Parameters Controlled on Indonesian *Sargassum duplicatum* Extraction Process To Obtain a Water-Soluble Alginate

Joddy Arya Laksmono[1*)], Robert Ronal Widjaya[1], Firman Triajie[2], Santoso Sastrosoeparno[3]
[1]The Research Centre for Chemistry, Indonesian Institute of Sciences, Kawasan PUSPIPTEK, Serpong 15314, Indonesia, ph. +62-21-7560929, fax. +62-21-7560549
[2]The Center for Innovation, Indonesian Institute of Sciences, Cibinong Science Center, Cibinong, Indonesia
[3]Balai Besar Tekstil, Jl. Jend. A. Yani Nr. 390, Bandung, West Java, 40272, Indonesia
*) e-mail: joddy_arya_laksmono@yahoo.com

***Abstract*-- Alginate is a family of organic polymeric polysaccharides is composed of two monomer units of guluronic acid (G) and mannuronic acid (M) or both alternating (GGMM). Alginate compound found in the cell walls of brown seaweed in the form of crystals arranged in parallel on fine threads and cellulose in the cell fluid. This study was conducted to obtain alginate products are soluble in water and it has sufficient viscosity. Optimization does on the extraction process to obtain seaweed alginate. The main parameter to be achieved is the efficiency of the process, both technically and economically. In this research, the extraction process has been carried out in stages to make the pH controlled. pH controlled has been carried out with the addition of HCl and Na_2CO_3. From the research, product characterization and analysis of physical chemistry by analyzing the moisture content, ash content, viscosity, solubility in water and colors. Then do the validation for the resulting alginate compounds using FTIR, LCMS and NMR. Results of ^1H and ^{13}C NMR analysis showed ties between the H and C atoms to form a compound with a structure like guluronic acid and mannuronic acid. This data is reinforced by the results of the FTIR analysis showed that functional groups contained in the alginate, and the results are showing molecular weight LCMS analysis of the alginate. From these results it can be concluded that the sample obtained from the extraction of the seaweed is alginate.**

***Index Term*-- alginate, polysaccharide, extraction, guluronic acid, mannuronic acid.**

1. INTRODUCTION

Alginate is a family of organic polymeric polysaccharides are composed of two monomer units of β-D guluronic acid (G) and α-L mannuronic acid (M) or both alternating (GGMM) as shown in Figure 1 (Truus *et al*. 2001; Soares *et al*. 2004). Alginate was first discovered by a British chemist ECC STANFORD extracted from brown seaweed (*Phaeophyta*) in the form of alginic acid. Then patented in 1881 (Draget *et al*. 2006; Ali, 2001; McHugh 1987).

Alginate compound found in the cell walls of brown seaweed in the form of crystals arranged in parallel on fine threads and cellulose in the cell fluid (Truus et al. 2001). Alginates in brown seaweed commonly bound with sodium, potassium, calcium and magnesium and are not soluble in water (Ali 2001; Higuera *et al*. 2002; McHugh 1987). Alginate extraction techniques performed in a variety of ways, Ali (2001) claims there are 20 ways to extract alginates from brown algae. Extraction of alginate can be made a variety of products such as alginic acid, sodium alginate, calcium alginate, potassium alginate, ammonium alginate and propylene glycol alginate. Sodium alginate mainly used in the food industry, the pharmaceutical and medicine that serves as a "thickening, stabilizing and emulsifying agent" (McHugh, 1987; Chapman & Chapman, 1980). The niche for the alginate industry in Indonesia from 2008 - 2011 approximately 2,000 tons per year and all are imported. Use of the biggest is textile industry (50%), whereas only 3% for food (Murtini, *et al*. 2000).

