Part-I

Chemical investigation of the polysaccharides of some brown seaweed species

Chemical studies on the alginate of the brown seaweed species: Sargassum tenerrimum, Sargassum wightii, Cystoseira indica and Padina tetrastromatica

Alginic acid is the major structural polysaccharide of brown algae belonging to the family Phaeophyceae. It is a linear block copolymer of two monomeric units, namely $\beta$-D-mannuronopyranosyl and $\alpha$-L-guluronopyranosyl units. These monomers occur in three types of blocks one containing mostly mannuronic acid (M), one mostly guluronic acid (G) and a third of intermediate composition (MG). The M/G ratio can vary from 0.4 to >20 (Painter, 1983). The main brown seaweeds that are processed commercially for the alginate worldwide are Macrocystis pyrifera, Laminaria spp, Ascophyllum spp, Ecklonia spp, Lessonia spp. None of these occur in the Indian waters. Extraction of alginic acid/alginate from brown algae and characterization has been reported by many authors (Haug & Larsen 1962; Haugh, Larsen & Smidsrod, 1974; Nishide et al. 1987). The Indian production of alginate is mainly from Sargassum spp. There are a few reports on alginate from Indian waters (Mody et al. 1992; Alankararao, 1988; Redekar and Raje 2000 and Ganesan et al. 2001). There exists, however, no report on the detailed systematic chemical investigation of alginates of Indian brown seaweeds. Therefore, Sargassum tenerrimum, Sargassum wightii, Cystoseira indica and Padina tetrastromatica were selected for detailed chemical studies for their alginates.

Alginates of Sargassum tenerrimum, Sargassum wightii, Cystoseira indica and Padina tetrastromatica were extracted and characterized. Alginates from these seaweeds were isolated following the method described by Nishide et al. (1996). M/G ratio of these alginates was determined by a rapid one-pot method of hydrolysis of
sodium alginate using microwave irradiation (Chhatbar et al. 2009a). Uronic acid sequence was determined by the method described by Haugh et al. (1974). The alginates were further characterized by XRD, FT-IR, $^{13}$C NMR (Figure 1; vide Chhatbar et al. 2009a), SEM and circular dichroism spectroscopy (CD-spectra) as well as determining their viscosity, molecular weight. The data of alginates obtained with these seaweeds were compared with Sigma alginate for bench marking. The properties of alginate samples studied are summarized in Table 1.

Table 1 Analytical data of the alginates

<table>
<thead>
<tr>
<th>Alginates of</th>
<th>Sargassum tenerrimum</th>
<th>Sargassum wightii</th>
<th>Cystoseira indica</th>
<th>Padina tetrastromatica</th>
<th>Sigma alginate (A2158-250G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield$^a$ (%)</td>
<td>18</td>
<td>20</td>
<td>10</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Viscosity (cps) (c 1.5 wt%)</td>
<td>120</td>
<td>110</td>
<td>80</td>
<td>95</td>
<td>200</td>
</tr>
<tr>
<td>M/G ratio</td>
<td>0.61</td>
<td>0.39</td>
<td>0.32</td>
<td>0.53</td>
<td>0.78</td>
</tr>
<tr>
<td>MG (%)</td>
<td>30</td>
<td>25</td>
<td>22</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>MM (%)</td>
<td>28</td>
<td>20</td>
<td>20</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>GG (%)</td>
<td>42</td>
<td>55</td>
<td>58</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

$^a$Yield was calculated on the basis of dry seaweed weight.
Chapter I.2 Chemical studies on the sulphated polysaccharides of the brown seaweed species *Cystoseira indica*, *Padina tetrastromatica* and *Sargassum tenerrimum*

**Chemical studies on the sulphated polysaccharides of *Cystoseira indica***

*Cystoseira indica* is a brown seaweed (Figure 2) species abundantly available in Indian waters, belonging to the Division-Phaeophyta (Class-Phaeophyceae, Order-Fucales, Family- Cystoseiraceae, Genus-*Cystoseira*, and Species-*indica*). Structural features and antiviral activity of sulphated fucans from the brown seaweed *Cystoseira indica* has been reported by (Mandal *et al.* 2007). The latter reports came out very recently from the same research group, when the work described in this dissertation on these polysaccharides was completed. *Cystoseira indica* used in the present study was collected in January 2008 from Diu (20° 42.727’ N, 70° 55.487’ E), from the inter-tidal zone in the west coast of India (Oza and Zaidi, 2001; www.algaebase.org). In this investigation, extraction, purification, fractionation and physicochemical characterization of the sulphated polysaccharide of *Cystoseira indica* have been studied. Sulphated polysaccharide of *Cystoseira indica* contained fucose as the major constituent (Table 2).

The sulphated polysaccharide of *Cystoseira indica* (CI*sps*) was extracted using the procedure described by Siddhanta *et al.* (2001), to yield crude cold (CWE) and hot water extracts (HWE). The yield of CWE was ca. 4% and that of HWE was ca. 6%. Carbohydrate profiling of both the extracts were done using their respective alditol
acetates (GC-MS). Crude hot water extract was fractionated into charged and neutral fractions, employing the method described by Sen et al. (2002), using CTAB. The fractions charged (CFCI<sub>sps</sub>) and neutral (NFCI<sub>sps</sub>) obtained from the crude HWE were freeze dried and the product was isolated and characterized. The CFCI<sub>sps</sub> was major in quantity (5.4%), and therefore, this was selected for detailed characterization by determining total sugar, sulphate, uronic acid, protein, and ash contents. Viscosity and optical rotation (-13.102°; 0.25%, H<sub>2</sub>O, 27°C) were measured, and elemental analysis was also done. Carbohydrate profiling of crude and fractionated (charged and neutral) sulphated polysaccharides were done by GC-MS technique according to the method of Siddhanta et al. (2001). The crude CI<sub>sps</sub> contained galactose, fucose, ribose, arabinose, xylose, and mannose while CFCI<sub>sps</sub> contained fucose, ribose, xylose, galactose, and glucose, and the NFCI<sub>sps</sub> contained fucose, ribose, arabinose, mannose, glucose and galactose units (Table 2). This investigation would provide crucial inputs for the characterization of charged and neutral polysaccharide fractions that were prepared.

