

Effects of Irradiation to Morphological, Physicochemical and Biocompatibility Properties of Carrageenan

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Abstract—The characterization of κ -carrageenan could provide a better understanding of its functions in biological, medical and industrial applications. Chemical and physical analyses of carrageenan from seaweeds, *Euchema cottonii* L., were done to offer information on its properties and the effects of Co-60 γ -irradiation on its thermochemical characteristics. The structural and morphological characteristics of κ -carrageenan were determined using scanning electron microscopy (SEM) while the composition, molecular weight and thermal properties were determined using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), gel permeation chromatography (GPC), thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). Further chemical analysis was done using hydrogen-1 nuclear magnetic resonance (^1H NMR) and functional characteristics in terms of biocompatibility were evaluated using cytotoxicity test.

Keywords—Biocompatibility, carrageenan, DSC, FTIR, GPC, irradiation, NMR, physicochemical, SEM, TGA.

I. INTRODUCTION

THE wide range of industrial applications of natural polymers such as phycocolloids and derivatives of polysaccharides are based on their versatile properties, and unlimited raw material supply [1]. The seaweed polysaccharides such as carrageenan represent one of the major ingredients in food industry. The interest in analyzing the structural, chemical and physical characteristics of carrageenan stems from its broad variety of functions in both biological systems and industrial operations. Although, there are several studies on characterization of this biopolymer [2, 3], different raw material source provides different properties and most of these data are given in range, hence extensive data analyses must be done prior their use.

Carrageenan and other galactans are produced by carragenophytes mostly Rhodophyta, thus presenting a chemical structure related to red algae. Generally, these seaweeds do not produce idealized and pure carrageenans but hybrid structures comprised of alternating 3-linked β -D-galactose and 4-linked α -D-galactose or 4-linked 3,6-anhydro-

D-galactose. The commercial carrageenan are normally divided into κ -carrageenan, ι -carrageenan and λ -carrageenan (Fig.1) depending on the number and position of the sulfate group, and has a molecular mass ranging from 400-600kDa [4]. Other carrageenan units include χ , β , μ and ν -carrageenan. The μ and ν -carrageenan are modifiable to κ and ι -carrageenan in alkali conditions, imparting a higher degree of regularity to the molecule through the formation of 3,6-anhydrogalactose bridge[5].

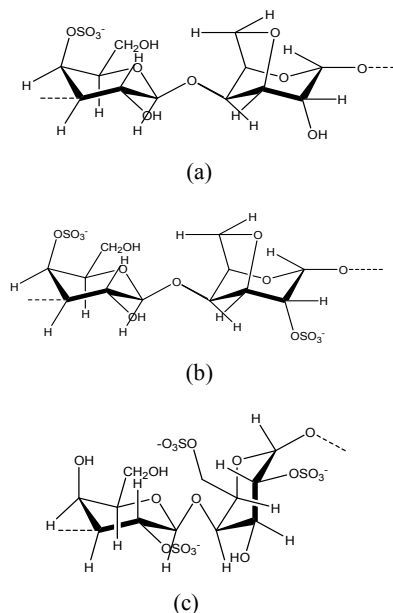


Fig. 1 Chemical structural units of (a) κ -carrageenan, (b) ι -carrageenan and (c) λ -carrageenan

These eclectic range of structures and properties of carrageenan result to broad applications, and with the increasing worldwide demand for new application developments, studies on its characterization gained momentum. This research evaluates the properties of carrageenan by analyzing not just its composition and chemical characteristics but as well as their surface morphology and functional properties, and the changes in these properties with irradiation treatment.

II. MATERIALS AND METHODS

Purification of the carrageenan samples was done as a pretreatment process to ensure that no raw materials from

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seaweed were included. The samples were grounded, dissolved in distilled water, filtered, precipitated and vacuum dried at 40°C for 12 hours. Irradiation using Cobalt 60 was done at a dose of 10kGy using the γ -rays irradiation facility of the Philippine Nuclear Research Institute.

