

## Fourier Transform Infrared Spectral Analysis of Agars of *Gelidium micropterum* and *Gracilaria fergusonii*

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The present work aims to make an analysis of the samples of agars obtained from *Gelidium micropterum* and *Gracilaria fergusonii* collected at summer and winter seasons by FTIR spectroscopic method. The qualitative analysis on the vibrational bands has been carried out. Further the spectra have been compared with that of Difco agar.

**Key Words:** Infrared, Agar, *Gelidium micropterum*, *Gracilaria fergusonii*.

### INTRODUCTION

Agars, a group of galactans, have little half-ester sulphate and are non-ionic for most practical purposes. In spite of the fact that the sulphated galactans are commercially important, the spectral studies on agars obtained from *Gelidium micropterum* and *Gracilaria fergusonii* are scanty. Infrared spectroscopy offers a suitable method to characterize agar with respect to the location of the sulphate group<sup>1-4</sup>. In present study, FT-IR spectroscopy has been used as an effective tool to identify the vibrational bands and type of polysaccharides.

### EXPERIMENTAL

Generally the cell wall polysaccharides are soluble in water. Therefore hot water extraction method was adopted. The polysaccharides of summer and winter collections from *G. micropterum* and *G. fergusonii* were dried in dessicator. The dried polysaccharide was powdered and passed through 1 mm IS sieve. The powdered polysaccharide was transferred to air tight glass bottle and stored for quality analysis. Fourier transform infrared (FT-IR) spectra were recorded in KBr disc using Bruker Vector 20 FT-IR double beam spectrophotometer in the range of 4000-600 cm<sup>-1</sup>. Spectra were taken under identical conditions and compared with spectrum of commercial agar.

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## RESULTS AND DISCUSSION

**Vibrational band assignments:** The probable assignment on the vibrational frequencies are summarized in Table-1.

TABLE-1  
FT-IR BANDS ( $\text{cm}^{-1}$ ) ASSIGNMENTS OF STANDARD AGAR AND AGARS OF  
*Gelidium micropterum* AND *Gracilaria fergusonii*

| Difco agar | <i>Gelidium micropterum</i> |         | <i>Gracilaria fergusonii</i> |         | Band assignments   |
|------------|-----------------------------|---------|------------------------------|---------|--|
|            | Summer                      | Winter  | Summer                       | Winter  |  |
| 688 s      | 662 vs                      | 668 w   | 671 mw                       | 669 s   | C-S link vibration/S-O stretch/<br>C-S deformation                       |
| 778 s      | 769 vs                      | 772 vw  | 774 w                        | 774 ms  | C-S link vibration/S-O stretch/<br>C-S deformation                       |
| -          | -                           | -       | 818 vw                       | 816 ms  | Ester-sulphate in C-6 link<br>vibration/C-C/C-O/ C-O-S stretch           |
| 890 s      | 890 s                       | 891 vw  | 890 vw                       | 892 ms  | Non sulphated $\beta$ -D galacto<br>pyranose residues/C-C/C-O<br>stretch |
| 931 s      | 931 s                       | 923 w   | 932 vw                       | 932 ms  | 3,6 anhydro galactose vibration/<br>C-C/C-O stretch                      |
| 1038 vs    | 1032 s                      | 1045 ms | 1045 ms                      | 1025 vs | Ester-sulphate link vibration/<br>C-C/C-O/C-O-S stretch                  |
| 1088 vs    | 1067 ms                     | 1084 ms | 1076 w                       | 1071 vs |  |
| 1158 s     | 1157 s                      | 1057 m  | 1161 mw                      | 1152 s  | Ester-sulphate link vibration/<br>C-C/C-O/C-O-S stretch                  |
| 1250 s     | 1257 ms                     | 1253 mw | 1256 m                       | 1232 s  | Ester-sulphate link vibration/<br>C-C/C-O/C-O-S stretch                  |
| 1382 s     | 1383 ms                     | 1384 m  | 1377 ms                      | 1384 ms | Methyl group vibration/S=O<br>symmetric stretch                          |
| 1434 ms    | 1424 ms                     | 1424 m  | 1419 m                       | 1424 m  | Methyl group vibration/S=O<br>asymmetric stretch                         |
| 1549 mw    | 1549 m                      | 1535 mw | 1574 m                       | 1545 m  | C=O symmetric stretch/<br>N-H deformation                                |
| 1641 ms    | 1620 ms                     | 1638 s  | 1643 ms                      | 1638 ms | C=O asymmetric stretch/<br>N-H deformation                               |
| 1723 w     | 1727 w                      | 1734 vw | 1727 vw                      | -       | C=O stretch  |
| -          | -                           | -       | 2854 ms                      | 2850 vw | Methyl group vibration/<br>C-H symmetric stretch                         |
| 2927 w     | 2926 mv                     | 2926 vw | 2926 s                       | 2928 w  | Methyl group vibration/<br>C-H asymmetric stretch                        |
| 3416 ms    | 3416 s                      | 3419 vs | 3391 vs                      | 3418 vs | OH/NH stretch  |
| 3470 ms    | 3476 s                      | 3470 vs | -                            | 3470 vs |  |

**O-H stretching vibrations:** The characteristic bands are observed in the spectra of alcohol and phenols resulting from O-H stretching<sup>5</sup> at  $3500\text{-}3300\text{ cm}^{-1}$ . In the present work, the strong bands at around  $3420$  and  $3470\text{ cm}^{-1}$  have been assigned to O-H stretching.