**Chemical studies on the sulphated polysaccharides of *Padina tetrastromatica***

*Padina tetrastromatica* is a brown seaweed (Figure 2) species abundantly available in Indian waters, belonging to the phylum-Phaeophyta (Class-Phaeophyceae, Order-Dictyotales, Family-Dictyotaceae, Genus-*Padina*, Species-*tetrastromatica*) (www.algaebase.org). Chemical studies on sulphated polysaccharide of *Padina tetrastromatica* has been reported by (Karmakar et al. 2009). The latter reports came out very recently from the same research group, when the work described in this dissertation on these polysaccharides was completed. *Padina tetrastromatica* used in this study was collected during January-February 2008 from Okha (22° 28.580’ N, 69° 04.254’ E) from the inter-tidal zone in the west coast and Valai island (09° 10.445’ N, 78° 55.55’ E) from the inter-tidal zone in the south east coast of India (Oza and Zaidi, 2001; www.algaebase.org). In this investigation, extraction, purification and physicochemical characterization of the sulphated polysaccharide of *Padina tetrastromatica* have been studied. Sulphated polysaccharide of *Padina tetrastromatica* contained galactose and fucose as the main monosaccharide constituents.

The sulphated polysaccharides of *Padina tetrastromatica* (PT<sub>sps</sub>) were extracted using the procedure described by Siddhanta et al. (2001), to yield crude cold (CWE) and hot water (HWE) extracts. The yields of CWE and HWE were ca.5% and ca.6.5% respectively. Carbohydrate profiling of both the extracts were done using their respective alditol acetates (GC-MS). Crude hot water extract was fractionated into
charged (CFPT\textsubscript{sps}) and neutral fractions (NFPT\textsubscript{sps}), employing the method described by Sen \textit{et al.} (2002), using CTAB. The fractions (charged and neutral) obtained from the crude HWE were freeze dried and the product was isolated and characterized. The major product was the CFPT\textsubscript{sps} (5.8%), and therefore, this was selected for detailed characterization by determining total sugar, sulphate, uronic acid, protein, and ash contents. Viscosity and optical rotation (-59.24°; c0.25%, H\textsubscript{2}O, 27°C) were measured and metal analysis was also carried out. Carbohydrate profiling of crude as well as charged and neutral fractions were done by GC-MS technique (cf. Siddhanta \textit{et al.} 2001). The crude PT\textsubscript{sps} contained fucose, xylose, mannose, galactose and glucose monosaccharide units, while CFPT\textsubscript{sps} contained fucose, xylose, mannose and galactose, and NFPT\textsubscript{sps} contained rhamnose xylose, mannose, galactose and glucose units (Table 2). This investigation would provide crucial inputs for the characterization of charged and neutral polysaccharide fractions that were prepared.

**Chemical studies on the sulphated polysaccharides of \textit{Sargassum tenerrimum}**

\textit{Sargassum tenerrimum} is a brown seaweed (Figure 2) species abundantly available in Indian waters belonging to the Division-Phaeophyta (Class-Phaeophyceae, Order-Fucales, Family-Sargassaceae, Genus-\textit{Sargassum} and Species-\textit{tenerrimum}) (www.algaebase.org). Chemical studies on the sulphated polysaccharide of \textit{Sargassum tenerrimum} has not been reported in the literature. \textit{Sargassum tenerrimum} used in this study was collected during March 2008 to April 2008 from Veraval (20° 54.875’ N, 70° 20.832’ E), Diu (20° 42.727’ N, 70° 55.487’ E), from the inter-tidal zone in the west coast of India (Oza and Zaidi, 2001, www.algaebase.org). In this investigation, extraction, purification and physicochemical characterization of the sulphated polysaccharide of \textit{Sargassum tenerrimum} have been studied. Sulphated polysaccharide of \textit{Sargassum tenerrimum} contained fucose and galactose as the main monosaccharide constituents (Table 2).

The sulphated polysaccharide of \textit{Sargassum tenerrimum} (ST\textsubscript{sps}) was extracted by using the procedure described by Siddhanta \textit{et al.} (2001), to yield crude cold (CWE) and hot water (HWE) extracts. The yields of CWE and HWE were 5% and 7.5 % respectively. Crude hot water extract was further fractionated into charged and neutral fractions employing the method described by Sen \textit{et al.} (2002), using CTAB.
Table 2 Carbohydrate profile (GC-MS) of crude sulphated polysaccharides and fractions

