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# Technological process for preparing low molecular weight sulfated polyguluronate from brown algae *Turbinaria ornata* grown in Nha Trang, Vietnam

Nguyen Dinh Thuat [1], Le Truong Trung Lien [1] and Dang Xuan Cuong [2,*]

[1] *Nha Trang Institution of Technology Research and Application, 650000 Khanh Hoa, Vietnam.*
[2] *Ho Chi Minh City University of Industry and Trade, 700000 Ho Chi Minh, Vietnam.*

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## Abstract

Brown seaweed contains many biologically active ingredients such as fucoidan, alginate, phlorotannin (polyphenol), iodine, and laminarin with antioxidant, antibacterial, cancer cell inhibition and anti-ageing properties. Sodium alginate is present in large quantities in brown seaweed and is used to prepare low molecular weight polyguluronate sulfate, a substance with diverse biological activities used in medicine and functional foods. This study uses physical and chemical methods in chemistry to study the preparation of alginate from brown seaweed and low molecular weight polyguluronate sulfate from alginate. The results of preparing alginate and low molecular weight polyguluronate sulfate from the brown seaweed *Sargassum* have been summarized in the current study. Gel chromatography exhibited the molecular weight from 10 kDa to 60 kDa for polyguluronate sulfate. Alginate and low molecular weight polyguluronate sulfate was prepared. The results will be the basis for the production and commercialization of alginate and low molecular weight polyguluronate sulfate and their application in pharmacology and functional foods.

**Keywords:** Alginate; Low molecular weight; Sulfated polyguluronate; Technological process; *Turbinaria ornata*

## 1. Introduction

Brown seaweed *Turbinaria ornate* (Fig. 1) contains 20-40% alginate (raw material for producing low molecular weight polyguluronate sulfate (LPG)) [6]. Alginate is a natural anionic polymer of seaweed that has been widely researched and used in many biomedical applications thanks to its biocompatibility, low toxicity, relative cost, and ease of gel formation with chemicals. divalent cation [4]. Alginate is a linear copolymer containing linked blocks of (1,4) β-D-mannuronate (M) and the remaining α-L-guluronate (G). The blocks consist of consecutive G residues (GGGGGG), consecutive M remainders (M) and alternating M and G remainders (GMGMGM) [15]. In addition, seaweed has other valuable ingredients such as endo and exo laminarin 1→3-β-D-glucan, endo 1→6-β-glucan, fucoidan, and other metabolites, low molecular weight including molecules such as mannies, free amino acids, polyphenols, iodine-containing compounds, vitamins and polyunsaturated fatty acids [1,2,7]. Alginate appears in different fields, for example, food, biotechnology, medicine, paper, textile and raw rayon. Low molecular weight polyguluronate sulfate is prepared from sodium alginate (SA) through reaction with a rare sulfating agent ($N(SO_3Na)_3$) synthesized from sodium bisulfite ($NaHSO_3$) and sodium nitrite ($NaNO_2$) in water [5]. The low molecular weight sulfated polyguluronate of alginate in seaweed can reduce cholesterol levels and are then eliminated by the digestive system, effectively affecting high blood pressure, preventing the absorption of toxic chemicals, and helping the body protect against cancer-causing agent, clean the digestive system, protect the surface of the stomach and intestinal cell membranes, exhibit anti-coagulant, anti-inflammatory and antioxidant properties, and in particular, these polysaccharides do not exist in any plant on earth. The low molecular weight sulfated polyguluronate possess the heparin-like activities [3,6,7,15-17]. A study into LPG production is of great significance in improving the effectiveness of seaweed use, as well as creating a new medicinal herb capable of treating

* Corresponding author: Dang Xuan Cuong

several diseases such as anti-coagulation and anti-inflammation, preventing kidney stones at a low cost is extremely necessary.

## 2. Material and methods

**Figure 1** Brown seaweed *Turbinaria ornate*

### 2.1. Material

The collected seaweed (Fig. 1) in May 2021 in Nha Trang Bay, Khanh Hoa province of Vietnam is washed with seawater to remove mechanical impurities (mud, sand, non-brown algae), dried naturally, and chopped into small pieces before being used for study.

