J. Black Sea/Mediterranean Environment
Vol.10: 45-60

Metachromatic Properties of Agars and Carrageenan

Agar ve Karagenanın Metakromatik Özellikleri

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Abstract:

The metachromatic properties of agars obtained from Turkish coast of carrageenan were investigated after eluation on Sepharose 2B. The each 5ml eluate was separated and α-, β- bands were determined with azur A by spectrophotometer. The metachromatic band is shown amongst the volume of the eluates are: for agars; 15-55 ml for Phyllophora nervosa, Gracilaria verrucosa 20-45 ml, Ceramium rubrum 25-55 ml, 15-45 ml for Gelidium latifolium and 20-30 ml for Pasteur agar and 25-95 ml for carrageenan. The results show that the metachromatic properties of agars tested were not similar.

Keywords: Agars, carrageenan, Sepharose 2B, Azure A, metachromasy.

Introduction

Agars and carrageenan are of high molecular sulfated algal polysaccharides. They are found characteristically in the cell walls and intercellular matrix of algae.

Agar is not a unique compound. It is a complex mixture of agarose and agaropectine. Agarose is a repeating unit of agarobiose. It also contains pyruvated agarose, 4,6-O-(1'-carboxy) ethylidene - D-galactose. Percentage composition and proportion of agarose are differed of species of algal. The average content seems to be of the order of 55 to 66% (Araki, 1996). Agaropectine contains D-galactose, 3,6 anhydro-L-galactose, some ester sulfate (3.5 to 9.7%) and D-glucuronic acid, pyruvic acid (1%). Another agaropectine- type content L-arabinose, D-glucuronic acid and 5,3 % of ester sulfate (Percival and McDowell 1967).

Carrageenan is a polymer formed of alternating α-1,3 and β-1,4-linked D- galactopyranosyl unit. A major variation occurs in κ- and i-carrageenan where 3,6- anyhydro- D- galactose units replace the 1,3-D galactose unit. The 3,6 anhydro bridges are formed by the elimination of sulfate from the C6 sulfate ester (OSO^{-}_{3}).

Metachromasy is a reaction of mucopolysaccharides with special dyes. It was first demonstrated by Ehrlich (1877) in histological staining on tissue elements. The metachromatic phenomenon of algal polysaccharides was studied by various workers (Michaelis, 1947; Shubert and Levin, 1953; Stone *et al.*, 1963; Suzuki *et al.*, 1969; Graham, 1971; Stone, 1972; Gangolli *et al.*,1973). The mechanism of this phenomenon was explained by Lison (1935) as the change of absorption band of the dyes from long to shorter wavelengths. Lison (1935) showed that agar gave metachromatic reaction with cresyl blue and carrageenan with toluidine blue and brilliant cresyl blue. Bank and De Jong (1939) used toluidine blue for carrageenans. Pal and Schubert (1963) tested toluidine blue, acridine orange and methylene blue,crystal viole for λ-carrageenans and at the dilute concentration of dyes visible absorption spectra as the dye have a single high pick (called the α-band), at higher concentration the α-band becomes depressed and a new band (β-band) appears at a shorter wavelength and at the highest concentration that can be reached in aqueous solution both α-bands and β-bands are usually depressed and a third band (γ-band and μ-band, bathochromic band) has emerged at a still shorter wavelength. Stone *et al.*, (1963) used acridine orange, methylene blue, proflavine and neutral red for λ and κ carrageenans Stone and Bradley (1967) studied neutral red for carrageenans. Agar formed an aggregate with metachromatic dyes (Michaelis and

Granick, 1945).

Dyes showed metachromasy are: Aniline blue, Parme blue, anilin violet, Methyl violet, Dahlia, Gentiane violet, Methylene violet, Thionine, Toluidine blue, Cresyl blue, Cresyl violet, Oxonine, Acridine red.

The other studies on this subject are: Güven and Güvener 1985(a,b) studied metachromatic reaction of alginic acid, agar, carrageenans of iota, kappa and λ types and showed characteristic, metachromatic, β-band for identification of algal polysacharides. Güven *et al.*, (1988) were studied for identification of carrageenan in tooth paste.

