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Physico-Chemical Characterization of Carrageenan at Different Temperatures, Isolated from *Hypnea musciformis* from Karachi Coast Pakistan

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Abstract: This study describe the basic tools like viscosity measurements and thermodynamic parameters of the polysaccharide isolated from *Hypnea musciformis* (red algae) of Karachi Coast (Pakistan) showed characteristics of κ-carrageenan. An IR spectrum showed a fairly sharp band at 1210, 1225 cm^{-1} corresponding to sulphate ester and is in accordance with the high sulphate content of κ-carrageenan. Viscosity measurements revealed a linear relationship with increase in concentration and decreased with the rise in temperature of aqueous solutions of κ-carrageenan. Thermodynamic parameters were determined by the change in viscosity data as a function of temperature and concentration. The free energy change of activation (ΔG_η) increased regularly as the concentration of aqueous κ-carrageenan increased, as well as rises in temperature. Higher values of free energy change of activation, (ΔG_η) showed the higher association of κ-carrageenan with water at given temperature. The values of entropy change of activation (ΔS_η) of viscous flow also increased with the increase in concentration and temperature of aqueous κ-carrageenan solution. The high negative values of entropy change of activation (ΔS_η) showed that the solution of κ-carrageenan was more ordered in initial state than the activated one.

Key words: *Hypnea musciformis* (red algae), κ-carrageenan, energy of activation, latent heat of vaporization, entropy of activation

INTRODUCTION

Carrageenan is a collective term for polysaccharides prepared by alkaline extraction (and modification) from red seaweed (*Hypnea musciformis*) of Karachi Coast, Pakistan mostly of genus *Chondrus*, *Eucheuma*, *Gigartina* and *Iridaea*. Different seaweeds produce different carrageenans (Lee *et al.*, 2004).

Carrageenan can be used in desert, ice cream, milk shakes, sauces-gel to increase viscosity, payday bars contain carrageenans, beer-clarifier to remove haze-causing proteins, Pâtés (Blandino *et al.*, 2001; Zumbado *et al.*, 1999) and processed meat substitute fat to increase water retention and increase volume, toothpaste-stabilizer to prevent constituents separating, fire fighting foam-thickener to cause foam to become sticky, shampoo and cosmetic Creams-thickener, air freshener gels, Shoe polish-gel to increase viscosity, thermo reversible gels on cooling in the presence of appropriate counter ions, biotechnology-gel to immobilize cells/enzymes (Onsoyen, 1997; Lee *et al.*, 2003). Personal lubricants-laboratory studies suggest that carrageenans might function as a topical microbicides, blocking sexually-transmitted viruses such as HIV, HPV and herpes.

The viscosity method is employed for determination and confirmation of compounds and their behaviour in different solvents. Viscosity is affected by a number of parameters such as molecular mass, shape and size of molecules, concentration, temperature and intermolecular attractions i.e. ion-ion and ion-solvent interactions (Billmeyer, 1962). Ample data is available in literature on rheological studies of carrageenan (Gomez-Diaz and Navaza, 2002; Ming *et al.*, 2000). However, little attention has been paid on viscometric and thermodynamic behavior of the carrageenan obtained from red sea weeds of Karachi coast.

