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Functional group dependent dielectric properties of sulfated hydrocolloids extracted from green macroalgal biomass

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ABSTRACT

Dielectric properties of aqueous solutions of sulfated hydrocolloids (ulvan and rhamnan sulfate) extracted from green macroalgal biomass were studied in a frequency range of 100 MHz–10 GHz. Counterion exchange of native hydrocolloids (mixture of Na^+, Mg^{2+} and Ca^{2+}) to H^+-form showed significant increase in loss factor due to ionic conduction. On the other hand, desulfations decreased their loss factors. The results suggested that ionic conduction of H^+ has significant contribution to loss factors. Additionally, H^+-form hydrocolloids showed significant improvement in hydration, which might also affect the dielectric property of the solution by reducing the amount of free water. The viscosity, however, did not show apparent relevance with the dielectric property.

1. Introduction

Microwave heating is widely used in domestic cooking, food industry and medical cares by applying its rapid, direct and internal heating properties. Unique heating mechanism of microwave is also capable of rapid hydrothermal conversion of terrestrial and aquatic biomass (Biller, Friedman, & Ross, 2013; Quitain, Kai, Sasaki, & Goto, 2013; Tsubaki & Azuma, 2011). Microwave-assisted hydrothermal treatment is effective for utilization of biomass by preserving sugars from unexpected degradation by the wall effect of reactors (Tsubaki, Oono, Onda, Yanagisawa, & Azuma, 2013a). Microwave heating significantly depends on the dielectric property of irradiated material (Gabriel et al., 1998), therefore, a composition of reaction system (a mixture of biomass substrate, solvent and catalyst) is significantly relevant to microwave energy transfer. Particularly, in the case of hydrothermal reaction, dielectric loss of water decreases with increase in temperature (Dallinger & Kappe, 2007), therefore, addition of microwave absorbing material such as electrolytes and activated carbon is very effective for higher microwave energy transfer into the reaction system (Matsumoto, Tsubaki, Sakamoto, & Azuma, 2011; Tsubaki, Oono, Onda, Yanagisawa, Azuma, & 2012; Tsubaki et al., 2013b).

Green macroalgae are fast growing biomass, and sometimes green tide of *Ulva* spp. become big environmental issue at eutrophic oceans (Hiraoka, Ichihara, Zhu, Ma, & Shimada, 2011). Green macroalgae can be converted to bio-gas and bio-oils through biological and physicochemical conversions including microwave process (Kim, Li, Jung, Chang & Lee, 2011; Zhuang et al., 2012). Water and electrolytes abundantly contained in macroalgae are good microwave energy sensitizers, therefore, microwave heating has high compatibility with heating macroalgaes. In addition to cellulose and starch, green macroalgae accumulate unique hydrocolloids called ulvan and rhamnan sulfate, which are predominantly composed of rhamnose and glucuronic acids with high substitution of sulfate groups on rhamnose (Lahaye & Robic, 2007; Lee, Koizumi, Hayashi, & Hayashi, 2010). These hydrocolloids are potential biomass feedstock to produce chemicals as well as value added compounds by applying their biological effects (Holdt & Kraan, 2011).

In this study, we have focused on dielectric properties of aqueous solutions of crude sulfated hydrocolloids of *Ulva meridionalis* and *Monostroma lattisimum* as a representative substrate of green macroalgal biomass. The effects of sulfation and their counterions

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on dielectric property were investigated, due to high substitution of sulfated group in their hydrocolloids.

2. Materials and methods

2.1. Collection of macroalgaes and extraction of polysaccharides

U. meridionalis (stain no. E16 in Horimoto, Masakiyo, Ichikawa, & Shimada, 2011) was supplied from Usa Marine Biological Institute, Kochi University. *M. lattisimum* was cultivated in Shimanto estuary, Kochi Prefecture Japan in 2011–2012. The algal bodies were thoroughly washed with distilled water, lyophilized and powdered.

Hydrocolloids were extracted according to Alves, Sousa, and Reis (2013). Namely the algal bodies were depigmented by extraction with 75% aqueous ethanol for 30 min for three times. The depigmented macroalgaes were, then, extracted with hot water at 100 °C for 1 h for three times. High molecular weight components in the supernatant were collected by ethanol precipitation and lyophilized.

