3.0. CHAPTER 1
EXTRACTION AND CHARACTERISATION OF SODIUM ALGINATE
FROM BROWN SEAWEED SARGASSUM POLYCYSTUM

3.1. INTRODUCTION

Seaweeds are macroscopic marine algae found attached on the rocky bottom in relatively shallow coastal waters. They are growing in the metrical, shallow and deep sea areas up to 180 meters depth and also in estuaries. Seaweeds are the major source of polysaccharides such as agar agar, carageenan, fucoidan, laminaran and alginates, which are extensively used in various industries such as food, confectionary, textiles, pharmaceutical, dairy and paper, mostly used as gelling, stabilizing and thickening agents (Percival and McDowell, 1967). The polysaccharides contain large proportions of L-fucose and sulfate, together with minor amounts of other sugars like xylose, galactose, mannose and glucuronic acid (Percival and McDowell, 1967; Duarte et al., 2001).

Laminarans, fucoidans and alginic acids are the most abundant polysaccharides of brown seaweeds; their contents generally vary from 40 to 80% of dry weight of defatted algal biomass. The content of water-soluble polysaccharides from different sources and their structures were shown to depend on the environment and some other factors (Percival and McDowell, 1967; Elyakova and Zvyagintseva, 1974; Painter, 1983; Zvyagintseva et al., 1994; Nagumo and Nishino, 1997; Honya et al., 1999). Chemical structure of polysaccharides from seaweeds has been investigated extensively in the past (Haug, 1964; Percival and McDowell, 1967; Fleury and Lahaye, 1991; Patankar et al., 1993; Chizhov et al., 1998; Duarte
et al., 2001). Their structures vary according to the seasons, age of population, species and geographic locations (Graham and Wilcox, 2000). Alginate is one of the polysaccharides, composed of mannuronic (M) and guluronic (G) acid with β (1, 4)-linkages and the structure varies according to the monomer position on the chain, forming either homopolymeric or heteropolymeric segments. The molecular weight of alginate ranges generally between 500 and 1000KDa. Its solubility is influenced by various factors such as pH, concentration of ion in solution, the presence of divalent ions like calcium (Morris and Norton, 1983).

The major structural polysaccharide of brown seaweeds (Phaeophyta) is alginate, a linear copolymer of 1, 4-linked β-D-mannopyranuronic acid (M) and 1,4-linked α-L-gulopyranuronic acid (G) residues, arranged in heteropolymeric and homopolymeric blocks (Painter, 1983; Larsen et al., 2003). The content of uronic acids varies with species and tissue types and partial acid hydrolysis of alginic acids allows the preparation of fractions enriched in hetero- and homopolymeric blocks (Haug et al., 1974; Craigie et al., 1984). Alginates should be distinguished from other phycocolloids, they are extracted from brown seaweeds (Phaeophyta). Alginic acid is a linear polymer based on two monomeric units, β-D mannuronic acid and α-L guluronic acid (Wilma, 1990).

Alginates consist of (1,4) linked β-D-mannuronic acid with 4 C1 ring conformation. The two uronic acid residues, in pyranosic conformation offer three varying sequences identified after partial acidic hydrolysis, as the blocks by consisting of nearly equal proportion of both monomers with a high number of MG dimers, the blocks GG and MM (Haug and Larsen, 1966). Although seaweeds are
the main sources of alginates, acetylated alginates are excreted by some telluric bacteria such as several species of the genus *Pseudomonas* and *Azolobacter* (Sabra *et al.*, 2001), However commercial alginates are mainly extracted from the seaweeds like *Ascophyllum nodosum*, *Laminaria* spp, *Lessonia nicrescens*, *Ecklonia marimba*, *Macrocystis pyrifera*, *Durvillea antartica*, etc., where the yield will be reached up to 40% of the dry weight (Draget *et al.*, 2002; Rinaudo, 2007). They are employed notably in food industry for their thickening characters and for their gel forming abilities (Sabra *et al.*, 2001). The major structural polysaccharide of brown seaweeds in alginic acid, a ten ear copolymer of (1→4) linked β-D-mannopyramanuronic acid (m) and (1→4) linked α-L-gulopyramuronic acid (G) residues, arranged in heteropolymeric and homo polymeric blocks (Painter *et al.*, 1983; Larsen *et al.*, 2003). The content of uronic acids with species and tissue types, and partial hydrolysis of alginic acid allows the preparation of fractions enriched in water and homopolymeric blocks (Haug *et al.*, 1974; Craigie *et al.*, 1984).

Alginate is located in the cell wall and the matrix of the algae cementing the cells together and giving certain mechanical properties to the algae (Baardseth, 1966; Vreeland, 1972). The extraction of alginate may thus be regarded as a process in two steps viz transformation of insoluble alginate into a soluble form namely sodium alginate, followed by diffusion of the soluble glycouronan into solution (Haug *et al.*, 1974). For commercial and scientific purposes, the most important property of alginates in their ability to form viscous solutions in water, and these alginate samples are usually characterized by means of their intrinsic viscosity (Haug and Smidsrod, 1962). The ability of alginates to form gels in the presence of calcium ions is one of their main nonfunctional properties, and in also of greater
industrial interest. The formation of gels depends mainly upon auto cooperatively formed junctions between chain regions enriched in GG sequences (Smidsrod and Haug, 1972; Smidrrod et al., 1972). Considering the importance of the above, in this chapter, a study was carried out to extract and characterize the sodium alginate of brown seaweed *Sargassum polycystum* with the following objectives

**Objectives**

1. To extract the sodium alginate from brown seaweed *S. polycystum* and to quantify the yield of sodium alginate.
2. To analyze the physicochemical as well as phytochemical properties of extracted sodium alginate.
3. To study the structural characteristics of extracted sodium alginate by UV-vis spectral analysis, FT-IR analysis, $^{13}$C and $^{1}H$ NMR analysis.
3.2. MATERIALS AND METHODS

