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## Chemical and Thermodynamic Studies of K-carrageenan Isolated from *Hypnea musciformis* (Red Algae) of Karachi Coast

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**Abstract:** Physical and chemical analysis of the polysaccharides isolated from *Hypnea musciformis* (red algae) of Karachi coast showed the characteristics of k-carrageenan. The observed yield for the isolated carrageenan was about 36%. Optical rotation and sulphated ash contents were found as  $[\alpha]_D = 52^\circ$  and 16.20%, respectively. FTIR spectra showed a characteristic band at  $930\text{ cm}^{-1}$  represents the anhydrogalactose where as the band at  $840\text{ cm}^{-1}$  represent the galactose-4-sulphate. Viscosity measurements revealed a linear relationship with increase in concentration and decreased with the rise in temperature of aqueous solution of carrageenan. Thermodynamic parameters such as activation energy, latent heat of vaporization, free energy change of activation and entropy change of activation was evaluated by the change in viscosity data as a function of temperature and concentration. The free energy change of activation ( $\Delta G_a$ ) increased regularly as the concentration of aqueous carrageenan solution and temperature of the system increased. Higher values of free energy change of activation, showed the higher association of carrageenan with water at particular temperature. The values of entropy change of activation ( $\Delta S_a$ ) of viscous flow also increased with the increase in concentration and temperature of carrageenan solution. The high negative values of entropy change of activation ( $\Delta S_a$ ) showed that the solution of carrageenan was more ordered in initial state than the activated one.

**Key words:** *Hypnea musciformis*, polysaccharides, carrageenan, energy of activation, latent heat of vaporization, entropy of activation

### INTRODUCTION

Carrageenans are the highly sulphated polysaccharides extracted from red seaweed and widely used as functional ingredients in the food and dairy products as stabilizing thickening and gelling agents (Stanley, 1990; Nishizawa, 2002). They have a strong electrolyte character due to their sulphate groups. There are three major types of carrageenans i.e., k, I and  $\lambda$  carrageenans which mainly differ in the number of sulphate groups in the polygalactose backbone (Hilliou *et al.*, 2006). Because of the position of sulphate group only k and I carrageenans have the ability to form gels under certain conditions, whereas  $\lambda$  carrageenan does not undergo conformational ordering nor form gels therefore this type is used as non-gelling, thickening agent (Lahaye, 2001).

Rheological investigation have shown that many factors including concentration of polymers, temperature, electrical charge, previous thermal and mechanical treatments and presence of electrolytes may affect the rheology of a fluid food containing gums/mucilages (Gomez-Diaz *et al.*, 2006; Marcotte *et al.*, 2001). Viscosity of solutions is affected by a number of parameters such as molecular mass, shape and size of molecules and intermolecular attractions i.e., ion-ion and ion-solvent

interactions (Reed, 2003; Billmeyer, 1984). Karachi has a large coastal area and produces a huge amount of marine algae, unfortunately the seaweeds are not utilized in Pakistan either as sea vegetables or for extracting commercial compounds (Hussain *et al.*, 2001). Not much work is reported on physical and chemical characterization of carrageenan isolated from red algal plants of Karachi Coast. The present work is related to the chemical and physical characterization, spectral and thermodynamic study of the carrageenan isolated from *Hypnea musciformis* (red algae) of Karachi coast.

In this study, viscosity measurement of aqueous solution of carrageenan at various dilutions and temperature indicated the water interaction and behavior of carrageenan in solution. Various thermodynamic parameters such as energy of activation ( $E_a$ ), latent heat of vaporization ( $L_v$ ), free energy change of activation ( $\Delta G_a$ ) and entropy change of activation ( $\Delta S_a$ ) for viscous flow of aqueous carrageenan solutions are studied as a function of temperature and concentrations. The activation energy of flow gave information about the behavior of carrageenan in water. FTIR spectral analysis helped to study the structure of carrageenan. The information presented here is vital and will be used in suggesting the utilization of carrageenan in food, pharmaceutical and in other areas of application.

## MATERIALS AND METHODS

### Isolation of k-Carrageenan

*Hypnea musciformis* of class Rhodophyceae (red algae) was collected from Cap Monz of Karachi coastal area in March 2005. The plant material was cleaned from epiphytes, washed with tap and distilled water, dried and ground to a fine powder in a domestic model mixer. Algal powder (50 g) was de-pigmented and extracted with hot water by the method described previously (Knutsen *et al.*, 1995). The grayish-white gel (carrageenan) was obtained after alcohol (1:2 v/v) treatment and dried on low temperature at 45-50°C.

