New extraction technology and characterization of sodium alginate

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Abstract. In this paper, the experiment was based on the principle of producing sodium carbonate by the combined alkali method, simulating seawater to absorb carbon dioxide in the air, and producing an alkaline extract solution. The effect of the extract on the extraction rate of sodium alginate was studied and then characterized by Fourier transform infrared spectroscopy and TGA. The viscosity and average molecular weight of sodium alginate were measured by viscometer.

1. Introduction

Sodium alginate is a substance of a white or light-yellow powders extracted from kelp (Laminaria digitate) and other algae[1]. It is a polysaccharide carbohydrate polymer composed of 1,4-poly-β-D-mannuronic acid (M) and α-L-guluronic acid (G), which is one of alginic acid derivatives[2]. The structure of sodium alginate is similar to cellulose and chitosan. It is a natural linear polymer that could be dissolved in water and not dissolved in organic solvents. Sodium alginate can be arranged in homopolymer (GG and MM) and hetero-polymer blocks (MG)[3], and the M/G ratio depends on the biological source, growth, and stationary conditions[4]. Sodium alginate has many properties, such as thickening, emulsifying, gel formation and good bio-compatibility. Therefore, it has been widely using in food[5], medicine, contaminated water treatment[6] and other fields.

Sodium alginate is mainly derived from brown algae, such as kelp, macrocystis, and Sargassum. It mainly extracted in the cell-matrix and cell wall[7], and the cell wall reinforces certain mechanical properties to the cells. Myklestad[8] found that alginate was mainly in the form of calcium alginate, and some are in the form of magnesium alginate, potassium alginate, and sodium alginate. The study found that the molecular weight of alginic acid was discrepant with different types of seaweed. The content of alginic acid was about 20% of dry weight, which is a kind of abundant renewable resources[9].

The amount of sodium alginate was thirty thousand tons per year in the industry, which was less than 10% of the amount of biosynthesized sodium alginate. In the existing production of sodium alginate, one ton of sodium alginate needs to consume one thousand tons of freshwater, and the water consumption is very large[10]. Today, the lack of freshwater has become one of the global challenges. Although 72% of the Earth’s surface is covered by water, freshwater resources account for 0.5% of all water resources, and the oceans account for 97.2%. Therefore, I have an idea that using seawater instead of freshwater prepare extraction solution by a Combined Alkali Process to extract sodium alginate from Laminaria digitate or other algae.
This paper is based on the principle of Combined Alkali Process, simulating seawater to absorb carbon dioxide in the air to prepare alkaline extraction solution. Extraction solution is produced by seawater to absorb ammonia gas form an ammonia-containing sodium chloride solution, which absorbs carbon dioxide of the air. In China, Laminaria digitate is the main raw material of extracting sodium alginate. In general, the raw material is pretreated with inorganic acid, then extracted with extraction solution. It is necessary to add inorganic acid for purifying and precipitate to form alginate. The acid is added to the sodium carbonate solution to transform a sodium alginate. In this paper, the preparation of alkaline solution be used seawater instead of freshwater in the extraction process of sodium alginate, which greatly reduces the use of freshwater. It also makes full use of seawater resources and basically solves the problem of large consumption of freshwater.

2. Materials and methods

2.1. Materials
Kelp (Laminaria digitate) was soaked in fresh water for 4 hours to remove impurities, such as sand, salt and epiphytes[11], then washed three times with seawater. The kelp was dried until constant weight in Electric thermostatic-drying oven (DHG-9053A). The dried kelp was pulverized into powders in pulverizer. Kelp (2.0000 g) was soaked in 2% (w / v) formaldehyde solution overnight to eliminate pigments[12] in the same way according to Fertah[13]. The insoluble portion was washed 3 times with MilliQ water, then supplemented 80 mL of 0.5 M HCl before to be incubated at 40 ℃ for 1 h under stirring (250 rpm). The acid pretreatment process was a very important step in the conversion of insoluble salt into alginic acid[11]. The suspension was centrifuged (6000 rap / min for 15 min at 20 ℃) and pellets with alginic acid were washed 3 times with MilliQ water.

2.2. Preparation of extraction solution
Seawater contains about 3.5% sodium chloride. Because carbon dioxide has a low solubility in sodium chloride, the ammonia gas is absorbed in the sodium chloride solution to make the solution alkaline, and then a large amount of carbon dioxide gas was absorbed. Therefore, a 3.5% sodium chloride solution was prepared using 28% ammonia water as a solvent to absorb carbon dioxide. The extraction solution was divided into five parts: solution A: a solution in which 3.5% of sodium chloride is dissolved in aqueous ammonia; solution B: a solution in which 3.5% of sodium chloride is dissolved in ammonia water to completely absorb carbon dioxide; solution C: 3.5% sodium chloride dissolved in ammonia water completely absorbs carbon dioxide and adjusts the solution pH to 11 with sodium hydroxide; solution D: 3.5% sodium chloride dissolved in ammonia water solution after completely absorbing carbon dioxide and adjust the pH to 11 with potassium hydroxide solution; solution E: 3.5% sodium chloride dissolved in ammonia water solution after completely absorbing carbon dioxide and adjust the pH to 11 with a sodium carbonate solution. Observing the effect of different extraction solution for the extraction yield.