3.2.1. Collection of seaweed

The brown seaweed *Sargassum polycystum* (Plate 3.1) was collected from the coastal villages of Kanyakumari District, Tamil nadu, India. The taxonomical position of *S. polycystum* is given below

Division : Phaeophyta  
Class : Phaeophyceae  
Order : Fucales  
Family : Sargassaceae  
Genus : *Sargassum*  
Species : *polycystum*

3.2.2. Extraction of sodium alginate

The sodium alginate was extracted from the brown seaweed *S. polycystum* by the modified method of Torres *et al.* (2007). The collected seaweed was washed thoroughly and dried under shade at room temperature. The dried seaweed was ground well by using mixer grinder and sieved using nylon sieve in order to remove seaweed fibre. 100g of milled seaweed sample was weighed and soaked in 2% formaldehyde taken in air tight conical flask for 24h. After 24h, the formaldehyde solution was filtered out and the residue was washed with distilled water for 2 to 3 times. Then 0.2 M HCl solution was added to the residue and kept at room temperature for 24h. After 24 h, the solution was removed and the residue was washed with distilled water for 2 to 3 times. The residue was extracted with 2% sodium carbonate for overnight. The extract was filtered through muslin cloth bag
and the filtrate was bleached with 2.5% sodium hypochloride. Then the solution was evaporated and dried at 60º C in hot air oven. The final product was scraped from the beaker and made into powder. The powdered product was weighed to calculate sodium alginate yield (Plate 3.2).

\[
\text{Yield of sodium alginate (\%) = } \frac{\text{Weight of sodium alginate}}{\text{Weight of milled seaweed}} \times 100
\]

3.2.3. Determination of purity of sodium alginate (phytochemical analysis)

To determine the purity of sodium alginate, tests for alkaloids, carbohydrates, flavonoids, steroids, phlobatannins, glycosides, terpins, saponins, tannins and phenols were carried out using standard methodologies (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993).

3.2.3.1. Alkaloids

0.5g of sodium alginate was dissolved in 10ml of diluted HCl (0.1N) and filtered. The filtrate was used to test the presence of alkaloids. Dragendorff’s reagent was added to the filtrate, formation of red colored precipitate indicated the presence of alkaloids.

3.2.3.2. Saponins

0.5 g of sodium alginate was dissolved in 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. An appearance of creamy mass of small bubbles indicated the presence of saponins.
3.2.3.3. Tannins

0.5 g of sodium alginate was boiled with 10 ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration indicated the presence of tannins.

3.2.3.4. Phlobatannins

0.5 g of sodium alginate was dissolved in 5ml of distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate indicated the presence of phlobatannins.

3.2.3.5. Flavonoids

0.5 g of sodium alginate was dissolved in diluted NaOH and then HCl was added. A yellow solution that turns colorless indicated the presence of flavonoids.

3.2.3.6. Steroids

2ml of acetic anhydride was added to 0.5 g of sodium alginate with 2 ml of H₂SO₄. The colour changed from violet to blue or green in samples indicated the presence of steroids.

3.2.3.7. Terpenoids (Salkowski method)

0.5 g of sodium alginate was added to 2 ml of chloroform. Then 3ml of concentrated H₂SO₄ was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids.

3.2.3.8. Cardiac glycosides (Keller-Killiani test)

0.5 g of sodium alginate was dissolved in 5 ml of distilled water. Then 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This
was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the
interface indicated the presence of deoxysugar which is the characteristic of
cardenolides. A violet ring may appear below the brown ring, while in the acetic
acid layer a greenish ring may form just above the brown ring and gradually spread
throughout this layer.

3.2.3.9. Phenolics

100mg of sodium alginate was boiled with 1ml of distilled water and filtered.
Then 2ml of filtrate was taken and 2ml of 1% ferric chloride solution was added in a
test tube. Formation of bluish black color indicated the presence of Phenolic
nucleus.

3.2.3.10. Carbohydrates (Molisch’s test)

500mg of sodium alginate was dissolved in 5ml of distilled water and
filtered. The filtrate was used to test the presence of carbohydrates. To 1ml of
filtrate, 2 drops of Molisch’s reagent was added in a test tube and 2ml of Conc.
H₂SO₄ was added carefully along the side of the test tube. Formation of violet ring at
the junction indicated the presence of carbohydrates.

3.2.4. Physical characteristics of sodium alginate

The physical properties such as organoleptic characters, pH, particle size,
moisture content, boiling point and solubility of sodium alginate were analyzed by
the following methodology.
3.2.4.1. Organoleptic evaluation

The organoleptic evaluation is to find out the color, odour, taste and texture of extracted sodium alginate. The above characters were evaluated based on the methodologies described by Kumar et al. (2011).

3.2.4.2. Estimation of pH (Schofield and Taylor, 1955)

1g of sodium alginate was dissolved in 100ml of distilled water. The pH of sodium alginate was then determined with the help of a digital pH meter (Digital pH meter, model 2001, Digisum electronics system).

3.2.4.3. Particle size measurement

The ocular micrometer was used to measure the size of individual particle by using a microscope. The powdered sample of sodium alginate was dispersed in glycerin and a smear of the dispersion was made on a glass slide and examined under microscope. The size of 500 particles was measured using a calibrated ocular micrometer (Kumar et al., 2011).

3.2.4.4. Moisture content

Moisture content is the loss in mass of the sodium alginate on heating at 105 ± 1°C under the specified operating conditions.

Apparatus

Metal dishes 7-8cm diameter and 2.3cm width provided with tight slip on covers.