<table>
<thead>
<tr>
<th>Carbohydrate moieties</th>
<th>Rha</th>
<th>Fuc</th>
<th>Rib</th>
<th>Ara</th>
<th>Xyl</th>
<th>Mann</th>
<th>Gal</th>
<th>Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude CI&lt;sub&gt;sps&lt;/sub&gt; (% area)</td>
<td>ND</td>
<td>29.63</td>
<td>19.08</td>
<td>7.29</td>
<td>6.55</td>
<td>4.43</td>
<td>33.01</td>
<td>ND</td>
</tr>
<tr>
<td>CFCI&lt;sub&gt;sps&lt;/sub&gt;</td>
<td>ND</td>
<td>80.25</td>
<td>7.08</td>
<td>ND</td>
<td>6.24</td>
<td>ND</td>
<td>4.16</td>
<td>2.28</td>
</tr>
<tr>
<td>NFCI&lt;sub&gt;sps&lt;/sub&gt;</td>
<td>ND</td>
<td>28.23</td>
<td>21.95</td>
<td>13.86</td>
<td>ND</td>
<td>7.79</td>
<td>2.84</td>
<td>6.27</td>
</tr>
<tr>
<td>Crude PT&lt;sub&gt;sps&lt;/sub&gt; (% area)</td>
<td>1.19</td>
<td>13.81</td>
<td>ND</td>
<td>ND</td>
<td>6.56</td>
<td>27.21</td>
<td>17.17</td>
<td>34.07</td>
</tr>
<tr>
<td>CFPT&lt;sub&gt;sps&lt;/sub&gt;</td>
<td>ND</td>
<td>32.65</td>
<td>ND</td>
<td>ND</td>
<td>9.16</td>
<td>14.53</td>
<td>43.66</td>
<td>ND</td>
</tr>
<tr>
<td>NFPT&lt;sub&gt;sps&lt;/sub&gt;</td>
<td>48.24</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>7.59</td>
<td>7.30</td>
<td>15.48</td>
<td>9.89</td>
</tr>
<tr>
<td>Crude ST&lt;sub&gt;sps&lt;/sub&gt; (% area)</td>
<td>2.19</td>
<td>28.56</td>
<td>3.46</td>
<td>3.94</td>
<td>21.38</td>
<td>12.46</td>
<td>21.12</td>
<td>6.89</td>
</tr>
<tr>
<td>CFST&lt;sub&gt;sps&lt;/sub&gt; (0.5M NaCl)</td>
<td>6.47</td>
<td>41.34</td>
<td>ND</td>
<td>1.89</td>
<td>7.82</td>
<td>11.63</td>
<td>23.35</td>
<td>7.49</td>
</tr>
<tr>
<td>CFST&lt;sub&gt;sps&lt;/sub&gt; (1M NaCl)</td>
<td>ND</td>
<td>70.26</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>29.74</td>
<td>ND</td>
</tr>
<tr>
<td>NFST&lt;sub&gt;sps&lt;/sub&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>9.24</td>
<td>30.52</td>
<td>60.23</td>
</tr>
</tbody>
</table>

ND=Not detected; HWE=Hot water extract; CI<sub>sps</sub>=Sulphated polysaccharide of *Cystoseira indica*; CF=Charged fraction; NF=Neutral fraction; PT<sub>sps</sub>=Sulphated polysaccharide of *Padina tetrastromatica*; ST<sub>sps</sub>=Sulphated polysaccharide of *Sargassum tenerrimum*

In view of the higher yield of hot water extract, this was selected for detailed chemical studies. Crude HWE was also purified by fractionation on an anion exchange chromatography column (DEAE cellulose in chloride form), using a modified method involving gradient elution with sodium chloride solutions (0 to 2.0M) (cf. Siddhanta...
et al. 2001). The fractions obtained from the crude HWE were freeze dried and the product was isolated and characterized. The major product was obtained from 1M NaCl eluents, and therefore, these were selected for detailed characterization by determining total sugar, sulphate, uronic acid, protein and ash contents, measuring viscosity and optical rotation (-48.24°; c0.25% at 27°C), and by elemental analysis. The 1.5 and 2.0M eluents did not contain any products. Alditol acetates of crude and purified ST_sps were prepared and their sugar profiling was done by GC-MS technique (Siddhanta et al. 2001). The crude ST_sps contained fucose, ribose, arabinose, xylose, mannose, galactose and glucose monosaccharide units while CFST_sps contained fucose and galactose, and NFST_sps contained glucose, mannose and galactose units. This investigation would provide crucial inputs for the characterization of charged and neutral polysaccharide fractions that were prepared (Table 2). The data obtained from the fractions of CTAB method were comparable with those of DEAE ion exchange column method.

Chapter I.3 Chemical studies on the cellulose of some brown seaweed species

Cellulose is a naturally occurring polysaccharide and the most abundant organic substance on earth, which consists of a chain of β-(1 → 4)-linked glucose residues (Klemm et al. 2005). Cellulose from green algae of Valonia, Halicystis and Cladophora species and from brown algae Laminaria were reported earlier (Whistler R L 1953). Cellulose polymer obtained from algal species has a porous or sponge like network and this is considerably different from the higher plant cellulose (Stromme et al. 2002 and Zuluaga et al. 2007). The latter reports also described preparation of micro-fibrils and micro-crystals cellulose from banana rachis. Recently, cellulose powder having high surface area and crystallinity were reported from the green alga Cladophora sp. (Ek et al. 1998). Mihranyan et al. (2004) studied the relation between moisture sorption and crystallinity of cellulose powder of Cladophora sp. stating that the degree of moisture sorption decreased with the increase in crystallinity of cellulose powder. This was looked upon as a useful alternative to commercially available dispersible grade cellulose. To our knowledge, there existed no report on the cellulose of any seaweed of Indian waters. Therefore, it was decided to study the cellulose contents of some Indian seaweed species.