2.2. Chemical composition of hydrocolloids

Contents of neutral sugar and uronic acids were determined by the phenol sulfuric acid and the m-hydroxydiphenyl methods. Monosaccharide compositions of polysaccharides from *U. meridionalis* (ulvan) and *M. lattisimum* (rhamnan sulfate) were determined by the alditol acetate method. Namely, the ulvan and rhamnan sulfate were hydrolyzed by 2 M TFA for 3 h at 100 °C, reduced by $NaBH_4$ for 12 h to alditol, and acetylated by anhydrous acetic acid and pyridine for 1 h at 100 °C to produce alditol acetate derivatives. The product was analyzed by GC equipped with a column of CBP-1 (25 m × 0.22 mm × 0.25 μm, Shimadzu GLC Ltd.). Oven temperature was programmed from 140 °C to 220 °C at 2 °C/min, and detection was performed by FID. The content of glucuronic acids in the TFA hydrolysates described above were determined by HPLC (LC-2000 system) equipped with column of Aminex HPX-87H (300 mm × 7.8 mm, Biorad Laboratories, Inc., CA, USA) at 40 °C with an eluent of 0.008 N H_2SO_4 (0.6 mL/min) and detection by RI. myo-Inositol was used as an internal standard to normalize the neutral sugar composition and glucuronic acid.

Ulvan and rhamnan sulfate were desulfated according to Nakagawa, Inoue, and Kamata (1977). The native polysaccharides were passed through Dowex 50 × 8 (100–200) by water to produce H^+-form hydrocolloids, then, neutralized by pyridine and lyophilized. The sulfate group was removed by solvolysis by a mixed solution of DMSO:pyridine:methanol = 3:25:0.3 (v/v) at 100 °C for 2 h. The product was dialyzed and lyophilized to yield desulfated hydrocolloids.

The elemental analyses were conducted by Flash EA1112 CHNS analyzer (Thermo Fisher Scientific Inc., MA, USA). The protein contents were estimated by the Lowry's method. FT-IR spectra of native and desulfated polysaccharides were obtained by FT-IR from 400 to 4000 cm^{-1} by the KBr method at 4 cm^{-1} resolution (FT-IR 6200, Jasco Co., Tokyo, Japan). The contents of ash were determined by dry ashing, and the composition of Na, Ca, and Mg in ashes was analyzed by ICP-AES (Optima 4300 DV CYCRON, PerkinElmer Inc., MA, USA) after solubilization of ashes in 1% aqueous HNO_3 solution. Size exclusion chromatography (LC-2000 system) was conducted by a column of Shodex OHpak SB-804 HQ (8.0 mm × 300 mm, Showa Denko K.K., Tokyo, Japan) at 30 °C with an eluent of 50 mM aqueous $NaNO_3$ solution containing 0.02% NaN_3 (0.7 mL/min) and detection by RI. The molecular weights of the hydrocolloids were estimated by calibration using the Shodex pullulan standards (Showa Denko K.K.).

Table 1
Chemical composition of ulvan and rhamnan sulfate.

Component	Ulvan (*Ulva meridionalis*)	Rhamnan sulfate (*Monostroma lattisimum*)
Composition (wt%)		
Neutral sugar	58.0	46.2
Uronic acid	13.1	4.9
N	2.1	2.6
S	4.0	6.5
Protein	9.5	2.4
Sulfate	10.0	16.3
Ash	12.9	21.2
Monosaccharide composition (wt%)		
Rha	16.4	72.2
Xyl	9.8	20.9
Man	1.1	0.1
Glc	49.5	3.1
Gal	19.2	1.1
GlcA	4.0	2.6
Inorganic compound composition (wt%)		
Na	0.57	2.07
Ca	1.05	0.76
Mg	1.99	1.06

2.3. Measurement of physical properties of hydrocolloid solutions

Relative permittivity and loss factor of aqueous solutions of ulvan and rhamnan sulfate (0.5–2.0 wt%) at 40 °C, 60 °C and 80 °C were measured by the coaxial probe method using Agilent Technologies 5242A Network Analyzer and an Agilent high temperature probe in a range of 100 MHz–10 GHz (Agilent Technologies Inc.). DC conductivity and pH of the solutions were measured by electric conductive meter (ES-51, Horiba, Ltd., Kyoto, Japan) and pH meter (Seven Go SG2, Metler-Toledo, OH, USA) at 40 °C, respectively. Viscometry was conducted by using VM-10AL (Sekonic Co., Tokyo, Japan) at 40 °C.