Procedure

0.1g of sodium alginate was weighed and kept in a previously dried and tarred dish. Then the sodium alginate was heated in an oven at 105 ± 1°C for 1h.
Then the dish was taken out from the oven and immediately closed the lid and cooled in a desicator containing phosphorus pentoxide. Then the weight was taken and again heated the dish with sample in oven for a further period of 1h, cooled and reweighed. Repeated this process until change in weight between two successive observations did not exceed 1mg. The experiment was carried out in triplicate to determine the mean value.

\[
\text{Moisture and volatile matter present by weight} = \frac{W_1 \times 100}{W}
\]

Where, \(W_1\) = Loss in g of material on drying

\(W\) = Weight in g of the material taken for test

3.2.4.5. Solubility of sodium alginate

Solubility of sodium alginate was checked with different organic solvents and acids. For this, 0.1g of sodium alginate was dissolved in 1ml of individual solvents such as water, ethanol, methanol, Dimethylsulfoxide (DMSO), acetone, ether, chloroform, Dichloromethane (DCM) and acids such as hydrochloric acid (HCl) and sulphuric acid (\(H_2SO_4\)). Then the solubility was observed.

3.2.5. Biochemical constituents of sodium alginate

The biochemical components such as protein, carbohydrate, fucose, sulfate and ash content (total ash, acid insoluble ash and water soluble ash) of sodium alginate were estimated by the following procedures.
3.2.5.1. Protein estimation (Lowry et al., 1951)

Principle

The carbonyl group of protein reacts with the copper ion present in the alkali solution and then this complex reacts with phosphomolybdic acid present in folin phenol reagent and get reduced with tyrosine and tryptophan.

Reagents

80% Ethanol

80ml of ethanol was dissolved in 20ml of distilled water

NaOH (0.1 N)

For 0.1 N NaOH solution, 400mg of NaOH was dissolved in 100ml distilled water.

NaOH (1N)

For the preparation of 1N NaOH solution, 4g of NaOH was dissolved in 100ml distilled water

Solution A

Solution A was prepared by dissolving 2g of sodium carbonate in 100ml of 0.1N NaOH.

Solution B

Solution B was prepared by dissolving 500mg of copper sulphate in 1% sodium potassium tartarate (1g of sodium potassium tartarate in 100ml distilled water).

Solution C

Solution C was prepared by mixing 50ml solution A with 1ml solution B
**Folin phenol reagent**

Folin phenol reagent was prepared by mixing 1ml folin phenol with 1ml distilled water.

**Blank**

For blank, 0.5ml of 1N NaOH, 5ml of solution C and 0.5ml of folin phenol were taken.

**Procedure**

A known amount of sodium alginate was taken and ground it well with 80% ethanol. Then it was centrifuged at 5000 rpm for 15 min. Then the precipitate was taken and dissolved in 1N NaOH and made up to 5ml. From this, 0.5ml was taken, followed by 5ml of solution C was added and kept it for 10min. Finally, 0.5 ml of folin phenol reagent was added and the intensity of colour developed was read at 640 nm in a spectrophotometer.

**Calculation**

Protein present in the sample (%) = \( \frac{\text{OD of the sample} \times \text{Concentration of the std}}{\text{OD of the standard}} \times \frac{100}{\text{Weight of the sample}} \)

**3.2.5.2. Carbohydrate estimation (Seifter et al., 1950)**

**Principle**

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxyl methyl furfurol. This compound forms green coloured products with Anthrone reagent.
Reagents

Anthrone reagent

200mg of Anthrone was dissolved in 100ml of ice cold 95% sulphuric acid.

Stock standard glucose

100 mg of glucose was dissolved in 100ml distilled water.

Working standard

10 ml of stock standard glucose was diluted in 100 ml distilled water.

Procedure

The carbohydrate content of sodium alginate was estimated by Anthrone method. To an aliquot of sodium alginate sample, 4ml of anthrone reagent was added and incubated in a boiling water bath for 15min. Tubes were then cooled to room temperature at dark condition. Then the optical density was measured at 750 nm by using spectrophotometer. Here glucose (100 mg / 100 ml distilled water) was used as the standard.

Calculation

Carbohydrate present in the sample (%) = \( \frac{\text{OD of the sample} \times \text{Concentration of the std} \times 100}{\text{Weight of the sample}} \)

\[ \text{OD of the standard} \]

3.2.5.3. Lipid estimation (Folch et al., 1957)

Principle

Quantitative determination of sulpho-phosphovanillin method depends on the reaction of lipid extracted from the sodium alginate using chloroform methanol with sulphuric acid, phosphoric acid and vanillin to give a red color complex.
Reagents

Chloroform methanol (2:1)

This reagent was prepared by mixing 200 ml of chloroform with 100 ml of methanol

Sodium chloride (0.9%)

900 mg of NaCl was dissolved in 100 ml of distilled water.

Sulpho-phosphovannilin reagent

200 mg of vannilin powder was dissolved in 100ml of 80% orthophosphoric acid.

Standard

8 mg of cholesterol was dissolved in 4 ml of chloroform methanol mixture (2:1).

Blank

5ml of chloroform methanol (2:1) was taken as a blank.

Procedure

Known weight of sodium alginate was taken and homogenized well with 4ml of chloroform methanol (2:1) mixture. After mixing well, 0.2 ml of 0.9% sodium chloride was added and kept the mixture overnight at room temperature. The lower layer of lipid was collected carefully and dried in a vacuum desiccator. The dried total lipid was dissolved by using concentrated sulphuric acid by keeping in boiling water bath for 10min. From the above prepared total lipid sample, 0.2 ml was taken in a test tube and 5ml of sulpho-phosphovannilin reagent was added, shaken well and kept for 30 min. The intensity of red color was measured at 520nm by using spectrophotometer.
Calculation

Lipid present in the Sample (%) = OD of the sample \times \text{Concentration of the std} \times 100
\[\frac{\text{OD of the standard}}{\text{Weight of the sample}}\]

3.2.5.4. Estimation of fucose content

The fucose content of sodium alginate was determined by phenol sulphuric acid method proposed by Dubois et al. (1956).

Reagent preparation

1. 89% phenol: 80g of phenol was dissolved in 20ml of distilled water and dissolved at 50° C.