Cellulose of Cystoseira indica, Sargassum tenerrimum, and Padina tetrastrumatica were extracted and characterized. Cellulose from these seaweeds was isolated following the method described by Mihranyan et al. (2004). Characterization of the cellulose was done by XRD, FT-IR, CP MAS 13C NMR, SEM and TGA. The alpha (α) and beta (β) celluloses were fractionated from the crude cellulose, employing the
method reported in the literature (Whistler 1963; Siddhanta et al. 2009). The ratios of alpha to beta cellulose varied e.g. 4.0 for *Cystoseira indica* and 8.0 for *Padina* spp. The data of cellulose obtained from these seaweeds were compared with those of Whatman filter paper No. 4, which was used as a standard. Whatman filter paper No. 4 contained major amount of α-cellulose and ≤ 1.0% β-cellulose. The properties of cellulose samples that were isolated are summarized in Table 3.

Table 3 Analytical data of cellulose of some brown seaweeds

<table>
<thead>
<tr>
<th>Cellulose of</th>
<th>Yielda (%)</th>
<th>Crystallinity Index (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude cellulose</td>
<td>α-cellulose</td>
</tr>
<tr>
<td>Whatman filter paper No. 4</td>
<td>94</td>
<td>88</td>
</tr>
<tr>
<td><em>Cystoseira indica</em></td>
<td>9.0</td>
<td>6.8</td>
</tr>
<tr>
<td><em>Sargassum tenerrimum</em></td>
<td>10.0</td>
<td>8.5</td>
</tr>
<tr>
<td><em>Padina tetrastromatica</em></td>
<td>9.5</td>
<td>8.2</td>
</tr>
</tbody>
</table>

a Yield was calculated on the basis of dry seaweed weight

Part-II

Modification of seaweed polysaccharides

This part consists of five chapters.

Chapter II.1: Review on modification of alginate

*Introduction*

This review compiles the information available in the literature on modification of alginate. Recently, natural polymers or polysaccharides have been receiving a great deal of attention because of their wide range of applications in food, pharmaceuticals, cosmetic etc. industries and their availability at a low cost. Several reactions have been performed for modification of alginate. Types of reactions done on alginates reported in the literature are given below

*Chemical modification of alginate*

Hybrid of sodium alginate with other biopolymers would widen the field of applications, such type of products which have shown improved swelling and barrier properties. The mucoadhesive properties of alginate can be improved by the covalent attachment of cysteine (Schnürch et al. 2001). Amphiphilic derivatives of sodium
alginate, prepared by covalent binding of long alkyl chains on to the polysaccharide backbone via ester functions, formed strong hydrogels in aqueous solutions (Leonard et al. 2004).

There has been increasing interest in the study of alginate-chitosan microcapsules as carriers for controlled release of proteins and drugs (Huguet et al. 1994; Hari et al. 1996; Ramdas et al. 2000; Vandenberge et al. 2001; Wheatley et al. 2001 and Mi et al. 2002). Chitosan–alginate complexes, being a pH sensitive hydrogel, have been studied for the development of oral delivery of peptide or protein drugs (Hari et al. 1996). The polyelectrolyte complex between chitosan and alginate has been widely used in order to obtain microcapsules for cell encapsulation and devices for the controlled release of drugs or other substances (Polk et al. 1994; Gasered et al. 1998; and Kim et al. 1999). Polymeric blend beads of PVA with sodium alginate were prepared by cross-linking with glutaraldehyde and were used to deliver a model anti-inflammatory drug, diclofenac sodium (Sanlı et al. 2007). Calcium crosslinked alginate purity and composition are critical in determining both rat marrow cell proliferation and mechanical behaviour of alginate gels. This work demonstrated that it has potential to act as a tissue-engineering scaffold on which tissues may be formed (Wang et al. 2003). Sodium alginate and their derivatives are used for wound pad, useful as blood styptic agent and as adhesive for joining of body tissue (Wegmann et al. 2008), it is also used as bone-repair composite (Wel et al. 2008). The hydrophilic polymers (i.e. xanthan gum, karaya gum, locust bean gum, guar gum, gelan gum, gum arabic, tragacanth, carrageenan, alginic acid, sodium alginate, hydroxypropylcellulose.) comprise controlled release formulation for treating diabetes mellitus in patient (Vishnupad et al. 2008).

**Physical modification**

This is basically done by mixing or blending the parent polysaccharides with suitable substrates which may be a monomer or oligomer or even a polysaccharide/polymer. In these cases, the change in the characteristics is brought about not by breaking and/or forming chemical bond but by the virtue of association of compounds facilitated by weak forces (van der Waals’ force) and hydrogen bonds as well as charge transfer complexes imparting supramolecular structural orientations. This phenomenon can give rise to improved properties in the parent polysaccharides, which may be used for newer applications. Sugar-induced gel thickening of agar (Meena et al. 2006), development of robust hydrogel systems based on agar and sodium alginate blend (Meena et al. 2008) as well as grafted blend of sodium alginate and agar (Chhatbar et al. 2009b) were reported from the author’s laboratory. Cellulose/alginate blend membranes were successfully cross-linked by Ca$^{2+}$ bridge in
5 wt% CaCl₂. The strong hydrogen bonding interaction between cellulose and alginate exists in the cellulose/alginate blend membranes (Yang et al. 2000). Carbohydrate polymeric blend microspheres of sodium alginate and methylcellulose were prepared for controlled release of nifedipine (Ramesh Babu et al. 2007). Improved swelling behavior of barium ions-crosslinked polymeric beads composed of sodium alginate and carboxymethyl guar gum were reported by Bajpai et al. (2006).

