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Effect of salts on the structure-function relationships of sodium kappa-carrageenan

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EFFECT OF SALTS ON THE STRUCTURE-FUNCTION RELATIONSHIPS OF SODIUM KAPPA-CARRAGEENAN

For the degree of Master of Science

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EFFECT OF SALTS ON THE STRUCTURE-FUNCTION RELATIONSHIPS OF
SODIUM KAPPA-CARRAGEENAN

A Thesis

Submitted to the Faculty

Of

Purdue University

by

Lohit Myneedu

In Partial Fulfillment of the

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West Lafayette, Indiana

Dedicated to my family

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ABSTRACT

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Carrageenans are sulfated marine polysaccharides used in a wide variety of food applications such as bodying, gelling, thickening and emulsion stabilization in water- and milk-based systems. They also find promising pharmaceutical usage due to anti-inflammatory, anti-tumor and anti-coagulant activities, to name a few. Among them, kappa-carrageenan is favored owing to its desirable properties such as high gel strength and stability. Though the significance of cations on the structure-function relationships is well-documented, the role of anions is still elusive. This investigation aims at providing the pertinent details, especially in the presence of halide ions such as Cl^- , Br^- and I^- . In this regard, sodium salts as well as sodium kappa-carrageenan have been chosen as the model systems. In addition, the effect of chaotropic salts urea and guanidinium chloride has been studied to understand their influence.

Dynamic rheological measurements and melting properties were obtained for 1.5% and 2% w/w solutions in the presence of 0, 50, and 100 mM salts. X-ray diffraction has been carried out on oriented fibers to assess the extent of association among the carrageenan chains towards network formation. The addition of sodium chloride, sodium bromide or

guanidinium chloride appears to promote aggregation among the carrageenan chains leading to increased elastic moduli, whereas urea has marginal effect. However, sodium iodide promotes soft gelling solutions as well as well-oriented and crystalline fibers. Furthermore, melting peak temperature and associated enthalpies are higher with sodium iodide than with the other salts, suggesting the important role of iodide ions in preventing the carrageenan chain aggregation leading to ordered networks. The outcome indeed aids in the enhanced utility of kappa-carrageenan, especially towards the design and development of novel functional materials such as carriers of bioactive compounds.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction to carrageenans

Carrageenans are a group of sulfated linear galactans extracted from a variety of seaweeds. The word "Carrageen" is a common term in Ireland, meaning "Little Rock". Their first use dates back to early 1800 to cure respiratory ailments (Turner, 1809). Following the successful commercialization of *Kappaphycus alvarezii* (referred as "cottonii") and *Eucheuma denticulatum* (known as "spinosum") in the mid-1960s, cultivation of red seaweeds for the carrageenan extraction began in Philippines and quickly spread to other warm-water countries such as Indonesia and Tanzania. The FAO report (2002) states that annually 150,000 tonnes (dry) of seaweed are utilized in producing 28,000 tonnes of carrageenan with a market value of \$270 million. Carrageenan has no nutritional value, but finds a myriad of applications in food and non-food industries (Burtin, 2003).

The general chemical structure comprises of a disaccharide repeat of an O3-substituted β -D-galactopyranosyl unit followed by an O4-substituted α -D-galactopyranosyl unit. Their classification was introduced using Greek alphabets and letter codes (Knutsen, Myslabodski, Larsen, & Usov, 1994) in addition to (1) the number and the position of the sulfate ester groups, and (2), the addition of alkali or enzymes resulting in the formation

Of an anhydro ring in the 4-linked unit. A total of 15 forms have been identified, however only iota- kappa- and lambda-carrageenan have found industrial uses. Based on Knutsen's nomenclature, the dimeric structures of κ , ι and λ -carrageenan are known as G4S-DA (Carrageenose 4'-sulfate), G4S-DA2S (Carrageenose 2,4'-disulfate), and G2S-D2S,6S(Carrageenan 2,6,2' trisulfate), respectively.

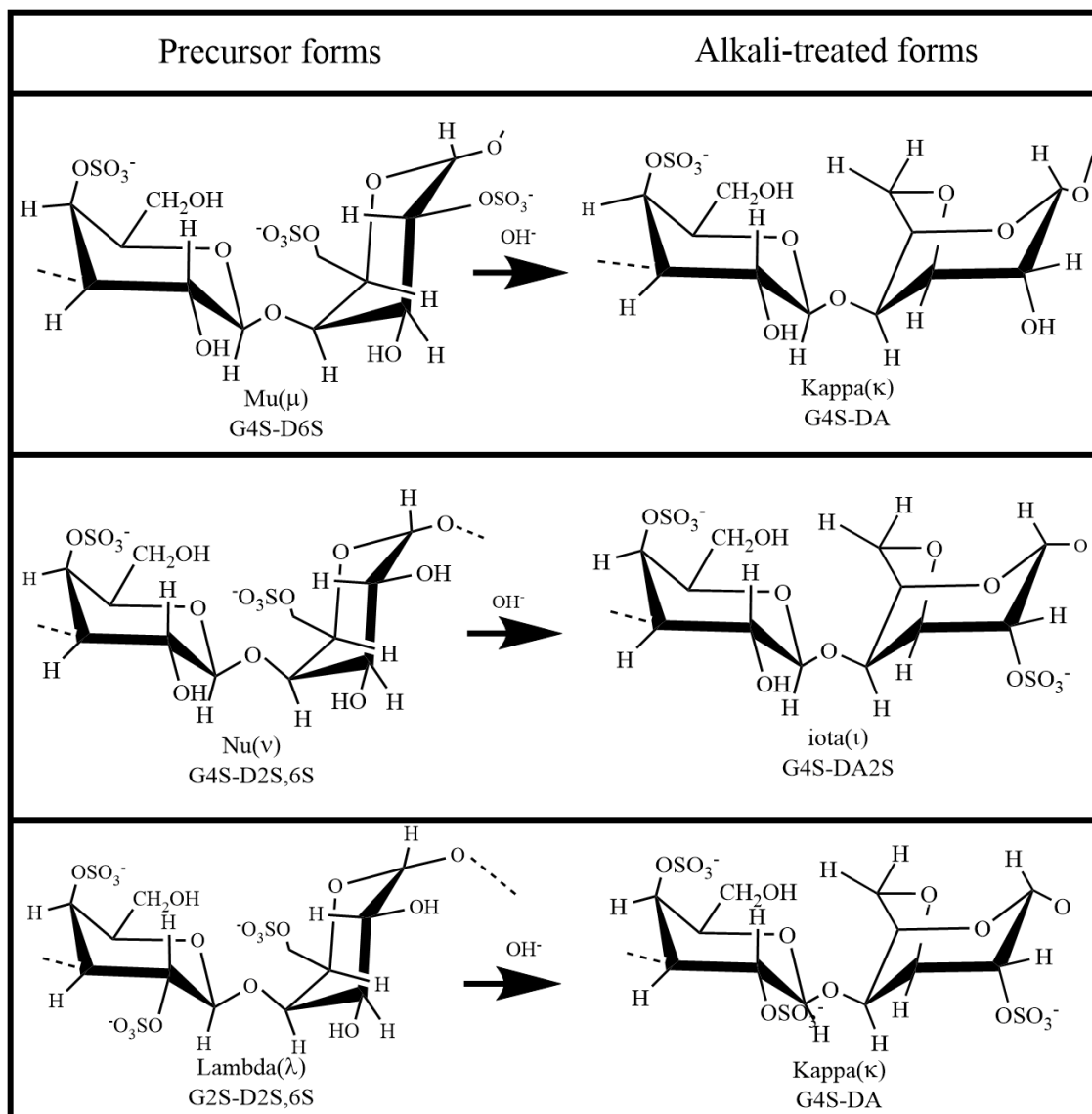


Figure 1. Commonly used forms of carrageenan. (Knutsen et al., 1994)

1.2 Identification of carrageenan

The yield of carrageenan varies with species variation, geographical location, seasonal changes etc. (Black, Blakemor, Colquhou, & Dewar, 1965). For example, during the summer comparatively higher percentages are obtained than the winter. Most identification methods call for the degradation or fractionation of carrageenans to analyze the structural details. However, a common and more convenient protocol is the NMR spectroscopy. The usual pre-treatments adopted include sonication of the sample and performing the measurements at high temperatures for reducing the higher solution viscosity to reduce line broadening (van de Velde, Knutsen, Usov, Rollema, & Cerezo, 2002). Carrageenans display well-resolved spectra with distinguishable signals in the anomeric region. The spectrum contains three peaks, with two of them representing the neocarrabiose sulfate repeating-unit and the third the bond between the 3,6-anhydro- α -D-galactose and the unsulfated D-galactose residue (Bhattacharjee, Yaphe, & Hamer, 1978).

ι -carrageenan and κ -carrageenan produced signals at 93.1ppm and 96.2 ppm, respectively. Carrageenans from the following red algae, *Eucheumacottonii* (Solieriaceae), *Eucheumaspinosum*, *Chondruscrispus gametophytes*, and *Gigartina stellata* (Gigartinaceae) have been fractionated in order to understand the nature of their precursors and the molecular structures. Results show ν -carrageenan (precursor of ι -carrageenan) in the *Eucheuma* species. *E.cottonii* is identified as a very good source of ι -carrageenan, while μ -carrageenan (precursor of κ -carrageenan) is the sole precursor found in the gametophyte portion of *C.crispus* (Bellion, Brigand, Prome, Welti, & Bociek, 1983).

Chromatographic methods are also used for their analysis. Multi-angle laser light scattering (MALLS) coupled with gel permeation chromatography (GPC) is used for separating the high mass fractions from the low mass fractions (van de Velde *et al.*, 2002). The weight-average molecular weight of the helical state was double compared to the coiled state implying the formation of double-helices (Viebke, Borgstrom, & Piculell, 1995).

High performance liquid chromatography (HPLC) has been used primarily for the detection and quantification of carrageenans in commercial foods or in a food matrix. Methanolysis is a commonly used pretreatment that produces 3,6 anhydro-galactosedimethylacetal, and the molar ratio of sulfates and anhydro-galactose forms the basis for differentiation of the carrageenans. The molar ratios for ι - and κ -carrageenan are 2.5 and 1.3, respectively (Voragen, Schols, & Pilnik, 1982). Quemeneret *et al.* (1995) evaluated the methanolysis step and concluded that while the yields of the anhydrogalactosyl residues was 100% for ι -carrageenan, it was only about 70% and 80% for κ -carrageenan and agarose, respectively.

FTIR has also been in use for certain kinds of structural analyses such as identification of the sulfate groups and anhydro rings. Rees (1969) reported that ι -carrageenan is easily distinguishable from κ -carrageenan by a pronounced band at a wavenumber of 805 cm^{-1} . In particular, FTIR is a simple and effective way to identify the types of carrageenan present in a seaweed sample. The occurrence of distinct peaks, or lack thereof, associated

with bonds/chemical groups is characteristic of certain carrageenans and allows identifying the carrageenan type.

Table 1. FTIR data for chemical bonds/groups characteristic of types of carrageenan (Bhattacharjee *et al.*, 1978).

Vibrations		Occurrence (+) or absence (-) of the peak, according to type of carrageenan				
Wave numbers	Bonds/group(s)	κ	μ	ι	ν	λ
1240	S=O of sulfate esters	+	+	+	+	+
930	C-O of 3-6-anhydro-D-galactose	+	-	+	-	-
845	C-O-S of axial secondary sulfate on C4 of galactose	+	+	+	+	-
830	C-O-S of equatorial secondary sulfate on C2 of galactose	-	-	-	+	+
820	C-O-S of equatorial primary sulfate on C6 of galactose	-	+	-	+	+
805	C-O-S of axial secondary sulfate on C2 of 3,6-anhydro-D-galactose	-	-	+	-	-

Size exclusion chromatography (SEC) is another method used, coupled with other measurements such as "low angle laser light scattering detector" (LALLS), refractive index, viscosity measurements etc. (Lecacheux, Panaras, Brigand, & Martin, 1985).

(Hjerde, Smidsrod, & Christensen, 1997) analyzed the conformational properties of κ -carrageenan solution with 0.2M LiI using SEC combined with LALLS. Elution profiles, molecular weight (M_w) and polydispersity index (M_w/M_n) are the parameters studied.

Upon achieving ordering by cooling κ -carrageenan samples, there is a shift towards lower elution volumes and doubling of M_w from $200,000 \pm 10,000$ (disordered) to $400,000$ (ordered) suggesting the formation of double-stranded species. An increase in the

polydispersity index value from 1.4 to 1.7 suggests the formation of multiple-stranded structures.

SEC is also used as part of a rapid method for the separation and analysis of carrageenan oligosaccharides released by iota and kappa-carrageenase (Knutsen *et al.*, 2001). Peaks for a wide range of oligosaccharides were obtained in 20 minutes on an analytical scale, hence the method could be used to monitor degradation in carrageenans.

Other methods such as rapid colorimetric procedures (Soedjak, 1994; Yabe, Ninomiya, Tatsuno, & Okada, 1991), immunological determination/detection (Arakawa, Ishihara, Nishio, & Isomura, 1991) were also used.

1.3 Sources of carrageenan

Carrageenan is extracted from various red seaweed species such as *Chondrus crispus*, *Gigartina stellate*, *G. radula*, *G. acicularis*, *G. pistillata*, *Eucheuma spinosum*, and *polyidesrotundus* (Black et al., 1965; Hirst & Jones, 1947). The yield and composition vary with seasonal changes and geographical location (Black et al., 1965; S. W. Fuller & Mathieson, 1972). *Kappaphycus alvarezii* (commonly referred to as *Eucheuma cottonii*) is known for its homogenous yield of κ -carrageenan when subjected to alkali treatment. *Eucheuma denticulate* (commonly known as spinosum) is another commercially important species for producing ι -carrageenan. *Kappaphycus alvarezii* and *Eucheuma denticulatum* account for about 85% of the raw materials used in industrial applications, while *Chondrus* has 5% share and *Gigartina* holds the remaining 10% (McHugh, 2002).

The potassium ions induced gelation is being utilized for separating iota- and kappa-carrageenan fractions (Smith, 1953).

1.4 Carrageenans in foods

Carrageenan was first reported to form complexes with K-Casein (Grindrod & Nickerso-Ta, 1968). Further research revealed its interactions with β -lactoglobulins at acidic pH conditions (Hidalgo & Hansen, 1969). Subsequently, several applications have been developed over the years, with a majority of them being in either dairy- or meat-based products.