The degree of hydration of the hydrocolloid solutions were estimated from the amount of freezable water and non-freezable water by DSC (SII Exstar 6200, Hitachi High-Tech Science Co., Tokyo, Japan) according to the method of Yudianti, Karina, Sakamoto, and Azuma (2009). DSC thermograms of 0.5% hydrocolloid solutions were obtained from −30 to 50 °C with heating rate of 8 °C/min in a sealed aluminum pan. Aluminum pan was used as reference. Non-freezable water content was estimated from the ratio of the endothermic peak area of hydrocolloid solution and pure water according to the following equation;

$$W_b(\%) = W_r - \left(\frac{Q_{endo}}{Q_{pure}}\right) \times 100 \quad (1)$$

where W_b, W_r, Q_{endo} and Q_{pure} were the amounts of non-freezable water and equilibrium water, and enthalpy of hydrocolloid solution and pure water (ΔH = 371 J/g), respectively.

3. Results and discussion

3.1. The chemical composition of ulvan and rhamnan sulfate, and their desulfation

U. meridionalis is a kind of green-tide forming *Ulva* spp. and its daily growth rate attains 112% (Hiraoka, 2012). *Ulva* sp. contain sulfated glucuronoxylorhamnan called ulvan. *M. latissimum* is a commercially cultivated edible macroalgaes, and they are also a good feedstock to produce rhamnose as a functional rare sugar in rhamnan sulfate (Holdt & Kraan, 2011). The compositions of ulvan and rhamnan sulfate were listed in Table 1. Yield of ulvan from *U. meridionalis* attained 18.7 wt% and it was abundantly composed of neutral sugar and uronic acid. Their predominant monomeric

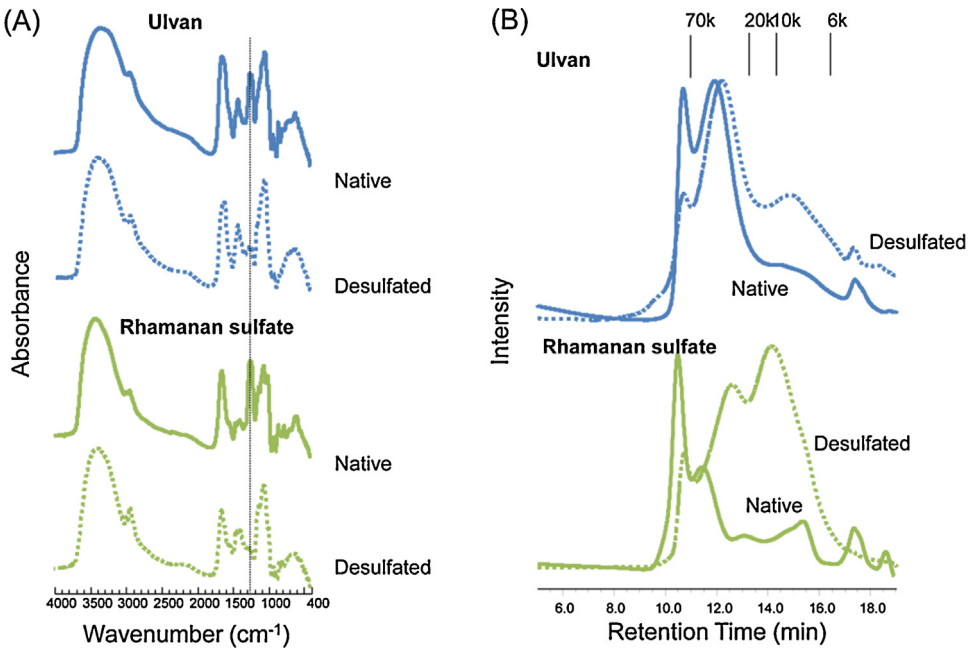

Fig. 1. Effects of desulfation on ulvan and rhamnan sulfate. (A) FT-IR spectra, and (B) size-exclusion chromatograms. The molecular weight was estimated by the elution time of pullulan standard (6k–70k).

composition was glucose, galactose, rhamnose and glucuronic acid. The rhamnose content of *U. meridionalis* was lower than the typical ulvan composition obtained from *Ulva lactuca* and *Ulva rigida* with high rhamnose content (Lahaye & Robic, 2007). Sulfate content attained 10.0% of the hydrocolloid. Ash content was 12.9%, mostly composed of divalent cations of Mg and Ca, which were presumed to be associated with the sulfate groups as counterions.