In this paper metachromatic reaction is reported of azur A with various agars and carrageenan after fractionation on Sepharose 2B.

Material and Method

The examined agars and carrageenan are:

Agar		Collection areas
Gracilaria verrucosa	(Huds.) Papenfuss	Büyükçekmece
Phyllophora nervosa	(D. C. Grev) (Güven *et al.*,1972)	Şile
Ceramium rubrum	(Huds.) C. Ag.	Bosphorus
Gelidium latifolium	(Glem.) Born. Et Thus	Şile

Pasteur agar obtained from market

Carrageenan type LC Pectin Factory (Copenhagen)

Sepharose 2B (Pharmacia)

The dye solution was 0.01% azür A in water.

The concentration of agar and carrageenan solution was 0.15% in water.

Metachromatic band (β-bands) of tested polysaccharides and λ max. of dye (α-band) are determined in UV spectrophotometer (Shimadzu 1601). In the present work the agars obtained from the algae collected from the Turkish coast were studied beside a sample of commercial agar and also one carrageenan type LC. The investigation was made with these algal polysaccharides after fractionation on Sepharose 2B. They were missed in the earlier work.

Solutions of agar and carrageenan were applied to a Sepharose 2B column. It was eluted with water. The measurement of absorption of dye (α-band) and metachromatic properties (β-band) of each 5 ml eluate were investigated at 620 (α-bands) and at 546 nm (β-band).

Results

The eluates that showed metachromatic reaction are:

Agar

Phyllophora nervosa	15-55 ml
Gracilaria verrucosa	20-45 ml
Gelidium latifolium	15-40 ml
Ceramium rubrum	25-55 ml
Pasteur agar	20-30 ml
Carrageenan Type LC	25-95 ml

The α- and β- bands of agars and carrageenan eluates obtained on Sepharose 2B are listed in Table 1.

Table 1. λ max. of agars and carrageenan

	α-band (nm)	β-band (nm)
P. nervosa	*620*	*546*
G. verucosa	*617*	*553*
G. latifolium	*626*	*546*
C. rubrum	*626*	*556*
Pasteur Agar	*623*	*545*
Carrageenan Type LC	614	544

The spectra of eluates of agars and carrageenan on Sepharose 2B are shown in Fig. 1-6.

Analysis of algal polysaccharides were made by infrared spectrophotometry (Stanley, 1963; Bellion *et al.*, 1981; Rochas *et al.*,1986; Sur and Güven 2002).The technique based on the presence of ester sulfate (1240 cm^{-1}), and 3,6- anhydro galactose (928-940 cm^{-1}), the position of esterification (820 cm^{-1}) primarily hydroxyl 6-sulfate (830 cm^{-1}), equatorial secondary 2-sulfate (840-850 cm^{-1}), axial secondary 4- sulfate. The absorption band near 805 cm^{-1} is atributed to 3,6 anhydro -D- galactose-2-sulfate (Anderson *et al.*, 1968) and it is characteristic for carrageenans (Craigie and Leigh 1978).