2. Concentrated sulphuric acid.

Procedure

20 mg of sodium alginate was dissolved in 2 ml of distilled water taken in a test tube. Then 0.10 ml of 89% phenol reagent was added to the test-tube. Then 6.0ml of concentrated sulfuric acid was added to the test tube and mixed well. Then the solution was kept at room temperature for 10min and the optical density was measured at 490nm by using spectrophotometer (Techcomp 8500). Simultaneously a blank was also set by using distilled water instead of sample. For fucose analysis, the commercial L-fucose was used as a standard.

Fucose content of sodium alginate (%) = OD of the sample \times \text{Conc. of the std} \times 100
\[\frac{\text{OD of the standard}}{\text{Weight of the sample}}\]
3.2.5.5. Ash content

The ash content of sodium alginate was determined by incinerating 1g of sodium alginate taken in a silica crucible and kept in a muffle furnace at 600°C. After incineration, the net content was cooled and weighed and expressed in terms of percentage.

\[
\text{Ash content (\%)} = \frac{(w_1 - w_2)}{w} \times 100
\]

\(w_1\) = Fresh sample and weight of silica crucible

\(w_2\) = Incinerated samples and silica crucible

\(w\) = Weight of the sample

a. Acid insoluble ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for five minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a silica crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried sample.

b. Water-soluble ash

The ash obtained as described for the determination of total ash was boiled for 5 min. with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was then transferred into silica crucible, ignited for 15 min, and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of the total ash. The difference of weight was considered as water-soluble ash. The
percentage of water-soluble ash was calculated with reference to the air dried sample.

3.2.5.6. Estimation of sulfate content (Dodgson and Price, 1962)

Estimation of the sulphate content of polysaccharide involves acid hydrolysis, followed by determination of liberated inorganic sulphate by turbidometric method. In this method sulphate is estimated turbidometrically as barium sulphate, light absorption at 360nm being measured and gelatin being used as a cloud stabilizer.

Reagents preparation

1. **Barium chloride reagent:** 2g of gelatin was dissolved in 400ml of hot water (60-70°C) and allowed to stand at 4°C for 6h. Then 2g of barium chloride was dissolved in the semi gelatinous fluid and the reagent was stored at 4°C for overnight before use.

2. **3% trichloroacetic acid:** 3g of trichloroacetic acid was dissolved in 100ml of distilled water.

Procedure

A known amount of sodium alginate was dissolved in sufficient amount of N- hydrochloric acid in glass test tube. Then it was sealed in an oxygas flame and kept in an oven at a temperature of 105 - 110°C for 5h. After being cooled, the content of the tube was mixed before opening. From this, 0.2ml was taken in a test tube containing 3.8ml of 3% trichloroacetic acid. Then 1 ml of barium chloride-gelatin reagent was added and mixed well. Then the tube was kept at room temperature for 15-20min and the optical density was measured at 360nm by using spectrophotometer (Techcomp 8500). Simultaneously a blank was also set by using
0.2ml of hydrochloric acid instead of sample. For sulphate analysis, the commercial 
K$_2$SO$_4$ was used as a standard.

Sulfate content of

\[
\text{sodium alginate (\%)} = \frac{\text{OD of the sample} \times \text{Concentration of the std} \times 100}{\text{OD of the standard} \times \text{Weight of the sample}}
\]

3.2.6. Purification of sodium alginate

250mg of sodium alginate was dissolved in 25ml of distilled water and
heated at reflux with 0.75 ml of 3.0M HCl for 3h. After cooling, the mixture was
centrifuged at 3000g and the supernatant solution was neutralized with 1.0M NaOH
and poured over 100ml of ethanol. Then the precipitate was dissolved in distilled
water and freeze dried (Fraction). Then the freeze dried sodium alginate was
hydrolyzed by the method proposed by Rioux et al. (2007).

3.2.7. Hydrolysis of sodium alginate

In order to reduce the viscosity of the sample as well as to convert the
polysaccharide into monosaccharide, the purified sodium alginate sample was
subjected for hydrolysis. For this, 20mg of purified sodium alginate was dissolved
in 5ml of distilled water and heated at 90°C for 1h. Then 1ml of 0.1 N HCl was
added to the sample and heated at 90°C for 2h. Then the sample was freeze dried
and kept for further analysis (Marais and Joseleau, 2001).
3.2.8. UV-vis spectral analysis

The sodium alginate fraction was scanned within the range between 190 and 1100nm in UV spectrophotometer (Techcomp, UV VIS 8500) for determining maximum absorbance (λ max).

3.2.9. FT-IR analysis

The qualitative analysis of the active principles of the sodium alginate was done by Fourier Transmission Infra Red (FTIR) method described by Kemp (1991).

Procedure

Preparation of KBr discs

KBr discs were prepared by grinding the sodium alginate (0.1 – 2.0 by weight) with KBr and compressing the whole into a transparent wafer or disc. The KBr was dried, and it is an advantage to carry out grinding under an infrared lamp to avoid condensation of atmospheric moisture, which gives to broad absorption at 3500 cm⁻¹.

- The particle size of grinding was achieved by grinding KBr sample complex to < 2 µm to avoid wavelength scaling.
- Given high pressure to KBr disc to condense fairly to 13 mm in diameter and 0.3 mm in thickness.

Infra Red analysis

The frequency of the spectra set to analysis was between 4000 - 400 cm⁻¹ wave number and the vibration spectrum was recorded as graphical chart. The instrument used to FTIR analysis was Shimadzu, Japan.
Applications of IR Spectroscopy to organic molecules

Organic functional groups differ from one another both in the strength of the bond(s) involved, and in the masses of the atoms involved. For instance, the O – H and C = O functional groups each contain atoms of different masses connected by bonds of different strengths. According to equation (1), we therefore expect the O – H and C = O groups to absorb IR radiation at different positions in the spectrum. The presence of a strong, broad band between 3200 and 3400 cm\(^{-1}\) indicates the presence of an O – H group in the molecule, while the presence of a strong band around 1700 cm\(^{-1}\) confirms the presence of a C = O group.