The aim and objective of present investigations is the chemical characterization, spectral and thermodynamic study of the carrageenan isolated from red sea weeds of Karachi coast for the use of this polymer in industrial and pharmaceutical level, as marine algae are very important utilization in human life. Various thermodynamic parameters such as energy of activation (E_η), free energy
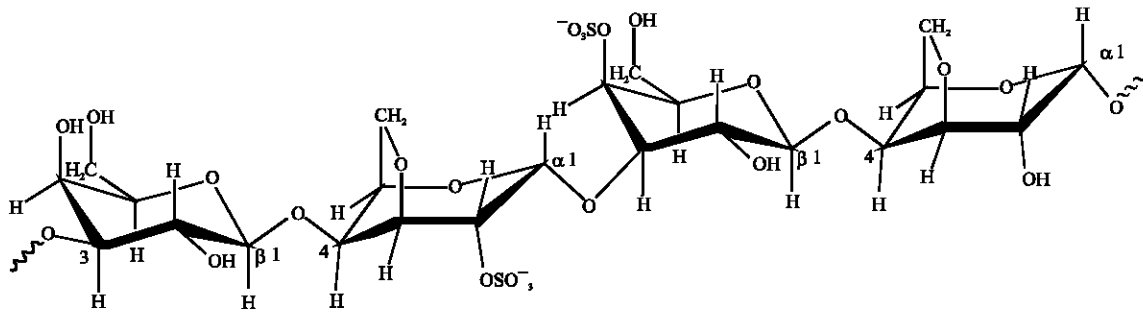
Carrageenan consists of alternating 3-linked- β -D-galactopyranose and 4-linked- α -D-galactopyranose units

κ-carrageenan (kappa-carrageenan) -(1→3)- β -D-galactopyranose-4-sulfate-(1→4)-3,6-anhydro- α -D-galactopyranose-(1→3)-

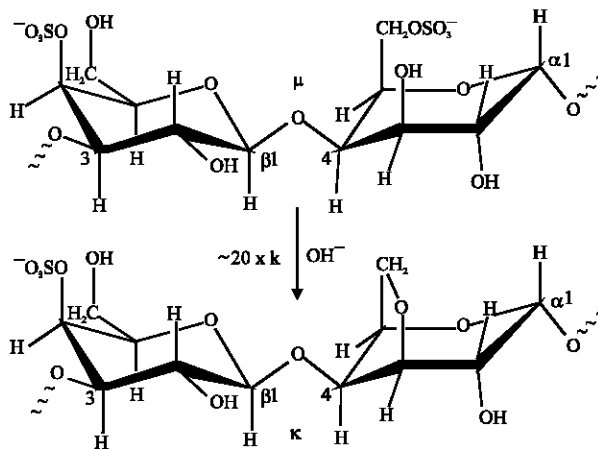κ-carrageenan is produced by alkaline elimination from µ-carrageenan isolated mostly from the red seaweed Hypnea musciformis. The experimental charge/dimer is 1.03 rather than 1.0 with 0.82 molecules of anhydrogalactose rather than one.

change of activation (ΔG$_\eta$), latent heat of vaporization (L$_\eta$) and entropy change of activation (ΔS$_\eta$), for the viscous flow of aqueous carrageenan solutions were studied as a function of temperature and concentration. The activation energy of flow gave information about the behavior of κ-carrageenan in water and FTIR spectral analysis helped to study the structure of carrageenan (Percival and Mcdowell, 1990).

MATERIALS AND METHODS

Collection of red algae: Marine red algae were harvested from Bullagee Coastal area of Karachi Coast in the morning from free floating low tide season in February 2006. The algae was transported in PCSITR Labs Complex Karachi where it washed with normal and then deionized water. Algae dried and ground at room temperature.

Isolation of carrageenan: After the separation of fatty material, the residual plant was dried at 25°C and subjected to hot water extraction (1 g/30 mL) twice. Extracts were obtained with constant stirring at 70-80°C for 6 h. Supernatant were collected and precipitated with

95% ethyl alcohol (1:3). The precipitates were dried at low temperature as κ-carrageenan.

Analytical method: Moisture, ash and total sugar were determined as before (Bi Fatima and Iqbal, 1999). Sulphate content was determined by modified method (Dodgson and Price, 1962). Optical rotation of aqueous solution of carrageenan of known concentration (1%) was determined with digital polarimeter (Jas. Co. Dip. 360) using 50 mm tubes and sodium D line at 589 cm wavelength. FTIR analysis was performed on Nicolet Avatar 370 DTGS Fourier Transform Infra Red Spectrometer (Bi Fatima and Iqbal, 1999).