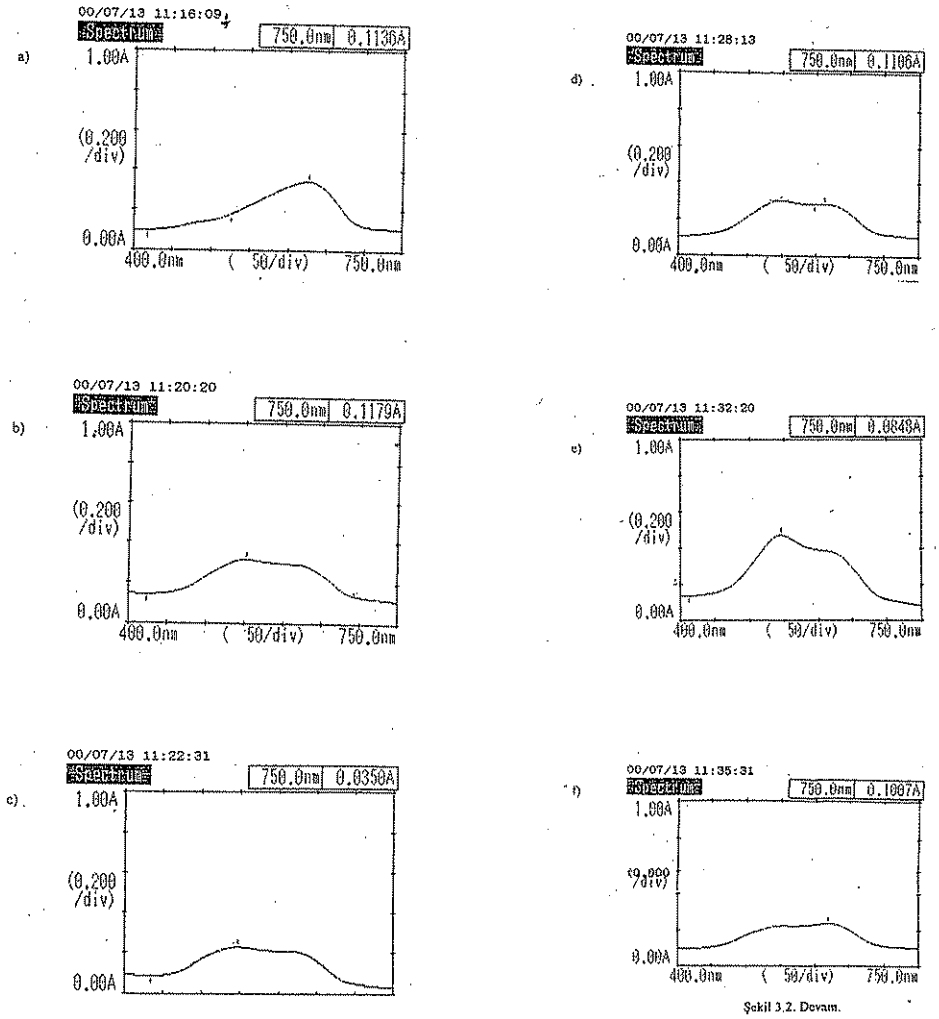

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Fig.1

The spectrum of *Phyllophora nervosa* agar eluates on Sepharose 2B

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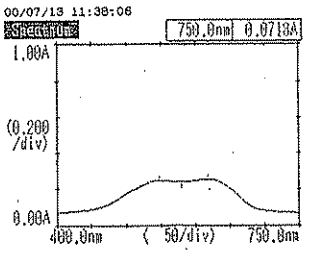

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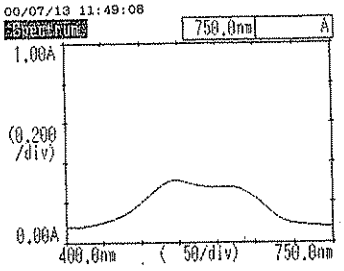

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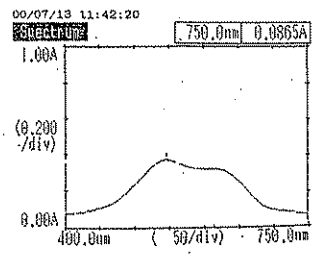

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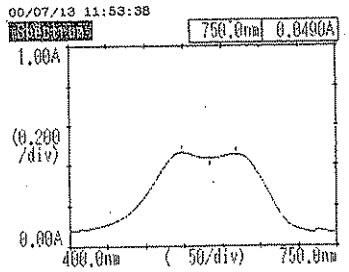

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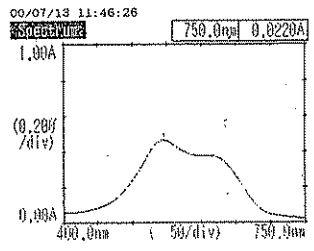