For organic molecules, the infrared spectrum can be divided into three regions. Absorptions between 4000 and 1300 cm\(^{-1}\) are primarily due to specific functional groups and bond types. Those between 1300 and 909 cm\(^{-1}\), the fingerprint region, are primarily due to more complex interactions in the molecules, and those between 909 and 650 cm\(^{-1}\) are usually associated with the presence of benzene rings in the molecules. Some particularly important regions are indicated below in the table as vibrational frequencies for organic molecules.

Vibrational frequencies for organic molecules

<table>
<thead>
<tr>
<th>Bond type</th>
<th>Specific context</th>
<th>(V_1) cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>C – H</td>
<td>C(_{sp})-3-H</td>
<td>2800 – 3000</td>
</tr>
<tr>
<td></td>
<td>C(_{sp})-2-H</td>
<td>3000 – 3100</td>
</tr>
<tr>
<td></td>
<td>C(_{sp})-H</td>
<td>3300</td>
</tr>
<tr>
<td>C=C</td>
<td>C-C</td>
<td>1250 – 1450</td>
</tr>
<tr>
<td></td>
<td>C=C</td>
<td>1600 – 1670</td>
</tr>
<tr>
<td>Bond</td>
<td>Species</td>
<td>Wavenumber Range</td>
</tr>
<tr>
<td>------------</td>
<td>---------</td>
<td>------------------</td>
</tr>
<tr>
<td>C≡C</td>
<td></td>
<td>2100 – 2260</td>
</tr>
<tr>
<td>C-N</td>
<td>C-N</td>
<td>1030 – 1230</td>
</tr>
<tr>
<td></td>
<td>C≡N</td>
<td>1640 – 1690</td>
</tr>
<tr>
<td></td>
<td>C≡N</td>
<td>2210 – 2260</td>
</tr>
<tr>
<td>C-O</td>
<td>C-O</td>
<td>1020 – 1275</td>
</tr>
<tr>
<td></td>
<td>C=O</td>
<td>1650 – 1800</td>
</tr>
<tr>
<td>C-X</td>
<td>C-F</td>
<td>1000 – 1350</td>
</tr>
<tr>
<td></td>
<td>C-Cl</td>
<td>800 – 850</td>
</tr>
<tr>
<td></td>
<td>C-Br</td>
<td>500 – 680</td>
</tr>
<tr>
<td></td>
<td>C-I</td>
<td>200 – 500</td>
</tr>
<tr>
<td>N-H</td>
<td>RNH₂, R₂NH</td>
<td>3400 – 3500 (Two)</td>
</tr>
<tr>
<td></td>
<td>R₂NH₂⁺, R₂NH₃⁺, R₃NH⁺</td>
<td>2250 – 3000</td>
</tr>
<tr>
<td></td>
<td>RCONH₂, RCONHR’</td>
<td>3400 – 3500</td>
</tr>
<tr>
<td>O-H</td>
<td>ROH</td>
<td>3610 – 3640 (free)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3200 – 3400 (H-bonded)</td>
</tr>
<tr>
<td></td>
<td>RCO₂H</td>
<td>2500 – 3000</td>
</tr>
<tr>
<td>N-O</td>
<td>RNO₂</td>
<td>1350 – 1560</td>
</tr>
<tr>
<td></td>
<td>RONO₂</td>
<td>1620 – 1640</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1270 – 1285</td>
</tr>
<tr>
<td></td>
<td>RN=O</td>
<td>1500 – 1600</td>
</tr>
<tr>
<td></td>
<td>RO-N=O</td>
<td>1610 – 1680 (Two)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>750 – 815</td>
</tr>
</tbody>
</table>

Conti…
<table>
<thead>
<tr>
<th>Structure</th>
<th>Wavenumber Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=N-OH</td>
<td>930 – 960</td>
</tr>
<tr>
<td>R₃N-O⁺</td>
<td>950 – 970</td>
</tr>
<tr>
<td>R₂SO</td>
<td>1040 – 1060</td>
</tr>
<tr>
<td>R₂S(=O)O</td>
<td>1310 – 1350</td>
</tr>
<tr>
<td></td>
<td>1120 – 1160</td>
</tr>
<tr>
<td>R-S(=O)₂-OR’</td>
<td>1330 – 1420</td>
</tr>
<tr>
<td></td>
<td>1145 – 1200</td>
</tr>
<tr>
<td>Cumulated systems</td>
<td></td>
</tr>
<tr>
<td>C=C=C</td>
<td>1950</td>
</tr>
<tr>
<td>C=C=O</td>
<td>2150</td>
</tr>
<tr>
<td>R₂C=N=N</td>
<td>2090 – 3100</td>
</tr>
<tr>
<td>RN=C=CO</td>
<td>2250 – 2275</td>
</tr>
<tr>
<td>RN=N=N</td>
<td>2120 – 2160</td>
</tr>
<tr>
<td>Out of plane bending vibrations</td>
<td></td>
</tr>
<tr>
<td>Alkynes</td>
<td></td>
</tr>
<tr>
<td>C≡C-H</td>
<td>600 – 700</td>
</tr>
<tr>
<td>Alkenes</td>
<td></td>
</tr>
<tr>
<td>RCH=CH₂</td>
<td>910, 990</td>
</tr>
<tr>
<td>R₂C=CH₂</td>
<td>890</td>
</tr>
<tr>
<td>Trans-RCH=CHR</td>
<td>970</td>
</tr>
<tr>
<td>Cis-RCH=CHR</td>
<td>725, 675</td>
</tr>
<tr>
<td>R₂C=CHR</td>
<td>790 – 840</td>
</tr>
<tr>
<td>mono-</td>
<td>730 – 770, 690 – 710 (two)</td>
</tr>
<tr>
<td>o-</td>
<td>735 – 770</td>
</tr>
<tr>
<td>m-</td>
<td>750 – 810, 690 – 710 (two)</td>
</tr>
<tr>
<td>p-</td>
<td>810 – 840</td>
</tr>
<tr>
<td>1,2,3-</td>
<td>760 – 780, 705 – 745 (two)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>1,3,5-</td>
<td>810 – 865, 675 – 730 (two)</td>
</tr>
<tr>
<td>1,2,4-</td>
<td>805 – 825, 870, 885 (two)</td>
</tr>
<tr>
<td>1,2,3,4-</td>
<td>800 – 810</td>
</tr>
<tr>
<td>1,2,4,5-</td>
<td>855 – 870</td>
</tr>
<tr>
<td>1,2,3,4</td>
<td>840 – 850</td>
</tr>
<tr>
<td>Penta-</td>
<td>870</td>
</tr>
</tbody>
</table>