Viscosity measurement: All glass wares used were of Pyrex A grade quality. A 0.5% stock solution was prepared in 100 mL volumetric flask by dissolving the 0.5 g of isolated κ-carrageenan in deionized water having conductivity less than 1 µS cm^{-1} with constant stirring till clear solution formed, then volume was made to the mark. This stock solution was used to prepare further dilutions ranging from 0.05 to 0.25%. Relative viscosities were measured with Ubbehlode Viscometer type

techniconomial constant 0.1 cS/s capillary ASTM D 445. Viscometer was placed inside a glass tube connected with thermostatic water bath (type VWP Scientific, model 1120, Ser 9143791) having a constant circulation of water throughout the course of experiment. The temperature of the water bath was kept constant with the help of temperature control device type Haake Delab Instrument (Electro Laboratoriet, Copenhagen) with±0.1 °C. A known volume of solvent (water) and solution was placed in the viscometer for at least 15 min to attain the required temperature and their time of flow was recorded (Dubois, 1956). Viscosity data at different temperatures were used to calculate thermodynamic parameters such as energy of activation, free energy change of activation, entropy change of activation etc.

Densities of solvent and solutions were measured with the help of a relative density bottle (10 mL) at different temperatures. The reproducibility of results was checked by repeating the measurements three times. The uncertainty in the experimental data was found to be ±0.001 g mL^{-1} and ±0.002 cP, respectively.

RESULTS AND DISCUSSION

Carrageenan yield was 30% of dry plant, *Hypnea musciformis*. The yield was much higher than reported earlier i.e., 23.7% (Dodgson and Price, 1962). Physico chemical properties of isolated cerageenan and commercial sample purchased from local market were compared. There were significant differences were observed in the optical rotations and in sulphated ash contents were shows in Table 1. Isolated product showed a rotation of 52° and found in the range as described earlier (Bitter and Muir, 1962). However optical rotation cannot be taken as a criteria of quality or a

reliable index of purity. Chemical analysis revealed that isolated sample exhibited 55.6% sugar and sulphate content (35.4%). Sometimes variables used during extraction procedures like type of solvents, concentration of solvents and also the seasonal variations influence and affect the yield, apparent viscosity, colour and chemical composition of the product (Smith and Montgomery, 1959; Zhenjin and Wanqiao, 1997). Infra red spectroscopy was used for the characterization of carrageenan (Vincent *et al.*, 1955; Jhonson *et al.*, 1997). An IR spectrum showed a fairly sharp band at 1210, 1225 cm^{-1} corresponding to sulphate ester and is in accordance with the high sulphate content of carrageenan (Fig. 1) (Zumbado *et al.*, 2000).

Viscosity measurements of aqueous solution of carrageenan were determined from 25 to 50°C at the rate of 5°C at various dilutions ranging from 0.1-0.5 g dL^{-1}. Solution properties of chemical substances in the region of extreme dilution are of great importance, both theoretically and experimentally. Viscosities of various aqueous solutions of isolated κ-carrageenan at 6 temperatures are recorded in Table 2. Results showed a regular increment in viscosity with the increase of concentration of solutes at each temperature i.e., 298 K, 303 K, 308 K, 313 K, 318 K and 323 K. The viscosity of the solutions seems to decrease with the rise of temperature as reported earlier (Ming *et al.*, 2000; Hussain *et al.*, 2004). The increase in viscosity on the addition of carrageenan to water is understood, to be due to increase in the degree of solvation. Representative plot of η_{sp}/C (reduced viscosity) versus concentration of aqueous carrageenan solution at 313 K (Fig. 2). The values of reduced viscosity (η_{sp}/C) of each sample of aqueous carrageenan solution were studied systematically.