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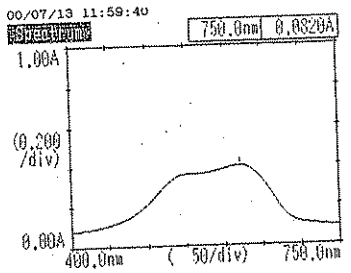
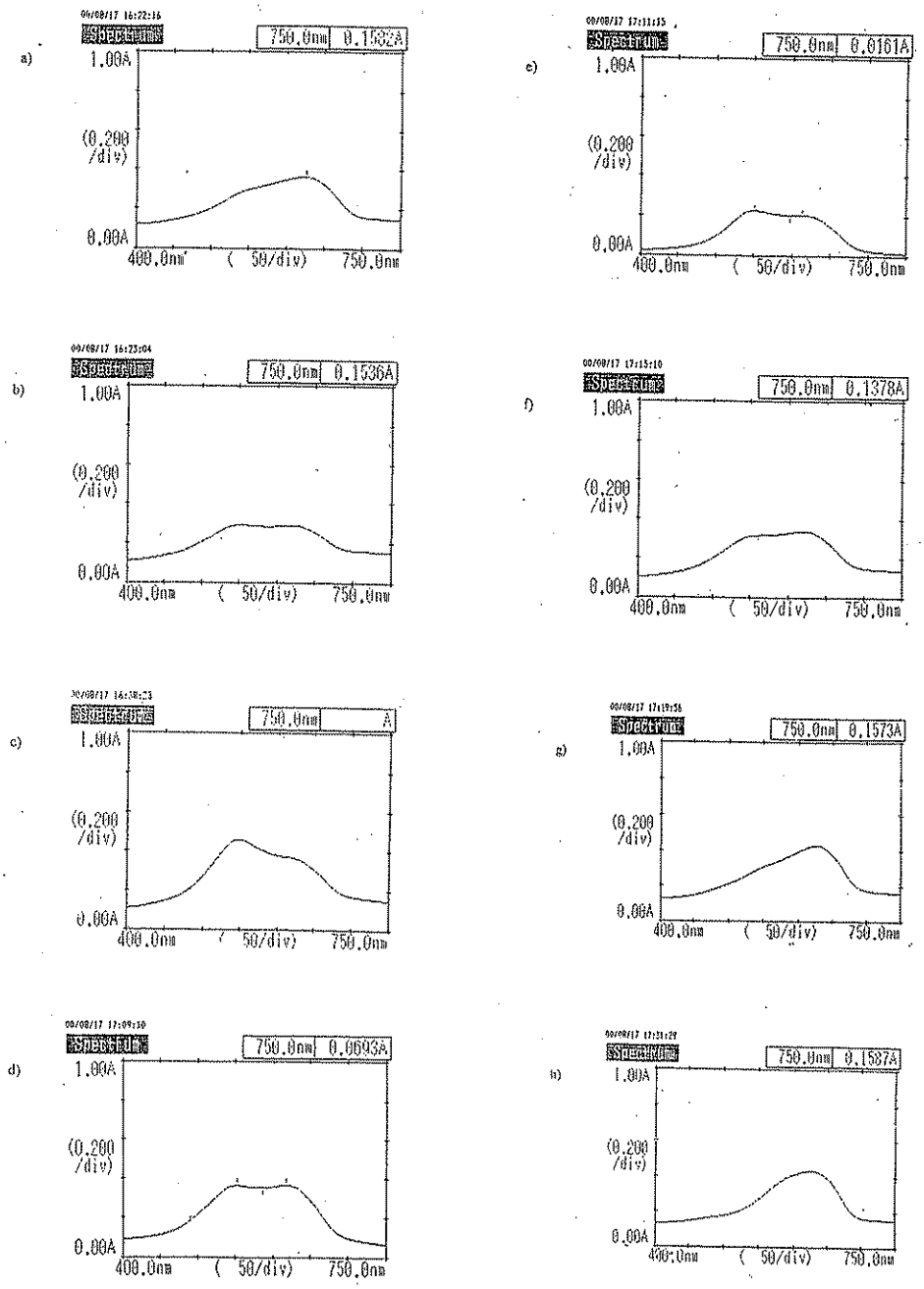


Fig.1. Continued.



Fig,2.