Alginate is a viscous organic polymer that is non-toxic and so widely used in industry, resulting from brown macroalgae, especially Sargassum. Potential raw material (alginofit) available in Indonesian waters is relatively abundant, so it can be modified extraction process. The purpose of this extraction process is to obtain optimum extraction process conditions so as to produce sodium alginate sodium alginate with better characteristics. Another component that affects the price is the viscosity. The higher viscosity gives the more expensive (SIGMA 2004). Viscosity is influenced by the degree of acidity (pH), the pH 5-10 stable viscosity, but the pH below 4.5 and above 11 viscosity will easily degraded (labile). We have been obtained modification processing methods that produce high-viscosity alginate.

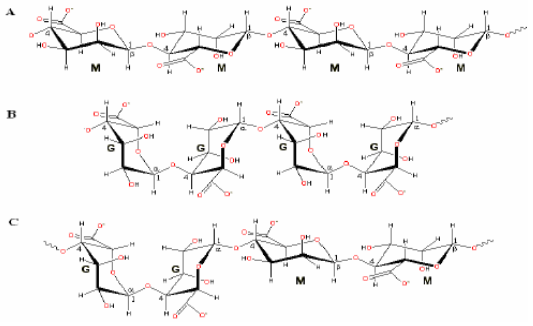

Fig. 1. Molecular structure of alginic acid (A) M = Mannuronic segment; (B) G = guluronic segment, and (C) GGMM = guluronic and Mannuronic segment.

Potential sources of alginate can be found in marine brown algae (*Phaeophyta*) and most common in temperate regions. These algae are species of *Ascophyllum*, *Macrocystis*, *Laminaria*, *Durvillaea*, *Ecklonia*, *Laessonia*, *Sargassum* and *Turbinaria*. In the Philippines *Sargassum* and *Turbinaria* exported to Japan for animal feed and fertilizer. In Indonesia brown algae *Sargassum*, *Turbinaria*

grow abundantly, while relatively few genera *Hormophysa* encountered. Brown algae *Sargassum* species *duplicatum* C. J. Agardh grown in abundance on the reef flats on the beach Pameungpeuk, Garut facing the Indian Ocean. Habitat, in the area around the edge of a strong choppy with a width of about 20-50 meters, elongated parallel to bluff. 1352.25 g/m2 wet biomass densities were associated with 9 other types of algae (Yulianto, 2005). The alginate extraction study aimed to obtain the optimal viscosity alginates from brown algae feedstock species *Sargassum duplicatum* Pameungpeuk origin, Garut, West Java. The results are expected to be a reference to the extraction of alginate in the future.

This study was conducted to obtain alginate products are soluble in water and have sufficient viscosity as a raw material in the textile process, especially in the dyeing process. This is done by optimizing the extraction of alginate from seaweed.

2. METHODS

Optimization does on the extraction process to obtain seaweed alginate. The main parameters to be achieved are the efficiency of the process, both technically and economically. Therefore, in this study conducted a few steps by doing some variation of process parameters that can be shown as below,

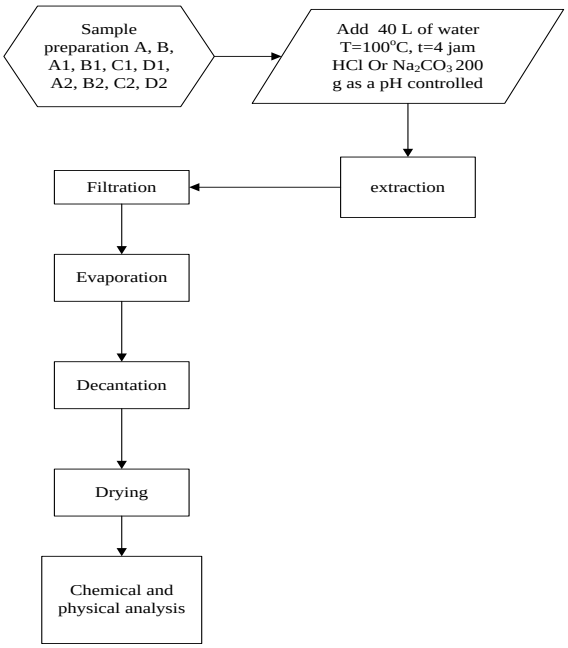

Fig. 2. Experimental flow diagram for alginate extraction process.