Carbonyl stretching frequencies

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldehydes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCHO</td>
<td>1725</td>
<td></td>
</tr>
<tr>
<td>C=CCCHO</td>
<td>1685</td>
<td></td>
</tr>
<tr>
<td>ArCHO</td>
<td>1700</td>
<td></td>
</tr>
<tr>
<td>Ketones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2C=O</td>
<td>1715</td>
<td></td>
</tr>
<tr>
<td>C=C-C=O</td>
<td>1675</td>
<td></td>
</tr>
<tr>
<td>Ar-C=O</td>
<td>1690</td>
<td></td>
</tr>
<tr>
<td>Four-membered cyclic</td>
<td>1780</td>
<td></td>
</tr>
<tr>
<td>Five- membered cyclic</td>
<td>1745</td>
<td></td>
</tr>
<tr>
<td>Six- membered cyclic</td>
<td>1715</td>
<td></td>
</tr>
<tr>
<td>RCOOH</td>
<td>1760 (Monomer), 1710 (Dimer)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1720 (Monomer), 1690 (Dimer)</td>
<td></td>
</tr>
<tr>
<td>RCO₂</td>
<td>1550-1610, 1400 (two)</td>
<td></td>
</tr>
<tr>
<td>Esters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCOOR</td>
<td>1735</td>
<td></td>
</tr>
<tr>
<td>C=C-COOR</td>
<td>1720</td>
<td></td>
</tr>
<tr>
<td>ArCOOR</td>
<td>720</td>
<td></td>
</tr>
<tr>
<td>γ-lactone</td>
<td>1770</td>
<td></td>
</tr>
</tbody>
</table>

Conti.....
3.2.10. $^{13}$C and $^1$H NMR analysis

After hydrolysis, the sample was dissolved in 0.5ml D$_2$O (Deutrium dioxide) and the proton number and carbon number of sodium alginate were identified and confirmed by $^1$H and $^{13}$C NMR experiments using a Bruker Biospin Avance 400 NMR spectrometer ($^1$H frequency = 400.13 MHz, $^{13}$C frequency = 100.62 MHz) at 298 K using 5-mm broad band inverse probe head equipped with shielded z-gradient and XWIN-NMR software version 3.5 using TMS as an internal reference. One-dimensional $^1$H and $^{13}$C spectra were obtained using one pulse sequence. One-dimensional $^{13}$C spectra using Spin Echo Fourier Transform (SEFT) and Quaternary Carbon Detection (QCD) 42sequences were also performed to aid the structure identification (Jayaprakash and Kalaiselvi, 2007).
3.3. RESULTS

The present study was undertaken to extract and characterize the sodium alginate from seaweed *S. polycystum* through UV, FT-IR, $^{13}$C & $^1$H NMR analysis. The detailed results are given below.

3.3.1. Sodium alginate yield

The yield of sodium alginate extracted from brown seaweed *S. polycystum* was $15.908 \pm 0.453 \%$ (Table 3.1).

3.3.2. Determination of purity of sodium alginate by phytochemical analysis

The phytochemical results indicated the absence of alkaloids, saponins, tannins, phlobatannins, flavonoids, steroids, terpenoids, cardiac glycosides and phenols. Only carbohydrate as well as sugar derivative of saponins were found to be present in the sodium alginate, which confirmed the better purity of the extracted sodium alginate (Table 3.2).

3.3.3. Physical properties of sodium alginate

The physical properties such as organoleptic characters, pH, particle size, moisture content, boiling point and solubility of extracted sodium alginate determined are given in Table 3.3.

3.3.3.1. Organoleptic characters evaluation

The organoleptic characters such as colour, odour, taste and texture of sodium alginate of *S. polycystum* were in the order of whitish yellow colour, odourless (seaweed odour), salty taste and powdery appearance, respectively.
3.3.3.2. pH of sodium alginate

The pH of 1% sodium alginate of *S. polycystum* solution was 9.6 ± 0.20.

3.3.3.3. Particle size of sodium alginate

Totally 500 particles of sodium alginate were measured by using ocular micrometer. The particles were grouped into different size ranges (0-10µm to 61-70µm). Among these, a maximum of 27.6% of particles were within the size range between 11 and 20µm, whereas minimum of 6.4% of particles were within the size range from 51 to 60µm.

3.3.3.4. Moisture content of sodium alginate

The moisture content of sodium alginate observed was 12 ± 0.42 %.

3.3.3.6. Solubility of sodium alginate

The solubility behavior of sodium alginate indicated that it was readily soluble in hydrochloric acid and sulphuric acid, but it was insoluble in all the other tested organic solvents such as distilled water, ethanol, methanol, DMSO, acetone, ether, chloroform, dichloromethane and n- butanol.

3.3.4. Biochemical composition of sodium alginate

The result on the biochemical composition of sodium alginate is given in Table 3.4. The major component recorded in sodium alginate was carbohydrate (43.57 ± 1.72 %), with little amount of protein (4.62 ± 0.12) and lipid (3.78 ± 0.14%) contents. The fucose content of sodium alginate was 27.29 ± 0.82%. The ash values such as total ash, acid insoluble ash and water soluble ash of sodium
alginate were 1.83 ± 0.063, 0.121 ± 0.0068 and 0.982 ± 0.028%, respectively. The sulphate content of sodium alginate recorded was 13.43 ± 0.481 %.