Smaller quantities of kappa-carrageenan are sufficient to prevent the separation of whey protein in cottage cheese (McHugh, 2003), and both iota and kappa-carrageenan increase the yield for cottage cheese up to 20% by preventing the protein loss through the water (Kailasapathy, Hourigan, & Nguyen, 1992). Cottage cheese could also be fortified with calcium ions but after masking the resulting bitterness through 1.5% carrageenan (Puspitasari, Lee, & Greger, 1991). Similarly, kappa-carrageenan is effective for suspending cocoa solids in chocolate milk. In ice-creams, carrageenan addition not only prevents the whey protein separation but also controls the meltdown (Cottrell, Pass, & Phillips, 1979). The addition of kappa-carrageenan to milk at concentrations higher than 0.03% results in sharp increase in pseudoplasticity and viscosity as well as better flavor-releasing properties (Yanes, Duran, & Costell, 2002). Liquid coffee whiteners are also improved by the addition of 0.2-0.3% lambda-carrageenan that stabilizes the fat and

prevents separation. Lesser quantities can be used for the same purpose in ultra-high temperature (UHT) pasteurized milks. Lambda- and kappa-carrageenan are also useful in maintaining the lightness of whipped cream.

Carrageenan is added to the brine formulations containing various sodium salts in the preparation of restructured hams, for improving water retention and preventing the loss of soluble protein (Motzer, Carpenter, Reynolds, & Lyon, 1998). In low-fat pork products, the meat can be severely affected in terms of taste and tenderness. Low-fat restructured pork nuggets processed with the addition of iota-carrageenan and salt enormously improves pre-cooking yields, juiciness, and tenderness compared to the all-pork nuggets (Berry & Bigner, 1996). Kappa-carrageenan is used as a fat-replacer in frankfurters, with up to 50% of the fat successfully substituted. Coatings made with carrageenan were found to prolong the frozen storage life of fatty oil fish (Stoloff, Puncochar, & Crowther, 1948), poultry carcasses and poultry meat, respectively (Meyer, Winter, & Weiser, 1959; Pearce & Lavers, 1949). Meyer *et al.* (1959) concluded that the addition of antibiotics through an edible coating resulted in much more effective retardation of microbial growth compared to dipping the poultry in antibiotic saline solutions. The thermal stability of pork increased significantly with the addition of 0.8% kappa-carrageenan (Jarmoluk *et al.*, 2003).

Gelatin alternatives were actively sought after the incidence of bovine spongiform encephalopathy (BSE) and a polysaccharide mixture including carrageenan was found to be an excellent replacement, especially in hot tropical conditions (Anton *et al.*, 2005).

Carrageenan also finds applications in low-calorie jelly, where it could be a substitute for pectin. Mixtures of iota- and lambda-carrageenan have proved useful as an alternative to pectin (Guittard & Salou, 1987). Carrageenan also finds uses in "low-oil" or "no-oil" salad dressings where iota- or kappa-carrageenans are used to maintain the herbs in suspension and prevent settling (Fung, Wuesthoff, Fung, & Miller 1991; Bauer *et al.*, 1994). In the clarification process of beer, the interaction between carrageenan and protein is used as the basis for separation of wort (Montgomery & Morrison, 1993; Posada *et al.*, 1979). Carrageenan is also used as the base for immobilizing bacteria in producing malic acid. Results suggest it increases the production around 5.2 times compared to polyacrylamide (Takata, Yamamoto, Tosa, & Chibata, 1980).

1.5 Analysis of carrageenans

1.5.1 X-ray fiber diffraction

X-rays are important to molecular structure studies because of their wavelength range 0.1- 100Å. Wavelengths of around 1 angstrom unit are used since they are of the same magnitude as the interatomic distances. X-ray fiber diffraction is a technique used to determine the structure of molecules which have some form of ordered arrangement along the fiber axis. X-rays are scattered by the crystal structure, forming patterns of diffraction which are interpreted using Bragg's law.

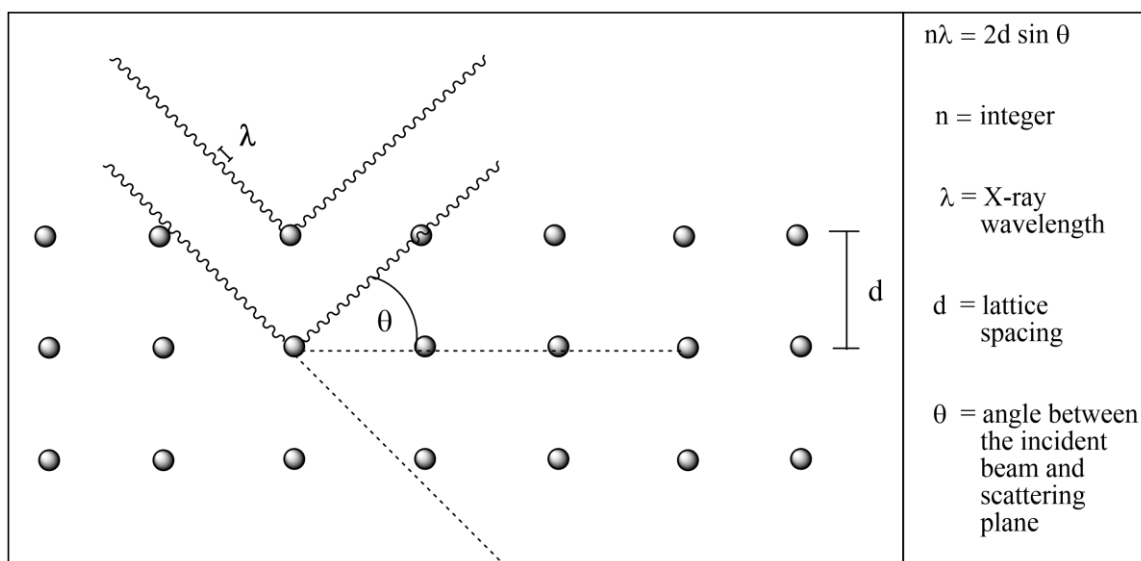


Figure 2. Schematic representation of Bragg's law.

Solutions of carrageenan lack crystallinity and hence the chains must be oriented uniaxially in space for a successful understanding of their three-dimensional structure. One method used to achieve alignment is to load a drop of warm polysaccharide solution between 2 glass beads and to draw it out while it cools down (Anderson, Campbell, Harding, Rees, & Samuel, 1969; Fuller, Hutchins, Spencer, & Wilkins, 1967). A humidity chamber is used to maintain the fibers at humidities between 58-76%.

The smallest divisible units that possess the properties and symmetry of the crystals are called unit cells, and their dimensions are usually represented in terms of a , b , c , α , β , and γ . The two common crystal lattice arrangements in carrageenan are orthorhombic and trigonal (Figure 3). The calculation of the unit cell dimensions is performed by computer programs which need the Bragg reflection coordinates and the fiber to detector distance.

Fibrefix is one such commonly used program (Rajkumar, Al-Khayat, Eakins, Knupp, & Squire, 2007).

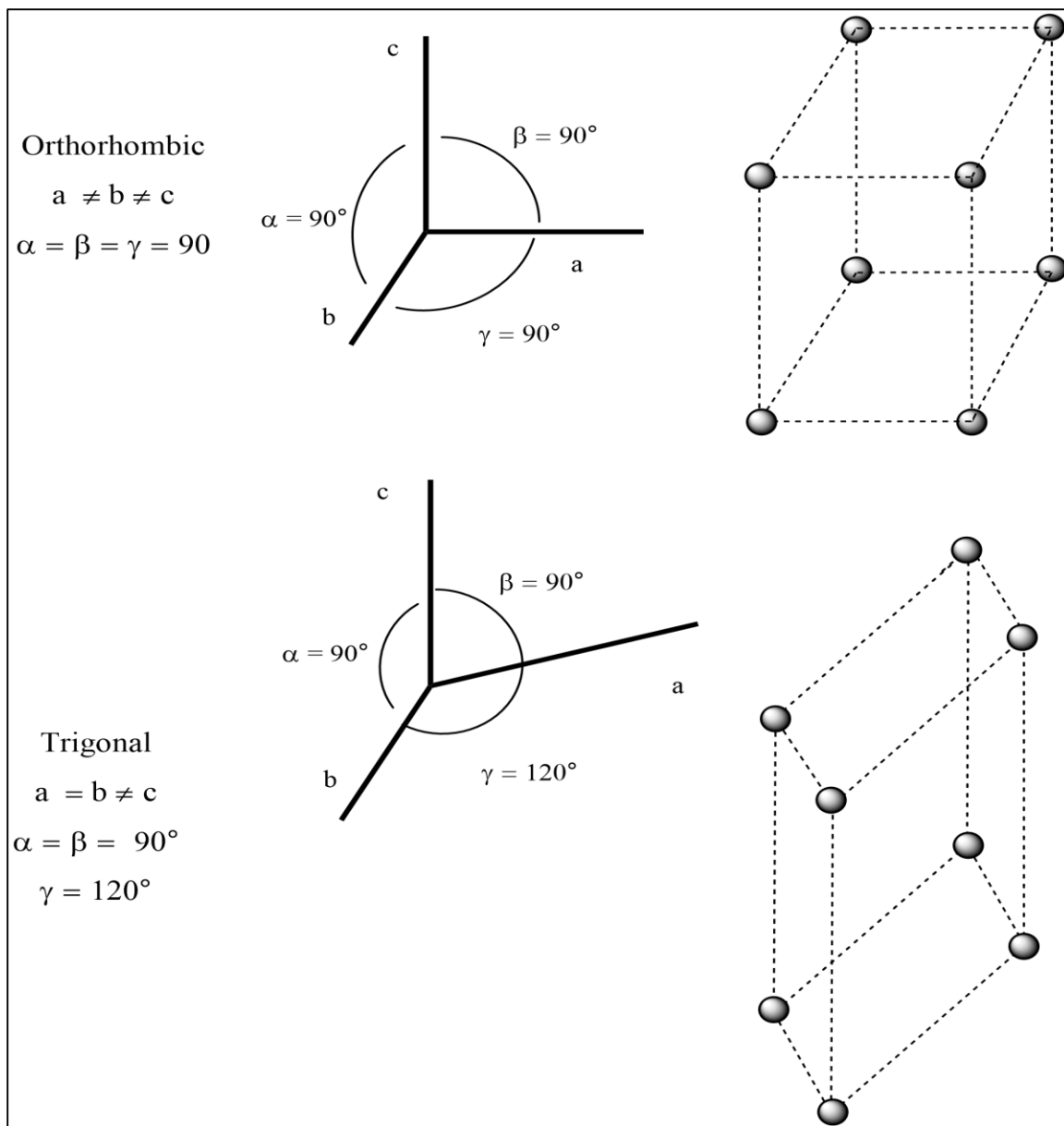


Figure 3. Common unit cell forms adopted by carrageenans.

1.6 Health and Pharmaceutical applications of Carrageenan

Carrageenan shows promise as a novel extrusion aid for spheronization of pellets in the place of the more commonly used microcrystalline cellulose (MCC)(Bornhoft, Thommes, & Kleinebudde, 2005). Recent research has shown promising antiviral and anti-tumor applications for carrageenan. Carrageenan gels from *Chondrus Crispus* species have demonstrated the ability to selectively inhibit the transmission of many enveloped viruses and stopped the transmission of viruses such as HIV, herpes simplex virus(HSV) (Carlucci, Scolaro, & Damonte, 1999), hepatitis-A (Girond, Crance, Vancuyckgandre, Renaudet, & Deloince, 1991), feline herpes virus and on experimentally induced herpetic conjunctivitis (Stiles, Guptill-Yoran, Moore, & Pogranichniy, 2008). Carrageenan has been reported to exhibit anti-coagulant and reduced platelet aggregation properties, with the most active form of carrageenan exhibiting anti-coagulant activity about one-fifteenth of heparin (Kindness, Williamson, & Long, 1979).

Despite the benefits of carrageenan, there has been scrutiny and wide-spread belief that it is carcinogenic to humans and animals. Some researchers have reported that the increasing consumption of gums, primarily 'degraded carrageenan', correlates positively to the increased incidence of ulcerative colitis (Watt & Marcus, 1971). A time-trend analysis was carried out to ascertain the correlation, if any, between the incidence of breast carcinoma and carrageenan consumption (Tobacman, Wallace, & Zimmerman, 2001). The results claim that due to the clear positive correlation, there is enough evidence to warrant further evaluations. Other researchers have concluded that dietary carrageenan acts as a co-carcinogen by enhancing the effect of other carcinogens on the

colon carcinogenesis (Watanabe, Reddy, Wong, & Weisburger, 1978). The aforementioned study used a carrageenan level of 15% total diet, an improbable scenario in terms of human consumption.

The large-bowel health of various animals upon administering carrageenan in native or degraded forms was investigated. Rat, hamster, guinea pig, rabbit, squirrel monkey and ferret were fed the native carrageenan diet (5%) and degraded carrageenan diet (2%) through drinking water. A basic study was also conducted on six malignant patients, who consumed 5g of degraded carrageenan for 10 days (Grasso, Sharratt, Carpanini, & Gangolli, 1973). Tissue samples were checked for ulcerations and tested chemically and histochemically for the presence of degraded carrageenan in the tissue. Ulceration was observed only in guinea-pigs and rabbits. There was no ulceration in human subjects or animals, leading to a conclusion that humans are resistant to carrageenan's harmful effects on the large-bowel seen in some animals. More recently, a 90-day study on kappa-carrageenan consumption was conducted to unravel its effects on the gastrointestinal (GI) tract (Weiner, 2014). Concentrations of 0, 25000 and 50000 ppm were used in the diet. Only soft feces were noted in the high dose groups, with no adverse effects on the organs or body weights of the rats.

Most concerns regarding the safety of carrageenan were due to its confusion with the 'degraded carrageenan'. The United States Adopted Names Council ("United States Adopted Names Council," 1988) assigned the name 'Poligeenan' to the substance

erstwhile referred to as 'degraded carrageenan'. Poligeenan is defined as having a molecular weight of 10000-20000 Da ("United States Adopted Names Council," 1988). There are rebuff concerns with poligeenan as it could be converted to carrageenan. Concerns were also expressed about the digestive acids in the body converting carrageenan to poligeenan. However, there is no literature support or evidence to the bodily conversion of carrageenan to poligeenan in animals or humans (McKim, 2014).

1.7 Research Goals

The goal of this research initiative was to understand the behavior of sodium-kappa carrageenan in the presence of sodium salts such as NaCl, NaBr and NaI as well as chaotropic agents such as urea and guanidinium chloride. The structural and functional changes have been characterized by X-ray fiber diffraction, rheology and DSC.