### Chemical Methods

Moisture content was determined as described earlier (Fatima and Iqbal, 1999). The analysis of sulphated ash was carried out by the conventional method of ashing in the presence of concentrated sulphuric acid. pH of 0.1% carrageenan solution was recorded by digital pH meter. Total sugar content was determined by the phenol sulphuric acid method (Dubois *et al.*, 1956). Assay for sulphate group was done by the 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 nm wavelength. FTIR analysis was performed on Nicolet Avatar 370 DTGS Fourier Transform Infrared Spectrometer.

### Viscosity Measurement

All glasswares used were of Pyrex A grade quality. A 1.0% stock solution was prepared in 100 mL volumetric flask by dissolving the 1.0 g of isolated carrageenan in deionized water having conductivity less than  $1 \mu\text{S cm}^{-1}$  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 0.1 to 0.5%. Relative viscosities were measured with Ubbelohde Viscometer type technical constant 0.1 cSt capillary ASTM D 445. The 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  $\pm 0.1^\circ\text{C}$ . 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 taken by stop watch (Advanced 85 Quartz) having least count of 0.25. Time of flow of water and aqueous solutions of carrageenan having concentration range 0.1-0.5% were recorded at 303-343 K temperatures.

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 of density and viscosity were found to be  $\pm 0.001 \text{ g mL}^{-1}$  and  $\pm 0.002 \text{ cP}$ , respectively.

## RESULTS AND DISCUSSION

The yield of the hot water extractable polysaccharides from *Hypnea musciformis* was 36% of the dry algae and similar in range as described earlier (Knutsen *et al.*, 1995). The physicochemical characterization of the isolated carrageenan compared with the commercial carrageenan (purchased from the local market) are summarised in Table 1. Results showed the characteristics of k-carrageenan. Significant differences were observed in total sugar and moisture contents. Isolated carrageenan exhibited high sugar 62.4%. It is documented that seaweeds belong to rhodophyceae (red algae) are commonly sulphated galactan (Miller and Blunt, 2002). Results also showed that both the samples were highly sulphated i.e contained 40.8 and 36.9%  $\text{SO}_4$ . Sometimes variables used during extraction procedures like concentration and type of solvents and also the seasonal variations influence and affect the yield, apparent viscosity, colour and chemical composition of the product (Bert *et al.*, 1989; Marcotte *et al.*, 2001). It was observed that the isolated product has an ability to form a thick and stable gel in 2% aqueous solution at  $4^\circ\text{C}$  whereas the commercial sample formed only a clear viscous solution. The reason could be that in market the carrageenan are found as blended material for different purposes rather a pure product (Thomas, 1997), so the isolated carrageenan showed high gelling strength as reported earlier (Puvanenthiran *et al.*, 2003).

Infrared spectroscopy is an alternative method to the expensive and time-consuming chemical and NMR methods for the characterization of carrageenan (Volery *et al.*, 2004). The IR spectra of the isolated carrageenan was recorded and compared with the market product (Fig. 1). It displayed a strong absorption band at  $1220 \text{ cm}^{-1}$ , general for sulphate ester (Chiovitti *et al.*, 1996) and is in agreement with the high sulphate content of these polysaccharides (Table 1). The diagnostic region ( $940\text{-}800 \text{ cm}^{-1}$ ) resembled that of k-carrageenan elaborated by red algal plant *H. musciformis* (Knutsen *et al.*, 1995). The characteristic bands at  $930$  and  $840 \text{ cm}^{-1}$  represented the anhydrogalactose and galactose-4-sulphate, respectively. A small band at  $805 \text{ cm}^{-1}$  showed a little contamination of l-carrageenan type as reported by Greer *et al.* (1984) who described that carrageenan extracted from *H. musciformis* contained 73% of k and 17% of l-carrageenan type structure.

In this study we describe viscosity measurements of aqueous carrageenan solutions from  $30$  to  $70^\circ\text{C}$  at the rate of  $10^\circ\text{C}$  increments at various dilutions ranging from  $0.1\text{-}0.5 \text{ g dL}^{-1}$ . Solution properties of chemical substances in the region of extreme dilution are of great importance, both theoretically and experimentally. Results in Table 2 showed a regular increment in viscosity with the increase of concentration of solutes at each temperature i.e.,  $303$ ,  $313$ ,  $323$ ,  $333$  and  $343 \text{ K}$ . 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  $\eta_{sp}/C$  (reduced viscosity) versus concentration of aqueous carrageenan solution at five different temperatures i.e.,  $303\text{-}343 \text{ K}$  is shown in Fig. 2. The values of reduced viscosity ( $\eta_{sp}/C$ ) of each sample of aqueous carrageenan solutions were studied systematically.