A. Surface Morphology

The morphological structure of the solid biopolymer samples, κ -carrageenan and its irradiated form were obtained at different magnification using SEM at electron beam of 5kV. The solid samples were initially coated with gold using ion sputter to avoid degradation and make the surface conductive.

B. Molecular Weight

The molecular weight determination was carried out using GPC using a Perkin Elmer apparatus equipped with isocratic LC pump 250 and a UV detector series 200. The instrument was equipped with a set of 10^3 , 10^4 and 10^5 Å PL-gel columns conditioned at 40°C and using GPC-grade tetrahydrofuran as mobile phase at 1mL/min flow rate. Polymer standards for carrageenan in a concentration range between 5.0 and 370.0kDa were used for calibration. Polymer samples were prepared by dissolution in the mobile phase of 10mg/mL followed by filtration using 0.22 μ m syringe filters directly into the glass vials.

C. Thermal Property Determination

The thermal behaviour of the polymer was evaluated using TGA and DSC. The TGA analysis of the samples weighing 3.0 to 4.0 mg was done in alumina cell at a N₂ flow rate of 50mL/min from 25°C to 1000°C at an increment of 20°C/min. The DSC analysis was obtained at 50mL/min N₂ from -50°C to 280°C at a ramp of 10°C/min for samples weighing 1.0 to 3.0mg along with an empty aluminium pan as reference. DSC curves were recorded and the transition temperature was estimated at the midpoint of the stepwise increase of the specific heat associated with the transition. The maximum peak of temperature of the endothermic was taken as the melting point, T_m, and the area under the endothermic peak as enthalpy of melting, ΔH_m .

D. Chemical Characterization

The molecular analysis of each solid biopolymer sample was determined using ATR- FTIR while the quantitative analysis was done using 1H NMR. The FTIR identification analysis was done in an ATR diamond window using approximately volume of 2mm³ dried powdered samples. The FTIR spectra of carrageenan sample was recorded on a spectrometer using single reflection diamond ATR system at 128 scans, a resolution of 2cm⁻¹ at 25°C. The analysis was done in triplicates and the resulting FTIR spectra were compared to literature.

Further chemical characterization of the biopolymer samples was done using 1H NMR analysis. The constantly dried samples of carrageenan were dissolved at 80°C in D₂O at 5mg/mL concentration containing 1mM 3-trimethylsilyl propionic- 2,2,3,3- d₄ acid sodium salt (TSP) and 20mM

Na₂HPO₄. The solution was subjected three times to sonication for 1 hour. The aliquots of the sonicated solutions was transferred to NMR tubes and analyzed. The analysis was run at 65°C operating at 500.13MHz in 256 scans.

E. Biocompatibility and Cytotoxicity Test

The lymphocyte culture obtained from fresh human blood sample from healthy individuals was collected in vacuum tubes. The erythrocytes were separated by centrifugation at 500rpm for 5 minutes and 5ml of the yellow plasma was overlaid onto 5mL of Histopaque and centrifuged at 2000rpm for 30minutes. The supplemented media containing fetal bovine serum, Penicillin-Streptomycin and Amphotericin B were added to obtain a final cell density of 2x10⁶cells/mL and then incubated at 37°C for 24 hours. The cytotoxicity test was done by dissolving the 0.1mg biopolymer samples to biological grade water. The solutions were then filtered and 50 μ L of the filtrate were dispensed in microcentrifuge tubes and 450 μ L aliquots of lymphocytes were added. The cultures were incubated at 37°C for 24 hours and prepared for cell counting. Seven μ L of incubated culture were mixed to 7 μ L of trypan blue, and was placed in a haemocytometer. The number of live lymphocytes and the number of dead lymphocytes were counted in all 25 squares within the 1 mm center grid.