3. RESULTS AND DISCUSSION

From the research, product characterization and analysis of physical chemistry, namely by analyzing the moisture content, ash content, viscosity, solubility in water and colors. Then do the validation for the resulting alginate compounds using FTIR, LCMS and NMR.

Analysis of physical chemical properties

Results of chemical analysis of the physical properties of alginate products from a variety of processes can be seen in Table 5.1. From the analysis of physical chemical properties can be seen that for each process with variations, have different characteristics.

Product specifications based on market conditions desire that the water content of alginate product value as low as possible. This is due to alginate hygroscopic or has the ability to absorb moisture from the air. Such circumstance is undesirable because it can affect the properties of alginate. In addition, if stored for long periods with high water content conditions, bacteria and fungi will live and breed in alginate products.

The physical chemical properties of alginate in the market as well as viscosity oriented. Based on data from existing products on the market that the viscosity of alginate ranged between 41,000-55,000 cPs. Thickening of existing products on the market are usually used Manutex. Based on Table 1 was shown that most of the products of alginate results of this study are in the range of 37,500-52,500 cPs. This value has been meeting to the value of the viscosity of the product thickener in the market.

From Figure 5.2. can be seen that for the sample 8 nlai C2 and B1 has a higher viscosity, which is 52.5 x 10^3 and 50 x 10^3 cPs. Therefore, the sample with C2 and B1 code is to be alginate product candidates in subsequent analyzes as well as the scaling up process.

Analysis of Functional Groups

The next stage is to analyze the functional groups contained in the alginate by using a Fourier Transform Infra Red (FTIR). Alginate has containing major components namely guluronic acid and mannuronic acid. The structure of these compounds can be seen in Figure 3 and 4 as follows,

Table I
Physical-chemistry analysis of the alginate products.

Parameters	Alginate's samples									
	A	**B**	**A1**	**A2**	**B1**	**B2**	**C1**	**C2**	**D1**	**D2**
Viscosity (cP) x 10^3	37.5	47.5	47.5	47.5	50	45	40	52.5	47.5	47.5
Solubility in water	Completely soluble in water									
Water content (%)	14.72	8.51	6.86	8.06	8.2	4.23	5.87	5.98	6.28	9.61
Ash content (%)	71.07	67.83	59.11	63.86	57.71	70.02	60.35	70.19	59.03	64.1
Color	Brown									

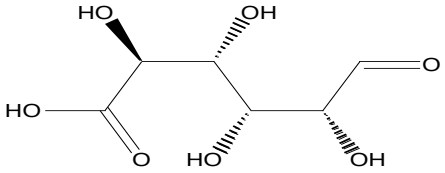

Fig. 3. Chemical structure of Guluronic acid.

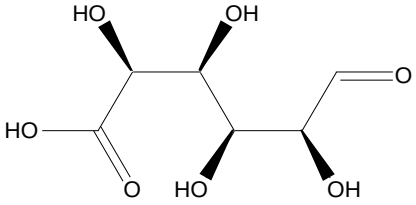

Fig. 4. Chemical structure mannuronic acid.

From Figure 3 and 4 it can be shown that the functional groups contained in the alginate is hydroxyl group ˉOH, C – C, C = O, CH – CH, CH_2 - CH_2, OH - C, so by using the FTIR instrument can detect cluster analysis and to declare the done earlier that samples containing alginate consisting of guluronic acid and mannuronic acid. By performing multiple processes on a variety of parameters, each sample was then analyzed for functional groups as follows,