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 (Jones and Dole, 1929). A representative plot of  $\eta_{sp}/C^{1/2}$  vs  $C^{1/2}$  at  $303\text{-}343 \text{ K}$  is shown in Fig. 3.

$$\frac{\eta_{sp}}{C^{1/2}} = A + BC^{1/2} \quad (1)$$

Table 1: Physicochemical properties of commercial and isolated samples of carrageenan from *Hypnea musciformis* (red algae)

| Tests                              | Commercial carrageenan  | Isolated carrageenan |
|------------------------------------|-------------------------|----------------------|
| Colour                             | Pinkish white           | Light brown          |
| Moisture (% w/w)                   | 3.1                     | 8.0                  |
| Ash (% w/w)                        | 15.7                    | 16.2                 |
| 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                    | 62.4                 |
| Sulphate content (% w/w)           | 40.8                    | 36.9                 |

Table 2: Viscosity ( $\eta_{CP}$ ) of aqueous carrageenan solution at various concentration and temperatures

| Concentration<br>(g dL <sup>-1</sup> ) | Temperature (K) |        |        |        |        |
|--|-----------------|--------|--------|--------|--------|
|  | 303             | 313    | 323    | 333    | 343    |
| 0.1                                    | 2.4705          | 2.1582 | 1.9509 | 1.8574 | 1.6855 |
| 0.2                                    | 2.9494          | 2.5338 | 2.2479 | 2.0085 | 1.8746 |
| 0.3                                    | 3.0909          | 2.8262 | 2.5079 | 2.2275 | 1.9638 |
| 0.4                                    | 3.4374          | 2.9722 | 2.7536 | 2.4880 | 2.2472 |
| 0.5                                    | 3.8855          | 3.2181 | 2.6703 | 2.5004 | 2.3554 |

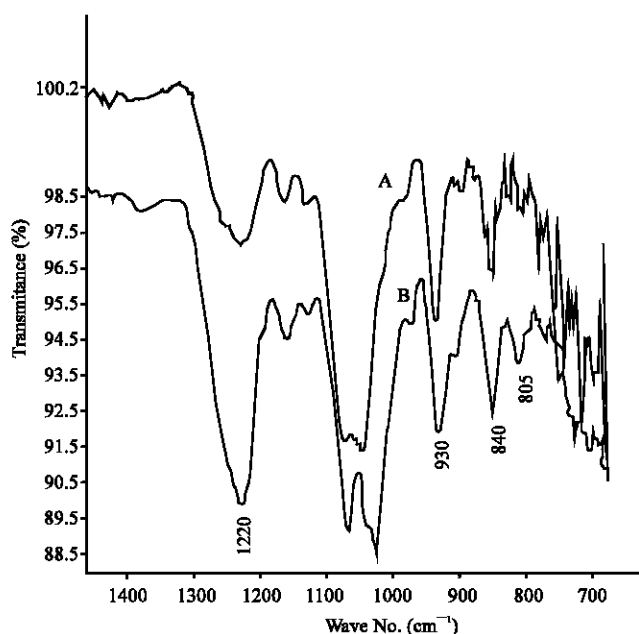


Fig. 1: Infrared spectra of isolated carrageenan (A) and commercial carrageenan (B)

Where  $\eta_{sp}$  is the specific viscosity, A and B are coefficients which represents ion-ion and ion-solvent interactions, respectively, C is the concentration of carrageenan solution in g dL<sup>-1</sup>. 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 negative values of A-coefficient do not have any significance (Table 3). In this case, A-values 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 are found to increase with the increase of carrageenan contents at all temperatures. A solute with a positive

