FT-IR, $^1$H- NMR AND $^{13}$C- NMR SPECTROSCOPY OF ALGINATE EXTRACTED FROM TURBINARIA DECURRENS (PHAEOPHYTA)

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ABSTRACT

The present study extracted and characterized the alginate from brown algae collected from the shores of Mandapam region, Gulf of Mannar, Southeast coast of India. Variations in yield and viscosity were observed in pre-treated material. The extraction for 5 hr exhibited high yield of alginate. The alginate extracted for 1 hr and 5 hr from Turbinaria decurrens was characterized by FT-IR, $^1$H NMR and $^{13}$C NMR spectroscopy.

KEY WORDS: Seaweeds, alginate yield, viscosity, FTIR, $^1$H NMR, $^{13}$C NMR, Spectroscopy.

INTRODUCTION

Brown algae (Phaeophyceae) contains large amounts of anionic polysaccharides in their matrix and cells.[1] Alginate is a (1→4) linked α-L-gulurono- β-D-mannuronans of broadly fluctuating composition and sequential structure.[2] The chemical composition changes according to seasonal growth behaviors such as season, age, geographic location.[3] The noteworthy property of alginites is the ability to form viscous solutions in water.[4] Alginates are extensively used in the pharmaceutical, cosmetic, food and biotechnology industries.[5,6] Low viscosity alginate is used in paper making and fruit industry whereas high viscosity is normally used in food and cosmetics. Sargassum and Turbinaria are common tropical genera, and more than 400 species of Sargassum occur worldwide.[7] Turbinaria is comparatively species-poor, with only 17 species described.[8]
The infrared spectra technique allows identifying the main polysaccharides\cite{9} while $^1$H NMR spectroscopy is used to characterize both the alginate composition and distribution sequence of the two uronic acid. It is mainly useful for quantitative work when only small amounts of material are available.\cite{10} The main focus of the present study was to extract the alginic acid from different brown seaweeds, to record the yield and viscosity and also to characterize the alginate obtained from the brown seaweed, *Turbinaria decurrens*.

**MATERIALS AND METHODS**

**Sample collection and preparation**

Seaweeds collected from Mandapam region of Gulf of Mannar were used for the extraction of alginic acid. The algae were washed with tap water to remove dirt, sand and then the same was shade-dried until constant weight was obtained. The sample was ground in an electric mixer and stored the powdered samples in refrigerator for future use.

**Extraction of alginic acid**

Pre-treatment of seaweed was done with different concentrations of formalin i.e 1%, 5%, 10%, 20%, 30% and 40% for 24 hours at room temperature, then dried and powdered. The alginic acid was extracted by the method described by.\cite{11,12} The material without formalin treatment formed the control in this experiment. The viscosity of 1 % alginate samples was determined using Ostwald’s viscometer. The values are expressed in mPa.

**Chemical characterization of the alginate extracted from *Turbinaria decurrens***

The moisture content was determined by drying 0.1 g of the sample at 105°C until constant weight was obtained. For ash determination, sample was heated at 600°C. The total carbon, hydrogen and nitrogen contents were determined using PE 2400 series II CHNS/O analyzer EA1112 (CE Instrument, Italy), using aspartic acid as reference standard. The sulfate content and uronic acid content was estimated using standard methods.\cite{13,14}

**FTIR spectra of alginic acid**

FTIR spectra were recorded on a Perkin Elmer Spectrometer (Perkin Elmer Ltd., Beaconsfield, BUCKS-HP9 2FX, UK). The spectra were collected from 2000-800 cm$^{-1}$ range in the transmission mode with 4 cm$^{-1}$ resolution over 40 scans.\cite{15}

**Nuclear magnetic resonance (NMR)**

The $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker 500 (Bruker Biospin AG, Fallanden, Switzerland) and Bruker 600 spectrometer operating at 500 and 600 MHz,
respectively. The 100 mg of alginate sample with 10 ml of distilled water was heated at 80°C for 1 hour, then 10 ml of 0.1 M HCl (20°C for 60 minutes), dialysed and the resulting solution was lyophilized. The freeze-dried sample was deuterium-exchanged by lyophilization with D₂O and then examined in 99.96 % D₂O.[16]