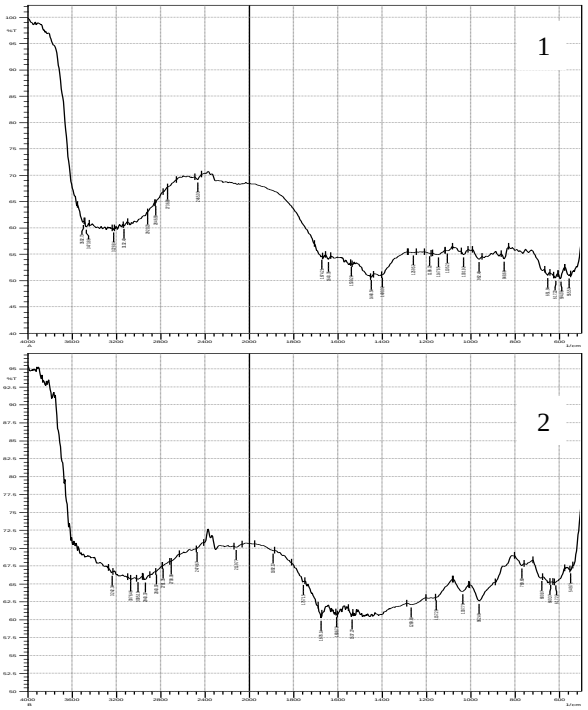

Fig. 5. Spectrum IR alginates for 1) sample A, 2) samples B.

Based on Figure 5, the functional groups can be detected guluronic and mannuronic acid. Visible at a wavelength (λ_{max}) 569 - 620 cm^{-1} represents a group C - C = O, the λ_{max} 650 – 678 cm^{-1} shows C - OH group, C – C group shown in λ_{max} 875 cm^{-1}, group C = O is shown in λ_{max} 1,741 cm^{-1}, CH_3 groups indicated by λ_{max} 2,852 – 2,924 cm^{-1}, whereas for the ˉOH group is shown at a wavelength (λ_{max}) 3,200 – 3,500 cm^{-1}.

Based on Figure 6 functional groups can be detected guluronic and mannuronic acid. Visible at a wavelength (λ_{max}) 569 - 620 cm^{-1} represents a group CC = O, the λ_{max} 650 - 678 cm^{-1} shows C – OH group, CC group shown in λ_{max} 875 cm^{-1}, group C = O is shown in λ_{max} 1,741 cm^{-1}, CH_3 groups indicated by λ_{max} 2,852 – 2,924 cm^{-1}, whereas for the ˉOH group is shown at a wavelength (λ_{max}) 3,200-3,500 cm^{-1}.