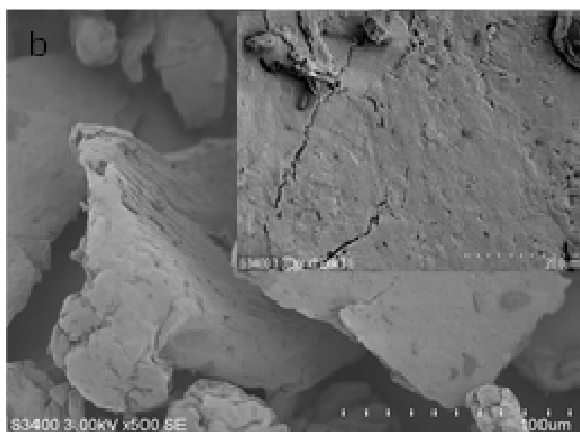
III. RESULTS AND DISCUSSION

A. Morphological Study

The surface properties of materials including biopolymers are relatively different from the bulk, and since the surface of the material is generally the one in contact with other materials, surface characterization is critical. The surface topography of the solid samples of κ -carrageenan prior and after irradiation at 10kGy was obtained using SEM (Fig.2). Comparison of the SEM images showed that the surface of carrageenan was relatively rough and amorphous prior irradiation.



(a)



(b)

Fig. 2 SEM image of (a) non-irradiated κ -carrageenan and (b) irradiated κ -carrageenan

B. Physicochemical Characteristics

The molecular weight (MW) of the non-irradiated carrageenan ranged from 958-963kDa with an average Mn of 461kDa. It was found that after gamma irradiation, the Mw of the samples decreased to 576-590kDa. Possible dehydration and degradation of the samples could account for the 39.3% decrease in MW. High MW carrageenan has been found to have a higher viscosity and lower solubility, irradiation using natural source could therefore be used to produce lower MW samples to better control the rheological properties of κ -carrageenan.

In addition, the changes in the thermal properties as provided by the considerable difference in the heating profile and melting point after irradiation. It was observed that irradiated samples have a higher transition temperature, and requires 165.87% more energy than the non-irradiated κ -carrageenan (Fig. 3). The weight loss during heating was achieved in three stages for non-irradiated up to 997.59°C while almost the same weight loss was achieved in a single stage for irradiated samples with a corresponding temperature of 586.13°C (Fig. 4).

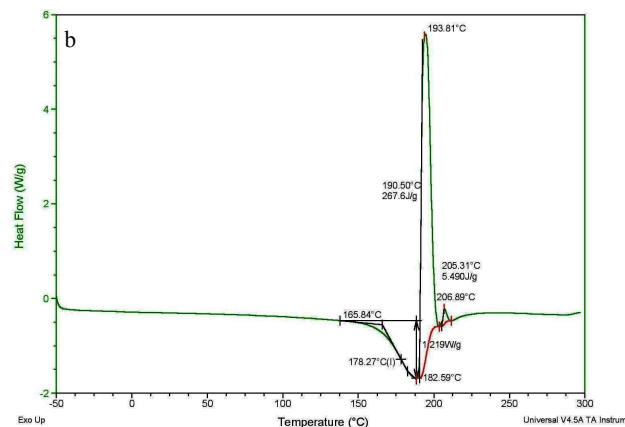
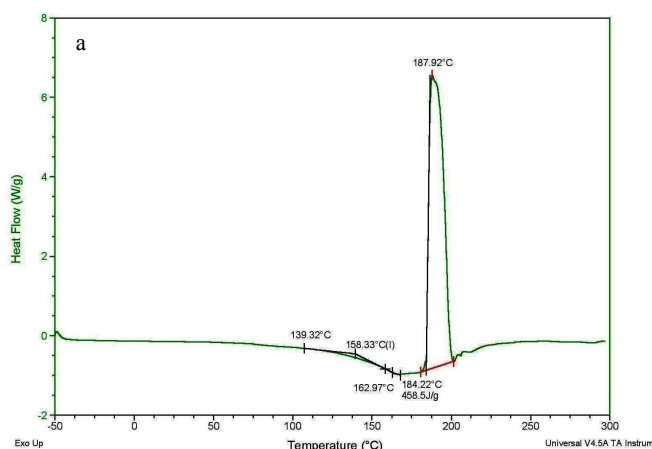