CHAPTER 2. EFFECT OF SODIUM SALTS ON THE THREE DIMENSIONAL STRUCTURE AND FUNCTIONAL BEHAVIOR OF KAPPA-CARRAGEENAN

2.1 Abstract

Carrageenans are sulfated polysaccharides extracted from marine algae and are recognized as GRAS materials. Depending on their source of extraction, and presence or absence of sulfate groups as well as anhydro group fifteen carrageenans are known to date. They have a niche in food and pharmaceutical industries due to their ability to form thickeners, viscosifiers and solutions. In particular, their food applications include bodying, gelling, thickening and emulsion stabilization in water- and milk-based systems. Only kappa-, iota- and lambda-carrageenan have so far been exploited industrially and are subjected to extensive studies toward delineating their structure-function relationships. In this set, kappa-carrageenan has been well studied; however, details about its junction zone architecture and effect of anions on the structure-function relationships are still elusive. In this regard, herein a systematic investigation about the effect of salts on the three-dimensional structure, viscoelastic and thermal properties of sodium kappa-carrageenan (NaKC) has been attempted. X-ray fiber diffraction patterns and dynamic rheological and thermal measurements are obtained for 1.5% and 2% w/w solutions in the presence of 0, 50, and 100 mM of NaCl, NaBr, and NaI. The dilute polysaccharide concentrations are to better represent a practical scenario encountered in formulations, and the halide ions are intended to unravel their effect on the structure and functionality

of kappa-carrageenan, if any. X-ray results suggest that the carrageenan fibers are uniaxially oriented, but with less crystallinity. Stronger gels are seen in the presence of NaBr compared to NaCl and NaI, however iodine ions are more effective in yielding well-oriented and crystalline diffraction patterns, along with better thermal stability. Overall, it appears that iodine ions favorably promote interactions among the kappa-carrageenan chains compared to chloride and bromide ions.

2.2 Introduction

Kappa-carrageenan is a popular gelling and viscosifying agent for the food industry owing to its thermoreversible nature and strong gelation properties in the presence of specific salts (Viebke, Piculell, & Nilsson, 1994). Various theories have been proposed to explain the mechanisms of gelation upon cooling (Morris, Rees, Norton, & Goodall, 1980; Morris, Rees, Thom, & Welsh, 1977; Rochas & Rinaudo, 1984; Viebke *et al.*, 1994).

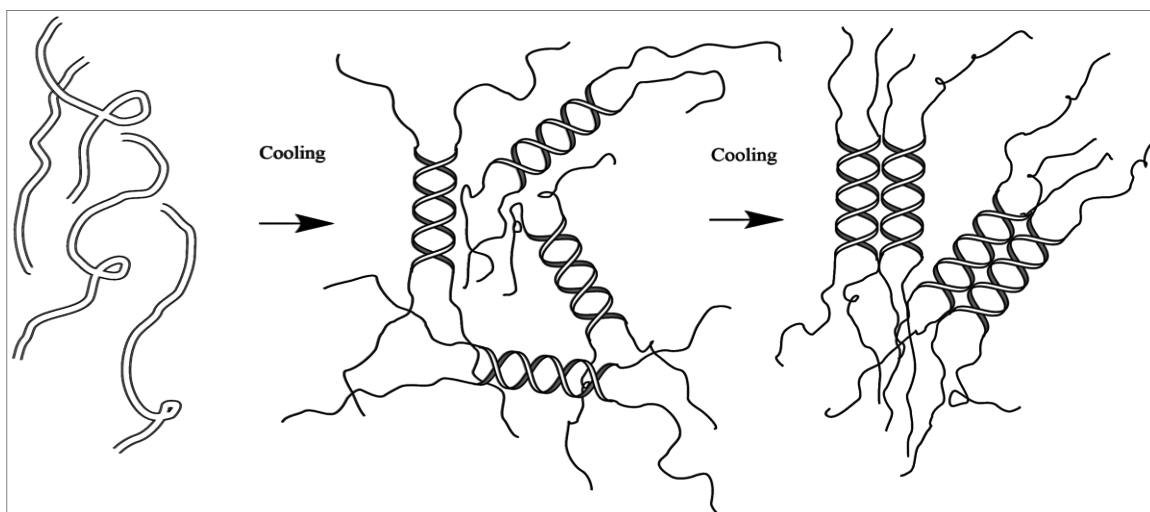


Figure 4. Formation of carrageenan coils upon cooling, coil-to-helix transition, followed by helix-to-dimer.

As highlighted in Fig. 4, in the solution state kappa-carrageenan chains exist as random coils. However, upon cooling they aggregate as double-helices, and finally as dimers. Interestingly, in the presence of KCl rigid rods are noticed (Hermansson, Eriksson, & Jordansson, 1991). The nature of counterions has also been studied, and more ordered network formation is noticed in the presence of Rb^+ and gel-forming ability is as follows : $\text{Li}^+, \text{Na}^+, \text{NR}_4^+ \ll \text{NH}_4^+ < \text{K}^+, \text{Cs}^+ < \text{Rb}^+$ (Ciancia, Milas, & Rinaudo, 1997; Norton, Goodall, Morris, & Rees, 1983; Rochas & Rinaudo, 1980).

Morris *et al.* (1980) reported that iota-carrageenan forms a substantial helix network, but does not gel in the presence of Li^+ , Na^+ or $(\text{CH}_3)_4\text{N}^+$. However, strong gel formation by the aggregation of helices is noted upon the addition of K^+ , Rb^+ , and Cs^+ ions. It is also observed that for kappa-carrageenan, substantial helix formation is noted only in the presence of conditions that promote helix aggregation in iota-carrageenan. The formation of superhelical networks was also theorized, with Viebke *et al.* (1994) in the presence of salts such as KCl and/or CsCl.

Apart from cations, anions are also thought of having influence on the gelation process of kappa-carrageenan. Gradalsen *et al.* (1981) reported that presence of iodide anions, even in conjunction with strongly gelling potassium ions, prevents helix aggregation, *i.e* gelation. They also proposed the formation of single helices as the conformational transition temperature is found to be independent of anion concentration. However, this theory has been contradicted by Viebke *et al.* (1981) due to the observation of doubling

in the molecular weight of the coil state with a conformational transition into the helix state.

The focus of this study is to understand the effect of anions Cl^- , Br^- and I^- on the three-dimensional structure, viscoelastic and thermal properties of sodium kappa-carrageenan. The solution concentrations 0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 have been studied in the presence of with 0, 50, and 100 mM salt amounts of NaCl, NaBr, and NaI. The dilute polysaccharide concentrations are to better represent a practical scenario encountered in formulations.

2.3 Materials and methods

2.3.1 Solutions

Kappa-carrageenan was from FMC Biopolymer (Philadelphia, PA). Lab grade NaCl, NaBr, and NaI were purchased from VWR International. Reagent grade isopropyl alcohol and double distilled water were used as necessary. Pure sodium-form of kappa-carrageenan was prepared by dissolving 0.1% w/w of polysaccharide in distilled, deionized water of 400 mL and heated to 95 °C with constant stirring until the solution reached homogeneity. Subsequently, 100mM of NaCl was added and further heated for 45-60 min. The hot solution was then poured into cold isopropanol (4 °C), the precipitate was separated. Later, the precipitate was re-dissolved in 200 mL distilled water and heated for 45-60 min. The hot solution was poured into cold isopropanol (4 °C) and the precipitate collected. The second round heating without adding any excess salt is the key for obtaining pure sodium kappa-carrageenan without any free cations. The final product

was dried at 60 °C for 24 hours, powdered and stored for further use. Solution concentrations of 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0% were prepared, and stored at 75 °C for further analysis.

Appropriate amounts of NaCl, NaBr and NaI were added to obtain 0, 50 and 100mM solutions. They were heated at 90 °C for 45-60 min with intermittent vortexing to ensure the formation of homogenous solutions.

2.3.2 Fiber preparation and X-ray diffraction

The polysaccharide solutions were heated at 80 °C for 5-10 minutes so that they in the fluid state. Approximately 30 μ L was loaded in between two glass rods in a fiber puller. The relative humidity was controlled by maintaining an air-seal and using RH solutions of 75% or 66% RH, as deemed necessary. Once the droplet is dried partially, it was stretched to about 1.0 - 1.5 mm in length carefully to obtain well-oriented, polycrystalline fibers. Synchrotron X-ray diffraction data were collected at Argonne National Laboratory (ANL) Chicago, IL. The wavelength of the beam was set to 0.979 \AA and the exposure time was 5 seconds. Calcite powder with a characteristic d-spacing of 3.035 \AA was used for internal calibration. Fiberfix 1.3.1 was used to analyze the patterns.

2.3.3 Rheology

The viscoelastic behaviors of the prepared solutions were analyzed using the DHR-3 Rheometer from TA instruments (Newcastle, DE). The geometry used was a 40 mm, 2° cone and plate with a solvent trap. Linear viscoelastic region was identified from the strain sweep measurements in the range 0.01-10% strain at 1 Hz and 5 °C. Frequency

sweeps in the range 0.1 to 100 Hz at 2.0% strain (linear region) were obtained at three selected temperatures 5, 25 and 45°C. Temperature ramp was conducted in the range 5-70 °C with a heating rate of 2 °C/min at 1Hz frequency with 2% strain (linear region) Tests were conducted in duplicate and results were analyzed statistically. Average values have been reported.

2.3.4 Thermal Behavior

The melting behaviors were studied using the model Q2000 Differential scanning calorimeter (TA instruments, Newcastle, DE). Approximately 50-60 µL of the sample was loaded into the aluminum pan and sealed hermetically using TZero press (TA instruments, New Castle, DE), and heated at 10°C/min from -20 to 95°C. Universal Analysis 2000 software (TA instruments, Newcastle DE) was used to estimate onset temperature, peak temperature and the enthalpy of melting endotherms. Duplicate measurements were recorded and average values are reported.

2.4 Results and Discussion

2.4.1 Rheology

2.4.1.1 Effect of polysaccharide concentration

In order to understand the effect of polysaccharide amount on the viscoelastic properties of sodium kappa carrageenan, solutions of concentration 0.25-2.0% w/w have been prepared.

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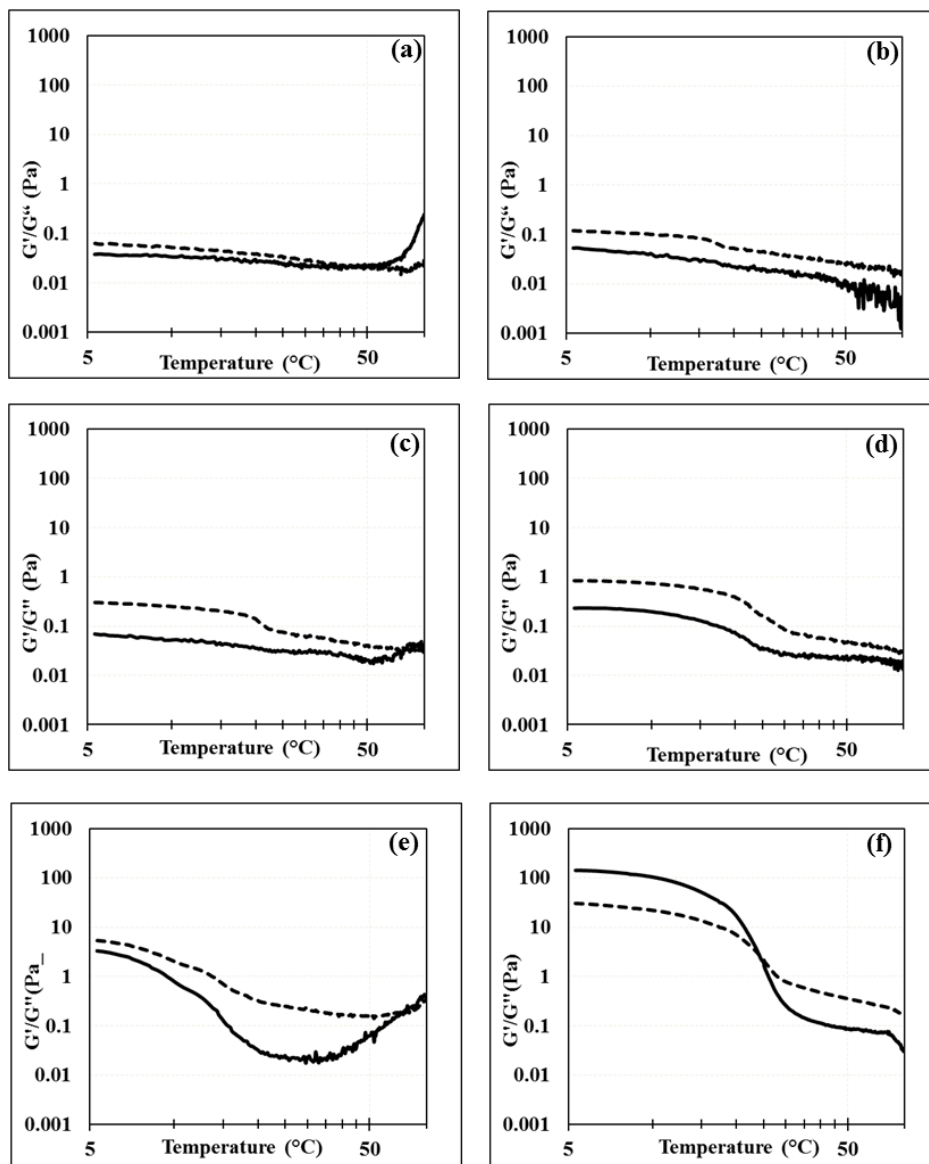


Figure 5. Comparison of G' (—) and G'' (---) temperature ramp values for Na kappa-carrageenan with polysaccharide concentrations of (a) 0.25, (b) 0.5, (c) 0.75, (d) 1.0, (e) 1.5 and (f) 2.0%.

The temperature ramp measurements in the range 5 – 70 °C (Fig. 5) suggests that at lower carrageenan concentrations, 0.25 through 1.50%, there is no gel formation.

However, at 2% the elastic moduli (G') is greater than the loss moduli (G''). For example, the G' and G'' are 103.4 and 22.1 Pa, respectively, at 10 °C, indicating the gel formation.

The gel-to-sol transition occurs at around 24 °C.

2.4.1.2 Effect of sodium chloride:

The variations in the elastic moduli (G') as a function of frequency for 1.5% sodium kappa-carrageenan at 5, 25 and 45 °C in the presence of 0, 50 and 100 mM salt are compared in Fig.6. The value of G' at 5 °C and 1 Hz is 2.7 Pa (Fig. 6a) without any added salt, however, with salt addition it significantly increases to 407.2 and 211.4 Pa for 50 and 100 mM, respectively. The decrease in the elastic moduli with increased salt amount might be due to the outnumbering of the salt ions to the available charge groups.

However, more data involving various dilute polysaccharide concentrations and salt amounts are needed to establish this fact. At 25 °C the G' reduces substantially to 0.01, 0.02 and 32.7 Pa (Fig. 6b), for 0, 50 and 100 mM of salt, respectively, and at 45 °C it further decreases (Fig. 6c) signifying the effect of temperature in reducing the gel strength of kappa-carrageenan solutions. Increasing the polysaccharide concentration to 2.0% raises the G' to 110.2, 164.2 and 398.5 Pa (Fig. 7a) at 5 °C for the same salt combination, respectively. At higher temperatures (25 and 45 °C) these amounts have progressively plummeted to 0.01 Pa further demonstrating the important role of temperature in modulating the solution properties of kappa-carrageenan.

Similar trend is noticed in the temperature ramp measurements. For example, at 10 °C the G' values for the 1.5% solutions are 0.78, 322.4 and 94.5 Pa (Fig. 8a) for 0, 50 and 100 mM of salt, respectively. They further increase to 103.5, 340.2 and 410.4 Pa (Fig. 8b), in the same salt order. The gel-to-sol (T_g) transition temperature is observed at around 30 °C (Table 2) in all the solution combinations. Overall, it appears that addition of sodium chloride significantly alters the viscoelastic properties of kappa-carrageenan.

