Turkish J. Marine Science 8:143- 156(2002)

Infrared Studies on *Phyllophora nervosa* Agar and Comparison with Various Agars and Carrageenans

Phyllophora nervosa Agar ve Değişik Agarlar ile Karragenanlar Üzerinde İnfrared Araştırma

Murşide Sur and Kasım Cemal Güven

Istanbul University , Institute of Marine Sciences and Management, Vefa, 34470, Istanbul

Abstract

In this work were studied IR spectra of the type of sulfated polysaccharides as agar of *Phyllophora nervosa* and various agars and carrageenans. Agars used were from *Phyllophora nervosa, Gracilaria verrucosa, Pterocladia capillecea, Ceramium rubrum, Hypnea musciformis,* , agar (Pasteur and commercial) and carrageenans were κ –, ι-, type LC and carrageenan obtained from *Grateloupia dichotoma, Acanthophora delilei.* IR spectra of these agars were compared with those of carrageenans. The bands of 805 and 705 cm^{-1} characteristic for carrageenans were absent at the spectra of the agars tested. Agars showed the same bands at (736, 771, 927, 968, 1253cm^{-1}). It was proved that the sulfated polysaccharide obtained from *Phyllophora nervosa* was agar.

Keywords: *Phyllophora nervosa,* agars, carrageenans, IR spectra.

Introduction

Sulfated polysaccharides are obtained from algae. They are classified into 1)L-Fucan (in Phaeophyceae), 2) D- and L-Galactan (in Rhodophyceae), 3) D-Glucurono-D-xylo-L-

rhamnans (in Chlorophyceae) and 4) Sulfated-L-arabino-D-xylo-D-galactans (in Chlorophyceae).

Agar amongst these is an important product for various industries and medicine.It is extracted from certain marine algae of the class Rhodophyceae especially as *Gracilaria, Gelidium, Ahnfeltia, Phyllophora, Pterocladia, Acanthopeltis and Ceramium* sp. etc.

The extraetim was made from the algae with hot water and it was separated by freezing, thawing and drying techniques. Agar is not a single substance but a mixture of at least two polysaccharides as agarose and agaropectin.Agarose, a gelling agent consists of a chain alternating 1→4-linked 3,6-anhydro-α-L-galactopyranose and 1→3-linked β-D-galactopyranose residues (Araki, 1958).
Agarose polymers contain low concentration of methoxyl and high content of L-galactose-6-sulfate (Craigie *et al.*, 1984).

Agaropectin is D-galactose, 3-6-anhydro-L-galactose, half ester sulfate, and glucuronic acid. It contains pyruvic acid in acetal linkage with D-galactose.

Phyllophora agar (called as agar-like, Russian agar) is obtained from *Phyllophora nervosa* first by Russian workers and used in microbiology. It was also extracted from the alga collected from Turkish coast and investigated (Güven *et al.*, 1966, 1972; Güven and Güler 1979).

Carrageenans are obtained from certain species of red seaweeds of the Gigartinaceae, Solieriaceae and Hypneaceae families. They were also obtained from *Phyllophora* sp as *Ph. crispa, Ph. pseudoceranoides, Ph. truncata. Phyllophora nervosa* extract was shown to be a special type of carrageenan.(Yaphe, 1981 personal communication.)

Carregeenans was obtained from Grateloupia dichotoma and Acanthophora delilei (Güven *et al.*, 1984).

They are sulfated polysaccharides of D-Galactose and 3,6-anhydro-D-galactose. The structure is built up of alternating 1,3-linked β-D-galactopyranosyl and 1,4-linked-α-D-galactopyranosyl units. Carrageenans contain 3,6 anhydro galactose sulfate, galactose sulfate (2 or 6, especially 3,6 anhydro galactose 2-sulfate). They have various types as lambda, kappa, mu, nu, ioto, theta, xi-carrageenans. Its highly sulfated type was kappa containing about 35%, and lambda 25%.They are a mixture of polysaccharides. Carrageenan contains (1→3) linked and 4-sulfated and a small proportion (1→4) linked and 6 or 2,4 disulfated groups (Percival and McDowell, 1967). Carrageenans are used as thickening, suspending and gelling agents in toothpaste, chocolate, milk puddings, water-gel desserts, air-freshener gel etc.