RESULT AND DISCUSSION
Alginates are the most abundant polysaccharides, constituting up to 40% of the dry weight of brown algae.[17] The alginates are widely utilized as gelling agents in pharmaceutical and food applications. Alginates do not have any nutritional value; however, they are often employed as additives to change and stabilize the texture of foods.[18,19] The potentially beneficial role of alginate in the gastrointestinal tract makes it a commercially valuable product.

Before extraction, most authors suggested a pre-extraction step using acid[20], alkali[21] or formalin.[22,23] Formaldehyde can fix the phenolic compounds and leads to discoloration have been proved. However, addition of acid may lead to loss of alginate viscosity.[22] The work also observed that the yield and viscosity were always more in pre-treated material than control.

The yield and viscosity of alginate obtained from the seaweed treated with formalin is given in the Table 1. Slight variations in yield and viscosity were observed in pre-treated material. Table 2. Shows the yield and viscosity of alginate extracted from the selected brown seaweeds. The alginic acid extraction was monitored over different time period (1 hr and 5 hr) at 80°C. The 5 hr extraction showed slightly higher yield than the 1 hr of extraction. The alginate yield increased with time, was found to be similar to earlier reports.[24] The result obtained in the present study for Turbinaria decurrens was higher than that reported for alginate from Sargassum vulgare.[24] However, it was lower than alginate, extracted from other algal species.[25] Earlier studies were made on two range-extending brown algae from Tahitian coral reefs, Sargassum mangarevense and Turbinaria ornata for their alginate properties, mannitol and phenolic contents, antioxidant and antimicrobial activities. They have reported Turbinaria ornata having the richest alginate content with the highest extraction yield of 19.2± 1.3% dry weight.[26]
Table 1: Pre-treatment of fresh seaweed (*Turbinaria decurrens*) with different concentrations of formalin (duration of exposure 24 hr)

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Formalin treatment</th>
<th>Yield (%)</th>
<th>Viscosity (mPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>14.2</td>
<td>12.64</td>
</tr>
<tr>
<td>2.</td>
<td>2%</td>
<td>9.9</td>
<td>12.32</td>
</tr>
<tr>
<td>3.</td>
<td>10%</td>
<td>9.6</td>
<td>12.44</td>
</tr>
<tr>
<td>4.</td>
<td>20%</td>
<td>16.9</td>
<td>12.82</td>
</tr>
<tr>
<td>5.</td>
<td>30%</td>
<td>12.2</td>
<td>12.44</td>
</tr>
<tr>
<td>6.</td>
<td>40%</td>
<td>15.8</td>
<td>13.01</td>
</tr>
</tbody>
</table>

Table 2: Yield and viscosity of alginate extracted from selected brown seaweeds

<table>
<thead>
<tr>
<th>Seaweeds</th>
<th>Yield (%) 1 hours</th>
<th>Viscosity (mPa) 1 hours</th>
<th>Yield (%) 5 hours</th>
<th>Viscosity (mPa) 5 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. wightii</em></td>
<td>14.21</td>
<td>12.82</td>
<td>21.71</td>
<td>14.32</td>
</tr>
<tr>
<td><em>P. tetrastromatica</em></td>
<td>12.40</td>
<td>12.64</td>
<td>19.70</td>
<td>14.14</td>
</tr>
<tr>
<td><em>C. minima</em></td>
<td>12.70</td>
<td>11.99</td>
<td>20.20</td>
<td>13.49</td>
</tr>
<tr>
<td><em>H. triqueta</em></td>
<td>19.20</td>
<td>13.59</td>
<td>26.70</td>
<td>15.09</td>
</tr>
<tr>
<td><em>S. myriocystum</em></td>
<td>12.60</td>
<td>11.50</td>
<td>20.10</td>
<td>13.02</td>
</tr>
<tr>
<td><em>S. plagiophyllum</em></td>
<td>15.0</td>
<td>12.08</td>
<td>23.90</td>
<td>14.47</td>
</tr>
<tr>
<td><em>S. ilicifolium</em></td>
<td>14.26</td>
<td>11.06</td>
<td>22.76</td>
<td>12.76</td>
</tr>
<tr>
<td><em>T. decurrens</em></td>
<td>22.70</td>
<td>13.19</td>
<td>25.50</td>
<td>15.92</td>
</tr>
</tbody>
</table>