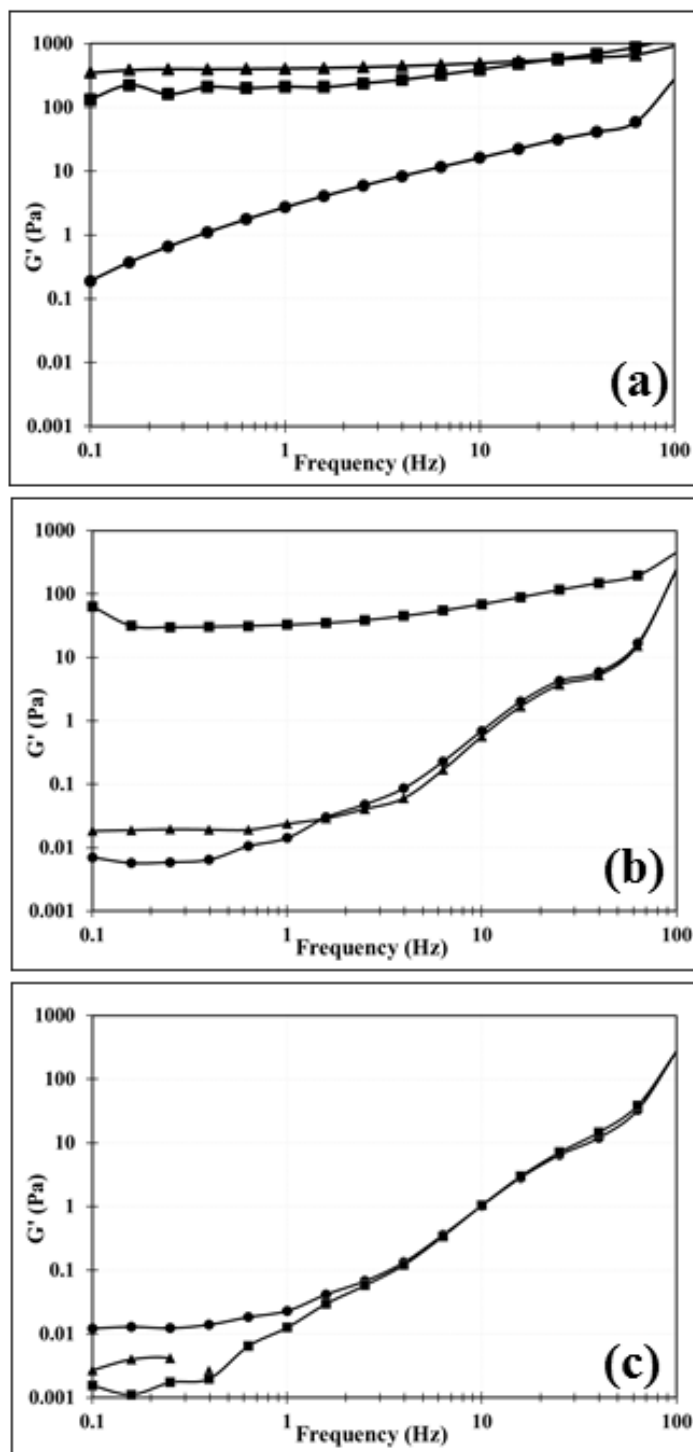


Figure 6. Comparison of the elastic moduli (G') for 1.5% Na kappa-carrageenan in the frequency range 0.1 – 100 Hz in the presence of (●) 0, (▲) 50 and (■) 100 mM NaCl at (a) 5, (b) 25 and (c) 45 °C.

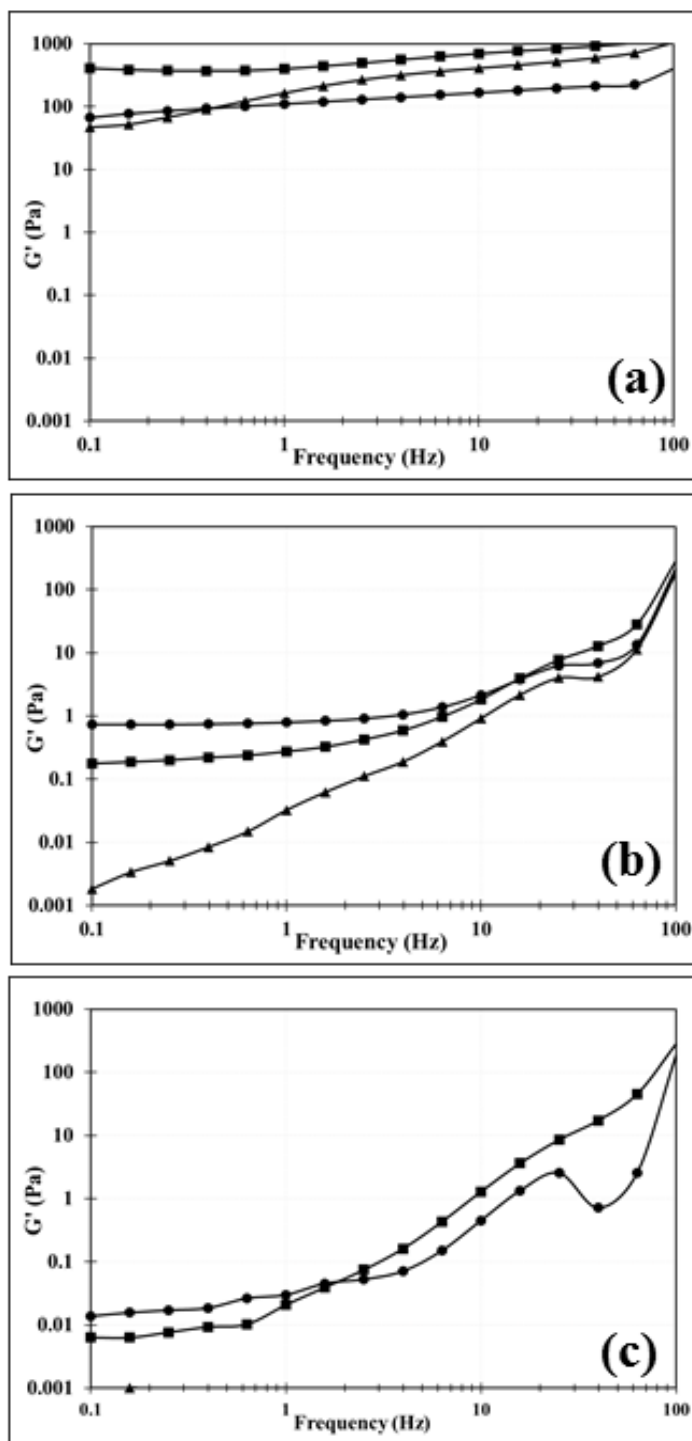


Figure 7. Comparison of the elastic moduli (G') for 2% Na kappa-carrageenan in the frequency range 0.1 – 100 Hz in the presence of (●) 0, (▲) 50 and (■) 100 mM NaCl at (a) 5, (b) 25 and (c) 45 °C.

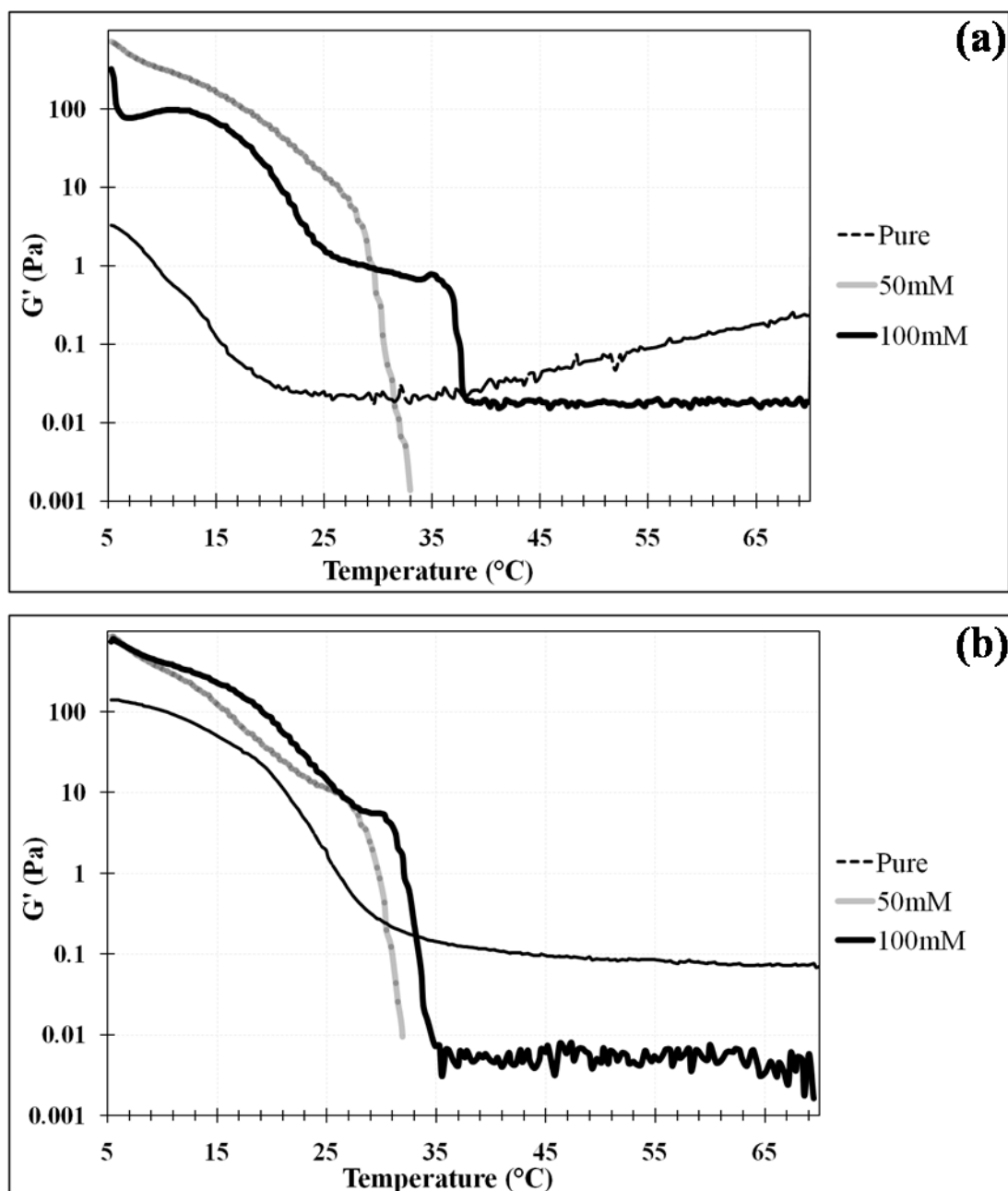


Figure 8. Comparison of the elastic moduli (G') for 0, 50 and 100 mM NaCl of (a) 1.5% and (b) 2.0% Na kappa-carrageenan in the temperature range 5 – 70 $^{\circ}\text{C}$.

2.4.1.3 Effect of sodium bromide:

The addition of sodium bromide has considerably increased the elastic moduli (G').

Fig. 9a compares the associated values as a function of frequency at 5 °C for 1.5% polysaccharide solution. At 1 Hz, the G' is 269.2 and 694.0 Pa for 50 and 100 mM of NaBr, respectively. Unlike NaCl in the presence of NaBr the G' is found to be relatively stable with the frequency increase. However, at higher temperatures (25 and 45 °C) the value decreases substantially to around 0.02 Pa (Fig. 9b and 9c). In the case of 2.0% solution, the values observed for the similar salt amounts are 799.4 and 245.8 Pa, respectively (Fig. 10a). The decrease in the G' at 100 mM further substantiates role of higher salts amounts in retreating the overall gel strength.

The temperature ramp data are quite interesting too (Fig. 11). At 10 °C, the G' values observed are 56.0 and 145.8 Pa for 1.5% sodium kappa-carrageenan with 50 and 100 mM salt, respectively. They increase to 480.1 and 410.8 Pa, respectively, for 2.0% solution for the same salt amounts. As highlighted in 2.4.1.2, in the presence of NaCl this trend in the individual values has been reversed, for the same salt amounts. It is also reflected in the gel-to-sol (T_g) transition. In the case of 1.5% solution, the T_g s are 25 and 30 °C for 50 and 100 mM salt, respectively, which further increase to 31 and 41 °C, in the same salt order, for the 2.0% solution. These findings clearly suggest the important role of anions in the gelation mechanism of kappa-carrageenan.

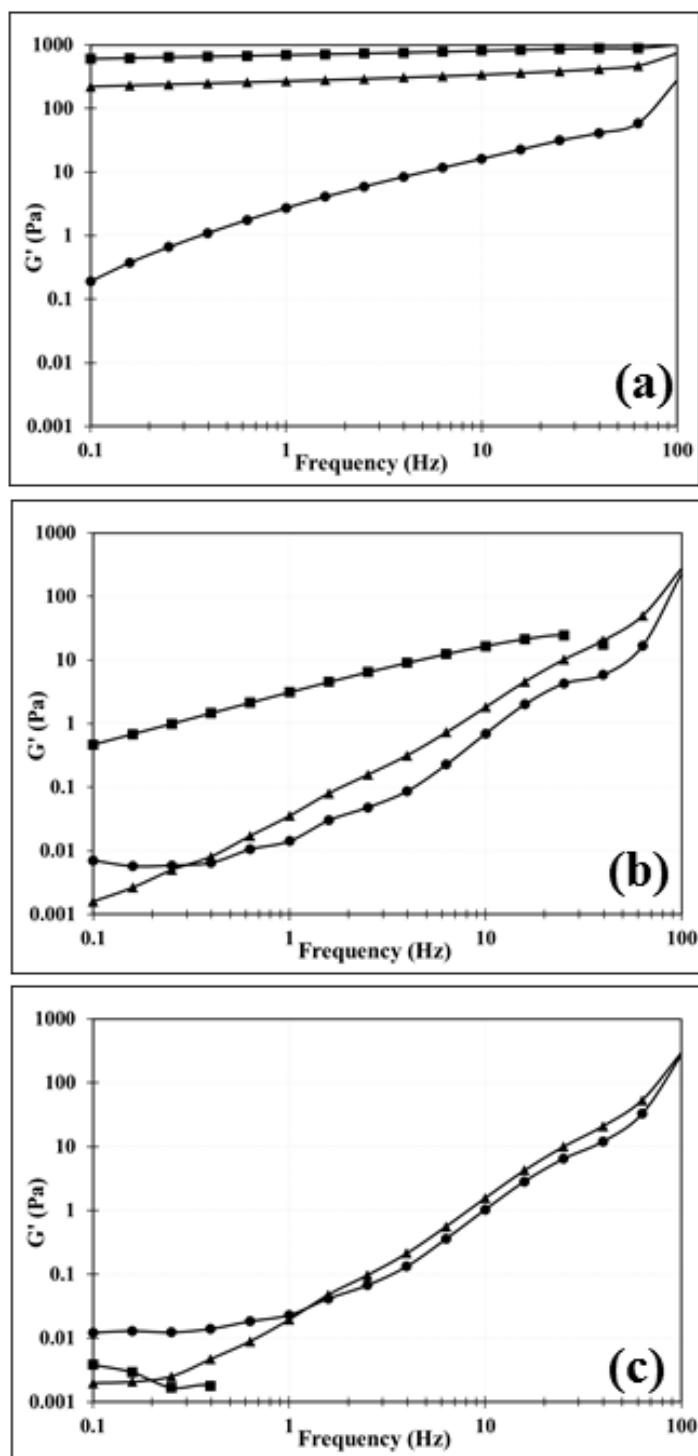


Figure 9. Comparison of the elastic moduli (G') for 1.5% Na kappa-carrageenan in the frequency range 0.1 – 100 Hz in the presence of (●) 0, (▲) 50 and (■) 100 mM NaBr at (a) 5, (b) 25 and (c) 45 °C.