Carrageenan is extracted from algae with hot water and precipitated with alcohol.Its molecular weight is approx. 200.000 Daltons.

IR spectrophotometry of these polysaccharides was investigated for agar by Laserna *et al.*, (1981) and Christiaen and Bodard (1983) and for carrageenans by Lloyd *et al.*, (1961), Zundel (1969), Cajipe *et al.*, (1980), McCandless *et al.*, (1981), Archbald *et al.*, (1981), Peats (1981), Craigie and Leigh (1978), Anderson *et al.*, (1968), Rochas *et al.*, (1986), Bellion *et al.*, (1983), Zablackis and Santos (1986).

The algae studied in this work were found in Turkish coasts as: Phyllophora *nervosa* first in Bosphorus by Dratzuyan, 1894-1895 (published by Öztığ, 1971) and Fritsch (1899), in Şile (Güven and Aktin, 1962), in Kefken-Karaburun (Birecik, 1973), in Yeşilköy and Sea of Marmara (Güven, unpublished), (Öztürk, unpublished), Gökçeada in Aegean Sea (Zeybek, 1966), in Inceburun, Sinop (Cirik and Cihangir, 1987).

Phyllophora sp. were largely found in the NW part of the Black Sea but diminished from 11.000 km^2 to 500 km^2 lately as a

result of pollution (Zaitsev, 1992; Zaitsev and Alexandrov, 1998; Petranu, 1997).

Metachromatic identification of agar and carrageenans was investigated by Güven and Güvener, 1985a,b.

Materials and Methods

Algae used in this study and their locations are;
(Güven and Öztiğ, 1971; Güven *et al.*, 1991).
For agar;
Phyllophora nervosa (D.C. Grev): Şile, Istanbul.
Gracilaria verrucosa (Huds) C.Ag.: Bosphorus.
Pterocladia capillacea(formerly Gelidium latifolium) Born et Thur.: Şile, Istanbul.
Ceramium rubrum (Huds) C.Ag.: Bosphorus
Hypnea musciformis (Wulfen) Lamx.: Izmir.

For carrageenans;
Grateloupia dichotoma (J.A. Agardh), Haydarpaşa breakwater, Istanbul.
Acanthophora delilei (Lamour), Izmir.

Tested compounds are:
Agar (Pasteur) and commercial agar
κ -carrageenan (Sigma)
ι-carrageenan (Sigma)
Genugel carrageenan type LC (Kobenhavns PektinFabrik).

Apparatus:
Infrared Spectrophotometer FTIR, Schimadzu P.C. 8601

Agar was obtained from algae as indicated above by hot water extraction followed by freezing, thawing and drying techniques (Güven *et al.*, 1966, 1972).
Carrageenan was extracted from *Grateloupia dichotoma* also with hot water and precipitated by isopropil alcohol (Güven *et al.*, 1984).

The tablet for infrared analysis was prepared with KBr and its spectrum taken in FTIR.

Results and Discussion

The IR spectra are shown for agar in Fig. 1. and for carrageenan in Fig. 2.
The infrared spectrum of sulfated polysaccharides were used for differentiation of types.The bands of IR spectrum were attributed to.
805 cm^{-1} 3,6-anhydrogalactose-2-sulfate (Anderson *et al.*, 1968),
820 cm^{-1} galactose-6-sulfate,
830 cm^{-1} galactose-2-sulfate,
805-810 cm^{-1} primary 6 sulfate in the 1,4 linked unit (Zablackis and Zantos, 1986),
920-930 cm^{-1} galactose-4-sulfate (Rochas *et al.*, 1986),
920-940 cm^{-1} other sulfated sugars (Archbald *et al.*, 1981),
930 cm^{-1} 3.6-anhydrogalactose-4-sulfate (Stanley, 1963).

The weak 850 cm^{-1} band denotes a low content of 4-sulfate in the 1,3-linked galactose unit and (a sharp peak) also 870 cm^{-1} (a shoulder), for carrageenan (Zablackis and Santos 1986).
845 cm^{-1} -C-O-S vibration C_4 of the 1,3-linked galactopyranosyl and no 845 cm^{-1} band indicates absence of 4 sulfate in the 1,3-linked unit (Zablackis and Santos, 1986).