Fig. 3 DSC analysis of κ -carrageenan (a) before and (b) after irradiation

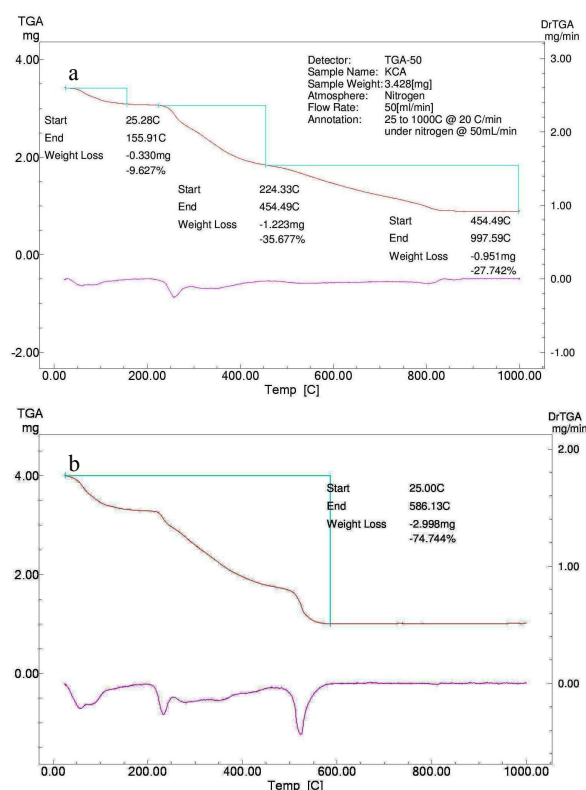


Fig. 4 TGA analysis of κ -carrageenan (a) before and (b) after irradiation

Infrared spectroscopy is commonly used to determine the physicochemical characteristics and chemical structures of polymers. In this study, the ATR FTIR spectra obtained were used to identify the presence of functional groups and differentiate the changes in the chemical structure of κ -carrageenan after irradiation treatment. The same bands of both samples were seen in the FTIR spectra (Fig.5). This indicated that both samples contain sulfate esters (S=O), 3,6-anhydrogalactose (C-O), galactose (C4, C-O-SO₃) and have the same sets of chemical structures despite having different thermal properties. Further analysis was done using 1H NMR and the detected chemical shifts for its oligosaccharides

protons were compared and verified with other literature values [6]. The first spin system describes the reducing end α -4-*O*-sulfolactopyranose anomer (α -Galp4S_{re}) with H-1 detected to be at 5.235ppm for non-irradiated and 5.21ppm for irradiated sample. However, the spectrum did not allow the detection of H-2, H-3 and H-4 for the same system. The signal for H-1 of the AnGalp residue which is directly linked to the α -Galp4S_{re} unit was only detected for irradiated sample at 5.011 ppm compared to the literature value of 5.120ppm. The next spin system corresponds to the β -Galp4S_{re}. H-1 and H-2 have only been detected for the non-irradiated samples while H-3, H-4 and H-5 for both. The H-2 signal at 3.54ppm corresponds to β -Galp4S_{re} which also shows correlation to H-1 at 4.65ppm, the same as the literature value. H-3, H-4 and H-5 were identified at 4.09ppm, 4.71ppm and 3.69ppm for non-irradiated κ -carrageenan while 3.99ppm, 4.90ppm and 3.79ppm respectively for irradiated sample. For the last spin which belongs to AnGalp, shifts have been detected to H-3 for both samples while H-4 and H-5 are seen in non-irradiated sample and H-2 and H-6b for irradiated sample. H-3 shifts for non-irradiated was at 4.67ppm and 4.51 for irradiated κ -carrageenan. H-4 and H-5 were detected at 4.68ppm and 4.69ppm while H-2 and H-6b of irradiated sample were both detected at 4.17ppm. These values were compared to the literature data and were within range for the κ -carrageenan. The chemical shift values were calculated with reference to 4.75 ppm for D₂O.

with cell viability at 67.12%. The biocompatibility was found to be not significantly affected by the irradiation treatment of the sample.