The physicochemical characteristics of sodium alginate obtained from *Sargassum crassifolium*, *Sargassum polycystum*, *Padina* sp., and *Sargassum echinocarpum*, from the coral coast of Gunung Kidul Yogyakarta Indonesia have been reported.[27] Here the average yield is 16.93 to 30.50 %. The physical properties such as viscosity and lightness are 25 to 39 cps and 46.2 to 52.3 (the color is yellow to light brown). In the present study, maximum viscosity (13.19 and 15.92 mPa; 1 hr and 5 hr respectively) was recorded in *Turbinaria decurrens* and minimum viscosity (11.06 and 12.76 mPa; 1hr and 5 hr respectively) was recorded in *Sargassum ilicifolium*. An increase in time of the extraction procedure resulted in higher solution viscosity, probably due to dissolution of high molar mass macro molecules. The effect of extraction time on viscosity was observed similar to that of *Sargassum vulgare* [24] but the values were very less when compared to other alginates.[27] The alginates studied from Red Sea brown algae (*Cystoseira trinode*, *Cystoseira myrica*, *Sargassum dentifolium*, *Sargassum asperifolium*, and *Sargassum latifolium*) have shown high molar fraction of G and a homopolymeric block type structure. The viscosity of alginates ranges from 8.6 to 15.2.[28] The analytical data obtained for 1 hr and 5 hr extracted alginate were more or less similar (Table. 3). The protein value determined was 1.72 % for 5 hr and it is similar to that reported in the literature.[24] In a previous study, alginate from *S. fluitans* and *S. siliquosum* contains no
detectable nitrogen content; however, *S. thumbergii* has 6.9 % of protein. Similarly, the % C and % H were more or less in the same range in the present study as reported previously.\[^{24}\]

It is interesting to note that very few works are able to identify the link between the structure and the rheological behavior of the polysaccharides.\[^{29}\] The chemical characterization, spectral, viscometric and thermodynamic study of the sodium alginates from brown algae of Karachi coast with 2, 1 electrolyte have been carried, so as to use this polymer in industrial and pharmaceutical industry.\[^{30}\]

### Table 3: Chemical characterization of alginate extracted from *Turbinaria decurrens*

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Analytical parameter</th>
<th>Extraction (1 hour)</th>
<th>Extraction (5 hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alginic acid yield</td>
<td>22.70%</td>
<td>25.50 %</td>
</tr>
<tr>
<td>2.</td>
<td>Viscosity mPa (1h &amp; 5h)</td>
<td>13.19 mPa; 14.43 mPa</td>
<td>13.96 mPa; 15.92 mPa</td>
</tr>
<tr>
<td>3.</td>
<td>Moisture %</td>
<td>12.06</td>
<td>9.81</td>
</tr>
<tr>
<td>4.</td>
<td>Ash %</td>
<td>5.43</td>
<td>4.30</td>
</tr>
<tr>
<td>5.</td>
<td>H %</td>
<td>4.60</td>
<td>4.17</td>
</tr>
<tr>
<td>6.</td>
<td>C %</td>
<td>32.02</td>
<td>29.65</td>
</tr>
<tr>
<td>7.</td>
<td>N %</td>
<td>0.36</td>
<td>0.56</td>
</tr>
<tr>
<td>8.</td>
<td>Sulphate %</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>9.</td>
<td>Uronic acids %</td>
<td>42.18</td>
<td>34.72</td>
</tr>
<tr>
<td>10.</td>
<td>Protein</td>
<td>2.03</td>
<td>1.72</td>
</tr>
</tbody>
</table>