Absorbance at 2845 cm^{-1} due to O-CH_3 occurs as a shoulder on the band at 2920 in spectro of highly methylated agars from *Gracilaria eucheumoides* (D.S 1:0.9) (Ji *et al.*, 1985).

Fig. 1. IR spectrum of *Phyllophora nervosa* agar.

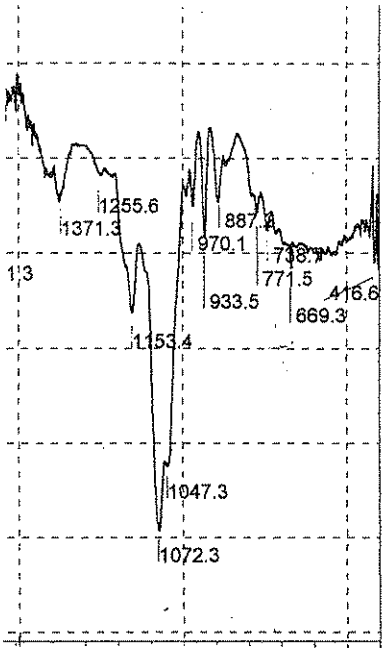


Fig. 2. IR spectrum of *Gracilaria verrucosa* agar.

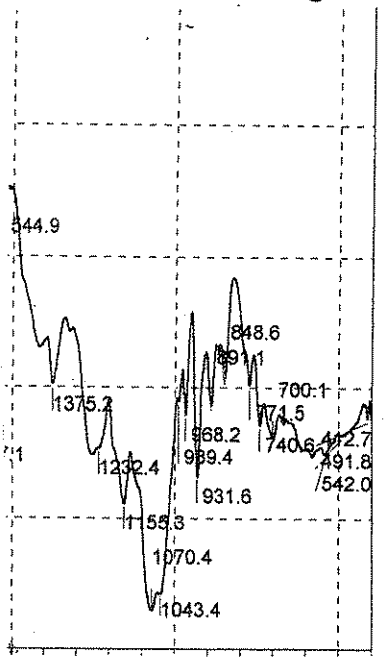


Fig. 3. IR spectrum of *Pterocladia capillacea* agar.

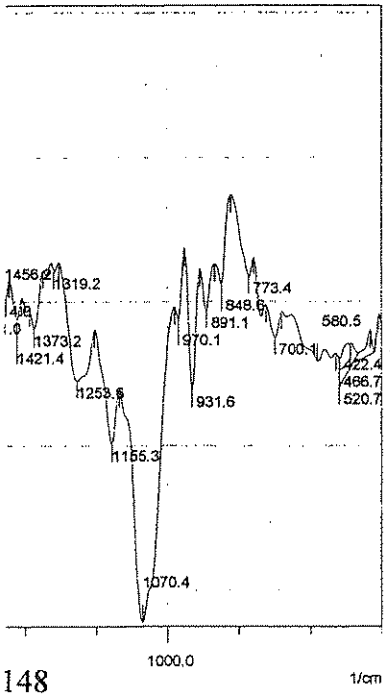


Fig. 4. IR spectrum of *Ceramium rubrum* agar.

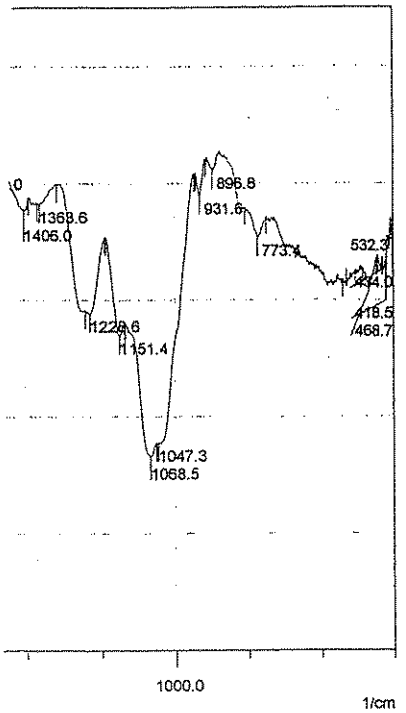


Fig. 5. IR spectrum of *Hypnea musciformus* agar.