2.3. Extraction and purification of sodium alginate
The optimal extraction process conditions were set according to previous researches[14]. After the acid pretreatment of Laminaria digitate was soaked in a different extraction solution, stirring at 65 ℃ for 2 hours. After the reactions were finished, the solution diluted twice with MilliQ water (the volume of the extraction solution: the volume of the MilliQ water = 1:1). The mixture was centrifuged (6000 rap / min for 20 min), and the supernatant was precipitated with HCl (1M) until pH=2–3. The precipitation was centrifuged (6000 rap / min for 20 min ), and we can obtain alginic acid. Purification was performed with 5% sodium carbonate solution for 30min until the pH increased to 10. Three volumes of a 95% aqueous ethanol solution were added to the mixture for 2 hours with constant stirring to form a sodium alginate gel. The samples were centrifuged, collected and frozen at -32 ℃ for 12 hours, and freeze-dried for 8 h using a SCIENTZ-10N vacuum freeze dryer.
2.4. Characterization of sodium alginate

The NaAlg were characterized by FTIR and Ubbelohde viscometer analysis.

3. Results and discussion

3.1. Effect of extraction conditions on the yield

The sodium alginate extraction was monitored over time in different conditions. As can be seen from Figure 1, the yield of sodium alginate is different in different extraction solutions. The extraction yield of other samples was lower than the extraction yield of the sample D. This can be explained by the fact that the algae cell wall can be better decomposed in the extraction D (an extraction solution in which 3.5% of sodium chloride is dissolved in aqueous ammonia). The extraction yields in extraction solution D is the largest, which can be as high as 40.46%. Extraction yields from sample B, C, D, and E are similar and approaching 36~40%. The content of alginate isolated from different seaweeds indicates that the extraction rate of alginate varies from 3.0% to 45%[15]. This significant difference in the amount of alginate extracted may be due to differences in species, growth environment, and separation methods[4]. Song Yanxian[16] et al. obtained higher yields for the same species, of 49.8%, and the yield depends on the alginate extraction method used. César G. Gomez[17] et al. obtained yields in three different extraction methods ranged from 25% to 34%, and Niloofar Jokar Borazjani[18] used different extraction methods (water, acid, alcalase and cellulase), the yield of sodium alginate obtained between 3.3% and 3.5%.

![Figure 1: Sodium alginate yield under different extraction solution.](image)

3.2. Intrinsic Viscosity

Intrinsic viscosity is the most commonly used expression for the viscosity of a macromolecule solution. It is defined as the reduced viscosity as the concentration of the macromolecule solution approaches zero[19]. That is to say, the contribution of a single molecule to the viscosity of a solution is a viscosity reflecting the properties of a macromolecule, and its value does not vary with concentration[17]. The value of intrinsic viscosity depends on the relative molecular mass, the structure of the macromolecule, the temperature of the solution and the characteristics of the solvent. When the temperature and the solvent are constant, the intrinsic viscosity of the same kind of macromolecule is relative to its molecular mass. Therefore, if a quantitative relationship between the relative molecular mass and the intrinsic viscosity can be established, the relative molecular mass of the macromolecule can be obtained by measuring the intrinsic viscosity.

Intrinsic viscosity is defined by the Huggins equation 1, where \(c\) and \(\eta_p\) are the concentration of the solution and the specific viscosity, respectively.

\[
[n] = \lim_{c \to 0} \frac{\eta_p}{c}
\] (1)
Based on equation 2, the classical method is to determine the viscosity of solutions with different concentrations and then extrapolate $\eta_s/c$ to zero. In a range of concentration variations, the relationship is linear and we can write Huggins:

$$\frac{\eta_s}{c} = [\eta] + k_H [\eta]^2$$

(2)

The relationship between viscosity and sodium alginate concentration is shown in Figure 2. The viscosity of sodium alginate was measured by using 0.1 M NaCl (containing 0.05% EDTA-2Na) as a solvent to prepare a sodium alginate solution having a mass fraction of 0.4%. The intrinsic viscosity $[\eta]$ of sodium alginate was measured under different conditions. The intrinsic viscosity $[\eta]$ of the different conditions calculated by the above equation were 1.84 dL/g, 5.77 dL/g, 5.18 dL/g, 5.39 dL/g, 4.80 dL/g, 3.69 dL/g, respectively. It can be seen from Table 1. that the extracted sodium alginate has a medium viscosity compared with other seaweed, especially the sodium alginate obtained from the extraction A has the lowest viscosity, which largely depends on the types of seaweed, extraction solution and extraction method. The low intrinsic viscosity may be due to the fact that the presence of reducing substances in kelp may cause degradation, which may cause a decrease in alkaline extraction. It is also possible that the electrostatic repulsion between the carboxylic on the molecular chain of sodium alginate was shielded, the flexibility of the polymer chain was increasing, and the shrinking random coils were formed, thereby reducing the fluid volume of sodium alginate, causing a drop in viscosity. Since the sodium alginate has a very low viscosity, the alginate sample may not have the gelling properties required for certain alginate applications. However, these features can be used to obtain polyelectrolyte complexes for the preparation of drug delivery microparticles and nanoparticles.