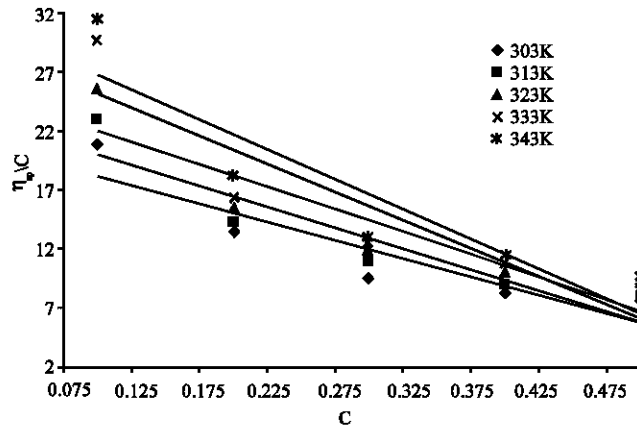


Fig. 2: Plot of  $\eta_{sp}/C$  versus concentration of the aqueous carrageenan solutions at five different temperatures

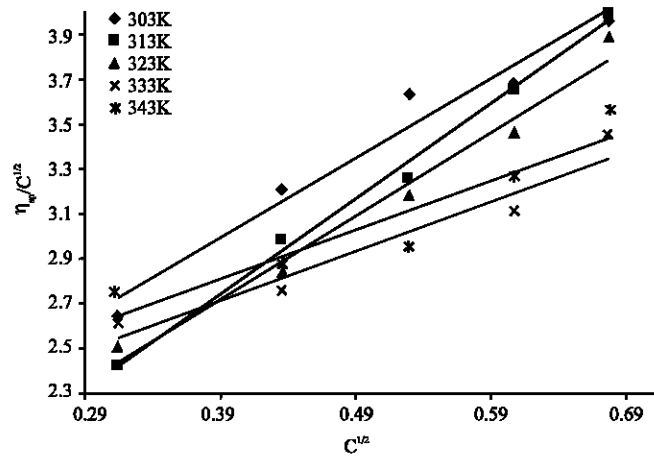


Fig. 3: Plot of  $\eta_{sp}/C^{1/2}$  versus square root of concentration of aqueous carrageenan solution at five different temperatures

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 degrees 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.

$$\eta = A \exp (E_{\eta}/RT) \quad (2)$$

Where  $\eta$  is the viscosity of the carrageenan solution.  $E_{\eta}$  is the energy of activation, R is the universal gas constant and T is the absolute temperature. The activation energy ( $E_{\eta}$ ) was evaluated

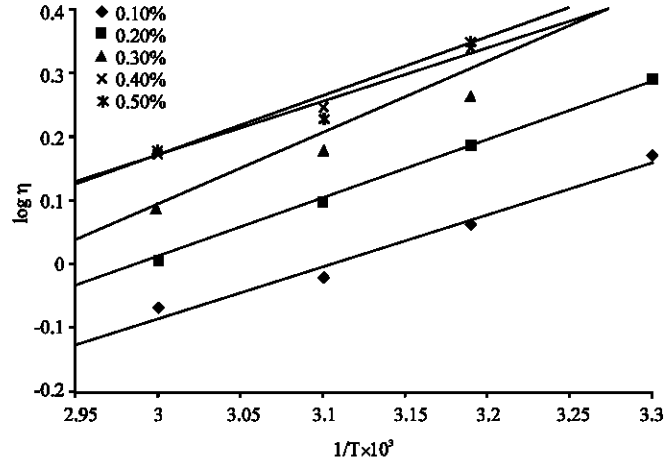


Fig. 4: Plot of log η versus 1/T for aqueous carrageenan solution at five different concentrations

from the Arrhenius relation (Saeed *et al.*, 2004) and the representative plot of log η versus 1/T as a function of carrageenan concentrations is shown in Fig. 4. The Latent heat of vaporization ( $L_{\eta}$ ) was evaluated from the following relation,

$$E_{\eta} = 0.4 L_{\eta} \quad (3)$$

Decrease in energy of activation and latent heat of vaporization, as temperature 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 (Table 4). 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 of Activation

The free energy change of activation for viscous flow ( $\Delta G_{\eta}^*$ ) is given by the following expression.

$$\Delta G_{\eta}^* = 2.303 RT \log \eta / 10^{-3} \quad (4)$$

Where R is the universal gas constant, T is the absolute temperature and η is the viscosity. The values of  $\Delta G_{\eta}^*$  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 (Table 5).