3.3.5. UV spectral analysis

The UV spectral analysis of purified sodium alginate is given in Fig. 3.1. The UV vis spectral analysis showed the presence of uronic acid and manuronic acid at the absorption maxima of 203, 205, 207, 302 and 305nm, respectively.

3.3.6. FT-IR analysis

The FT-IR analysis determined for the extracted sodium alginate is given in Table 3.5 and Fig. 3.2. In the wave number of 3500–1500 cm\(^{-1}\) region, four bands appeared with a broad band centered at 3428.32cm\(^{-1}\), it was assigned to hydrogen bond (O–H) stretching vibrations, the weak signal at 2934.86 cm\(^{-1}\)was due to C–H stretching vibrations, the wavelength at 2150.30 cm\(^{-1}\) indicated the presence of C=C=O and the asymmetric stretching of carboxylate O–C–O vibration at 1613.46 cm\(^{-1}\). The band at 1416.52 cm\(^{-1}\) may be due to C–OH deformation vibration with contribution of O–C–O symmetric stretching vibration of carboxylate group. The weak bands at 1088.87 cm\(^{-1}\) may be assigned to C–O stretching, and C–O and C–C stretching vibrations of pyranose rings; the band at 1033.10 cm\(^{-1}\) may also be due to C–O stretching vibrations. The spectrum showed a band at 947.96 cm\(^{-1}\), which was assigned to the C–O stretching vibration of uronic acid residues, and one at 895.65 cm\(^{-1}\) assigned to the C1–H deformation vibration of β-mannuronic acid residue. The band at 814.19 cm\(^{-1}\) seems to be characteristics of mannuronic acid residues. The band at 675.66 and 624.80cm\(^{-1}\) may be due to Cis-RCH=CHR and C≡C-H stretching vibrations.
3.3.7. $^{13}$C and $^1$H NMR analysis

The results on $^{13}$C and $^1$H NMR spectral analysis of purified sodium alginate are given in Fig. 3.3 and 3.4. The $^{13}$C NMR spectrum showed absorptions corresponding to a β-D mannanuronic acid at ppm 99.98 (C-1), 66.94 (C-2), 69.53 (C-3), 79.96 (C-4), 71.23 (C-5) and 175.43 (C-6). Similarly, the $^1$H NMR spectrum showed the correlation of these signals with the ppm of 5.551 (H-1), 3.996 (H-2), 3.722 (H-3), 3.911 (H-4), 3.801 (H-5) and 1.096 (H-6), respectively.
3.4. DISCUSSION

Alginate is one of the major polysaccharide components in brown algae (Percival, 1979). In the present study sodium alginate was extracted from brown seaweed *S. polycystum* and it was characterized through FT-IR and NMR analysis. The yield of sodium alginate observed was $15.908 \pm 0.453\%$. Usually the sodium alginate content of various seaweeds is varied much. Immanuel *et al.* (2012) have extracted the sodium alginate from *S. wightii* and observed its yield as 16.352%. Similarly, Torres *et al.* (2007) reported the yield of sodium alginate extracted from *S. vulgare* as 16.9%. Davis *et al.* (2004) found the yield within the range of 21.1 – 24.5% in *S. fluitans* and 16.3 – 20.5% in *S. oligocystum* with variations being depended on the alginate extraction method followed and also the variation in the species of seaweeds.

The phytochemical analysis was carried out in the present study to determine the purity of the extracted sodium alginate. This result indicated that the extracted sodium alginate of *S. polycystum* is pure form and it has no trace of alkaloids, tannins, phlobatannins, flavonoids, steroids, terpenoids, cardiac glycosides, phenols, but the only element carbohydrate and its derivative saponins were present, which confirmed its better purity. Similarly, Kumar *et al.* (2011) have reported the purity of tamarind seed polysaccharide by phytochemical analysis, which indicated the absence of alkaloids, steroids, flavonoids, saponins, tannins and phenols, however the only chemical component carbohydrate was found to be present, which confirmed the purity of polysaccharide of tamarind seed.
In the present study, the physical characteristic features such as organoleptic properties, pH, particle size, moisture content, boiling point and solubility of sodium alginate were determined. The color, odor, taste, texture, pH and moisture content of sodium alginate were in the order of whitish yellow color, odourless, salty taste, powder form, 9.6 and 12%, respectively. Xiamen JieJing Biology Technology Co., Ltd. (2007) have extracted the sodium alginate from marine alga and observed the organoleptic characters. They observed that the colour, odour, texture and taste of sodium alginate was white or light yellow, vagiform powder, odorless and tasteless, respectively. Likewise, Suqian Broad Seaweed Industry Co., Ltd, China, have extracted and characterized the sodium alginate from seaweeds. They reported that colour, taste, texture, pH and moisture content of sodium alginate were white or light yellow colour, tasteless, granule or powder, 6.0 – 8.0 pH and ≤ 15% moisture content, respectively. According to FAO (1995), the colour, texture and moisture content of sodium alginate of seaweeds were white to yellowish brown, filamentous, grainy, granular or powder forms and 15%, respectively. Moreover, Science Lab.com (Chemical and Laboratory equipment) (2005) have published the physical properties of sodium alginate and they quoted that the color, odor, taste and melting point of sodium alginate were respectively with white to off white color, odourless, tasteless and >300°C.

The particle size of sodium alginate was determined by using ocular micrometer. Totally 500 particles of sodium alginate of S. polycystum with different size groups were measured. Among these 6.4 to 27.6 % particles were within the size range of 0 – 10 to 61 - 70µm, respectively. Similarly, Kumar et al. (2011) measured 500 particles of tamarind seed polysaccharide by ocular micrometer.
method. They reported that 2% of particles were within the size range of 0 - 30µm, 10.4% of particles were within the size range of 30 - 60µm, 51.6% of particles were within the size range of 60-90µm, 34% of particles were within the size range of 90-120µm and only 3.6% of particles were in the size group of >120µm. Invariably, FMC Biopolymer (2008) have reported the size range of sodium alginate particles after extracted from brown seaweed was between 75 and 250µm.