Table 1: Physicochemical properties of κ-carrageenan and an isolated carrageenan from *Hypnea musciformis* (red algae)

Tests	Commercial carrageenan	Isolated carrageenan
Colour	Pinkish white powder	Light brown
Moisture (% w/w)	3.1	7.5
Ash (% w/w)	15.7	15.4
Aqueous gel strength (2% w/v)	Viscous solution at 4°C	Gel formation at 4°C
Optical rotation $[\alpha]_D^{25}$	53.8°	52.0°
Total sugar content (% w/w)	40.1	55.6
Sulphate content (% w/w)	40.8	35.4

Table 2: Values of densities and viscosities of an isolated carrageenan at different temperatures and concentrations (%w/v)

| Composition (g dL^{-1})	Density (g cm^{-3})						Viscosity (cP)					
	Temperature (K)						Temperature (K)					
	298	303	308	313	318	323	298	303	308	313	318	323
0.1	1.01505	1.01409	1.01313	1.01220	1.01112	1.00892	1.6676	1.4705	1.3006	1.2582	1.0495	0.9509
0.2	1.01654	1.01516	1.01401	1.01281	1.01157	1.01076	2.1920	1.9494	1.6742	1.5338	1.4141	1.2479
0.3	1.01766	1.01598	1.01435	1.01274	1.01163	1.01010	2.6871	2.1909	2.0962	1.8262	1.6251	1.5079
0.4	1.01789	1.01601	1.01448	1.01288	1.01210	1.01110	2.8417	2.5374	2.3374	2.1722	1.9578	1.7536
0.5	1.01795	1.01625	1.01499	1.01400	1.01280	1.01160	2.9511	2.8855	2.5801	2.2181	2.0186	1.9703
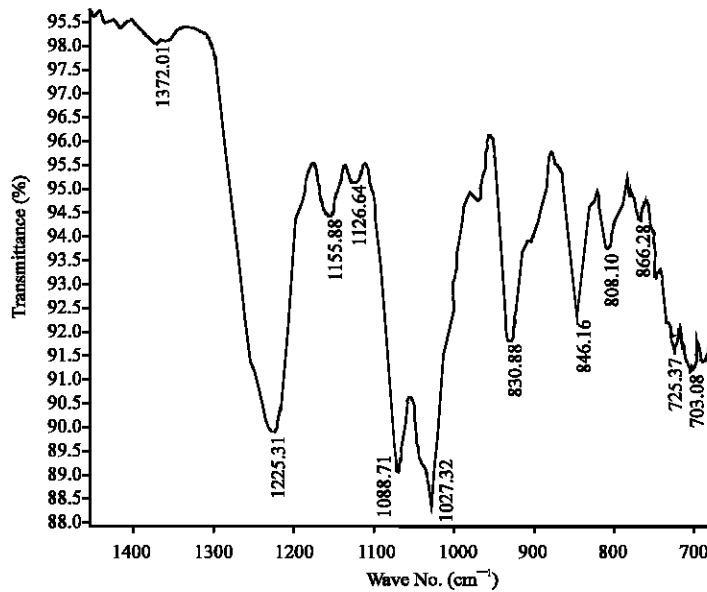
Fig. 1: FTIR Spectra of κ Carrageenan preparation from *Hypnea musciformis*
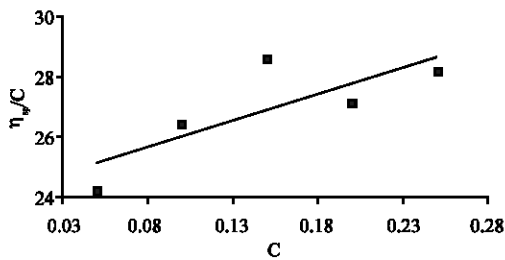

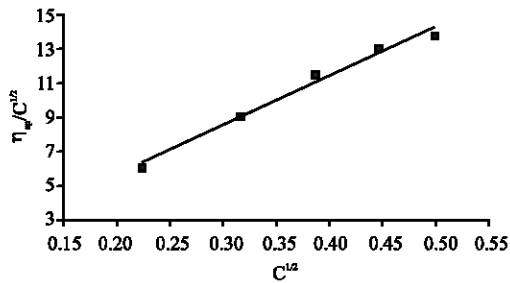Fig. 2: Plot of η_{sp}/C versus concentration for aqueous κ-carrageenan solution at 313 (K)