### 2.2. Methods

#### *2.2.1. Preparation of sodium alginate*

The sample was stirred with $Na_2CO_3$ solution (25 mL) and combined temperature and time, the extract collection was by centrifugation, and the residue treatment was a second time under similar conditions. The treatment of pooled soda extract was at room temperature with five drops (110 mg; 0.035 mL) of bromide (Br-) and stirred for a few minutes for the dissolved bromide and kept overnight. The decolourized solution was separated for three days with distilled water, transferred to a 500 mL flask, brought to the mark with distilled water and filtered (if necessary) through filter paper.

#### *2.2.2. Preparation of low molecular weight polyguluronate sulfate*

The separation of MG, M (β-D mannuronic acid), and G (α-L-guluronic acid) blocks was according to Fenoradosoa's method. For example, the pKa value of the M block is 3.38, while the *p*Ka of the G block is 3.65. The M blocks are soluble in water (pH 2.85), but the G blocks are not. Therefore, two polysaccharide fractions can be separated by pH solution after the hydrolyzed alginate. Alginate hydrolyzation is in 0.5mol/L HCl at 100°C for 8 hours. Polyguluronat hydrolyzation is by fractional precipitation at pH 2.85. Preparation of polyguluronate sulfate was according to Lihong *et al.* (2011) [5]. $NaHSO_3/NaNO_2$ agent used in sulfation. Low molecular weight polyguluronate sulfate is formed through the agent $H_2O_2$.

#### *2.2.3. Experiment design*

The design of experiments was according to the classical method. For example, fix other factors and run a factor.

#### *2.2.4. Analysis method*

- Alginate content: The sample solution was by colourimetric reaction with 3,5-dimethylphenol and sulfuric acid. Sodium alginate (100 mg/L, BDH, UK) was a standard substance.
- Molecular weight: Gel chromatography was used for determining the molecular weight of polyguluronate sulfate.
- Sensory targets: Sensory targets were evaluated according to National Standard [9].
- Sulfate content: Sulfate content was quantificated by the method of Priscila et al. (2021) [8].

- Heavey metal: Heavey metal quantification was according to Vietnam National Standard [10].
- Bacterial determination: Determination of bacterial number was based on Vietnam National Standard, such as total aerobic bacteria (CFU/g) [11], *E. coli* (CFU/g) [12], *Salmonella* (CFU/25g) [13], and fungi and mold (CFU/g) [14].

### 2.3. Data analysis

Data was analysed with descriptive statistics and removed outliers using the Duncan method.

## 3. Results and discussion

The results showed that the condition for the optimal sulfation agent preparation reaction is the concentration ratio $M_{NaHSO_3}/M_{NaNO_2}$ of 4.25, reaction at 90°C for 90 minutes. These conditions are suitable for further investigations to find optimal conditions for the polyguluronate sulfate preparation reaction. The ratio between the concentration of sulfation agent and polyguluronate is 2:198 mol/g. The reaction condition is chosen at 40°C for 4 hours as the optimal sulfation process. At the conditions, an exchange rate of 1.75 corresponds to a sulfate content of 14.9%. The preparation and creation of low molecular weight derivatives with specific and higher biological activity than the original alginate is a growing new trend. The conducted study will allow proposals for forming new seaweed processing and use technologies. Molecular weight was determined by gel chromatography (GPC) when short-circuiting using $H_2O_2$ at different concentrations, determined low molecular weight polyguluronate sulfate (Fig. 2) with values ranging from 10 kDa to 60 kDa (Fig. 6).