The spectrum of *Gracilaria verrucosa* agar eluates on Sepharose 2B

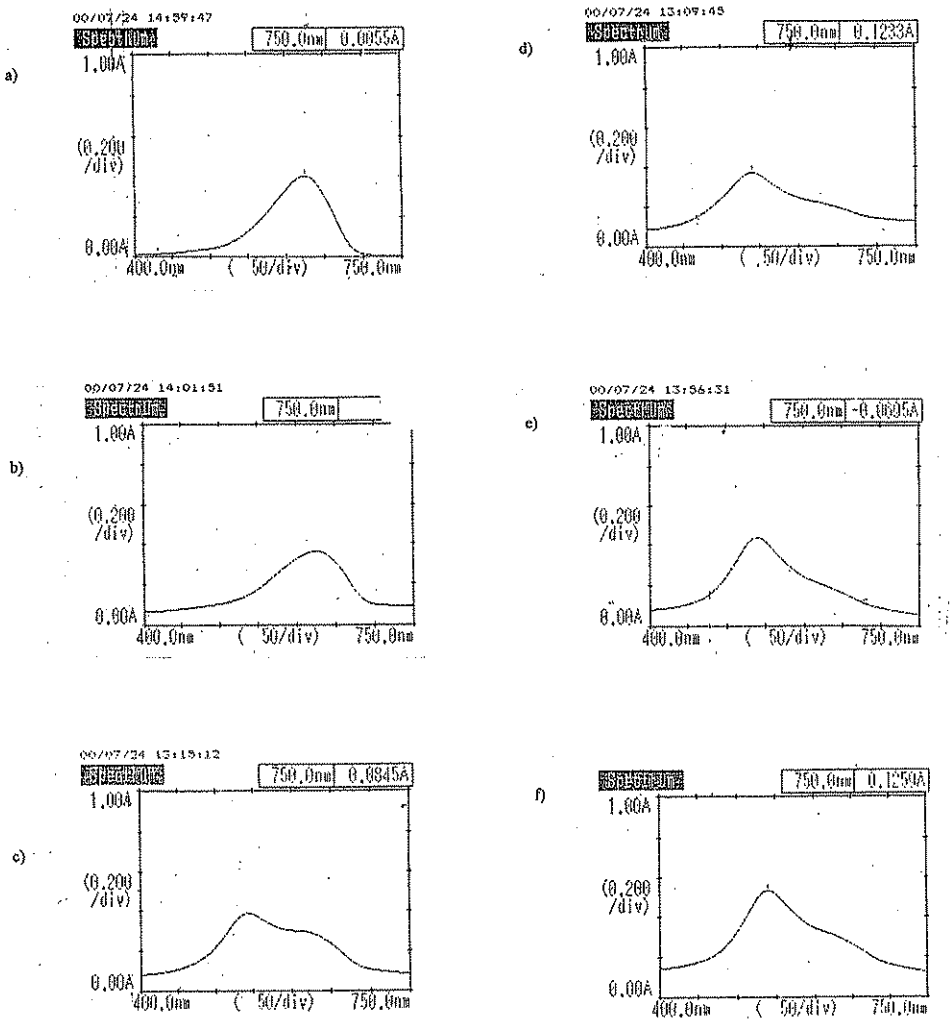
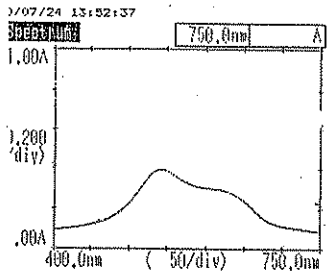


Fig.3

The spectrum of *Gelidium latifolium* agar eluates on Sepharose 2B

g)



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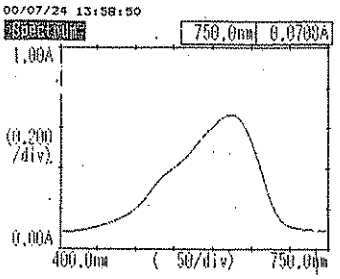