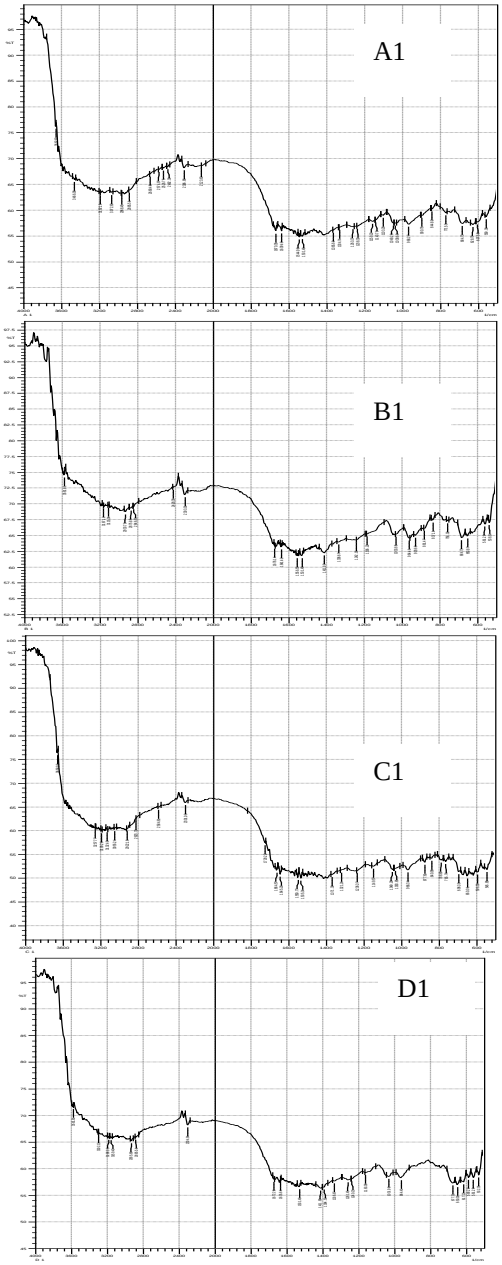

Fig. 6. IR spectra for compound alginate sample 1) A1, 2) B1, 3) C1, 4) D1.

Based on Figure 7. functional groups can be detected as guluronic and mannuronic acid. Visible at a wavelength (λ_{max}) 569 – 620 cm^{-1} represents a group CC = O, the λ_{max} 650 – 678 cm^{-1} shows C – OH group, CC group shown in λ_{max} 875 cm^{-1}, group C = O is shown in λ_{max} 1,741 cm^{-1}, CH_3 groups indicated by λ_{max} 2,852 – 2,924 cm^{-1}, whereas for the ˉOH group is shown at a wavelength (λ_{max}) 3,200 – 3,500 cm^{-1}.

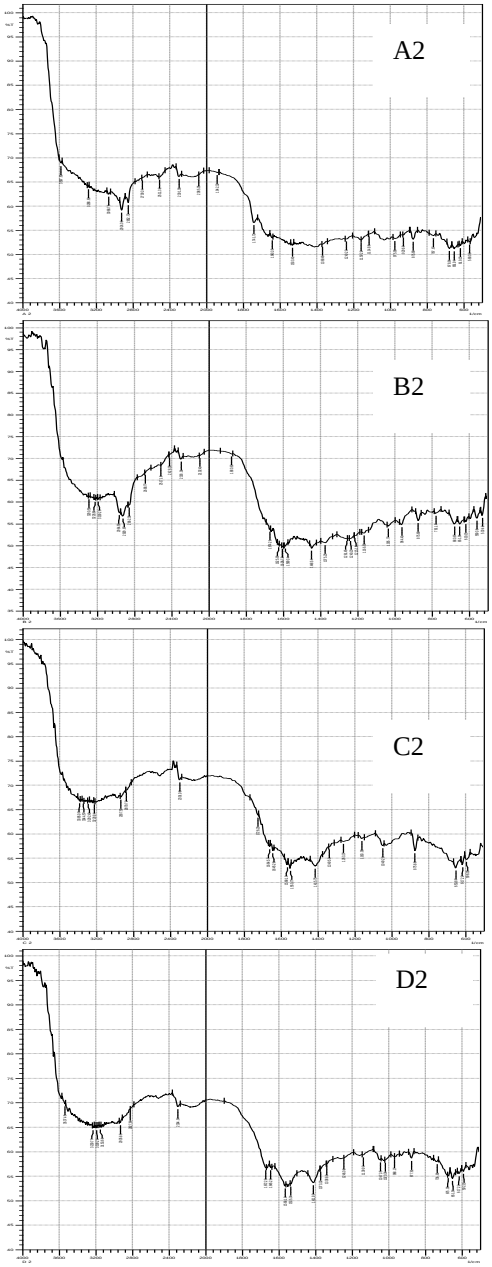

Fig. 7. IR spectra for compound alginate sample
1) A2, 2) B2, 3) C2, 4) D2.

Based on the results of FTIR analysis, it can be seen that the product contains alginic acid groups as guluronic and mannuronic acid. Thus as a preliminary analysis using FTIR, the resulting product is an alginate with the building blocks of guluronic acid and mannuronic acid.