Chapter II.2: Chemical modification of CI₉ps by grafting with synthetic polymers

The crude sulphated polysaccharide of Cystoseira indica CI₉ps was grafted with polyvinylpyrrolidone (PVP), polyacrylamide (PAAm) and polymethylmethacrylate (PMMA). The products were characterized. These reactions were carried out for the first time with these heteropolysaccharides (CI₉ps), with the objective of generating modified polysaccharides, which might have some useful properties and would exhibit new effects compared to the parent polysaccharide.

Grafting of CI₉ps with polyvinylpyrrolidone (PVP)

Grafting of CI₉ps (Crude-HWE) with (PVP, average molecular weight 10,000 D) in an aqueous medium at a pH ca.7.0 produced CI₉ps-graft-PVP product. The reaction was carried out under microwave irradiation in the presence of a water-soluble initiator, potassium persulfate (K₂S₂O₈). Optimum microwave irradiation conditions for obtaining greatest grafting % of the grafted product were achieved. The structural characteristics and thermal stability of the grafted product was studied by FT-IR spectrometry, and thermogravimetric analyses. Appearance of new IR bands at 1665, 1495, 1422, and 1427 cm⁻¹ in the grafted product and its greater thermal stability (TGA) indicated insertion of PVP moiety into the polysaccharide structure. CI₉ps-graft-PVP was grafted to a considerable degree, with 90.5 E% and 165 G% and 87 C%. Grafted product exhibited adhesive properties. The data are summarized in Table 4.

Grafting of CI₉ps with polyacrylamide (PAAm)

Grafting of CI₉ps (Crude HWE) with PAAm in an aqueous medium at a pH ca. 7.0 produced CI₉ps-graft-PAAm product. The reaction was carried out under microwave irradiation in the presence of a water-soluble initiator, potassium per sulfate. Optimum microwave irradiation conditions for obtaining highest grafting % (G %) of the grafted product was achieved. The structural characteristics and thermal stability of the grafted product was studied by FT-IR spectrometry, and thermogravimetric analyses. Appearance of new IR bands at 1654, 1452, and 1417 cm⁻¹ in the grafted
products and its greater thermal stability indicated grafting of PAAm on the polysaccharide structure. CI\textsubscript{sps-}graft-PAAm was grafted to a considerable degree, with 92.35 E% and 140 G% and 76 C%. Grafted product was more hydrophilic than the parent polysaccharides, as one would expect. The data are summarized in Table 4.

**Grafting of CI\textsubscript{sps} with polymethymethacrylate (PMMA)**

Grafting of CI\textsubscript{sps} (crude HWE) with PMMA in an aqueous medium at a pH ca. 7.0 produced CI\textsubscript{sps-}graft-PMMA product. The reaction was carried out under microwave irradiation in the presence of a water-soluble initiator potassium persulfate. Optimum microwave irradiation conditions for obtaining highest grafting % (G%) of the grafted product was achieved. The structural characteristics and thermal stability of the grafted product was studied by FT-IR spectrometry and thermogravimetric analyses. Appearance of new IR bands at 1735, 1624, and 1433 cm\(^{-1}\) in the grafted product and its greater thermal stability (TGA) indicated grafting of PMMA on the polysaccharide structure. CI\textsubscript{sps-}graft-PMMA was grafted to a considerable degree, with 78.22 E% and 158 G% and 86 C%. Powder X-ray diffraction studies showed enhanced crystallinity (C.I. 0.33) in the products compared to in the control polysaccharides as well as PMMA. The grafted product became hydrophobic compared to the parent polysaccharide, as expected. The data are summarized in Table 4.

<table>
<thead>
<tr>
<th>Products</th>
<th>Total conversion (C %)</th>
<th>Grafting efficiency (E %)</th>
<th>Grafting percentage (G %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI\textsubscript{sps-}graft-PVP</td>
<td>87</td>
<td>90.5</td>
<td>165</td>
</tr>
<tr>
<td>CI\textsubscript{sps-}graft-PAAm</td>
<td>76</td>
<td>92.35</td>
<td>140</td>
</tr>
<tr>
<td>CI\textsubscript{sps-}graft-PMMA</td>
<td>86</td>
<td>78.22</td>
<td>158</td>
</tr>
</tbody>
</table>

**Chapter II.3: Water based synthesis of super swellable chitosan-polyuronic acid adducts with controlled release properties.**

Chitosan has been widely used in diverse fields, ranging from waste management to food processing, medicine, pharmaceutical applications and biotechnology (Illum \textit{et al.} 1998; Borchard \textit{et al.} 2001; Karlsen \textit{et al.} 1991 and Thanou \textit{et al.} 2001). Polyelectrolyte complexes of alginate and chitosan have been reported in the literature George \textit{et al.} (2006). Alginate is a linear co-polymer of $\beta$-D-mannuronic acid (MA) and $\alpha$-L-guluronic (GA) acid. To our knowledge, there exists no report of synthesis of
adducts of individual polyuronic acids e.g. PMA and PGA with chitosan (CH). We report herein a one-pot water based synthesis of adducts of chitosan with PMA (CH-PMA), and PGA (CH-PGA), under microwave irradiation conditions. Studies on the controlled release properties of these adducts using the anti-inflammatory drug paracetamol (PCT) and indometacine (IND) have also been described.