![Figure 2: Specific viscosity of A, B, C, D, E and NaAlg samples in 0.1 M NaCl (containing 0.05% EDTA-2Na).](image)

| Alginate source | $[\eta]$ dL/g | Mw $\times 10^5$ (g/mol) | Ref. |
|-----------------|--------------|--------------------------|------|
| L. hyperborea   | 6.4          | 3.05                     | [20] |
| F. vesiculosus  | 2.5          | 1.17                     | [21] |
| A. nodosum      | 2.8          | 1.32                     | [21] |
| L. japonica     | 15.4         | 7.44                     | [21] |
| S. dentifolium  | 12.6         | 6.06                     | [22] |
| S. asperifolium | 15.2         | 7.34                     | [22] |
| S. latifolium   | 8.7          | 4.16                     | [22] |
L. digitate   2.43   1.14       [13]
L. digitate A 1.84   0.85       This study
L. digitate B 5.77   2.74       This study
L. digitate C 5.18   2.46       This study
L. digitate D 5.39   2.56       This study
L. digitate E 4.80   2.28       This study
NaAlg       3.69   1.74       This study

3.3. Molecular weight
Calculating the viscosity average molecular weight $M_v$ by the Mark-Houwink equation:

$$[\eta]=kM_v^a$$  \hspace{1cm} (3)

Where $[\eta]$ is the intrinsic viscosity, and the constants $a$ and $k$ are related to the temperature, the properties of the polymer and the solvent, and the values are determined by using other independent methods (such as osmotic pressure, light scattering or sedimentation balance.) to determine the intrinsic viscosity of the polymer of a known molar mass.

Clementi[20] et al. determined the empirical relationship between $[\eta]$ and weight-average molar mass ($M_w$):

$$[\eta]=0.023M_w^{0.984}$$  \hspace{1cm} (4)

Where the unit of $[\eta]$ is dL/g, and $M$ is given by kDaltons. $M_w$ values from kelp and alginate from other algal sources are estimated from intrinsic viscosity data using equation 4. As shown in Table 1, the values of all alginates have a magnitude of $10^5$ g/mol, including A, B, C, D, E and NaAlg samples. The molar mass values of all samples were lower than most of the values reported by Sargassum alginate. These samples have a medium-lower molecular weight (0.85×$10^5$~2.74×$10^5$ g/mol). Medium-low molecular weight sodium alginate could reduce blood lipid and has a health-care function for the production of healthy functional food and pharmaceutical raw materials.

3.4. Infrared measurements
Fourier Transform Infrared Spectroscopy (FT-IR) spectrum of sodium alginate from Laminaria digitate shows typical absorption bands in Figure 3. A broad band at 3146 cm$^{-1}$ was assigned to hydrogen bonded O–H stretching vibrations and the other at 2900 cm$^{-1}$ was attributed to C–H stretching vibrations. According to previous studies[17], the absorption at 1600 cm$^{-1}$ was carboxylate O–C–O asymmetric stretching vibrations, and at 1400 cm$^{-1}$ was assigned to C–OH deformation vibration with contribution of O–C–O symmetric stretching vibration of carboxylate group. The band measured at 1200 cm$^{-1}$ may be attributed to C–C–H (and O–C–H) deformation. The band measured at 900 cm$^{-1}$ was assigned to the $\alpha$-L-guluronic asymmetric ring vibration, and the band at 860 cm$^{-1}$ is C$_1$-H deformation vibration of $\beta$-mannuronic acid residues[23].

![Figure 3: FT-IR spectra of sodium alginate.](image-url)
4. Conclusion
In this paper, alginate was extracted, purified and characterized. First, we tried to establish a relationship between the extraction solution and the extraction yield. We have found that an alkaline solution formed by the introduction of ammonia gas into 3.5% sodium chloride is a suitable condition for obtaining a high extraction yield. Secondly, the average molecular mass of alginate is between $0.85 \times 10^5$–$2.74 \times 10^5$ g/mol, molecular weight sodium alginate could reduce blood lipid and has a health-care function for the production of healthy functional food and pharmaceutical raw materials. The intrinsic viscosity is medium, which may be due to the presence of reducing substances in kelp may cause degradation, which may cause a decrease in alkaline extraction.

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