**Figure 2** Low molecular weight sulfated polyguluronate product from brown algae *Turbinaria ornate* [6]

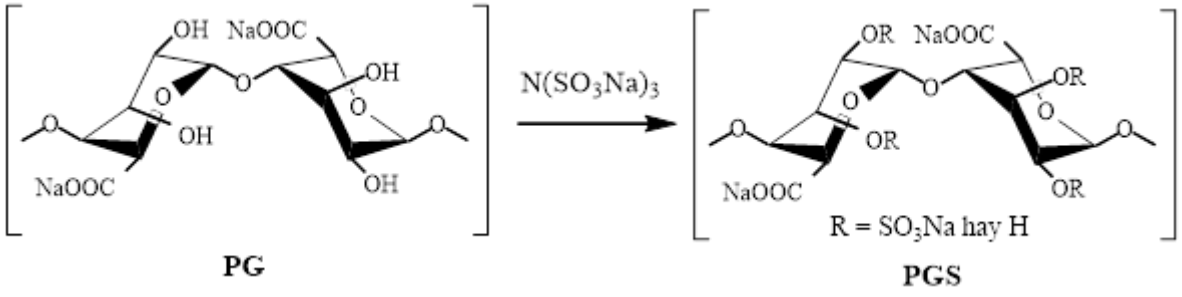


**Figure 3** Sulfation of polyguluronate [6]

The exchange rate increased as the ratio between the sulfation agent and polyguluronate concentrations increased from 1/198 to 2/198 but then decreased and remained unchanged even though the concentration ratio increase continuously. The concentration ratio of sulfate agent ($N(SO_3Na)_3$) to a hydroxyl group in an anhydroglucose unit is 1:2. Each anhydroglucose sodium alginate unit contains two hydroxyl groups. Thus, according to the stoichiometric ratio, each molecule of the sulfating agent will react with one unit of anhydroglucose, or in other words, calculated according

to the theoretical value of $M_{NaNO_2}/M_{NaAlg}$ is 1/198 (Fig. 3), which can lead to a complete reaction. However, the $NaNO_2$ prepared for the sulfating agent is less than 1M, and the activity of $N(SO_3Na)_3$ will gradually decrease with the reduction of the $SO_3Na$ group. Therefore, $N(SO_3Na)_2$ will not react completely until chemical kinetic equilibrium is established, so the amount of $NaNO_2$ required will be higher than the theoretical value. The technical process for producing sodium alginate and low molecular weight polyguluronate sulfate from brown algae *Turbinaria ornata* is shown and presented in Fig. 4.