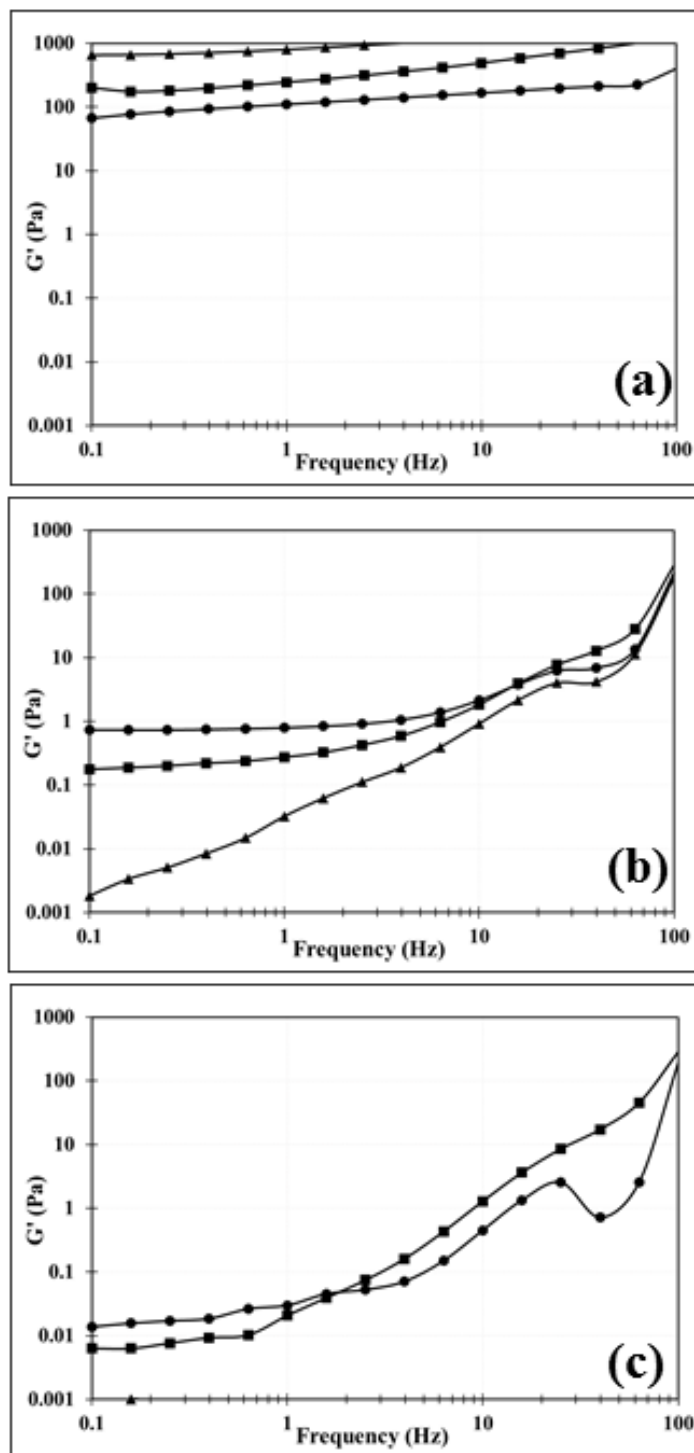


Figure 10. Comparison of the elastic moduli (G') for 2% Na kappa-carrageenan in the frequency range 0.1 – 100 Hz in the presence of (●) 0, (▲) 50 and (■) 100 mM NaBr at (a) 5, (b) 25 and (c) 45 °C.

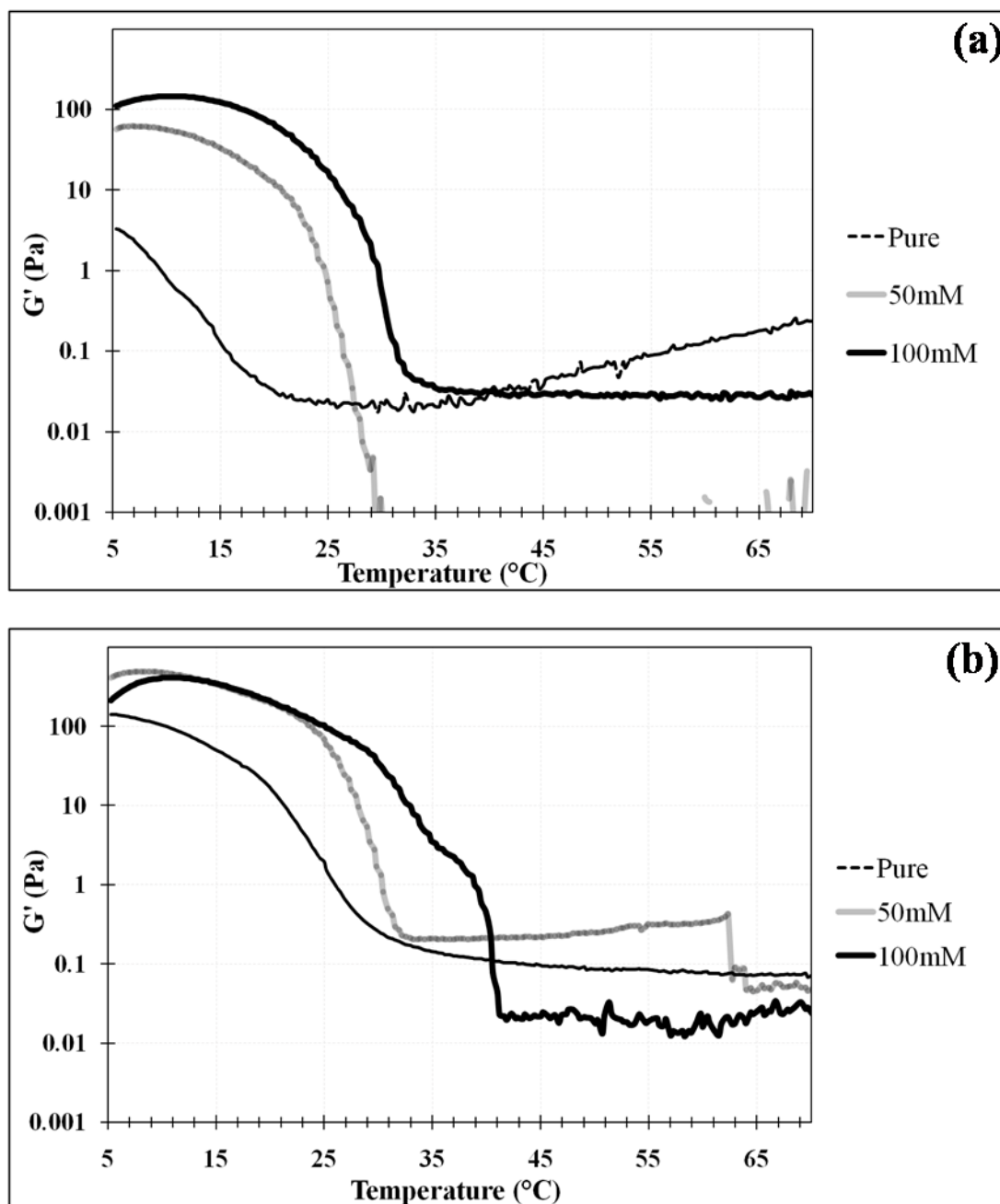


Figure 11. Comparison of the elastic moduli (G') for 0, 50 and 100 mM NaBr at (a) 1.5% and (b) 2.0% Na kappa-carrageenan in the temperature range 5 – 70 $^{\circ}\text{C}$.

2.4.1.4 Effect of sodium iodide:

The presence of sodium iodide has significantly altered the viscoelastic properties of sodium kappa-carrageenan. The gels are relatively soft compared to the sodium chloride and sodium bromide addition. Fig. 12a compares the elastic moduli (G') as a function of frequency for 1.5% sodium kappa-carrageenan measured at 5 °C. The observed G' values 40.0 and 71.0 Pa for 50 and 100 mM, respectively, at 1 Hz are far less compared to the sodium chloride or sodium bromide addition. These values further rise to 267.0 and 393.0 Pa for the same salt amount for 2.0% solution (Fig. 13a). Interestingly, even at 25 °C higher G' values such as 57.3 and 169.2 Pa are observed for the 2.0% solution suggesting the prevalence of gel structure in the kappa-carrageenan solutions in the presence of iodide ions. The temperature ramps also display similar behavior (Fig. 14) and little higher gel-sol (T_g) transition temperature of 43.5 °C for 2.0% solution with 100 mM NaI (Table 2). Overall, the study clearly shows that anions indeed have a significant effect on the viscoelastic properties of kappa-carrageenan and iodide ions are more effective in yielding relatively soft gels, which could be amenable in producing well-ordered network arrangements especially in oriented and crystalline fibers.

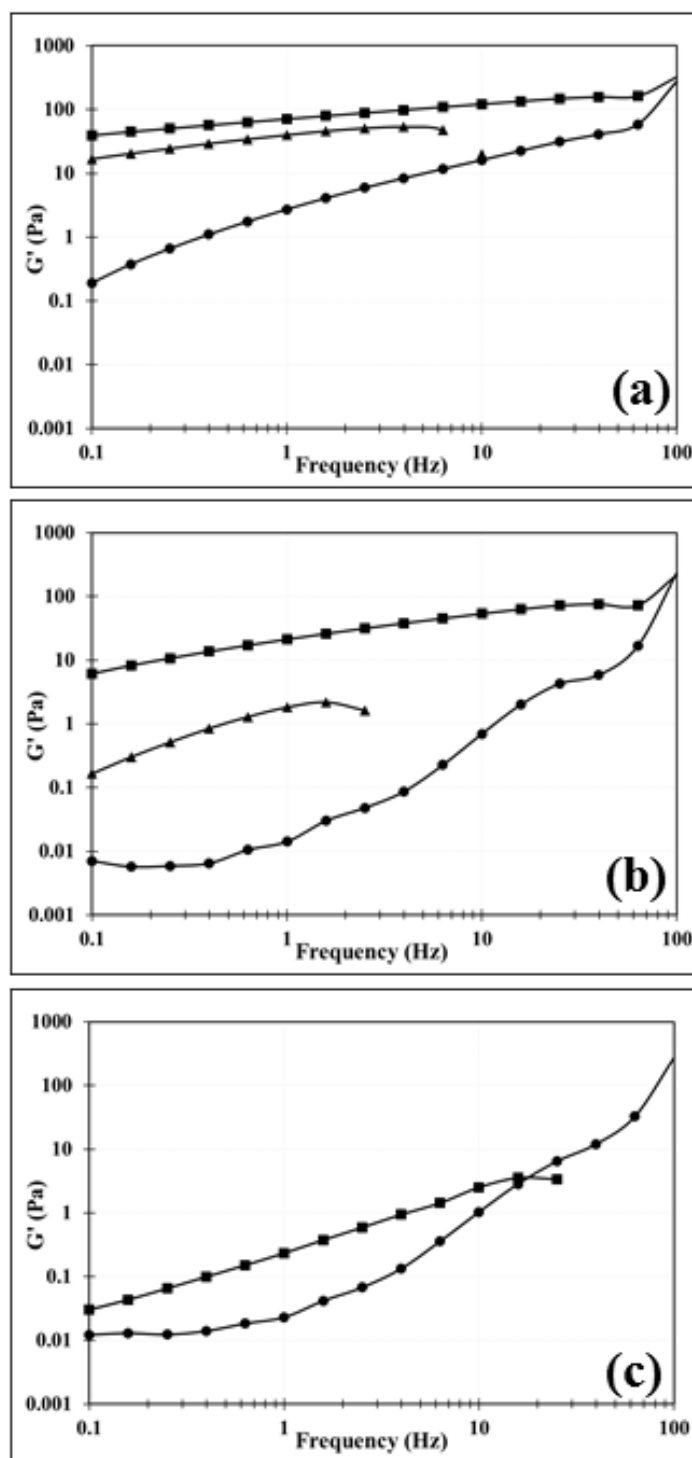


Figure 12. Comparison of the elastic moduli (G') for 1.5% Na kappa-carrageenan in the frequency range 0.1 – 100 Hz in the presence of (●) 0, (▲) 50 and (■) 100 mM NaI at (a) 5, (b) 25 and (c) 45 °C.

