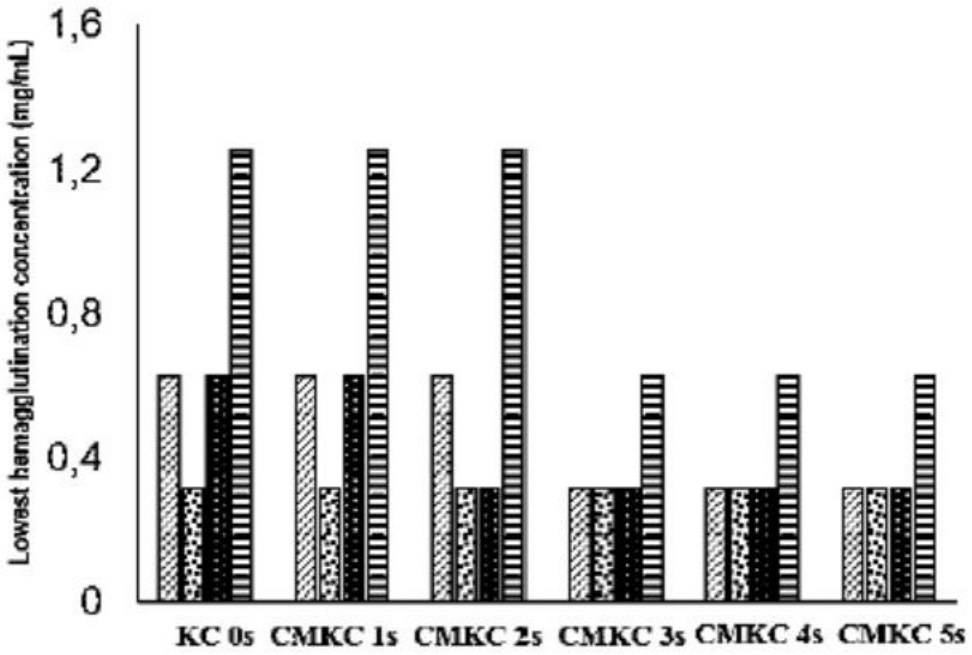



**Fig. 5** Lowest hemagglutination concentration (mg/mL) of kappa carrageenan was synthesized at same conditions without monochloroacetic acid (KC 0s) and carboxymethyl kappa carrageenan derivatives (CMKC 1s – 5s) on native human blood groups, A (▨), B (▩), AB (■) and O (▤)

**Figure 5**

See image above for figure legend

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Table2.pdf
- Scheme1.pdf