Molecular Weight Analysis

Analysis for further clarification that the alginate guluronic acid and mannuronic acid molecular weight analysis using tools Liquid Chromatography Mass Spectrometry (LCMS). Analysis results can be seen in Figure 8. as below :

The structure of guluronic acid (Figure 3) and mannuronic acid (Figure 4), Theoretically generated using software chemoffice while the alginate contained both types of acid so that the amount of molecular weight (MW) on theoretical alginate product was 388.28 g/mol. Seen from Figure 8 there is a peak which indicates the value of the molecular weight of 388.074 g/mol.

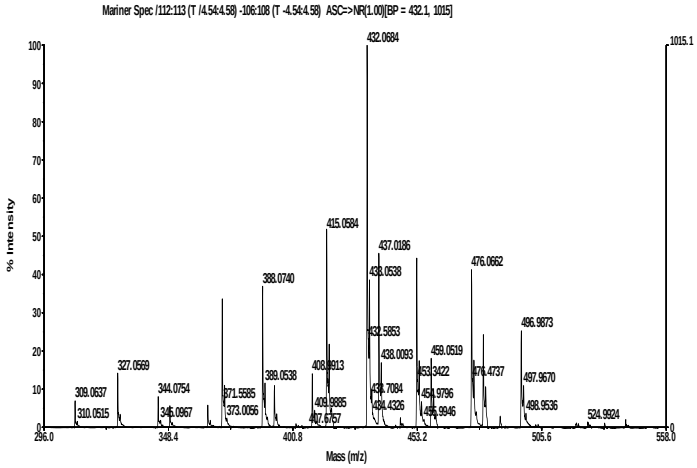

Fig. 8. Spectra of LCMS analysis of the spectra for alginate.

Structure Analysis of Compound Alginate

Further analysis to ascertain the structure of guluronic acid and mannuronic acid contained in alginate, alginate compound structure analysis using ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR).

Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH	9.72	9.60	CHO
		0.12	1 -C
CH	4.16	1.50	methine
		1.73	1 alpha -O
		0.86	1 alpha -C=O
		0.08	1 beta -O
		-0.01	1 beta -C
OH	2.0	2.00	alcohol
CH	3.60	1.50	methine
		1.73	1 alpha -O
		0.08	1 beta -O
		0.22	1 beta -C=O
		0.08	1 beta -O
		-0.01	1 beta -C
OH	2.0	2.00	alcohol
CH	3.70	1.50	methine
		1.73	1 alpha -O
		0.08	1 beta -O
		-0.01	1 beta -C
		0.08	1 beta -O
		0.32	1 beta -C(=O)O
OH	2.0	2.00	alcohol
CH	4.17	1.50	methine
		1.73	1 alpha -O
		0.87	1 alpha -C(=O)O
		0.08	1 beta -O
		-0.01	1 beta -C
OH	2.0	2.00	alcohol
OH	11.0	11.00	carboxylic acid

Where as for ^{13}C NMR analysis results can be seen in Figure 10. ^{13}C NMR analysis of the results showed a series of carbon bonds in alginate compound. Results of ^{1}H and ^{13}C NMR analyzes were then combined to determine the bonds so as to construct a compounds, in this case the target compound is guluronic acid and mannuronic acid.

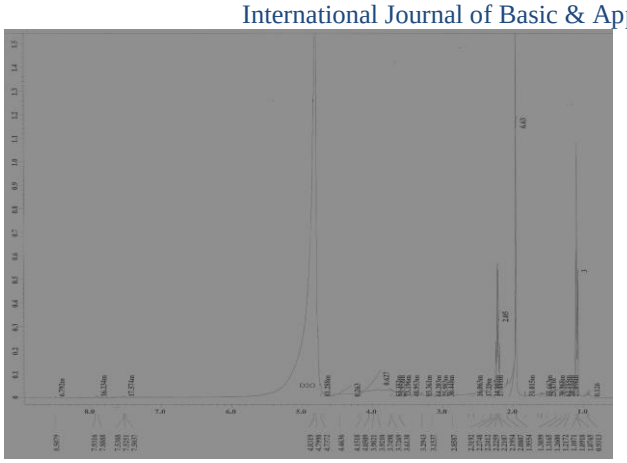

Fig. 9. ^{1}H NMR spectra of alginate products.