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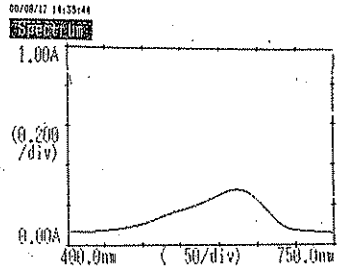
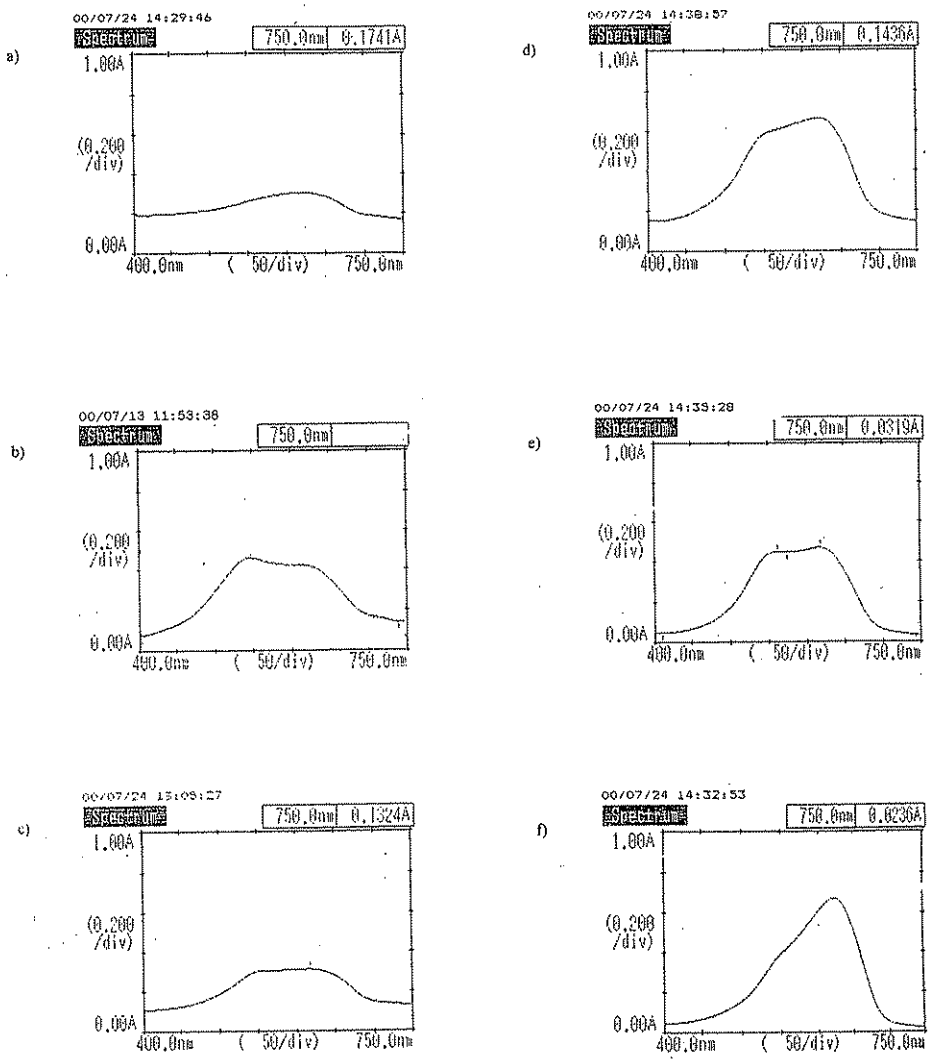


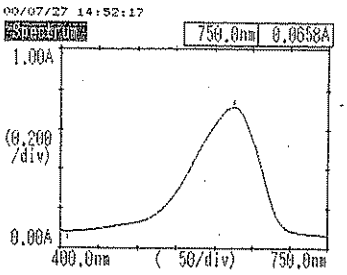
Fig.3. Continued.



Fig,4 .