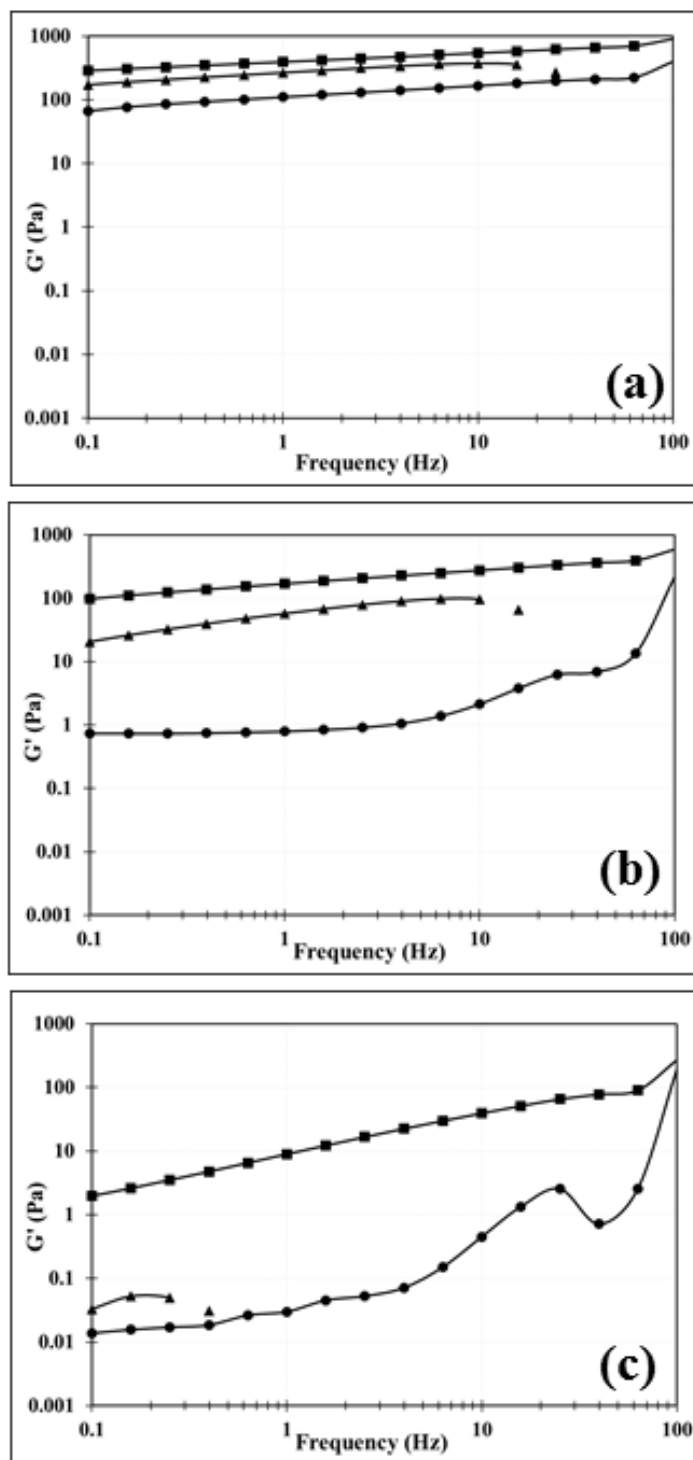


Figure 13. Comparison of the elastic moduli (G') for 2% Na kappa-carrageenan in the frequency range 0.1 – 100 Hz in the presence of (●) 0, (▲) 50 and (■) 100 mM NaI at (a) 5, (b) 25 and (c) 45 °C.

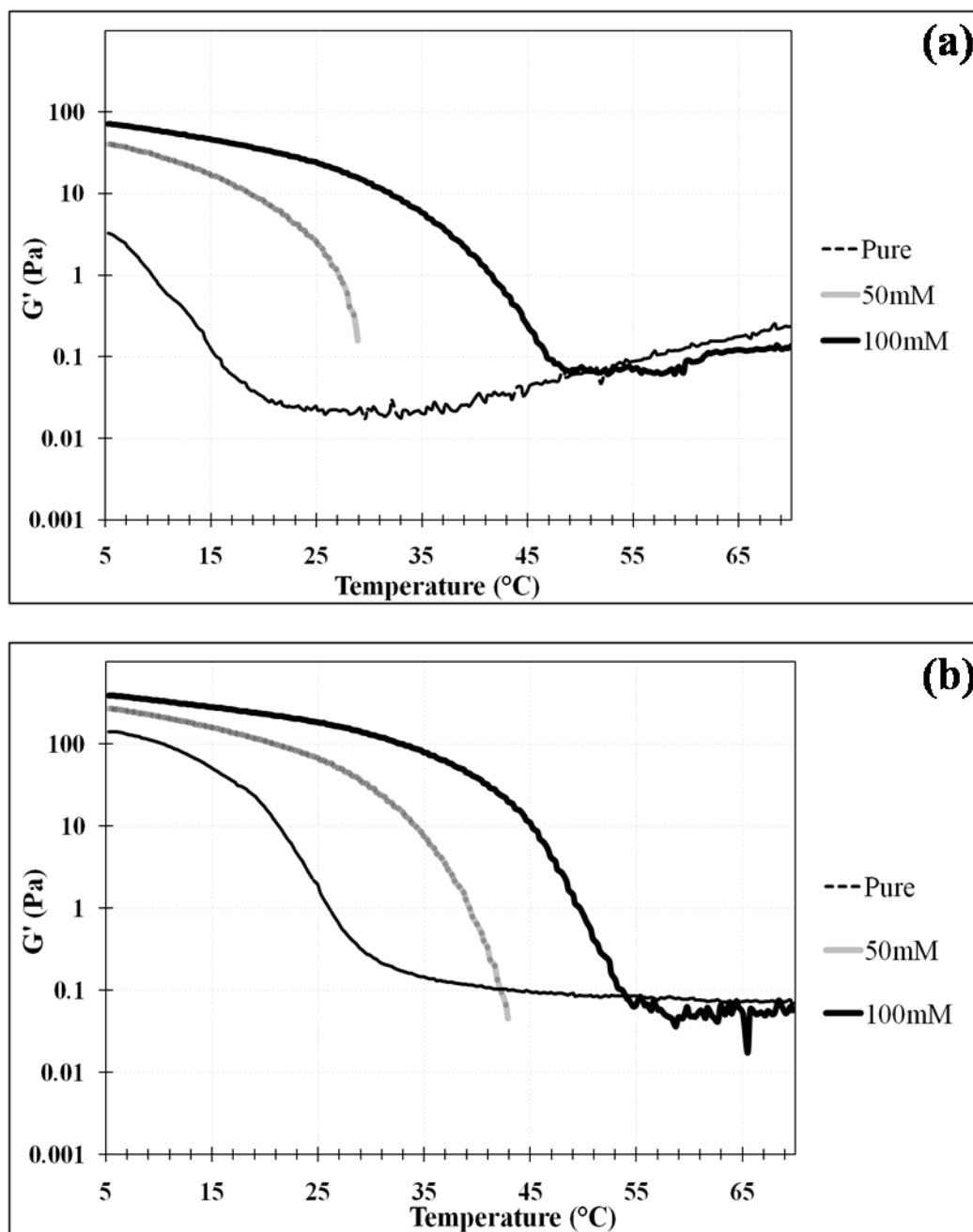


Figure 14. Comparison of the elastic moduli (G') for 0, 50 and 100 mM NaI at (a) 1.5% and (b) 2.0% Na kappa-carrageenan in the temperature range 5 – 70 $^{\circ}\text{C}$.

Table 2. The elastic moduli (G') at 5 °C and T_g (°C) of Na kappa-carrageenan in the presence of salts

Na-kappa (%w/w)	Salt (mM)	G' (Pa)	T_g (°C)
1.50	0	3.3	-
1.50	50 NaI(10)	40.4	16.7(2.8)
1.50	100 NaI(9)	71.3	31.3(0.9)
1.50	50 NaCl	723.0	31.3
1.50	100 NaCl	325.8	Unclear
1.50	50 NaBr	55.7	24.9
1.50	100 NaBr	110.2	29.9
2.00	0	129.1	-
2.00	50 NaI	269.4	31.3(0.9)
2.00	100 NaI	391.7	42.5(1.3)
2.00	50 NaCl	872.3	30.2
2.00	100 NaCl	899(209)	32.6(0.2)
2.00	50 NaBr	415.2	30.7
2.00	100 NaBr	211.6	40.6

Standard deviation values are added in parentheses wherever possible

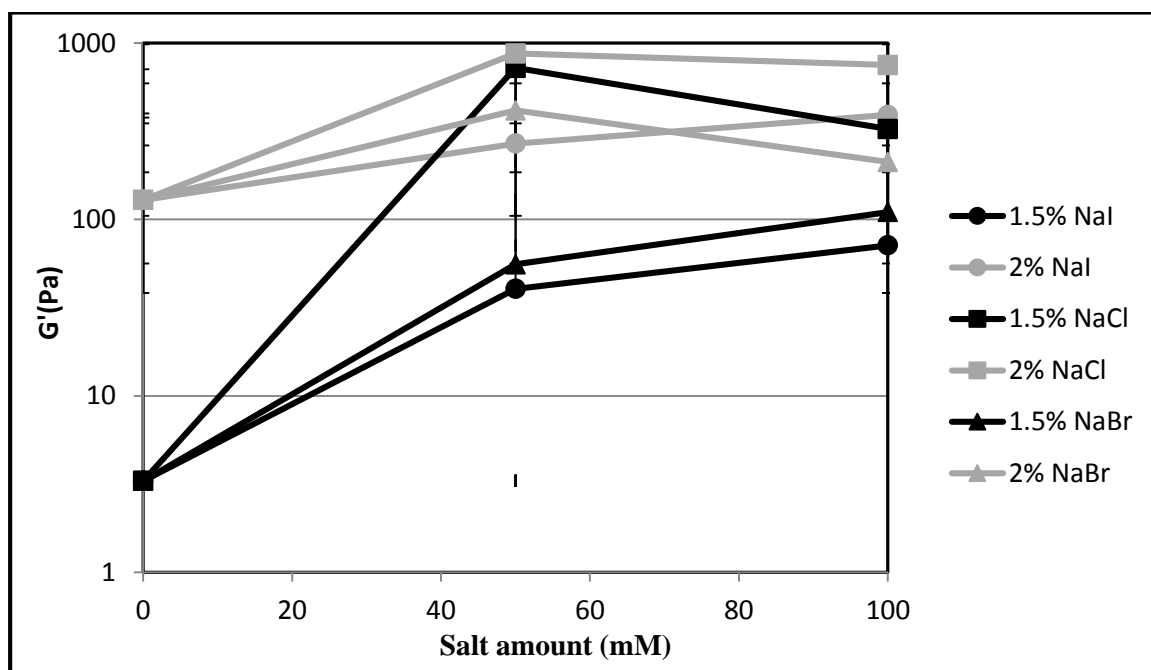


Figure 15. The elastic moduli (G') at 5 °C as a function of sodium kappa-carrageenan and sodium salts.

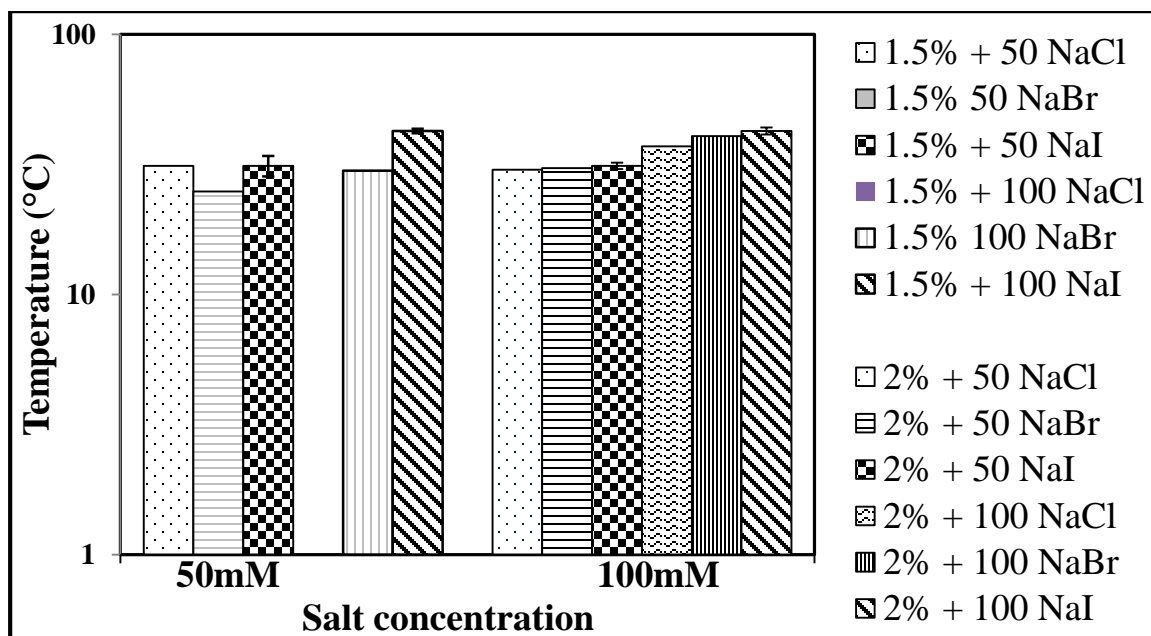


Figure 16. Comparison of gel-to-sol ($^{\circ}\text{C}$) values as a function of salt type, amount and sodium kappa-carrageenan (NaKC) concentration.

2.4.1.5 Discussion:

Figures 15 and 16 portray the variation in G' (measured at 5°C) and gel-sol transition temperature, respectively. As expected with increasing polysaccharide concentration the individual G' values have increased as the kappa-carrageenan is largely responsible for the gel formation that is largely by the presence of cations and further modulated by the anions. Overall, the order of G' is found to be $\text{NaCl} > \text{NaBr} > \text{NaI}$. The lower G' values in the presence of NaI are in agreement with the literature findings of (Grasdalen & Smidsrod, 1981; Hugerth, Nilsson, & Sundelof, 1999; Norton, Morris, & Rees, 1984)), wherein it has been noted that addition of iodide ions not only resulted in weaker gel, but also produced a higher helical content. The variation in the individual G' values and their

trend clearly suggest that iodide ions promote helix formation by preventing the aggregation among the kappa-carrageenan chains.

2.4.2 X-ray fiber diffraction

Figure 17 highlights the X-ray diffraction patterns from the fibers prepared using the 2.0% sodium kappa-carrageenan, 1.5% sodium kappa-carrageenan with 100 mM NaCl, 2.0% sodium kappa-carrageenan with 75 mM NaBr, and 2.0% sodium kappa-carrageenan with 50 mM NaI. The kappa-carrageenan without any added salt yields relatively less ordered diffraction patterns. In the presence of NaCl the diffraction pattern quality improved with the presence of diffuse and very broad Bragg reflections. The presence of NaBr in the carrageenan solution did not yield any ordered network. The high elastic moduli (G') observed could be due to severe aggregation of carrageenan chains in the presence of bromide ions. On the other hand, well-resolved and highly crystalline Bragg reflections are observed in the presence of NaI. The reflections extend up to 2.5 \AA on 0 to 8 layer lines. The meridional reflections are observed on 3rd and 6th layer lines, suggesting 3-fold helical arrangement for kappa-carrageenan chains. Thus, it appears that iodide ions are able to prevent aggregation among the kappa-carrageenan chains towards promoting ordered networks, certainly better than Cl or Br anions.