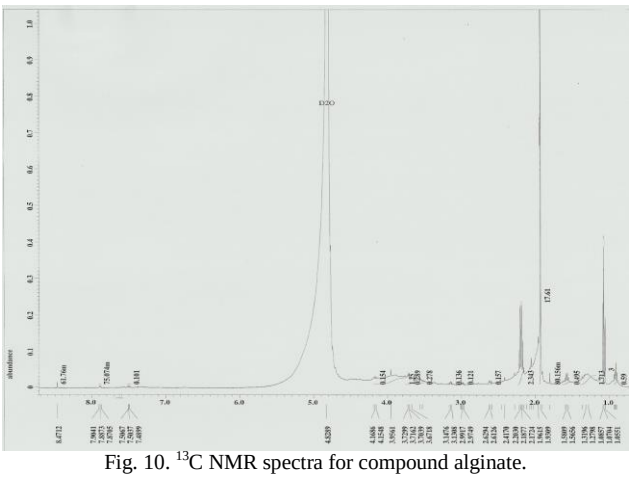

Fig. 10. ^{13}C NMR spectra for compound alginate.

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Protocol of the C-13 NMR Prediction:

Node    Shift     Base + Inc.    Comment (ppm rel. to TMS)

  CH    200.6        193.0       1-carbonyl
                       7.6       1 -C-C
  CH     88.3         -2.3       aliphatic
                      29.9       1 alpha -C=O
                       9.1       1 alpha -C
                      49.0       1 alpha -O
                       9.4       1 beta -C
                      10.1       1 beta -O
                      -2.5       1 gamma -C
                      -6.2       1 gamma -O
                       0.0       1 delta -C(=O)-O
                       0.3       1 delta -O
                      -8.5       steric corrections
  CH     67.9         -2.3       aliphatic
                      18.2       2 alpha -C
                      49.0       1 alpha -O
                      -0.6       1 beta -C=O
                       9.4       1 beta -C
                      20.2       2 beta -O
                      -2.8       1 gamma -C(=O)-O
                      -6.2       1 gamma -O
                     -17.0       steric corrections
  CH     70.6         -2.3       aliphatic
                      18.2       2 alpha -C
                      49.0       1 alpha -O
                       2.0       1 beta -C(=O)-O
                       9.4       1 beta -C
                      20.2       2 beta -O
                      -2.7       1 gamma -C=O
                      -6.2       1 gamma -O
                     -17.0       steric corrections
  CH     80.2         -2.3       aliphatic
                      21.8       1 alpha -C(=O)-O
                       9.1       1 alpha -C
                      49.0       1 alpha -O
                       9.4       1 beta -C
                      10.1       1 beta -O
                      -2.5       1 gamma -C
                      -6.2       1 gamma -O
                       0.0       1 delta -C=O
                       0.3       1 delta -O
                      -8.5       steric corrections
   C   177.0        166.0       1-carboxyl
                      11.0       1 -C-C-C
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^{1}H and ^{13}C NMR analysis can be shown that there is a distinctive ties to form a compound guluronic acid and mannuronic acid.

Based on Figure 10 to do an analysis of the structure as below,

4. Conclusion

Alginate is then performed to obtain physical chemistry test data with the data that will compare into alginate market. From the analysis of physical chemistry can be shown that alginate can be dissolved in water, has an average viscosity in the range of 37,500 - 52,500 cPs., and the colors is brown as well as similar to standard of alginate.

This data was reinforced by the results of the FTIR analysis showed that functional groups contained in the alginate, and the results are showing MW LCMS analysis of the alginate. Results of ^{1}H and ^{13}C NMR analysis showed ties between the H and C atoms to form compounds with structures such guluronic acid and mannuronic acid. From these results it can be concluded that the sample obtained from the extraction of the seaweed is alginate.

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