Yield of rhamnan sulfate from *M. lattisimum* attained 15.9 wt%, and the predominant constituent was neutral sugars (46.2%) followed by low amount of uronic acid. Rhamnan sulfate was mostly composed of rhamnose (72.2%) with larger amount of sulfate groups and ash than ulvan. The inorganic compound was constituted with Na followed by Mg.

Ulvan and rhamnan sulfate was desulfated by solvolysis, and their yields attained 54.0% and 40.9% form the native hydrocolloids, respectively. The degree of desulfation was investigated by FT-IR (Fig. 1A). Typical IR absorption of stretching vibration of sulfate at around 1250 cm^{-1} was significantly reduced by the desulfation. The change in the molecular weight distribution through desulfation process was monitored by SEC (Fig. 1B). Native ulvan showed predominant peaks larger than 70k, however, the desulfation slightly decreased them down to 40k–70k. Desulfation of rhamnan sulfate inevitably decreased its molecular weight from ≥70k down to broader range of molecular weight distribution from 10k to 70k. The susceptibility of degradation along with desulfation process seems to be dependent on each polysaccharide.

3.2. Dielectric property of the aqueous solutions of ulvan and rhamnan sulfate

Dielectric properties of aqueous solution of ulvan and rhamnan sulfate (0.5–2.0 wt% hydrocolloid solution) were investigated by the dielectric probe method in a range of 100 MHz–10 GHz (Fig. 2). Both ulvan and rhamnan sulfate showed quite similar temperature and frequency dependency. The permittivities showed gradual decrease with increase in frequency and temperature, while the loss factors showed U-shaped curve. Increases in loss factor at higher and lower frequencies were due to dielectric loss of water and conduction loss of electrolytes, respectively (Gabriel et al., 1998). Conduction loss of the solution may be relevant to electrolyte polysaccharides retaining sulfate and carboxyl groups as well as their counterions. Increase in temperature gradually decreased loss factor due to dielectric loss at higher frequency accompanied by decrease in dielectric constant of water (Dallinger & Kappe, 2007). Contrary, the loss factor due to ionic conduction at lower frequency increased with increase in temperature due to lower restriction of electrolytes from the surrounding environment.

The concentration dependent dielectric properties were also shown in Fig. 2. The higher concentrations of hydrocolloids slightly increased relative permittivity at lower frequency, while they drastically increased conduction loss of the solution at lower frequency. Conduction loss of hydrocolloid solutions also increased with increase in temperature up to 80 °C.

Dielectric properties of native form (a mixture of counterions of Na^+, Ca^{2+}, Mg^{2+}), H^+-form and desulfated hydrocolloids were shown in Fig. 3. Exchange of the counterion to H^+ form significantly improved conduction loss in both hydrocolloids. The counterion exchange of the hydrocolloids increased H^+ concentration by 1.0×10^3 fold (ulvan) to 4.1×10^4 fold (rhamnan sulfate) accompanied by increase in DC conductivity by 10.9 fold (ulvan) and 27.4 fold (rhamnan sulfate), respectively (Table 2). Therefore, the significant increase in conduction loss could be due to higher ionic conductivity of H^+ in aqueous solution rather than those of Na^+, Ca^{2+} and Mg^{2+} by Grotthuss mechanism. Different counterions (Ca^{2+}, Na^+ and K^+) have previously shown to affect dielectric property of alginate and carrageenan at frequencies below 10 MHz

Table 2
Values of pH and DC conductivity of 0.5% hydrocolloid solutions of ulvan and rhamnan sulfate.