Super swellable and pH (1.2-12.0) responsive adducts of acid soluble chitosan (CH) with alkali soluble poly-mannuronic (PMA) and poly-guluronic (PGA) acids, were synthesized by a rapid method under microwave irradiation. Greatest yields (95-97%), of the adducts (CH-PMA & CH-PGA) with swelling ratios 2700-3000% were obtained by this method (5 min, 100°C). The conventional heating method was also employed to synthesize these adducts, which required 60 min to afford greatest yields 74-80%, which got plateaued even after heating up to 120 min having swelling ratios 1450-1900%. The adducts were characterized by FT-IR, $^{13}$C-NMR CP-MAS, XRD, TGA and SEM analysis. The controlled release performance of the adducts using an antipyretic paracetamol (PCT) and anti-inflammatory drug indomethacin (IND) was investigated. CH-PMA and CH-PGA synthesized with 50 wt.-% of PMA or PGA, showed greatest (ca. 95%) release of PCT in 800 and 1200 min, respectively, at pH 1.2 (simulated gastric fluid) (Figures 3 & 4), while CH-PMA and CH-PGA showed greatest (ca. 95% and 65%) release of IND in 600 and 900 min respectively, at pH 7.4 (simulated intestinal fluid) (Figures 5 & 6)

![Figure 3 Controlled release of PCT from CH-PMA adducts](image)
Figure 4 Controlled release of PCT from CH-PGA adducts

Figure 5 Controlled release of IND from CH-PMA adducts
Chapter II.4: Polyacrylonitrile-graft-Agar/sodium alginate, a stable hydrogel system

Acrylonitrile was grafted onto various natural and modified polysaccharides (i.e., gum arabic, xanthan gum, sodium alginate, chitosan, sodium carboxymethyl cellulose, hydroxy-ethyl cellulose, methyl cellulose) by using ceric-carbohydrate redox initiating system (Pourjavadi et al. 2002). Meena et al. (2008), reported a robust hydrogel system based on agar/Na-Alginate blend polysaccharide graft with acrylamide (AAm).

Polyacrylonitrile grafted agar/sodium alginate (Agar/Na-Alg-graft-PAN) was synthesized in aqueous medium under reflux conditions in the presence of potassium persulphate (KPS), as a free radical initiator (Scheme 1). Varying the reaction parameters e.g. concentration of acrylonitrile monomer (AN) and KPS, reaction time and temperature, optimum grafting conditions were identified as the one having the highest grafting ratio (Gr 1.87), total conversion (Ct 1.05) and grafting efficiency (Ge 0.89). The blend and grafted product were characterized by FT-IR, X-ray diffraction, DSC and SEM imaging. The swelling capacity of Agar/Na-Alg-graft-PAN showed 8.5g/g in pH 1.2 and the swelled material was stable for over 24h. This copolymer hydrogel system may be exploited in various applications utilizing its swelling

Figure 6 Controlled release of IND from CH-PGA adduct
properties and stability in acidic medium. This work has recently been published (Chhatbar et al. 2009b).

\[
K_2S_2O_8 \xrightarrow{\Delta} 2SO_4^+ + 2K^+
\]

Agar (Idealized repeating unit)

\[
1,4\text{-alpha-L-guluronic acid} \quad 1,4\text{-beta-D-mannuronic acid}
\]

Sodium Alginate (Idealized repeating unit)

\[
\text{Agar/Na-Alginate Blend} \xrightarrow{2SO_4^+} \text{Agar/Na-Alg-graft-PAN copolymer}
\]

Scheme 1 Proposed mechanism for the formation of Agar/Na-Alg-graft-PAN copolymer
Chapter II.5: Synthesis of Alginic acid-biphenyl conjugate.

Modification of natural polymers is a promising method for the preparation of new material and potential applications. The development of polymer adhesion conjugates providing a specific binding to epithelia (Naisbett et al. 1994 and Bernkop-Schnurch et al. 1995). However, all these systems are based on the formation of non-covalent bonds such as hydrogen bonds, van der Waals forces, and ionic interaction. Bernkop-Schnurch et al. (2001) reported improvement in the mucoadhesive properties of alginate-cysteine conjugate. Ionescu et al. (2005) reported a novel pyrrole-alginate conjugate was prepared by coupling aminopropyl pyrrole with alginate via carbodiimine chemistry. We report herein for the first time the synthesis of alginic acid-ortho-tolidine (a biphenyl compound) conjugate, in an attempt to introduce aromatic systems on to the polysaccharide backbone for modifying the properties of the latter.