The spectrum of *Ceramium rubrum* agar eluates on Sepharose 2B

a)



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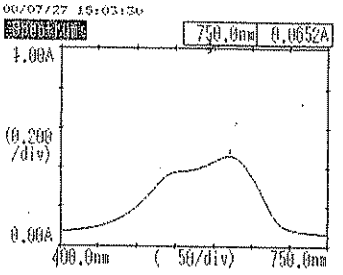
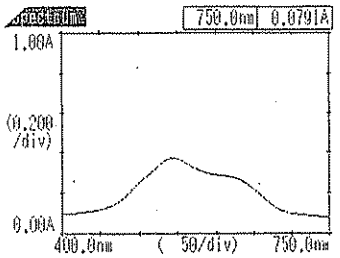
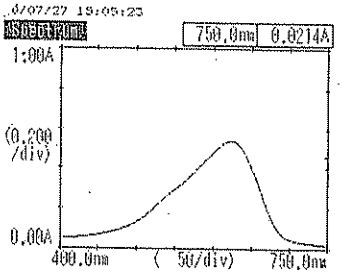


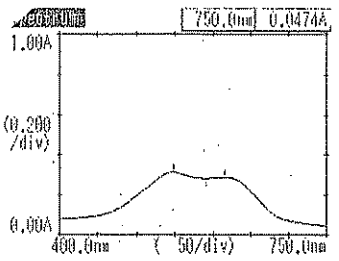
b)



c)



e)



f)

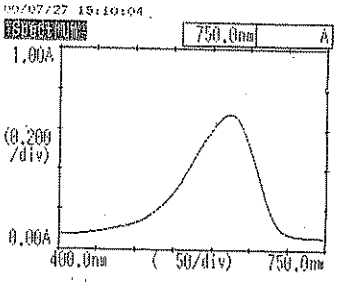
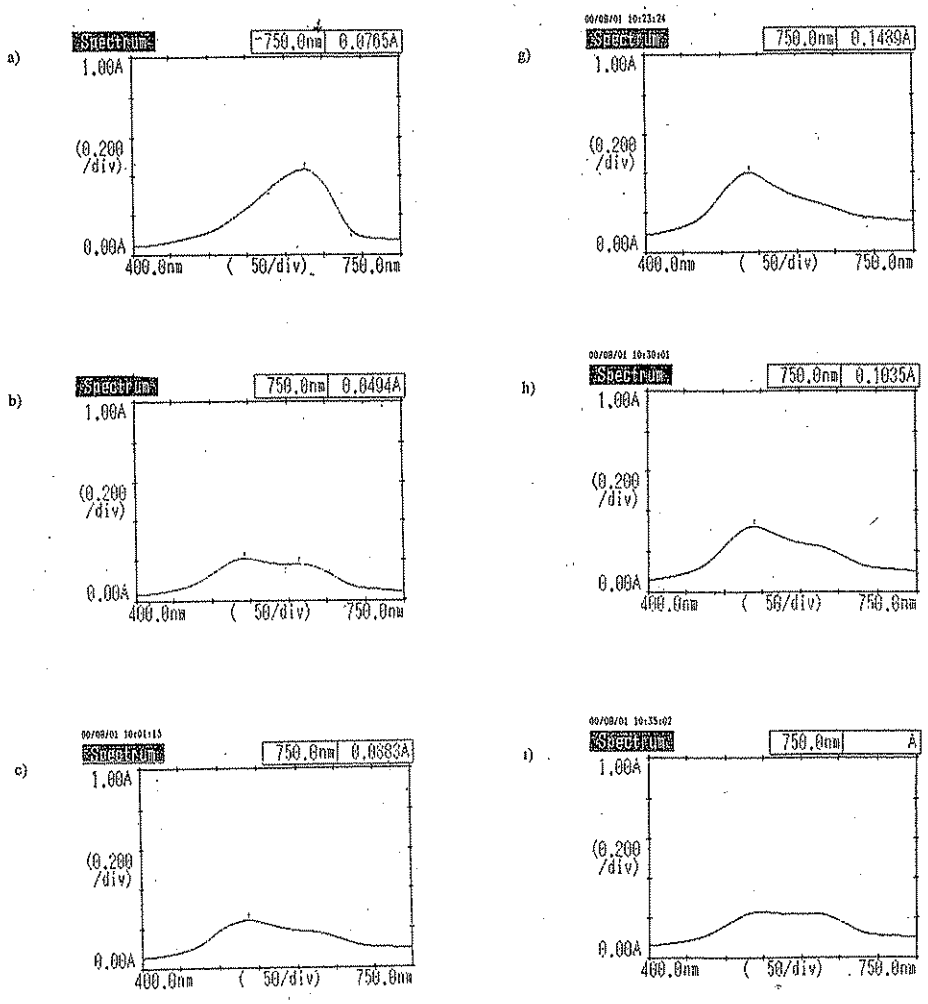