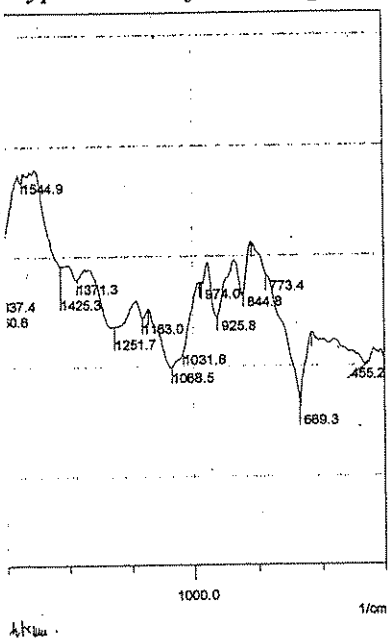


Fig. 6. IR spectrum of Pasteur agar.

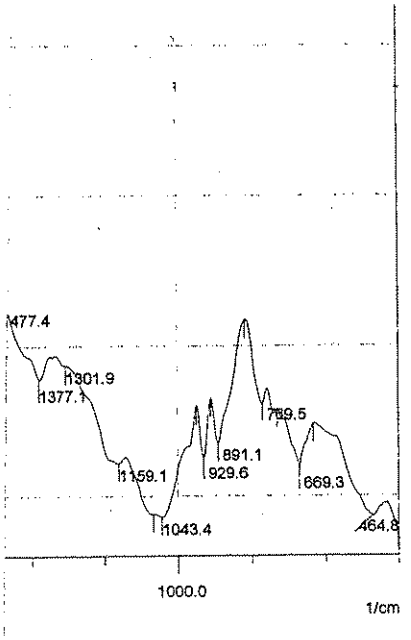


Fig. 7. IR spectrum of κ-carrageenan.

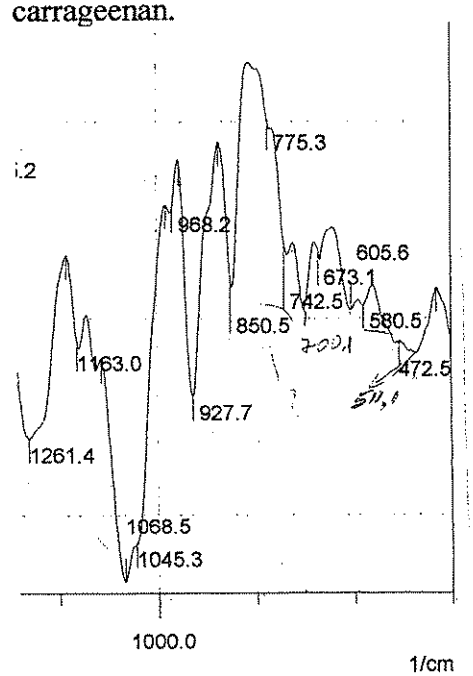


Fig. 8. IR spectrum of ι-carrageenan.

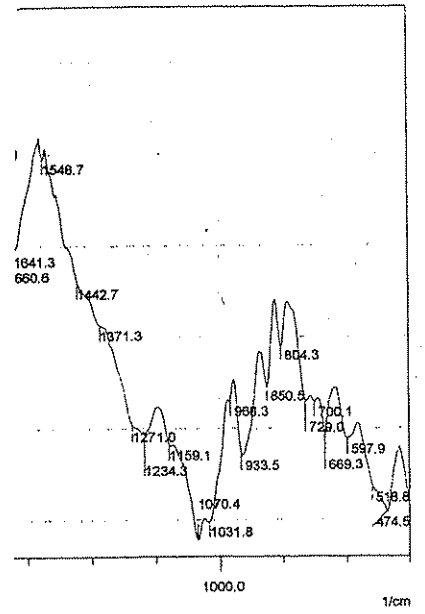


Fig. 9. IR spectrum of carrageenan type LC (Kobenhavns PektinFabrik).