Fig. 3: Plot of $\eta_{sp}/C^{1/2}$ versus square root of concentration of aqueous κ-carrageenan solution at 308 (K)

Viscosity is one of the most appropriate techniques used to study various types of interaction occurring in solutions. The viscosity data are computed by using Jones-Dole equation (Stephen and David, 1975). A representative plot of $\eta_{sp}/\, C^{1/2}$ vs $C^{1/2}$ at 308 K (Fig. 3).

$$\frac{\eta_{sp}}{C^{1/2}} = A + B\,C^{1/2} \qquad (1)$$

Where:

η_{sp} = The specific viscosity,

A and B = Coefficients which represents ion-ion and ion-solvent interactions, respectively,

C = The concentration of carrageenan solution in $g\ dL^{-1}$.

The values of A and B coefficients were determined from the intercept and slope of the linear plots of $\eta_{sp}/\, C^{1/2}$ versus $C^{1/2}$. The regression method was applied for this purpose and the resulting A and B coefficients are shown in Table 3. The negative values of A-coefficient do not have any significance (Hoogmoed *et al.*, 2003; Anastassiou *et al.*, 1987). In this case, negative value of A have been found to decrease with the rise of temperature, which is due to dielectric constant of the medium and decrease in the ion-ion interactions. The values of B coefficient calculated and are found to increase with the increase of κ-carrageenan contents at all six temperatures. A solute with a positive B coefficient is expected to have a structure making effect. Present data shows the regular variation of B values with the rise of temperature, this may be due to the different degree of hydrolysis in given solvents. In this case as our solutes are of high molecular weights, high B values show that the ion-solvent interactions is strong i.e., structure maker.

Energy of activation: The relationship between viscosity and energy of activation is given by following equation.

Table 3: Values of ionic interactions of aqueous carrageenan solution in terms of Jones-Dole coefficients A and B at various temperatures

Temperature (k)	A (mol$^{-1/2}$ dm$^{3/4}$)	B (mol^{-1} dm^3)
298	-2.0582±0.002	27.328±0.015
303	-2.0732±0.005	26.391±0.022
304	1.9396±0.008	23.726±0.013
308	-1.6776±0.005	22.555±0.009
313	-1.6790±0.001	21.788±0.004
318	-1.8695±0.004	20.155±0.154
323	1.8890±0.007	20.093±0.654

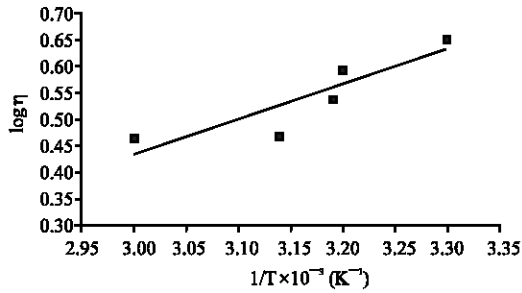Fig. 4: Plot of log η versus 1/T for κ-carrageenan in water at 303 (K)

$$\eta = A \exp (E_\eta / RT) \qquad (2)$$

Where:
η   = Viscosity of the algin solution,
E_η = Energy of activation,
R   = Universal gas constant,
T   = Absolute temperature.

The activation energy (E_η) was evaluated from the Arrhenius relation (Saeed *et al.*, 2004) and the representative plot of log η versus 1/T of 0.3% κ-carrageenan (Fig. 4). The Latent heat of vaporization (L_η) was evaluated from following relation,

$$E_\eta = 0.4 L_\eta \qquad (3)$$

The values of energy of activation and latent heat of vaporization are tabulated in Table 4. Decrease in energy of activation and latent heat of vaporization, as percent composition of aqueous κ-carrageenan rises is due to weakening of the inter molecular forces between the molecules of carrageenan by uncoiling aggregated molecules there by reducing entanglement between them. The energy of activation is related to the work needed to form a hole in the liquid. The holes are necessary for a liquid to flow. It is concluded that the values of activation energy are found high in pure water.