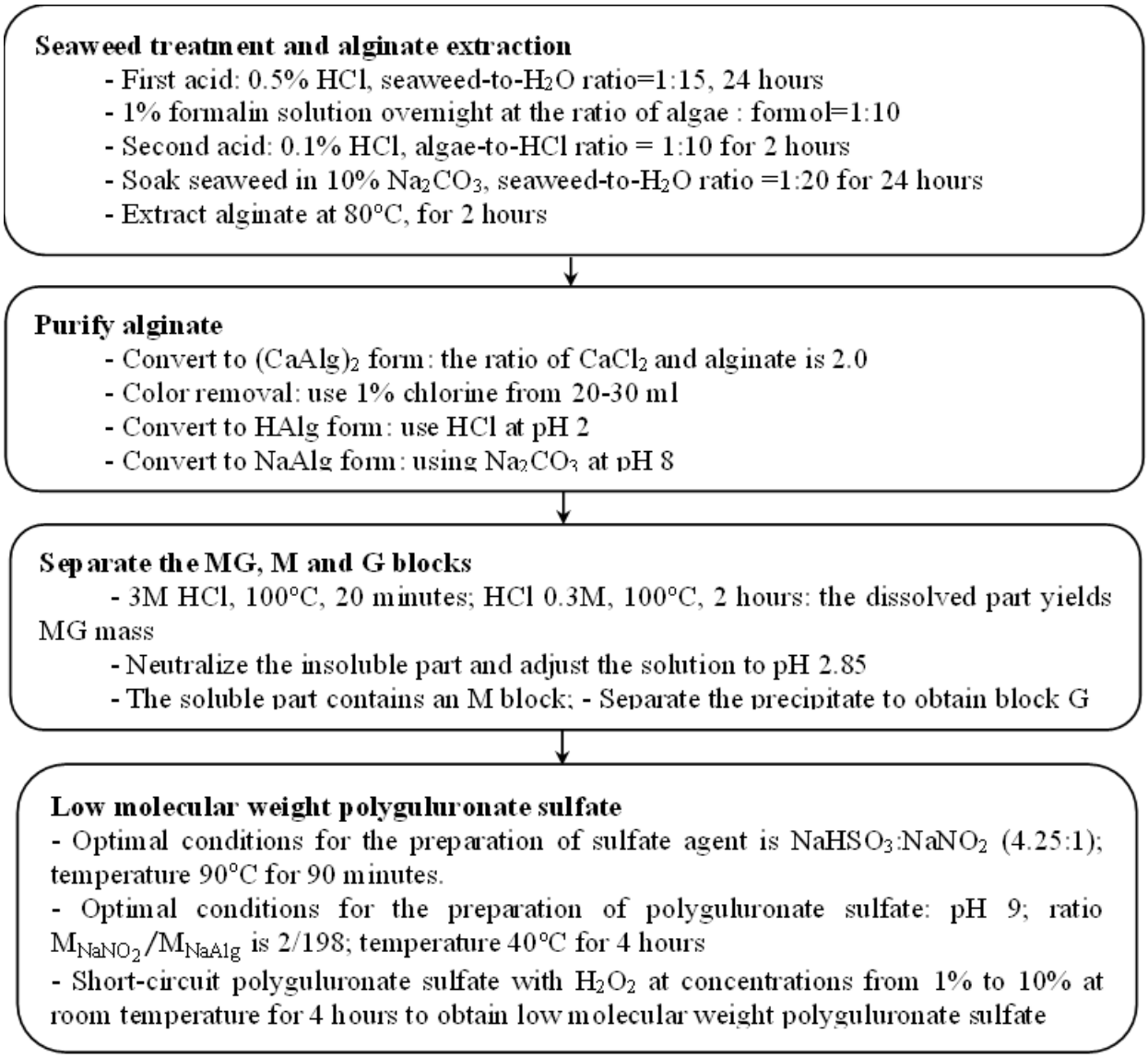


**Figure 4** Technological process for preparing low molecular weight polyguluronate sulfate

Wherein, process for separating alginate blocks was done according to Fig. 5.