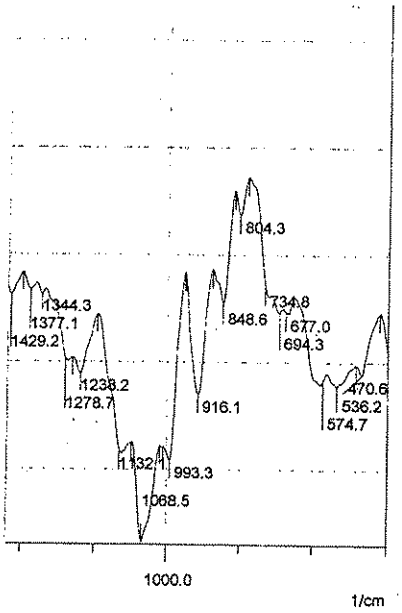


Fig. 10. IR spectrum of *Grateloupia dichotoma* carrageenan.

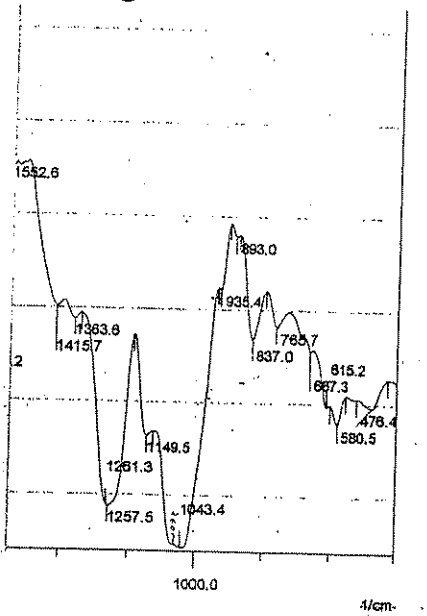
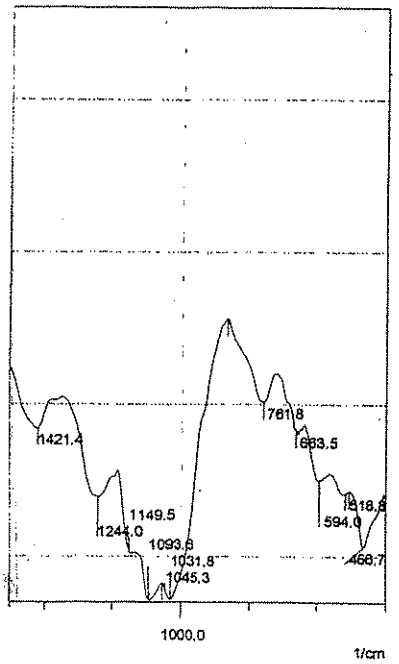


Fig. 11. IR spectrum of *Acanthophora delilei* carrageenan.



According to Laserna *et al.*(1981), the relatively low sulfate content (10%) of agar is further indicated in all the spectra by the very weak and poorly defined band in the region between 800-850 cm^{-1} where primary and secondary equatorial and axial sulfate groups absorb. The peak at 930 cm^{-1} is attributed to 3,6-anhydrogalactose moiety. The intensity of the peak at 1250 cm^{-1} decreased due to removal of a sulfate group, resulting in the formation of a 3,6,anhydro-galactose residue.

McCandley *et al.*, (1986) studied Phyllophora carrageenans and observed the peaks at 805, 845 and 930 cm^{-1}. The identified carrageenans were i- and λ- types.*Phyllophora nervosa* carrageenan was denoted as special type by Yaphe(1981, personal communication). It contains 4-linked 3,6- anhydro-D-galactose associated with a 3-linked D-galactose. The 3-linked D-galactose sugar differ from those in κ-carrageenan.

The bands at 1370 cm^{-1} and 1250 cm^{-1} are due to sulfate (Lloyd *et al.* 1981). Rochas *et al.*, (1986) determined sulfate content of carrageenan and agar by infrared spectroscopy. The IR spectra of agar and carrageenans in the literature are summarized as follows: For agar and carrageenan (Rochas, 1986; Peats, 1981): 2960(-CH_2), 2920 (CH), 2845 (O-CH3), 1370(SO4), 1250 (-SO4), 930 (3,6 anhydro galactose), 845 (Galactose-4- sulfate), 830 (Galactose-2- sulfate), 820 (galactose-6-sulfate), 805 (3,6-anhydrogalactose-2-sulfate), 705 (galactose C-4-sulfate) cm^{-1}.