Component	Ulvan		Rhamnan sulfate	
	pH	σ (mS/m)	pH	σ (mS/m)
Native	5.21	123	6.66	98
H^+ form	2.21	1345	2.05	2690
Desulfated	6.14	78	6.56	145

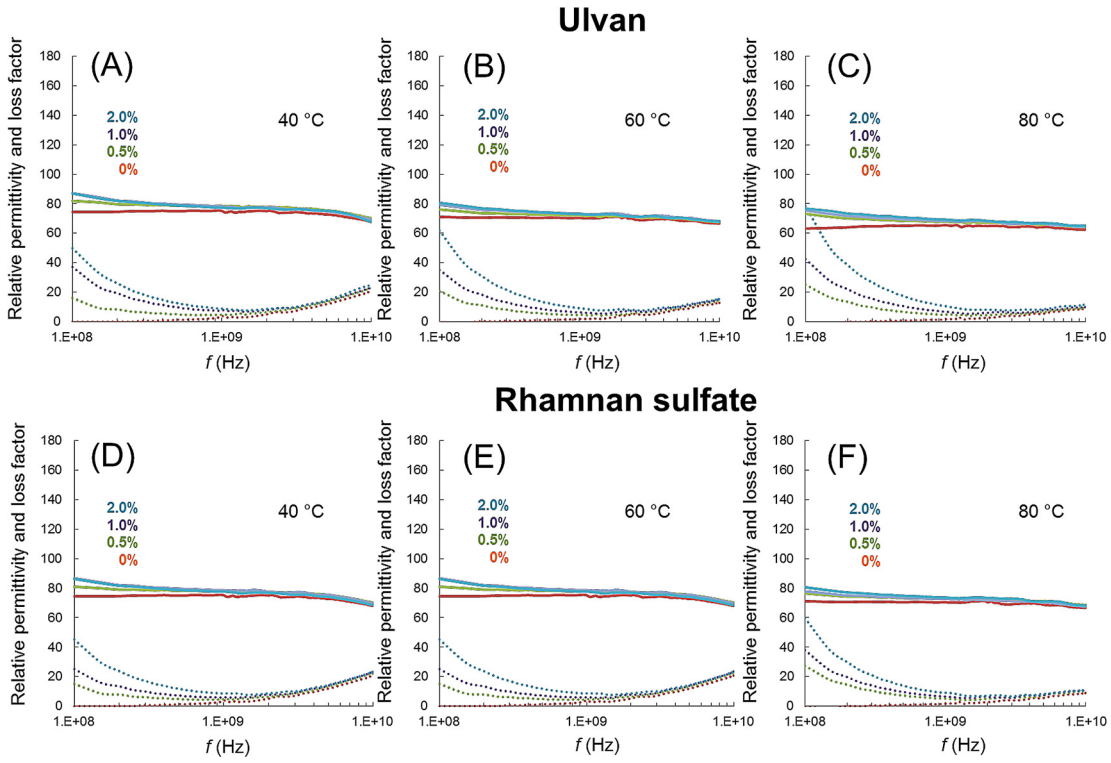

Fig. 2. Relative permittivity and loss factor of aqueous solutions of ulvan and rhamnan sulfate (0 ~ 2.0%) at 40 °C, 60 °C and 80 °C as a function of frequency. Solid line; relative permittivity (ε'), dashed line; loss factor (ε'').