Sodium alginate was extracted from the brown seaweed *Sargassum tenerrimum* of Indian waters (cf. Haug et al. 1967 and Sai Krishna Murthy 2000). This sodium alginate was used for the synthesis of a water soluble conjugate with ortho-tolidine (3,3′-diamino-4,4′-dimethyl-1,1′-biphenyl) under microwave irradiation at pH 4.0. The resulting conjugate was characterized by FT-IR spectrometry, $^{13}$C-NMR, SEM imaging, XRD, TGA, circular dichroism (CD), viscosity and optical rotation measurements, UV and fluorescence spectrophotometry as well as elemental analyses. The analysis of the results indicated that the amino groups of *o*-tolidine reacted with the carboxyl groups present in the polysaccharides to form the amide linkages. The UV-Vis [$\lambda_{\text{max}}$ 280 (log $\varepsilon$ 2.3319) (Figure 7b)] and fluorescence spectra of the product were recorded. Sharp emission maxima were observed in the fluorescence spectra for both *o*-tolidine and alginic acid/ortho-tolidine conjugate at ca. 385nm (Figure 7a). The fluorescence emission ($\lambda_{\text{max}}$ (ex) 280nm) of the modified alginic acid in 3x10$^{-4}$M solution in distilled water was significantly lower than that of *o*-tolidine at the same concentration in methanol. However, the enhancement in fluorescence emission ($\lambda_{\text{max}}$ (em) 381nm) in the product in 7.347 x 10$^{-4}$M concentration was significantly higher (ca. 25%) than that of the solution of pure *o*-tolidine in the same concentration, which is the amount of *o*-tolidine present in the product (i.e. 0.3mM in 7.347 x 10$^{-4}$M solution; $\lambda_{\text{max}}$ (em) 385nm). A hypochromic shift of the sharper emission maxima was detected in the fluorescence spectrum of the product in higher concentration (7.347 x 10$^{-4}$ M) in comparison to a lower concentration (3.0 x 10$^{-4}$ M) presumably due to enhanced intermolecular interactions facilitating energy exchange processes.
There was a total reversal of specific rotation value. The $[\alpha]_D$ of alginic acid was $-30.82^\circ$ (c 0.25%, 0.5Maq Na$_2$CO$_3$ at 30°C, 589nm), while o-tolidine was not optically active due to the presence of a plane of symmetry; the conjugate, however, exhibited an $[\alpha]_D +39.48^\circ$ (c 0.25%, H$_2$O at 30°C, 589nm), indicating a makeover in the optical properties in the adduct. This report demonstrates the successful introduction of an element of orderliness in the amorphous geometry of alginic acid polymer (XRD; C.I. of the adduct 0.53). Thus the product may be of utility in the domain of applications demanding surface activity e.g. catalysis. The plausible reaction mechanism for the formation of alginic acid adducts with o-tolidine shown in Scheme 2, which basically shows elimination of water leading to the formation of amide presumably involving a bimolecular mechanism. The scanning electron micrographs (SEM) of the modified and non-modified polysaccharides are depicted in Figure 8a-c. The SEM images indicated significant changes of the particle size and morphology of the conjugate when compared with alginate. The surface morphology of the conjugate looked relatively composed and porous. Pore size of the conjugate was in the range 1.5µm to 2.0µm. These results constitute a part of a Chapter coauthored by the candidate for a book to be published shortly (Siddhanta et al. 2010).

![Figure 7a Fluorescence spectra of o-tolidine and the conjugate at different concentrations](image1)

![Figure 7b UV-VIS spectra of o-tolidine and the conjugate](image2)
Evaluation of the catalytic activity of alginic acid/o-tolidine conjugate in Henry reaction

Alginic acid/o-tolidine conjugate evaluated as organocatalysts for the direct asymmetric nitroaldol reaction (Henry reaction) of nitromethane with electron-deficient aromatic aldehyde (Benzaldehyde). Standard procedure for Henry reaction is as follows: Benzaldehyde (1.0mmol) was stirred with nitromethane (1.5mmol) and alginic acid/o-tolidine conjugate (10mol% in 2.0ml water) was stirred at ambient temperature for 12-20hrs (Figure 9). The resulting product was characterized by FT-IR spectrometry, $^1$H-NMR, $^{13}$C-NMR, and HPLC The product might consist of a 50%-50% (or racemic) mixture of enantiomers, and yield was found ca. 85%.

Figure 8a-c. SEM imaging (a) o-Tolidine (b) Alginic acid (c) Alginic acid/o-tolidine conjugate

Figure 9 Nitroaldol reaction catalyzed by alginic acid/o-tolidine conjugate
Scheme 2 Proposed mechanism for formation of alginic acid/o-tolidine conjugate.
SUMMARY AND CONCLUSION

Value addition is the central theme of any R&D work. In this dissertation the results of the R&D efforts to value add Indian seaweeds, which were otherwise not known for any great value, have been documented.

The most important outcomes of the present study are:

[1] Alginites were extracted from the brown seaweeds Sargassum tenerrimum, Sargassum wightii, Cystoseira indica and Padina tetrastromatica of Indian waters. The yields and viscosity of the alginites of Sargassum tenerrimum and Cystoseira indica were the highest and the lowest respectively in this series. A rapid new microwave assisted method was developed wherein alginites were hydrolyzed to yield M- and G-acids. M/G ratio determined by this method was in good agreement with that obtained by conventional heating method (Chhatbar et al. 2009a). The M/G ratio of the alginate of Sargassum tenerrimum was higher than that of Cystoseira indica alginate – this was in line with the viscosity values obtained. This constitutes the first systematic chemical studies on alginate of Indian brown seaweeds, which will be useful in the bioprospecting works on alginophytes.

[2] Sulphated polysaccharides of Indian brown algae Cystoseira indica, Padina tetrastromatica and Sargassum tenerrimum contain similar types of monosaccharide units. Fucose and galactose were the major constituent monosaccharide units in all the sulphated polysaccharides extracted from Cystoseira indica, Padina tetrastromatica and Sargassum tenerrimum. Neutral fractions of these sulphated polysaccharides did not contain fucose whereas in charged fraction fucose was found as the major sugar which indicated that the fucose moiety was sulphated. These polysaccharides offer a classic example of heteropolymers containing most of the common naturally occurring carbohydrate moieties. These results would be useful in the studies on seaweed biodiversity as well as on chemical diversity of polysaccharides.

[3] Cellulose contents of the brown seaweeds viz. Cystoseira indica, Sargassum tenerrimum and Padina tetrastromatica were studied herein. The celluloses consisted of major amount of α-cellulose which had greater degree of crystallinity than the corresponding parent celluloses. Crystallinity of a polymeric material denotes its ordered structure, thus it holds great application potential wherein the merits of “symmetry” are demanded e.g. in the areas adsorption
related processes. The work on the celluloses of these seaweed species has been published (Siddhanta et al., 2009; cf. Siddhanta et al., 2010a).