In our work the spectra obtained from the sulfated polysaccharides tested are: The same peaks were observed on *Phyllophora nervosa* agar and various agar (Pasteur and commercial); 536/538, 588, 690/700, 736/742, 771, 841/893, 927/931, 968, 1066/1074, 1119, 1253/1261, 1384, 1378 cm^{-1} . The common peaks amongst the agar obtained from the algae tested are: 773, 925/931, 1045/1047, 1068/1070, 1151/1159/1163, 1363, 1425, 1552/1564 cm^{-1}; for commercial and Pasteur agar 2900 (CH2), 1375 (-SO4), 1261 (-SO4), 931 (3,6 anhydrogalactose). The peak at 930 cm^{-1} corresponds to 3,6-anhydrogalactose, intensity decrease at 1250 cm^{-1} is due to removal of sulfate group resulting 3,6-anhydrogalactose.

According to this similarity *Phyllophora nervosa* agar is equal to commercial agar. The special peaks belonging to carrageenans are: 604, 677, 705, 805, 848, 916, 993, 1238, 1278, 1332 cm^{-1}.

The same peaks were observed on the spectra of κ- and ι- and carrageenan type LC and *G. dichotoma* and *A. delilei* carrageenan as: 470/474/476, 805, 1045, 1070 cm^{-1}.

The similar peaks observed from *Phyllophora nervosa* agar with carrageenans are: 464, 482, 536, 588, 690, 742, 771, 868, 893, 931, 968, 987, 1074, 1159, 1261, 1375, 1415, 1475, 1643 cm^{-1}. The same peaks were noted from *Phyllophora nervosa, Gracilaria verrucosa, Pterocladia capillacea, Ceramium rubrum, Hypnea musciformis* agar: 2958 (CH2), 2852 (CH), 1641, 1371, 1377 (-SO4), 1249, 1253 (-SO4), 927,931 (3,6 anhydrogalactose).

Conclusion

IR spectra of carrageenan differed at the bands of 805 and especially 705 cm^{-1} which obtained missed in those of agar. Thus sulfated polysaccharide obtained from *Phyllophora nervosa* was proved to be agar.

Özet

Bu çalışmada *Phyllophora nervosa* 'nın sülfatlı polisakkaridinin IR analiz sonuçları verilmiştir. Sülfatlı polisakkaritlerden değişik menşeili agar ile *Phyllophora nervosa* agarı ve değişik tip ve menşeili karragenanın IR spektrumu ile karşılaştırılmıştır. *Phyllophora nervosa'* dan elde edilen agar evvelce mikrobiyolojik vasat olarak denenmiş ve kullanılmıştır. Bu çalışmada mukayese için, *Gracilaria verrucosa, Ceramium rubrum, Pterocladia capillacea,Hypnea musciformis*'den elde edilen agar ile Pasteur ve ticari agar ve karragenan için κ- , ι- , type LC ve *Grateloupia dichotoma* ve *Acanthophora delilei*'den elde edilen karragenan kullanılmıştır. Karragenan için IR spektrumunda karakteristik olan 805 ve 705cm^{-1} bantları yukarda belirtilen agar tiplerinde rastlanmamıştır.

Phyllophora nervosa agarı ile diğer agarlarda görülen benzer bantlar: 536/538, 588, 690/700, 736/742, 771, 841/893, 927/931, 968, 1066/1074, 1119, 1253/1261, 1384, 1378 cm^{-1}.dir. Karragenana ait bant ise: 604, 677, 705, 805, 848, 916, 993, 1238, 1278, 1332 cm^{-1}.dir. Bu şekilde *Phyllophora nervosa*'dan elde edilen polisakkaridin agar olduğu saptanmıştır.

Acknowledgement

The authors thank Prof. Dr. Öznur Ateş and Ms. Sc. Tuncay Gezgin for their kind help.

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Received 06.02.2002
Accepted 28.03.2002