Free energy and entropy change of activation: The free energy change of activation for viscous flow (ΔG^*_η) is given by the following expression.

$$\Delta G^*_\eta = 2.303 \, RT \log \eta / 10^{-3} \qquad (4)$$

Table 4: Value of energy of activation (E_η) and latent heat of vaporization (L_η) of aqueous carrageenan solution

Composition (g dL^{-1})	Energy of activation (J mol^{-1})	Latent heat of vaporization (J mol^{-1})
0.1	19.115	47.787
0.2	18.564	46.410
0.3	16.956	42.390
0.4	15.945	39.862
0.5	14.569	36.422

Table 5: Values of free energy change of activation (ΔG_η) of aqueous κ-carrageenan solution at various concentrations and temperatures

Temperatures (K)	Free energy change of activation (ΔG_η) (kJ mol^{-1})				
	0.1	0.2	0.3	0.4	0.5
			(g dL^{-1})		
298	19.0640	19.0860	19.16720	19.2675	19.2959
303	19.1060	19.1430	19.19600	19.3230	19.3570
308	19.1310	19.1860	19.23900	19.3610	19.4030
313	19.2610	19.2930	19.34600	19.4280	19.4590
318	19.3990	19.4110	19.46870	19.5170	19.6010
323	19.6090	19.7050	19.81800	19.8940	20.0330

Where:
R   =   Universal gas constant,
T   =   Absolute temperature,
η   =   Viscosity.

The values of ΔG^*_η control the rate of flow in fluid process. The flow process is governed by the activity of molecule to move into the prepared hole and the readiness with which the holes are prepared in the liquid. The values of free energy change of activation are calculated and shown in Table 5.

The entropy change of activation is given by

$$\Delta S_\eta = \Delta H_\eta - \Delta G_\eta / T \qquad (5)$$

Energy of activation (E_η) does not differ appreciably from enthalpy change of activation (ΔH_η)

$$\Delta S_\eta = \frac{E_\eta - \Delta G_\eta}{T} \qquad (6)$$

Data shows that in some cases the values of E_η and ΔS_η are found lesser than that of the pure solvent indicating that the solvent structure is broken by the presence of solutes. In some cases, these values are found higher which are attributed to the excess of energy necessary to break the hydrogen bonds in solution. When these values are found equal to that of solvent, the hydrogen bond breaking of the solvent is not significantly affected by the solutes (Table 6).

The Mark-Houwink equation is used for estimation of average molecular weight of alginic acid of sodium salt in water at 323 K.

$$[\eta] = 1.60 \times 10^{-2} \, M^{0.748} \qquad (7)$$

The average molecular weight is found approximately >998,000 where as commercially available carrageenan

Table 6: Entropy change of activation (ΔS_η) of aqueous carrageenan solution at various concentrations and temperatures

Temperatures (K)	Entropy of activation (ΔS_η) (kJ mol^{-1})				
	0.10	0.20	0.30	0.40	0.50
	(g dL^{-1})				
298	-63.909	-63.984	-64.261	-64.600	-64.699
303	-62.993	-63.116	-63.297	-63.719	-63.836
308	-62.051	-62.231	-62.409	-62.808	-62.949
313	-61.475	-61.579	-61.755	-62.019	-62.122
318	-60.943	-60.982	-61.166	-61.324	-61.592
323	-60.649	-60.948	-61.303	-61.541	-61.976

have molecular weight between 76000-110,000 and the degree of polymerization of carrageenan is 180-930.

Chemical analysis and other physical data such as optical rotation, FTIR spectral studies etc. revealed that isolated carrageenan is a representative of k-carrageenan. Viscometric study confirms that associative interaction between the isolated product and water exists at specific temperature. Rheological studies described carrageenan as gelling, thickening and viscosity increasing agent for the given solvent i.e., water (Bi Fatima *et al.*, 2006).

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