**Methyl group vibrations:** In alkanes, CH symmetric stretch band at  $2855\text{ cm}^{-1}$  and asymmetric C-H stretch band at  $2925\text{ cm}^{-1}$  occur with strong intensity<sup>6</sup>. In the FTIR spectra of *G. micropterum*, weak bands were exhibited at around  $2900\text{ cm}^{-1}$ . However, there was a strong absorptions in *Gracilaria fergusonii* collected in summer.

The bands observed at around 1450 and 1370  $\text{cm}^{-1}$  are attributed to asymmetrical and symmetrical bending vibrations of  $\text{CH}_3/\text{CH}_2$  group respectively<sup>7,8</sup>. In the present work, FTIR spectra of agars of different source showed bands ranging from strong to weak intensity at or around 1450 and 1380  $\text{cm}^{-1}$  for asymmetric and symmetric deformation of  $\text{CH}_3/\text{CH}_2$ , respectively.

**Ester-sulphate link vibrations:** The ester-sulphate link vibrations are exhibited by the peaks<sup>9,10</sup> at 1370, 1250, 1180 and 1060  $\text{cm}^{-1}$ . In this investigation, the intensity of the absorption in these regions vary from strong to weak or even very weak and depends on the species and the seasons. The bands at 1180-1060  $\text{cm}^{-1}$  are due to symmetric stretching of S-O/C-O and asymmetric vibration occurs at 1380-1250  $\text{cm}^{-1}$ . The sulphate ester in C-6 link vibration occurs at 820  $\text{cm}^{-1}$ . In the present study, this band was exhibited only in *Gracilaria fergusonii* as reported in previous work<sup>11</sup>.

The band at 890  $\text{cm}^{-1}$  in the spectra of agarophytes is due to non sulphated  $\beta$ -D galacto pyranose residues, typical of agar peak as reported in previous works<sup>2</sup>.

**Carbon-sulphur link vibrations:** The literature study reveals that the carbon sulphur link vibration of polysaccharides occurs in the region<sup>9,12</sup> 770-730  $\text{cm}^{-1}$ . The spectra of present study showed strong to weak band over the range 790-703  $\text{cm}^{-1}$  attributed to the deformation of sulphate. The peaks in the range 670-640  $\text{cm}^{-1}$  represent C-S deformation in the spectra of these two species.

**3,6-Anhydro-galactose bridge vibrations:** The strong to weak absorption bands occurring at 1070 and 930  $\text{cm}^{-1}$  in all the spectra of the present study are attributed to C-O ether bond of 3,6-anhydro-galactose vibration as observed in previous works<sup>9,12</sup>.

**Carbonyl vibrations:** Carboxylic acids and carboxylic esters show strong C=O stretching bands in the region<sup>13</sup> of 1700-1540  $\text{cm}^{-1}$ . In the present work, very weak band was obtained at around 1740  $\text{cm}^{-1}$  in both the species. The strong to weak absorption bands at 1640  $\text{cm}^{-1}$  was assigned to carbonyl vibration of carboxylic group (COO) due to asymmetric stretching (C=O). The medium to weak intensity of band at 1560-1520  $\text{cm}^{-1}$  was assigned to symmetrical stretching of C=O. These assignments are in agreement with previous observations<sup>14</sup>.

**NH<sub>2</sub> group vibrations:** The spectra of the present investigation show strong to medium band at 1640  $\text{cm}^{-1}$  to primary amide and medium to weak band of secondary amide stretching at 1540  $\text{cm}^{-1}$ , according to previous observations<sup>3</sup>. A sharp peak centered at around 3500  $\text{cm}^{-1}$  may be due to NH stretching vibrations<sup>15</sup>.

**C-C stretching vibrations:** The C-C vibrations are weak in the region<sup>16</sup> of 1200- 800  $\text{cm}^{-1}$ . In the present work the band observed in the region 1200-800  $\text{cm}^{-1}$  may be due to overlapping of C-C stretching and C-H bending modes.

**Qualitative analysis:** The FTIR spectra of Difco agar exhibited peaks at 3470, 3416, 2927, 1723, 1641, 1549, 1434, 1382, 1250, 1158, 1088, 1038, 931, 890, 778 and 668  $\text{cm}^{-1}$  and the results of FTIR spectra of samples of the present studies are given in Table-1. The spectra of these two samples, exhibited more or less common

constant peaks both in summer and in winter seasons as similar to those shown by Difco agar. Apart from these, additional peaks were exhibited in spectra of *G. fergusonii*.

The peak in the region 2926-2850  $\text{cm}^{-1}$  characteristic of methyl group occurs in *G. fergusonii*. The band at around 1720  $\text{cm}^{-1}$  is not exhibited in the spectrum of *G. fergusonii* obtained for winter collection. This may be due to developmental and seasonal variation associated with enzymes involved in synthesis of polysaccharides. The additional peak at around 820  $\text{cm}^{-1}$  was noticed in both the spectra of *Gracilaria fergusonii* and it is due to sulphate on C-6 galactose.

It is interesting to note that the band at around 1240  $\text{cm}^{-1}$  is characteristic of sulphate esters appear in each of the spectra during both the seasons. There appears to be a variation in the intensity of this band with algal source. The peak is represented as a shoulder in the spectra of both seasons in *Gelidium micropterum* as in Difco agar. The broad band is exhibited in *G. fergusonii*. This indicates that the phycocolloids from latter mentioned species contains a greater amount of sulphate esters.

### Conclusion

In the present study, it is concluded that agar obtained from *G. micropterum* is less sulphated than *G. fergusonii* and therefore gelling strongly, which could be useful for bacteriological and biomedical applications while *G. fergusonii* affords soft gelling agars suitable for food industries.

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