Fig.5。

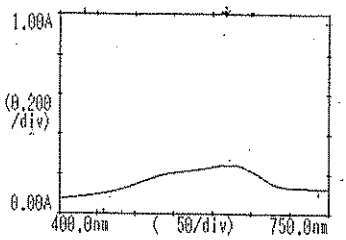
The spectrum of *Pasteur agar* eluates on Sepharose 2B



Fig,6

The spectrum of carrageenan type LC eluates on Sepharose 2B

j)



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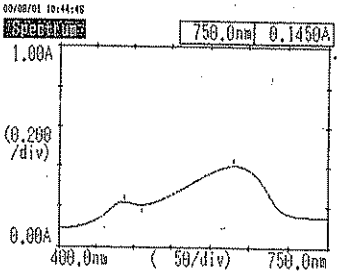

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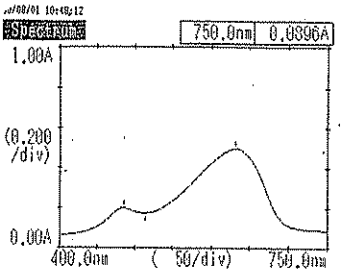

Fig.6. Continued.

The other method of analysis algal polysaccharides is based on metachromasy. Bank and De Jong (1939) investigated metachromatic reaction of agar, carragenan and other polysaccharides with various dyes. The α- and β- bands were 570-535 nm for agar and 560-525 nm for carrageenan with toluidine blue.

Toluidine blue and thionine gave best results on metachromasy (Michaelis and Granick, 1945). Contrary to the findings of Michaelis (1947) we did not observe bathochromatic band (γ- band 670 nm) in agar. In earlier studies the absorbance values of α- bands of agars and carrageenans were generally higher than those of β- bands, but in this work the reverse was observed for agars and carrageenan obtained on Sepharose 2B column.

According to our findings the eluates of *Phyllophora nervosa* agar showed higher absorption values than the tested other agars and carrageenan. The λ max. of eluates and non fractionated agar were the same. Güven and Güvener (1985 a,b) investigated the metachromasy with various dyes and also Azure A on unfractinated algal polysaccharides at 618 nm for α- and 557 nm for β-bands.b In this work the λ max values are 617 nm for α - and 553nm for β-band in agar and 614 nm for α- and 544 nm for β- band in carrageenan. The results obtained showed that agars tested were not similar for metachromatic properties.

In contrary to Pal and Schubert (1963) findings bathochromic band (γ-band) was not observed in eluates of algal polysaccharides on Sepharose 2B. In summary it may be noted that 25-95 ml eluates on Sepharose 2B of carrageenan showed metachromasy while the agar fractions did it between 20-45 ml. The maximum absorption value of β-band was higher than for α-band in some cases.

Özet

Türkiye sahillerindeki alglerden elde edilen agar ile ticari agar ve karagenanın metakromatik özellikleri Sepharose 2B de yapılan fraksiyonlama sonucu elde edilen eluatlar üzerinde incelendi. Her eluatın alpha ve beta bandlarının lamda maxları tayin edildi. Metakromatik özellik gösteren eluatların hacmi *P.nervoza* için 12-55 ml, *G. verrucosa* için 20-45 ml, *C. rubrum* için 25-55 ml, *G. latifolium* için 15-45 ml, *Pasteur agar* için 20-30 ve karagenan için 20-95 ml. dir. Bu sonuçlara gore agar çeşitleri ile karagenanın aynı metakromik özellik göstermediği tespit edilmiştir.

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Recevived:22.11.2002
Accepted:25.02.2003