IV. SUMMARY AND CONCLUSION

The use of several techniques such as microscopy, chromatography and spectroscopy in extensively characterizing carrageenan is an accurate way of determining structural and chemical properties of these polymers prior application. The morphological structure of the samples using SEM can be used to qualitatively assess the porosity and fibril properties of the κ -carrageenan. Although, it has not been correlated in this study, surface morphology of these biopolymers influence their activity and chemical properties and are therefore a significant set of data. In addition, the chemical, thermal and biological analyses of carrageenan before and after irradiation provide enough data to determine the changes in both the chemical and thermal properties of κ -carrageenan after subjecting to 10kGy γ -rays. It has been determined using the FTIR and NMR data that although the chemical composition for both samples remained similar, the thermal properties of the biopolymer changed radically. Moreover, the use of irradiation treatment was found to not affect its biocompatibility. The analyses obtained in this work can be utilized as supplementary data to evaluate their possible industrial and medical applications.

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REFERENCES

- [1] Usov, A. I., "Structural analysis of red seaweed galactans of agar and carrageenan groups," *Food Hydrocolloids*, vol. 12, pp. 301–308, 1998.
- [2] Yermak, I.M., Kim, Y., Titlynov, E., Isakov, V., Solov'eva, T., "Chemical structure and gel properties of carrageenans from algae belonging to the Gigartinales and Tichocarpales, collected from the Russian Pacific coast," *Journal of Applied Phycology*, vol. 11, pp. 41–48, 1999.
- [3] Michel, A.S., Mestdagh, M.M., and M.A. Axelos, "Physico-chemical properties of carrageenan gels in presence of various cations," *International Journal of Biological Molecules*, vol. 21, pp. 195–200, 1997.
- [4] Knutsen, S., Myslabodski, D., Larsen, B. and A. Usov, "A modified system of nomenclature for red algal galactans," *Botanica Marina*, vol. 37, no. 2, pp. 163–169, 1994.
- [5] Nosedá, M. D. and A. S. Cerezo, "Room temperature, low-field ¹³C-NMR spectra of degraded carrageenans: Part III. Autohydrolysis of a lambda carrageenan and of its alkali-treated derivative," *International Journal of Biological Macromolecules*, vol. 15, no. 3, pp. 177–181, 1993.
- [6] Knutsen, S. H. and H. Grasdalen, "The use of neocarrabiose oligosaccharides with different length and sulphate substitution as model compounds for ¹H-NMR spectroscopy," *Carbohydrate Research*, vol. 229, pp. 233–244, 1992.

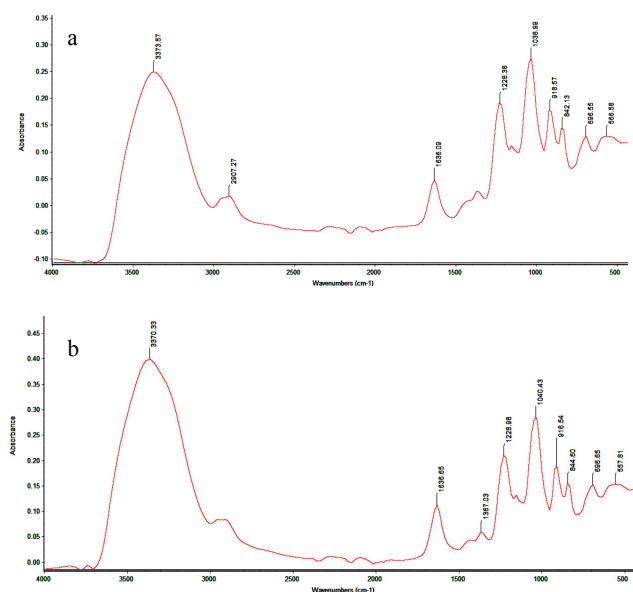


Fig. 5 ATR-FTIR spectral change in κ -carrageenan (a) before and (b) after irradiation

C. Biocompatibility

The biocompatibility was tested using cytotoxicity studies with fresh human blood samples. The 1mg/mL samples were incubated together with human leukocytes and the cells were counted after 24 hours of incubation. It was observed that both samples were biocompatible at 63.47 and 62.93% live cell count after incubation. This data is comparable to the control