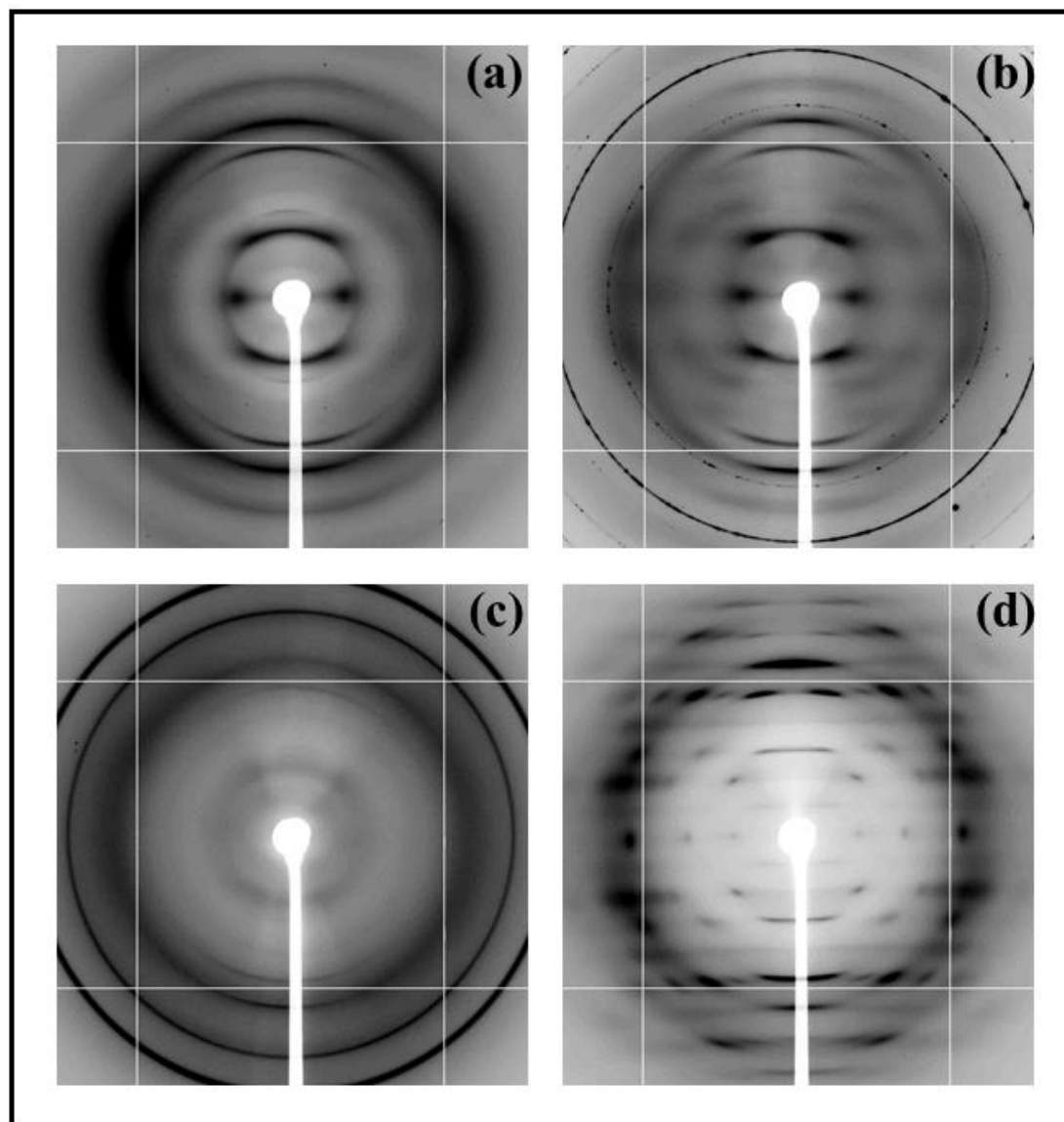


Figure 17. X-ray fiber diffraction patterns of (a) 2.0% sodium kappa-carrageenan, (b) 1.5% sodium kappa-carrageenan + 100 mM NaCl, (c) 2.0% sodium kappa-carrageenan + 75 mM NaBr, and (d) 2.0% sodium kappa-carrageenan + 50 mM NaI.

2.4.3 Thermal Properties

DSC thermograms are reported for different sodium salts with varying salt amounts (Figs. 18, 19, and 20). Both 1.5% and 2% polysaccharide concentration with 0 mM salts did not yield any melting peaks, thus data has been reported for samples with salt addition.

2.4.3.1 Effect of Sodium chloride:

The addition of sodium chloride (NaCl) produces small, broad peaks in the DSC thermograms (Fig. 18). At 1.5% polysaccharide concentration and 50 mM NaCl, there is a very subtle melting peak at about 30 °C, and exact measurement of peak values or enthalpy is not plausible (Fig.18a). The same holds true for 100 mM salt level, with a broad peak and a weak melting profile, but the onset temperature could be obtained at 32.2 °C, with the peak temperature at 38.7 °C (Fig. 18b). Increasing the polysaccharide concentration has a favorable effect, with small, but short and sharp peaks noticed in both 2.0 % NaKC with 50 mM NaCl, and 2.0% NaKC with 100 mM samples (Figs. 18c and d). The melting peak values are roughly around 40 °C for both.

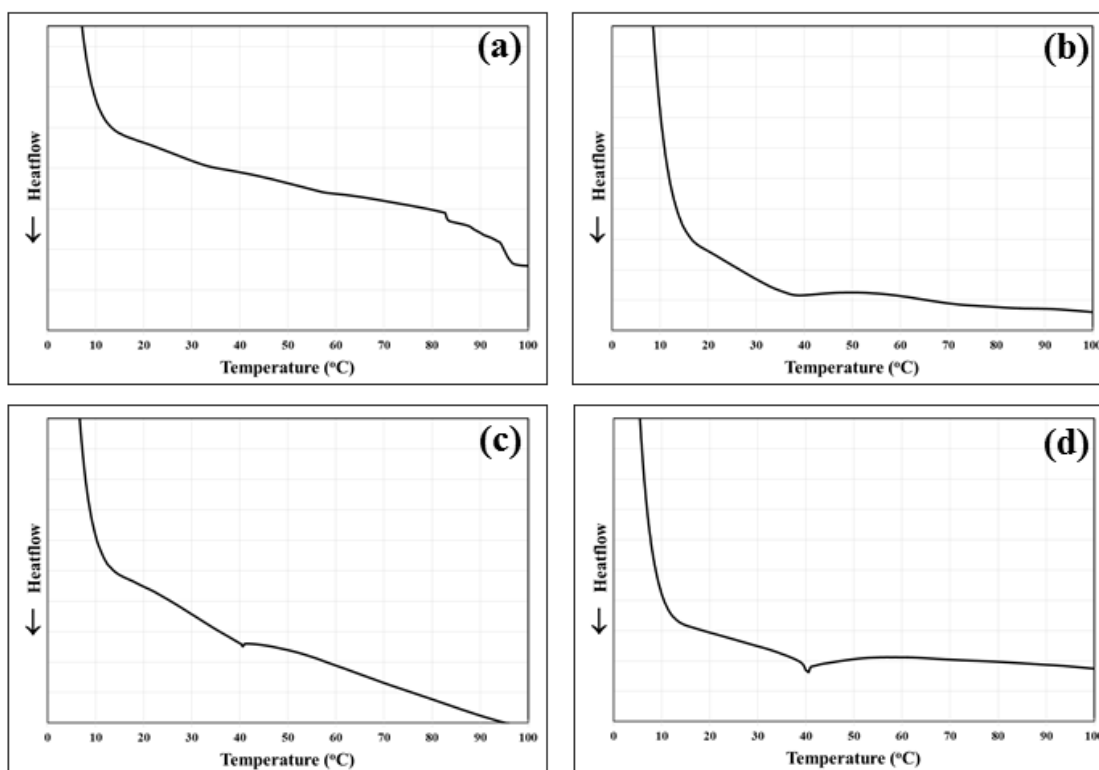


Figure 18. DSC thermograms of (a) 1.5% NaKC 50mM NaCl, (b) 1.5% NaKC + 100 mM NaCl, (c) 2.0% NaKC + 50 NaCl, and (d) 2.0% NaKC + 100 mM NaCl.

2.4.3.2 Effect of Sodium bromide:

The addition of sodium bromide produces noticeably larger peaks with clear enthalpies (Fig. 19). At 1.5% polysaccharide concentration and 50 mM NaBr, the onset and melting peak temperatures are 24.9 and 32.0 °C, respectively. The melting enthalpy is recorded as 0.37J/g (Fig. 19a, and Table 2). In the case of 1.5% polysaccharide and 100 mM NaBr salt amount, the onset and melting peak temperatures rise to 33.3 and 37.1 °C, respectively, but the melting peak is very short and hence, the enthalpy value reduces to 0.032 J/g (Fig. 19b). At 2% polysaccharide concentration and 50mM salt, the mean onset

and mean peak temperature values are 32.6 and 38.6 °C, respectively. In the case of 2.0% polysaccharide concentration with 100 mM NaBr addition, the onset temperature declines slightly to 31.6 °C. However, the melting peak temperature increases considerably to 43.9 °C. Compared to 2.4.3.1, NaBr seems to have higher melting peak temperatures, suggesting stronger aggregate/complex formation. The results clearly demonstrate the effect of various anions on the thermal properties of carrageenan.

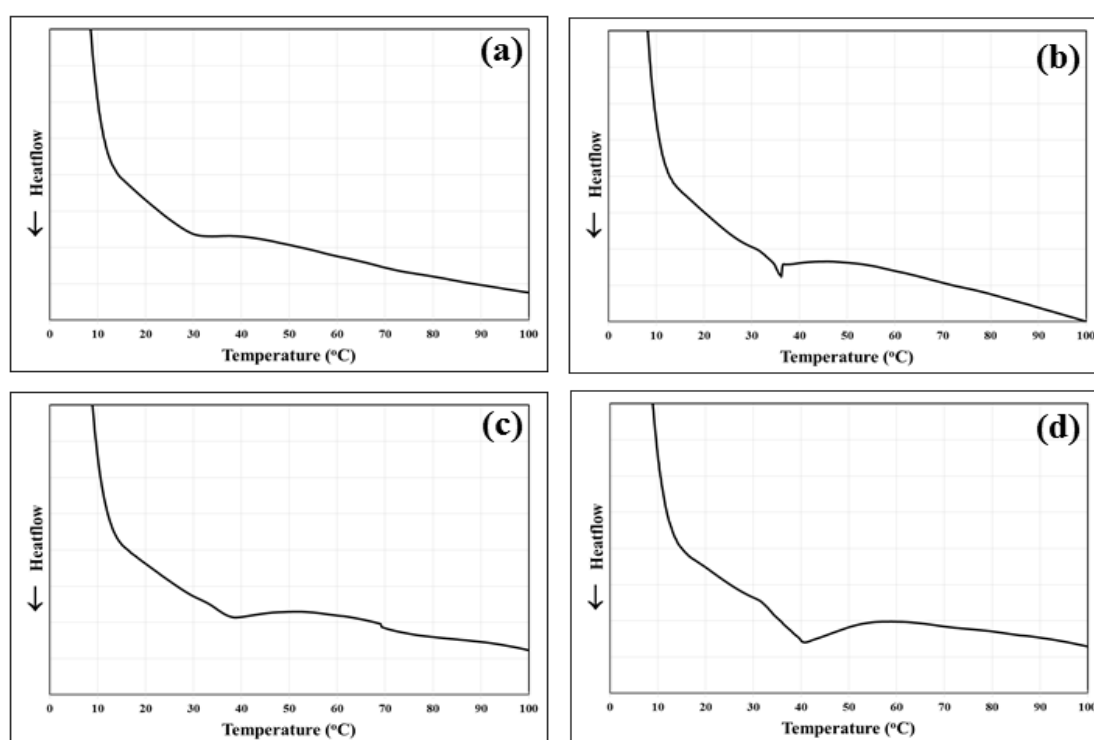


Figure 19. DSC thermograms of (a) 1.5% NaKC 50 mM NaBr, (b) 1.5% NaKC + 100 mM NaBr, (c) 2.0% NaKC + 50 NaBr, and (d) 2.0% NaKC + 100 mM NaBr.

2.4.3.3 Effect of sodium iodide:

In the case of iodide salts, well defined, sharp melting peaks are noticed (Fig. 20). At 1.5% polysaccharide concentration with 50 mM NaI, the onset temperature and melting peak values are 29.8 and 35.9 °C, respectively. The enthalpy is 0.11 J/g. At 100 mM salt amount, there is a sharp increase in both onset temperature and melting peak values at 42.1 and 45.7, respectively. However, the enthalpy drops from 0.2 J/g in the 50 mM sample to 0.16 J/g in the 100 mM sample. This could be due to the narrowing of the melting peak in the latter, whereas a broad peak is formed with the former.

Interestingly, the increase in polysaccharide concentration to 2.0% (w/w) and addition of only 50 mM salt results in a decline in the onset temperature and mean peak temperature to 25.7 and 36.3 °C, respectively. In comparison with other samples for the iodide salt system, this data seems to indicate that broader melting peaks are formed upon the addition of polysaccharide, whereas narrower and sharper melting peaks are formed consequent to an increase in sodium iodide concentration.

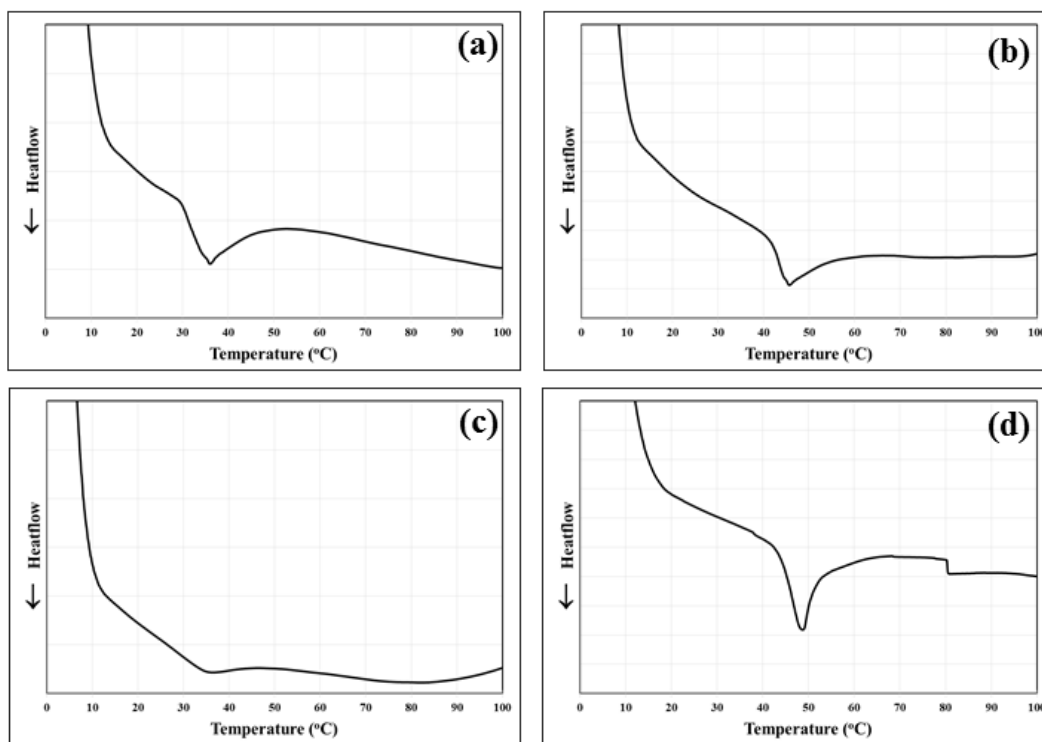


Figure 20. DSC thermograms of (a) 1.5% NaKC 50mM NaI, (b) 1.5% NaKC + 100 mM NaI, (c) 2.0% NaKC + 50 NaI, and (d) 2.0% NaKC + 100 mM NaI.