[4] Hetropoysaccharides of *Cystoseira indica* (CIsp) containing seven sugar residues were chemically modified by grafting with synthetic polymers for the first time. The PVP grafted product exhibited adhesive property; the PAAm grafted product was more readily dispersible in water than the parent polysaccharide. The intrinsically hydrophobic PMMA grafted product was hydrophobic, as expected. All the three grafted products were thermally more stable than their respective parent polysaccharides. No significant improvements in water holding capacity and viscosity were observed in the grafted products. This particular study did not yield products with great or phenomenal effects, except for CIsp-graft-PVP, which exhibited adhesive effect comparable to that of a commercially available one. However, this perhaps gives one the preview of the fact that heteropolymers are not good candidates for modification targeting hydrogel networks. This is presumably because of the structural vicissitudes in the heteropolymeric architecture as a result of branching of different carbohydrate monomeric units on the constituent backbone, which in this case is fucan. Homopolymers, on the contrary, tend to have linear backbone that is amenable to network formation.

[5] Biopolymer based super swellable materials, the chitosan-polyuronic acid adducts, have been synthesized employing a water based microwave-assisted green process. These adduct exhibited significant pH-responsive swelling and controlled-release performance with the drug paracetamol (PCT) and indomethacin (IND). The delivery profile presented a staggered pattern e.g. initially the drug got released at a faster rate reaching a plateau, thereafter a gradual release took place. The maximum amount of PCT absorbed into CH-PMA is 170.5mg/g CH-PMA and into CH-PGA is 148.6mg/g CH-PGA while, the maximum amount of IND absorbed into CH-PMA is 150.6mg/g CH-PMA and into CH-PGA is 135.2mg/g CH-PGA within 1.5h at pH 1.2 and 30°C. The release rates of PCT and IND were dependent on the pH values of the aqueous media employed. The release rate of PCT increased when pH of the decreased, and it was the greatest at a pH 1.2 and lowest at pH 7.4. Exactly reversed situation was obtained with the release rates of IND i.e. the greatest at a pH 7.4 and the lowest being at pH 1.2. This study showcases an array of biopolymer based materials which can be harnessed for catering to specific dosage schedules and would be of potential utility in pharmaceutical formulations (Meena et al. 2011).
[6] Sargassum tenerrimum alginate was blended with agar (Gelidiella acerosa) and the blend was grafted with polycrylonitrile (PAN), the product was a stable copolymer hydrogel. This hydrogel was stable in aqueous media over a wide range of pH (1.2, 7.0 and 12.5), and did not disperse even after 24h. The unmodified blend, however, swelled and dispersed after 12h in all pH media. The copolymer hydrogel would be useful in newer applications especially due to its resilience to acidic pH medium (Chhatbar et al. 2009b).

[7] A water soluble alginic acid-biphenyl (ortho-tolidine) conjugate was synthesized under microwave irradiation at pH 4.0. There was a total reversal of specific rotation value in the product; $[\alpha]_D$ of alginic acid was -30.82° (c 0.25%, 0.5M aq Na₂CO₃ at 30°C, 589nm), while o-tolidine was not optically active due to the presence of a plane of symmetry in the molecule; the conjugate exhibited an $[\alpha]_D$ +39.48° (c 0.25%, H₂O at 30°C, 589nm), indicating a makeover in the chirooptical properties in the product.

The fluorescence emission $[\lambda_{\text{max}}$ 381nm; $\lambda_{\text{max}}$ (ex) 280nm] of alginic acid/o-tolidine conjugate in the product in $7.347 \times 10^{-4}$M concentration was significantly higher (ca. 25%) than that of the solution of pure o-tolidine in the same concentration, which is the amount of o-tolidine present in the product i.e. 0.3mM in $7.347 \times 10^{-4}$M solution exhibiting $\lambda_{\text{max}}$ (em) 385nm. The fluorescence emission of the conjugate in $3 \times 10^{-4}$M solution in distilled water was significantly lower than that of o-tolidine at the same concentration in methanol, probably indicating lower occurrence of energy transfer phenomena at lower concentration. Conversely, a higher concentration of the conjugate exhibited enhanced fluorescence emission, as mentioned above. This study demonstrates successful introduction of an element of orderliness in the amorphous geometry of alginic acid polymer (XRD; C.I. of the adduct 0.53). The product may be of utility in the domain of applications demanding surface activity e.g. catalysis. Important advantages of this method of preparation were that the reaction was water based and ecofriendly and the polysaccharide did not undergo decomposition during the course of reaction. These results constitute a part of the Chapter entitled “Development of carbohydrate polymer based new hydrogel materials derived from seaweed polysaccharides” (Siddhanta et al. 2010b).

[8] Alginic acid/o-tolidine conjugate acts as a base catalyst (pKₐ ca. 11.0) in a nitroaldol reaction. It was found that only aromatic aldehydes (benzaldehyde) reacted efficiently with nitromethane catalyzed by alginic acid/o-tolidine conjugate, yielding ca. 85% aldol. The HPLC data showed that the aldol product
was a *racemic* mixture (ca. 50:50%), indicating that this was not an enantioselective catalyst. Reactions with aliphatic aldehydes, the said aldol condensation did not take place, presumably due to its relatively low basicity (pK₈ ca. 11.0).

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