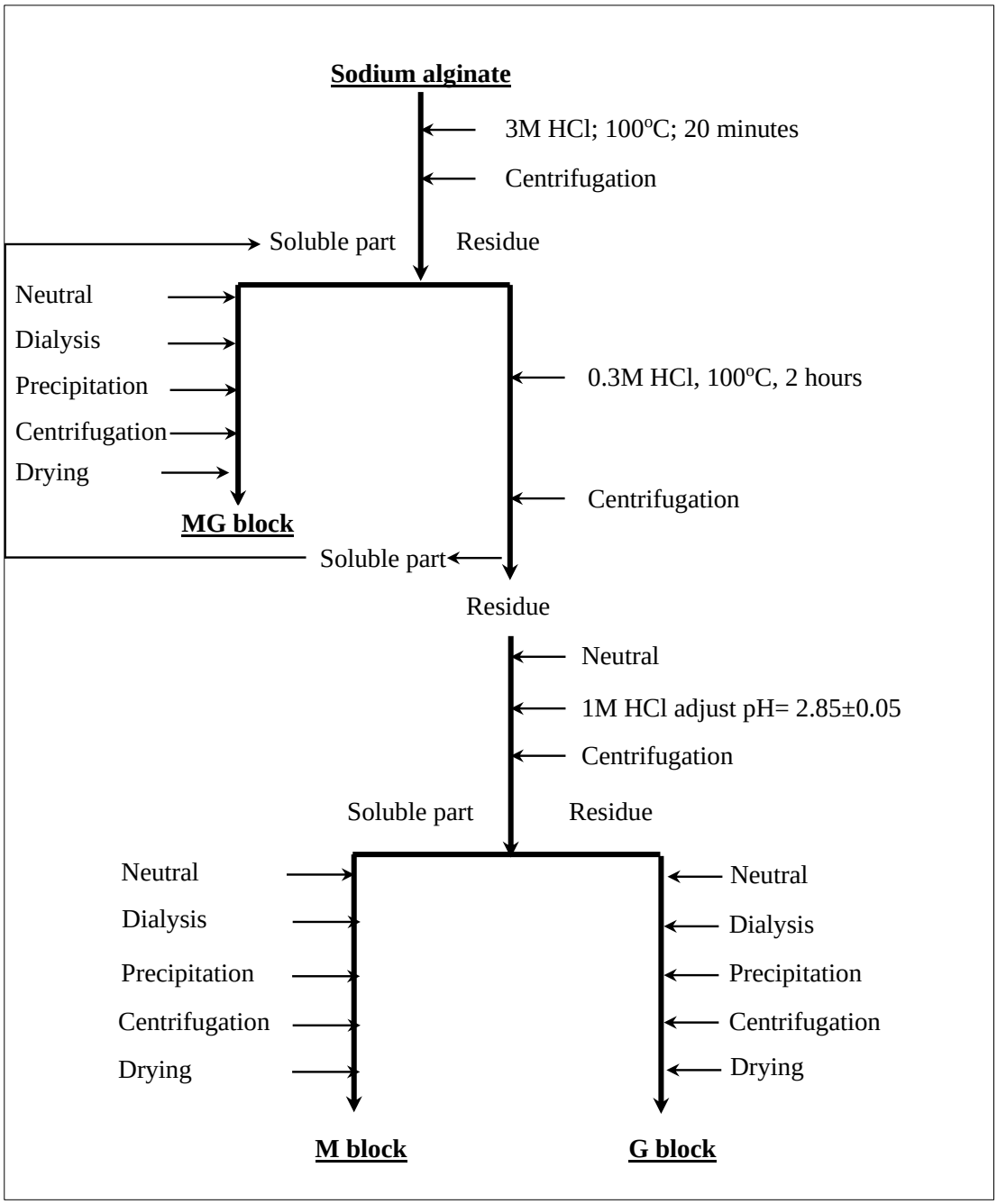


**Figure 5** Process for separating alginate blocks

For alginate to convert into a, it is necessary to go through basic steps, such as neutral, dialysis, precipitation, centrifugation, and drying (Fig. 5). The neutralized insoluble part was by 1M NaOH to pH 7, then adjusted with 1M HCl to pH 2.85 ± 0.05. After centrifuging at 10,000 rpm to obtain the soluble and insoluble parts. The neutralized soluble solution was by 1M NaOH, dialyzed for 72 hours, continuously precipitated with alcohol at a ratio of 1:1 (v:v), washed three times with alcohol, filtered, and dried to obtain the bulk of fraction M. The neutralized insoluble precipitate was by 1M NaOH, dialyzed for 72 hours, then precipitated with alcohol at a ratio of 1:1 (v:v), washed three times with alcohol, filtered, and dried to obtain segmented block G. The results showed molecular weight polyguluronate sulfate from 10 kDa to 60 kDa (Fig. 6).