In the present study, the solubility behavior of sodium alginate indicated that it is readily soluble in hydrochloric acid and sulphuric acid, but it is insoluble in certain organic solvents like ethanol, methanol, DMSO, acetone, ether, chloroform, Dichloromethane and n- butanol. According to FAO (1995) report, the solubility behavior of sodium alginate is that it is slowly soluble in water, forming a viscous solution and insoluble in ethanol and ether. Science Lab.com (Chemical and Laboratory equipment) (2005) have published the solubility behavior of sodium alginate, as it is readily soluble in cold and hot water and insoluble in diethyl ether.

The biochemical composition of sodium alginate was estimated. The biochemical components such as protein, carbohydrate, lipid, fucose, total ash, acid insoluble ash, water soluble ash and sulphate contents of sodium alginate were recorded as 4.62, 43.57, 3.78, 27.29, 1.83, 0.121, 0.982 and 13.43 %, respectively. Similarly, Torres et al. (2007) have reported that the biochemical composition of sodium alginate extracted from S. vulgare. They observed the protein content of 1.1 and 1.0 % for S. vulgare low-viscosity alginate (SVLV) and S. vulgare high-viscosity alginate (SVHV) samples, respectively. The moisture and ash content of SVLV and SVHV was 14 & 16% and 2 & 1%, respectively. Larsen et al. (2003)
have reported the biochemical composition of alginates of algae harvested from the Egyptian Red Sea coast. They observed that the total carbohydrate and fucose contents were 74.93 and 11.63%, respectively in *Cystophyllum trinode*, 57.87 and 5.62%, respectively in *S. dentifolium*, 32.16 and 4.15%, respectively in *S. asperifolium* and 42.26 and 8.24%, respectively in *S. latifolium*.

In the present study, the UV spectral analysis of purified sodium alginate showed the presence of uronic acid and manuronic acid at the absorption maxima of 203, 205, 207, 302 and 305nm and the FT-IR analysis revealed the presence of uronic acid (C-O stretching) at the wave length of 947.96 cm$^{-1}$ and the vibrations at 895.65 and 814.19 cm$^{-1}$ indicated the presence of β-mannuronic acid and mannuronic acid residues, respectively. In accordance with these, Leal *et al.* (2008) have reported the FT-IR analysis of sodium alginate in three species of brown seaweeds. They observed a spectral band at 948.5 cm$^{-1}$, which was assigned to be the C–O stretching vibration of uronic acid residues, and one at 888.3 cm$^{-1}$ assigned to the C1–H deformation vibration of β-mannuronic acid residues and the band at 820.0 cm$^{-1}$ seems to be characteristic of mannuronic acid residues. Zhang *et al.* (2008) have reported the -OH groups present in sodium alginate are clearly seen at 3400 cm$^{-1}$. They also suggested that the peaks attributed to the -CH2 groups present at 2931 cm$^{-1}$ and 2926 cm$^{-1}$ in sodium alginate and some distinct peaks such as carboxyl group showed strong absorption bands at 1614 cm$^{-1}$, 1416 cm$^{-1}$ and 1306 cm$^{-1}$, due to carboxyl anions. The band at 1648 cm$^{-1}$ is attributed to the absorption band of the carbonyl (-HC=O) stretching. The other band at 1041 cm$^{-1}$ that was assigned to the stretching vibration of CH-OH appeared at 1643 cm$^{-1}$ and 1045 cm$^{-1}$, respectively for the composite gel beads.
$^1$H and $^{13}$C NMR spectroscopies are reliable methods for the determination of the composition and also the block structures of alginate molecules (Panikkar and Brasch, 1996; Larsen et al., 2003). In the present study, the C and H NMR analysis were performed after hydrolysis of sodium alginate fraction. The result of C and H NMR indicated the presence of carbon and anomeric proton of guluronic acid and manuronic acid. Similarly, Torres et al. (2007) have reported the NMR analysis of S. vulgare alginate and they observed the guluronic acid anomeric proton (G-1) at 5.06 ppm; guluronic acid H-5 (G-5) at 4.4 ppm; and mannnuronic acid anomeric proton (M-1) and the C-5 of alternating blocks (GM-5) overlapped at 4.7 ppm. Larsen et al. (2003) have studied the NMR spectrum of alginates from algae harvested from the Egyptian Red Sea coast. They reported the presence of guluronic acid and manuronic acid protons at 3.50 to 3.56 and 3.80 to 4.80ppm, respectively and the carbon at 71.2 to 72.4 and 93.4 to 94.8ppm in S. asperifolium alginate. The present results clearly emphasized that the brown seaweed S. polycystum synthesized a polysaccharide composed of guluronic acid and manuronic acid, which constitute sodium alginate.
3.5. SUMMARY

The present study was undertaken to extract and characterize the polysaccharide sodium alginate from brown seaweed *S. polycystum*. The highlights of the study are summarized below.

- An initial investigation was carried out to extract sodium alginate from brown seaweed *S. polycystum* and the yield obtained was 15.908 ± 0.453 %.

- The organoleptic characters of sodium alginate indicated: whitish yellow color, seaweed odour, salty taste and the texture was in powder form. Likewise, the physical properties indicated that it has 9.6 pH and 12% moisture content. The particle size of the grains of sodium alginate was in between 0 to 70µm. It is readily soluble in hydrochloric acid and also in sulphuric acid.

- The biochemical components such as protein, carbohydrate, lipid, fucose, total ash, acid insoluble ash, water soluble ash and sulphate contents of sodium alginate of *S. polycystum* were 4.62, 43.57, 3.78, 27.29, 1.83, 0.121, 0.982 and 13.43%, respectively.

- UV vis spectral analysis and FT-IR analysis were carried out to assign the functional groups of purified sodium alginate after hydrolysis. These observations indicated the presence of uronic acid and manuronic acid residues in the extracted sodium alginate sample.
The result on $^{13}$C and $^1$H NMR analysis indicated the presence of carbon and anomeric proton of guluronic acid and β-D-mannuronic acid in purified sodium alginate.