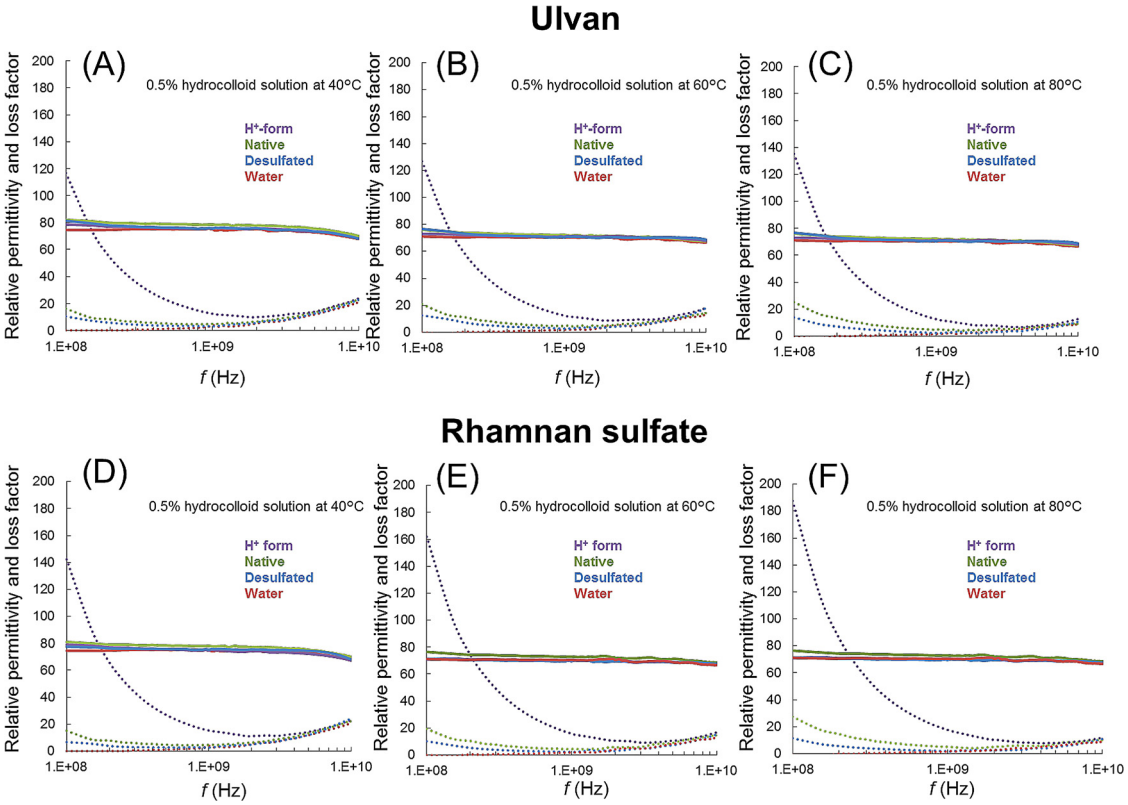

Fig. 3. Functional group dependent relative permittivity and loss factor of 0.5% aqueous solutions of ulvan and rhamnan sulfate at 40–80 °C as a function of frequency. Solid line; relative permittivity (ε'), dashed line; loss factor (ε'').

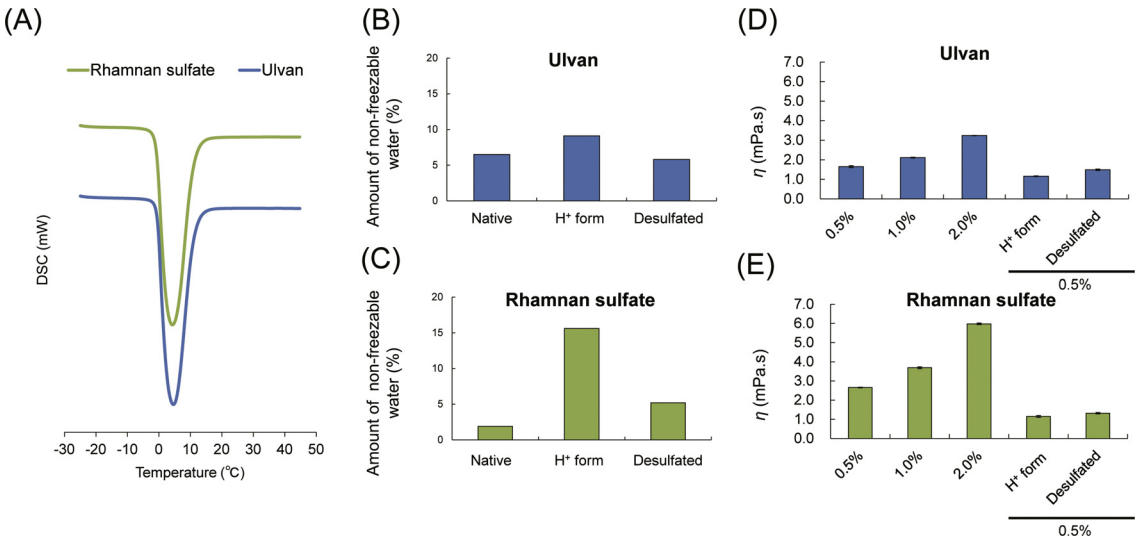

Fig. 4. Effects of counterion and desulfation of ulvan and rhamnan sulfate on the amounts of non-freezable water and viscosity of the hydrocolloid solutions. (A) DSC thermograms of 0.5% aqueous solution of rhamnan sulfate and ulvan; (B), (C) the amount of non-freezable water on 0.5% aqueous solution of ulvan and rhamnan sulfate; (D), (E) the viscosity of the aqueous solution of ulvan and rhamnan sulfate. Error bars indicate standard deviation ($n = 3$).