The FT-IR spectrum of sodium alginate (1hr and 5hr) from *Turbinaria decurrens* is presented in the Fig. 1 and 2. The FT-IR spectrum of sodium alginate from *S. turbinarioides* showed a broad band at 3,428 cm\(^{-1}\) was assigned to hydrogen bonded O–H stretching vibrations and two other at 2,929 and 1,608 cm\(^{-1}\) were attributed to C–H stretching and to carboxylate O–C–O asymmetric stretching vibrations, respectively. Similarly, in the present study also a broad band at 3,417.86 cm\(^{-1}\) was assigned to hydrogen bonded O–H stretching vibrations and two other at 2,926 and 1,629.25 cm\(^{-1}\) were attributed to C–H stretching and to carboxylate O–C–O asymmetric stretching vibrations, respectively.
Fig. 1: FT-IR spectra of sodium alginate (1 hr) from *Turbinaria decurrens*

Fig. 2: FT-IR spectra of sodium alginate (5 hr) from *Turbinaria decurrens*

According to [31, 32], the absorption at 1415 cm$^{-1}$ is assigned to C–OH deformation vibration with contribution of O–C–O symmetric stretching vibration of carboxylate group. The bands measured at 1315, 1090 and 1033 cm$^{-1}$ may be attributed to C–C–H (and O–C–H) deformation, to C–O stretching vibrations and C–O (and C–C) stretching vibrations of pyranose rings, respectively. Likewise, in the present study the absorption at 1413.82 cm$^{-1}$ was assigned to C–OH deformation vibration with contribution of O–C–O symmetric stretching vibration of carboxylate group. The bands measured at 1,317.38, 1,097.50 and 1,033.85 cm$^{-1}$ were due to C–C–H (and O–C–H) deformation, to C–O stretching vibrations...
and C–O (and C–C) stretching vibrations of pyranose rings, respectively. Comparable spectral characteristics have been reported erstwhile.\(^{[9]}\)

The band at 946 cm\(^{-1}\) is indicative of uronic acid presence by the C–O stretching vibration.\(^{[2,3,3]}\) More signals at 900 and 815 cm\(^{-1}\) are assigned to the \(\alpha\)-L-gulopyranuronic asymmetric ring vibration and to the mannnuronic acid residues, respectively.\(^{[2,3,1]}\) The presence of certain signals in the 13C NMR spectra supports the composition of various fractions. The signals of C-1 (100–102 ppm) and C-6 (175–178 ppm) are characteristic for alginic acids.\(^{[3,4]}\) In the present study also sodium alginate extracted from 1hr and 5 hr extraction period showed signals of C-6 (175.40 ppm) (Fig.3, 4 and Fig 5, 6).

Fig. 3. \(^1\)H- NMR spectra for sodium alginate (1 hr) from Turbinaria decurrens

Fig. 4: \(^1\)H- NMR spectra for sodium alginate for (5 hr) from Turbinaria decurrens
Pre use assignment of the bands in FT-IR of polysaccharide in general is not easy task, as most of the vibrations arise from highly coupled C-O, C-OH, C-C groups. However, in the present study the band characteristic of alginate was identified. Also, the characterization of alginate was complemented by $^{13}$C-NMR.

CONCLUSION
It can be concluded that alginate extracted from *Turbinaria decurrens* is of low viscosity. The present study shows that FTIR spectroscopy allows an easier and more accurate monitoring of alginate. The characterization of seaweed phycocollloids can be complemented by $^{13}$C-NMR and $^1$H-NMR. The data can be used for the development of the phycocollloid chemotaxonomy. And it can be used in future as a matrix for enzyme and drug encapsulation.
Since it is least utilized species, in future, it can be used for industrial application of stabilization of aqueous mixtures and emulsions.

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REFERENCES