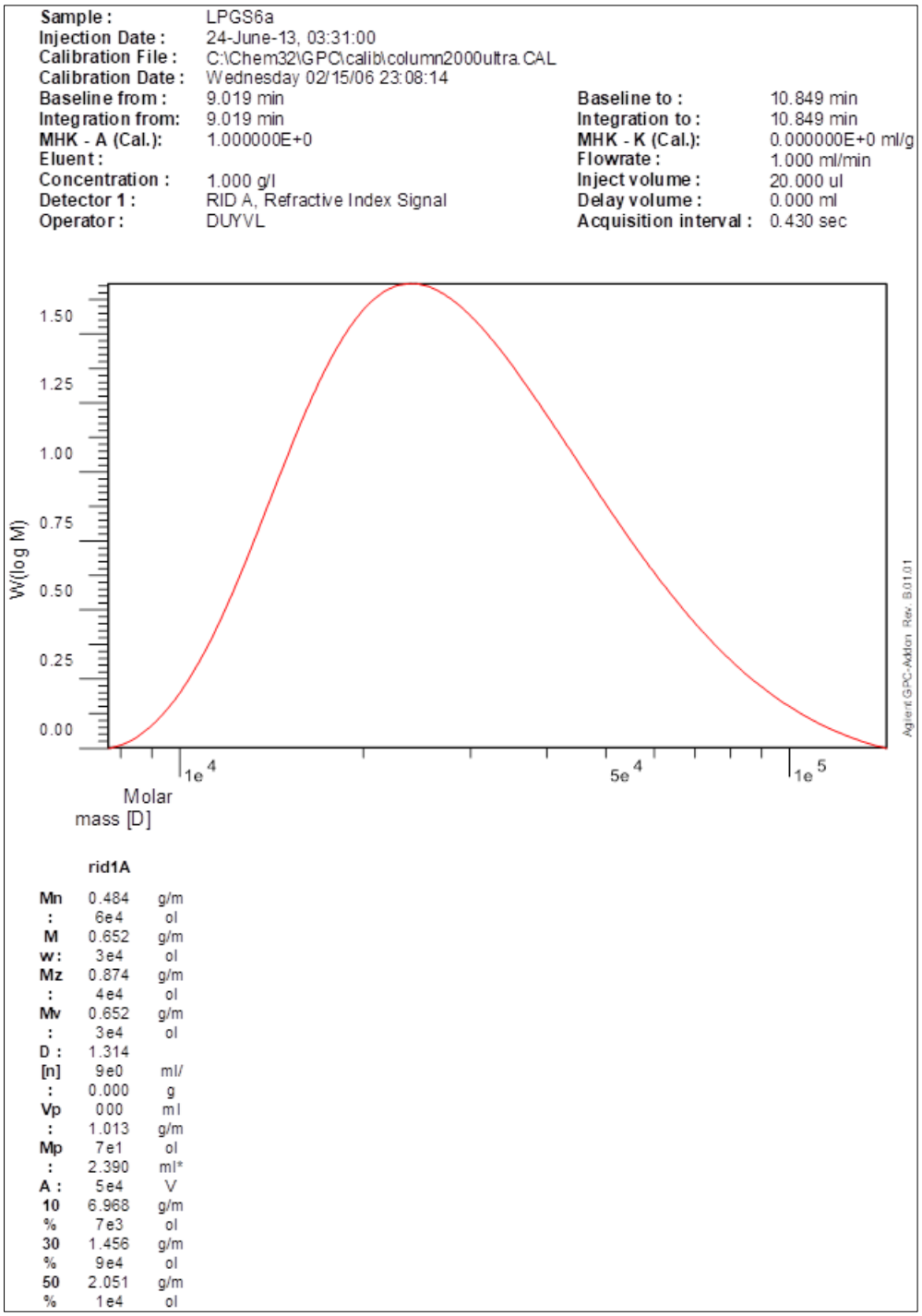


**Figure 6** Molecular mass distribution of low molecular weight sulfated polyguluronate from brown algae *Turbinaria ornate*

From low molecular weight sulfated polyguluronate, their sensory, bacterial and physical-chemistry targets were analysed (Table 1, 2 and 3). The results showed low molecular weight sulfated polyguluronate get the standard of Vietnam Ministry of Health. Low molecular weight sulfated polyguluronate has complete applicability and commercial viability. Production process a is to meet the requirements for manufacturing pharmaceutical products and functional foods.

**Table 1** Sensory properties of low molecular weight sulfated polyguluronate

| Order | Target | Resutls |
|---|---|---|
| 1 | Color | White to ivory yellow |
| 2 | Taste | No characteristic taste |
| 3 | Homogeneous | Powder State |

**Table 2.** Physicochemical indicators

| Order | Target | Resutls |
|---|---|---|
| 1 | Humidity | Max 10 % |
| 2 | Hg | < 0.5 ppm |
| 3 | As | < 2.0 ppm |
| 4 | Cd | < 0.5 ppm |
| 5 | Pb | < 0.5 ppm |
| 6 | Molecular weight | < 100kDa |
| 7 | Sulfate content | > 12% |

**Table 3.** Bacterial indicators

| Order | Target | Resutls |
|---|---|---|
| 1 | Total aerobic bacteria (CFU/g) | < 1,000 |
| 2 | Fungi and mold (CFU/g) | < 100 |
| 3 | *E. coli* (CFU/g) | (-) |
| 4 | *Salmonella* (CFU/25g) | (-) |

The humidity of LPS was lower than 10%, and its sulfate content was higher than 12%. *E. coli* and *Salmonella* were non-detection. The maximum limit of total aerobic bacteria (CFU/g) and fungi and mould (CFU/g) were lower than 1,000 and 100, respectively. Cd, Hg and Pb content of LPS were lower than 0.5 ppm. Maximum As content was lower than 2.0 ppm.

## 4. Conclusion

The results showed that alginate and LPS were created from the brown seaweed *Turbinaria ornata* growing in Nha Trang city, Khanh Hoa province, Vietnam. The molecular weight of LPS ranges from 10 kDa to 60 kDa. The sensory criteria, microorganisms, heavy metals, and sulfate content of LPS meet Vietnam Ministry of Health standards. The production conditions for alginate and LPS are favourable and easy with commercial chemicals and physicochemical techniques in the pharmaceuticals and functional foods field. From the results of this proposed process, it is possible to create alginate and LPS for wide application in functional foods and pharmaceuticals.

## Compliance with ethical standards

### *Acknowledgments*

Research on the production of low molecular weight polyguluronate sulfate from alginate in Vietnamese brown algae source to apply in pharmaceutical products. Code: Vast 06.05/12-13 - Direction: Science and Technology Marine Technology. Project leader: Dr. Nguyen Dinh Thuat.

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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