The entropy change of activation is given by:

$$\Delta S_{\eta} = \frac{\Delta H_{\eta} - \Delta G_{\eta}}{T} \quad (5)$$

Energy of activation ( $E_{\eta}$ ) does not differ appreciably from enthalpy change of activation ( $\Delta h_{\eta}$ )

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

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

| Temp. (K) | A and B coefficients of Jones-Dole equation |  |
|-----------|---|--|
|           | A (mol <sup>-1/2</sup> dm <sup>3/2</sup> )  | B (mol <sup>-1</sup> dm <sup>3</sup> ) |
| 303       | -2.0732                                     | +2.5391                                |
| 313       | -1.6776                                     | +2.9555                                |
| 323       | -1.8695                                     | +3.4155                                |
| 333       | -1.9332                                     | +3.8987                                |
| 343       | -1.4962                                     | +4.4385                                |

Table 4: Energy of activation (E<sub>a</sub>) and latent heat of vaporization (L<sub>v</sub>) of aqueous carrageenan solutions

| Composition of carrageenan (g dL <sup>-1</sup> ) | Energy of activation (E <sub>a</sub> ) (J mol <sup>-1</sup> ) | Latent heat of vaporization (L <sub>v</sub> ) (J mol <sup>-1</sup> ) |
|--|---|--|
| 0.1  | 15.8078   | 39.5797  |
| 0.2  | 17.0926   | 42.7315  |
| 0.3  | 18.7163   | 46.7907  |
| 0.4  | 18.9342   | 47.3355  |
| 0.5  | 19.0524   | 47.6310  |

Table 5: Free energy change of activation (ΔG<sub>a</sub>) of aqueous carrageenan solution at various concentration and temperatures

| Temp. (K) | Free energy change of activation (ΔG <sub>a</sub> ) at different concentration of carrageenan solution (KJ mol <sup>-1</sup> K <sup>-1</sup> ) |        |        |        |        |
|-----------|--|--------|--------|--------|--------|
|           | 0.1  | 0.2    | 0.3    | 0.4    | 0.5    |
| 303       | 19.683   | 20.129 | 20.248 | 20.515 | 20.824 |
| 313       | 19.981   | 20.398 | 20.683 | 20.814 | 21.021 |
| 323       | 20.348   | 20.729 | 21.023 | 21.274 | 21.191 |
| 333       | 20.842   | 21.059 | 21.345 | 21.651 | 21.665 |
| 343       | 21.191   | 21.494 | 21.627 | 22.011 | 22.145 |

Table 6: Entropy change of activation (ΔS<sub>a</sub>) of aqueous carrageenan solution at various concentration and temperatures

| Temp. (K) | Entropy change of activation (ΔS <sub>a</sub> ) at different concentration of carrageenan solution (K.J. mol <sup>-1</sup> K <sup>-1</sup> ) |         |         |         |         |
|-----------|--|---------|---------|---------|---------|
|           | 0.1  | 0.2     | 0.3     | 0.4     | 0.5     |
| 303       | -64.908  | -66.375 | -66.763 | -67.643 | -68.663 |
| 313       | -63.786  | -65.114 | -65.876 | -66.437 | -67.098 |
| 323       | -62.947  | -64.123 | -65.028 | -65.805 | -65.547 |
| 333       | -62.541  | -63.188 | -64.042 | -64.961 | -65.002 |
| 343       | -61.735  | -62.614 | -62.997 | -64.116 | -64.507 |

Data in Table 6 shows that in some cases the values of E<sub>a</sub> and ΔS<sub>a</sub> 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.

The Mark- Houwink equation is used for estimation of average molecular weight of carrageenan in water at 323 K

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

The average molecular weight of the isolated carrageenan is found approximately >100,000 where as reported molecular weight of commercially available carrageenan is greater than 250,000 and the degree of polymerization of carrageenan is 1100-1300 (Whistler and Bemiller, 1959).

It is concluded from the chemical analysis and other physical data that observed yield for the isolated carrageenan was about 36% and sulphated ash contents as 16.20%. Optical rotation of the product is  $[\alpha]_D = 52^\circ$  and FTIR spectral studies with a characteristic band at 930 and 840 cm<sup>-1</sup> for the



anhydrogalactose and galactose-4-sulphated group, respectively, tentatively identified the isolated product as k-carrageenan. Viscosity measurements revealed a linear relationship with increase in concentration and decreases with the rise in temperature of aqueous solution of carrageenan. Higher value of free energy change of activation ( $\Delta G^\ddagger$ ) showed the higher association of carrageenan with water at particular temperature. The high negative values of entropy change ( $\Delta S^\ddagger$ ) of activation showed that the solution of carrageenan was more ordered in initial state than the activated one. Viscometric study confirms that the 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 (Mamza and Folaranni, 1996).

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