(Ikeda, Kumagai, & Nakamura, 1997; Takemasa & Chiba, 2002). At frequencies between 100 MHz and 1 GHz, H^+ was suggested to contribute to dielectric property of hydrocolloid solution by significantly higher ionic conductivity. By increasing the temperature, the conduction loss became more prominent due to higher ionic conductivity. Rhamnan sulfate showed more intensive enhancement in conduction loss through counterion exchange, probably due to their higher contents in sulfate and ash.

Contrary, desulfations drastically decreased conduction loss and the values came closer to that of water. Desulfation significantly decreased H^+ concentration and DC conductivity. However, they still retained slight higher loss factor than water probably because of conduction loss related to carboxyl groups of uronic acids remained in the desulfated hydrocolloids. Although ulvan and rhamnan sulfate showed different molecular weight distribution after desulfation treatment, they showed very similar behavior in dielectric property. Therefore, the decrease in molecular weight of ulvan and rhamnan sulfate in a range of 10k–70k might not significantly affect dielectric property as sulfate groups.

3.3. *The hydration and viscosity of the aqueous solutions of ulvan and rhamnan sulfate*

Subsequently, the degree of hydration and viscosity relevant to dielectric properties of the hydrocolloid solution were investigated. The hydration states of the hydrocolloid solutions were estimated from the amount of non-freezable water measured by DSC. Typical DSC thermogram and the amount of non-freezable water in the hydrocolloid solutions were shown in Fig. 4A–C. The native hydrocolloid showed 6.5% (ulvan) and 1.9% (rhamnan sulfate) of non-freezable water bounded to hydrocolloid, however, H^+ form showed 9.1% (ulvan) and 15.6% (rhamnan sulfate) of hydration. Subsequent desulfation showed drastic decrease in the amount of non-freezable water. Dielectric dispersions of bound water and polysaccharide such as pullulan occur at around 100 MHz, thus, interpretation of ionic conduction and dipole polarization are complex (Kishikawa, Seki, Singai, Kita, Shinyashiki, & Yagihara, 2013; Tanaka, Morita, Mallikarjunan, Hung, & Ezeike, 2005), however, the high hydration of H^+-form hydrocolloids might also affect the dielectric property of the solution by reducing the amount of free water.

Viscosity of the hydrocolloid solution was investigated by viscometer at 40 °C. The viscosity of native hydrocolloids increased in proportion to their concentration. The viscosity of native solution may be relevant to ionic bond between hydrocolloids and divalent cations such as Mg^{2+} and Ca^{2+}. Rhamnan sulfate showed higher viscosity than ulvan probably due to higher degree of sulfation. By exchanging cations to H^+, the viscosity decreased by 30% (ulvan) and 57% (rhamnan sulfate). Native hydrocolloids seems to construct aggregated structure by cross-linking with cations (Robic, Gaillard, Sassi, Lerat, & Lahaye, 2009), while chains of H^+-form hydrocolloids may be dispersed in the solution by static repulsion of sulfate groups. The subsequent desulfation also showed low viscosity by losing ionic interaction of hydrocolloids. In the present study, the viscosity of the hydrocolloid solution did not show apparent relationship with dielectric property.

4. Conclusions

The present study demonstrated the association of sulfate groups of hydrocolloid obtained from green macroalgae with the dielectric property of the hydrocolloid solution. H^+ form hydrocolloids showed significant increase in loss factor, while desulfated ones showed reduced loss factor. The different behavior in the loss factor seems to be related to ionic conduction of acidic hydrocolloids and their counterions. Especially, ionic conduction of H^+ showed significant contribution to the loss factor. Hydration also progressed at H^+-form hydrocolloids, thus difference in proportion of freezable and non-freezable water might also affected the dielectric property of the hydrocolloid solution. On the other hand, viscosity of the solution did not show apparent contribution to the dielectric property.

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