Table 3. DSC Analysis of 1.5 and 2% sodium kappa-carrageenan (NaKC) solutions with added sodium salts

NaKC (% w/w)	Salt type & amount (mM)	Onset temperature (°C)	Peak temperature (°C)	Enthalpy (J/g)
1.5	NaCl 50	Unclear	Unclear	Unclear
	NaCl 100	32.2	38.7	Unclear
	NaBr 50	24.9	32.0	0.37
	NaBr 100	33.3	37.1	0.03
	NaI 50	29.8	35.9	0.20
	NaI 100	42.1	45.7	0.16
	2%	NaCl 50	30.3	40.6
NaCl 100		37.5	40.6	0.07
NaBr 50		32.6	38.6	Unclear
NaBr 100		31.6	43.9	0.29
NaI 50		25.7	36.3	0.11
NaI 100		44.1	48.8	0.38

Overall, with increasing concentrations of iodide salts there is a steady raise in the peak temperature and enthalpy, which certainly suggest enhanced gel strength as well as network stability and agree with the X-ray observations (Fig. 17).

2.5 Conclusions

This study reveals that the solution, thermal and structural behavior of kappa-carrageenan varies considerably with the type of anion present. Especially, iodide anions are suited well compared to chloride or bromide anions in controlling the aggregation among the carrageenan chains towards forming helical and stable network arrangements. However, the exact mechanism and role of iodide anions in stabilizing the gel network is unclear, and certainly needs further investigation.

CHAPTER 3. THE EFFECT OF CHAOTROPIC AGENTS ON THE STRUCTURE-FUNCTION RELATIONSHIPS OF KAPPA-CARRAGEENAN

3.1 Abstract

Chaotropic agents have long been hypothesized to alter the water structure by disrupting the hydrogen bonds between molecules. Thus, they are natural denaturants and the effects of several chaotropic agents such as urea, urea derivatives and guanidinium chloride on proteins have been well documented. However, the mechanism of their action and the effect on the structure-function relationships of polysaccharides is not clear. The current study presents details about the interactions of urea and guanidinium chloride (0, 50, 100, 150 mM) on polysaccharides with sodium kappa-carrageenan (1.5 and 2% w/w) as a model compound. Viscoelastic and melting properties reveal that the gel strength increases exponentially in the presence of guanidinium chloride, while the addition of urea produces only marginal increase. Nevertheless, it appears that both urea and guanidinium ions favorably promote interactions among the kappa-carrageenan chains.

3.2 Introduction

Guanidinium ion (Gdm^+) has been used as a model to understand the hydration of complex molecular substances in solution owing to its symmetry and rigid structure. It also serves as a functional analogue to arginine, a non-essential amino acid. Hence it is used as a tool to study the thermodynamics of protein folding and denaturation (Azuaga *et al.*, 2003). Similarly, urea, a small organic molecule, is also known a chemical denaturant of biopolymers. It has been used to assess protein stability and unfolding (Scholtz, Grimsley, & Pace, 2009). It is also found to indirectly affect protein structure by altering water structure and dynamics (Bennion & Daggett, 2003; Scholtz *et al.*, 2009).

The effects of urea on polysaccharides have been well documented. Large doses of urea prevent gelation of amylose (Welsh, Bailey, Chandarana, & Norris, 1982), xanthan (Frangou, Morris, Rees, Richardson, & Rossmurphy, 1982), iota-carrageenan (Morris & Belton, 1982), and kappa-carrageenan (Nishinari, Watase, Williams, & Phillips, 1990). More recently, the effects of urea on the structural, viscoelastic and thermal properties of iota-carrageenan were analyzed, wherein well-resolved fiber diffraction patterns along with increased onset temperatures were observed suggesting that urea readily participates in the junction-zone formation (Patel, Campanella, & Janaswamy, 2013).

Herein the focus is on understanding the effects of urea and guanidinium chloride on the solution and thermal properties of dilute sodium kappa-carrageenan solutions.

3.3 Materials and methods

3.3.1 Solution preparation

Kappa-carrageenan was from FMC biopolymer (Philadelphia, PA). Lab grade urea and guanidinium chloride were purchased from VWR International. Reagent grade isopropyl alcohol and double distilled water were used as necessary. Pure sodium-form of kappa-carrageenan was prepared by dissolving 0.1% w/w of polysaccharide in distilled, deionized water of 400 mL and heated to 95 °C with constant stirring until the solution reached homogeneity. Subsequently, 100mM of NaCl was added and further heated for 45-60 min. The hot solution was then poured into cold isopropanol (4 °C), the precipitate was separated. Later, the precipitate was re-dissolved in 200 mL distilled water and heated to 45-60 min. The hot solution was poured into cold isopropanol (4 °C) and the precipitate collected. The second round heating without adding any excess salt is the key for obtaining pure sodium kappa-carrageenan. The final product was dried at 60 °C for 24 hours, powdered and solution concentrations of 1.5 and 2% were prepared and stored at 75 °C for further analysis.

Appropriate amounts of urea and guanidinium chloride were added to obtain 0, 50, 100 and 150 mM solutions. Subsequently the mixtures were heated to 90 °C and held at this temperature for about 45-60 min with intermittent vortexing to ensure the formation of homogenous solutions.

3.3.2 Rheology

The viscoelastic behaviors of the prepared solutions were analyzed using the DHR-3 Rheometer from TA instruments (Newcastle, DE). The geometry used was a 40 mm, 2° cone and plate with a solvent trap. Linear viscoelastic region was identified from the strain sweep measurements in the range 0.01-10% strain at 1 Hz and 5 °C. Frequency sweeps in the range 0.1 to 100 Hz at 2% strain (linear region) were obtained at three selected temperatures 5, 25 and 45°C. Temperature ramp was conducted in the range 5-70 °C with a heating rate of 2 °C/min at 1Hz frequency with 2% strain (linear region). Tests were conducted in duplicate and average values have been reported.

3.3.3 Thermal behavior

The melting behaviors were studied using the model Q2000 Differential scanning calorimeter (TA instruments, Newcastle, DE). Approximately 50-60µL of the sample was loaded into the pans, which were sealed hermetically using a TZero press (TA instruments, New Castle, DE) and heated at a rate of 10 °C/min in the range -20 to 95 °C, cooled at 15°C/min to -20°C, and reheated at a rate of 10 °C/min to 95 °C. Thermal transitions were recorded in the range of 20-95 °C at a rate of 10°C/min. Universal Analysis 2000 software (TA instruments, Newcastle DE) was used to estimate onset temperature, peak temperature and the enthalpy of melting endotherms. Duplicate measurements were recorded and average values are reported.

3.4 Results and Discussion

3.4.1.1 Effect of urea:

The changes in the elastic moduli (G') as a function of frequency for 1.5% sodium kappa-carrageenan (NaKC) at 5, 25 and 45 °C in the presence of 0, 50 and 100 mM urea are compared in Fig 21. The G' value at 5 °C and 1 Hz is 2.7 Pa (Fig. 21a) for pure NaKC, and the addition of urea increases it substantially to 50.4, 76.3, and 166.4 Pa for 50, 100 and 150 mM, respectively. There is a small but steady increase in the G' values with an increase in urea amounts clearly suggesting favorable interactions between urea and kappa-carrageenan chains. At 25 °C the G' reduces substantially to 0.01, 0.3, 0.4 and 0.01 Pa (Fig. 21b), respectively, and at 45 °C it declines even further (Fig. 21c), underlining the effect of temperature on the gel strength of carrageenan solutions. Increasing the polysaccharide concentration to 2.0% rises the G' to 110.2, 422.2, 217.2 and 264.04 Pa (Fig. 22a) for 0, 50 and 100 and 150 mM of urea, respectively, at 5 °C. At 25 °C these amounts have reduced to 0.8, 0.4, 0.06, and 0.02 Pa further demonstrating the important role of temperature in modifying the solution properties of carrageenans. More interestingly, the heating steps resulted in a greater decline of G' especially for the higher urea amounts *e.g.* 150 mM suggesting the pronounced denaturant effect of urea.

Similar trend is noticed in the temperature ramp as well. For example, at 10 °C, the G' for the 1.5% solutions are 0.8, 13.8, 19.6 and 42.4 Pa (Fig. 23a) for 0, 50, 100 and 150 mM of salt, respectively. On increasing the polysaccharide concentration to 2% (w/w), the elastic moduli further increase to 103.5, 205.7, 656.5 and 357.8 Pa (Fig. 23b), in the same

salt order. Thus, it appears that urea participates in promoting the inter-molecular interactions among the kappa-carrageenan chains.

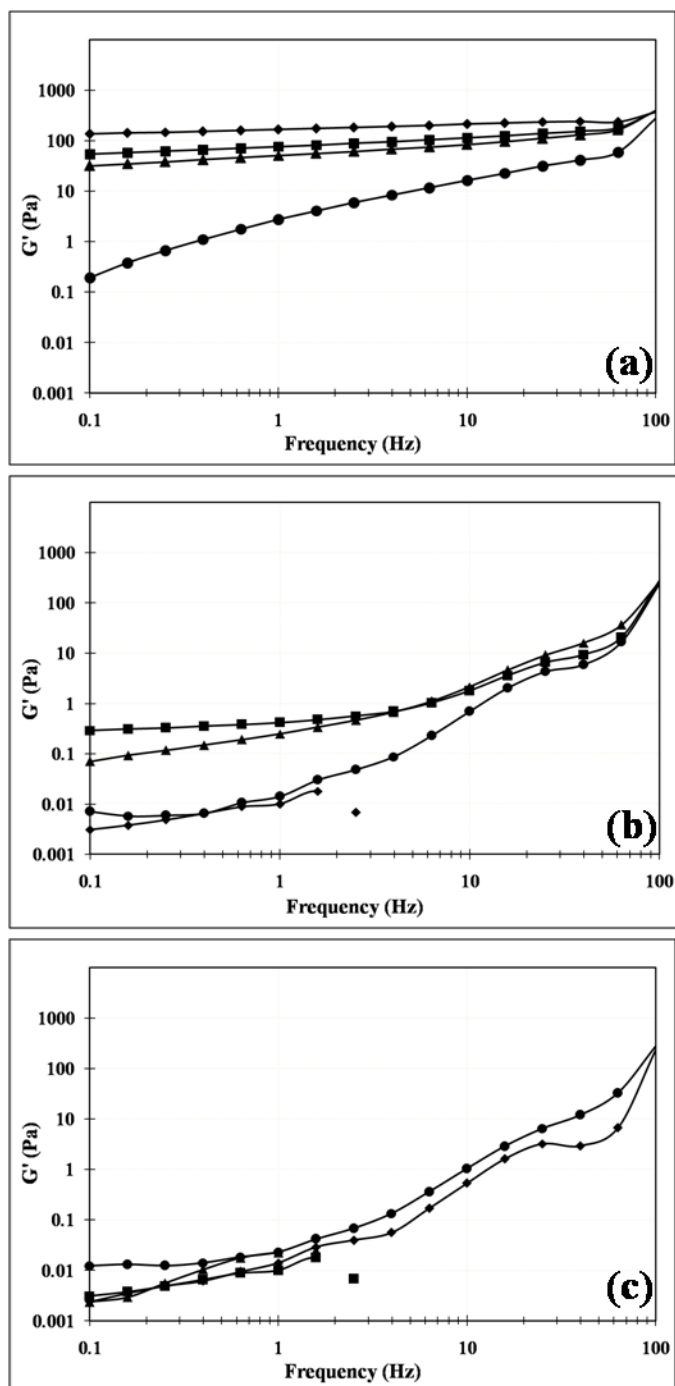


Figure 21. Comparison of the elastic moduli (G') for 1.5% Na kappa-carrageenan in the frequency range 0.1 – 100 Hz in the presence of (●) 0, (▲) 50, (■) 100, and 150 (◆) mM urea at (a) 5, (b) 25 and (c) 45 °C.

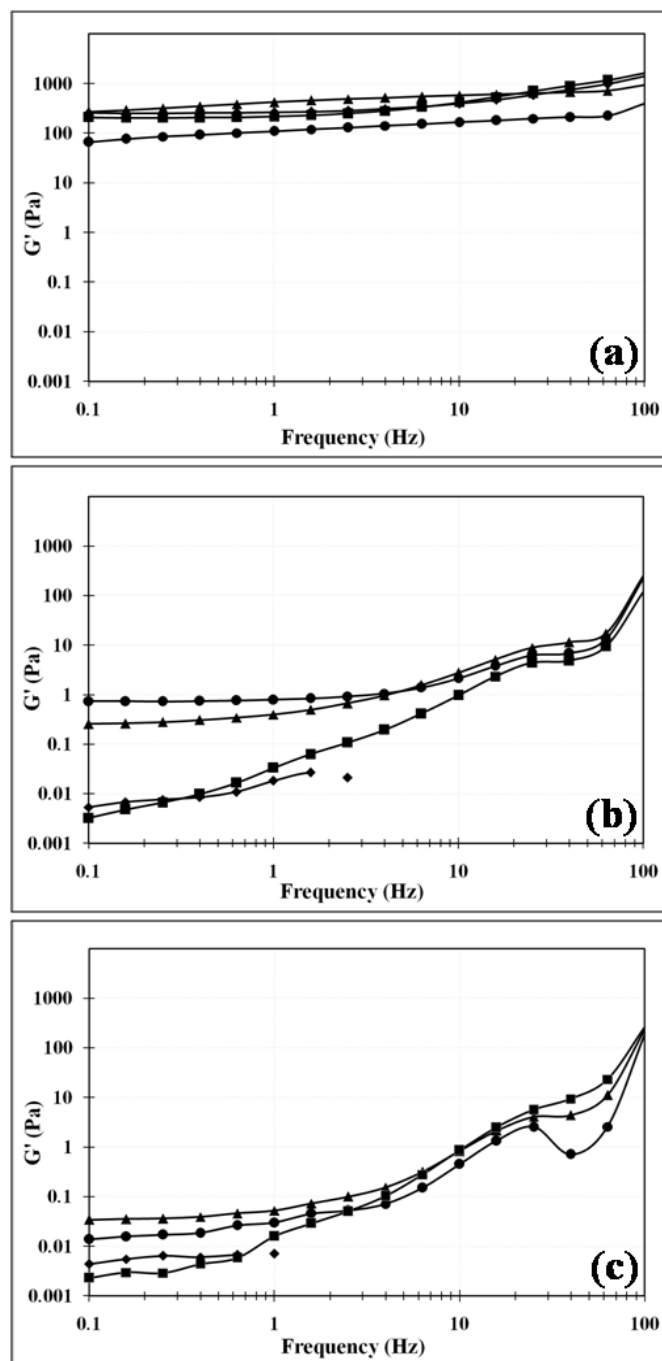


Figure 22. Comparison of the elastic moduli (G') for 2% Na kappa-carrageenan in the frequency range 0.1 – 100 Hz in the presence of (●) 0, (▲) 50, (■) 100, and 150 (◆) mM urea at (a) 5, (b) 25 and (c) 45 °C.

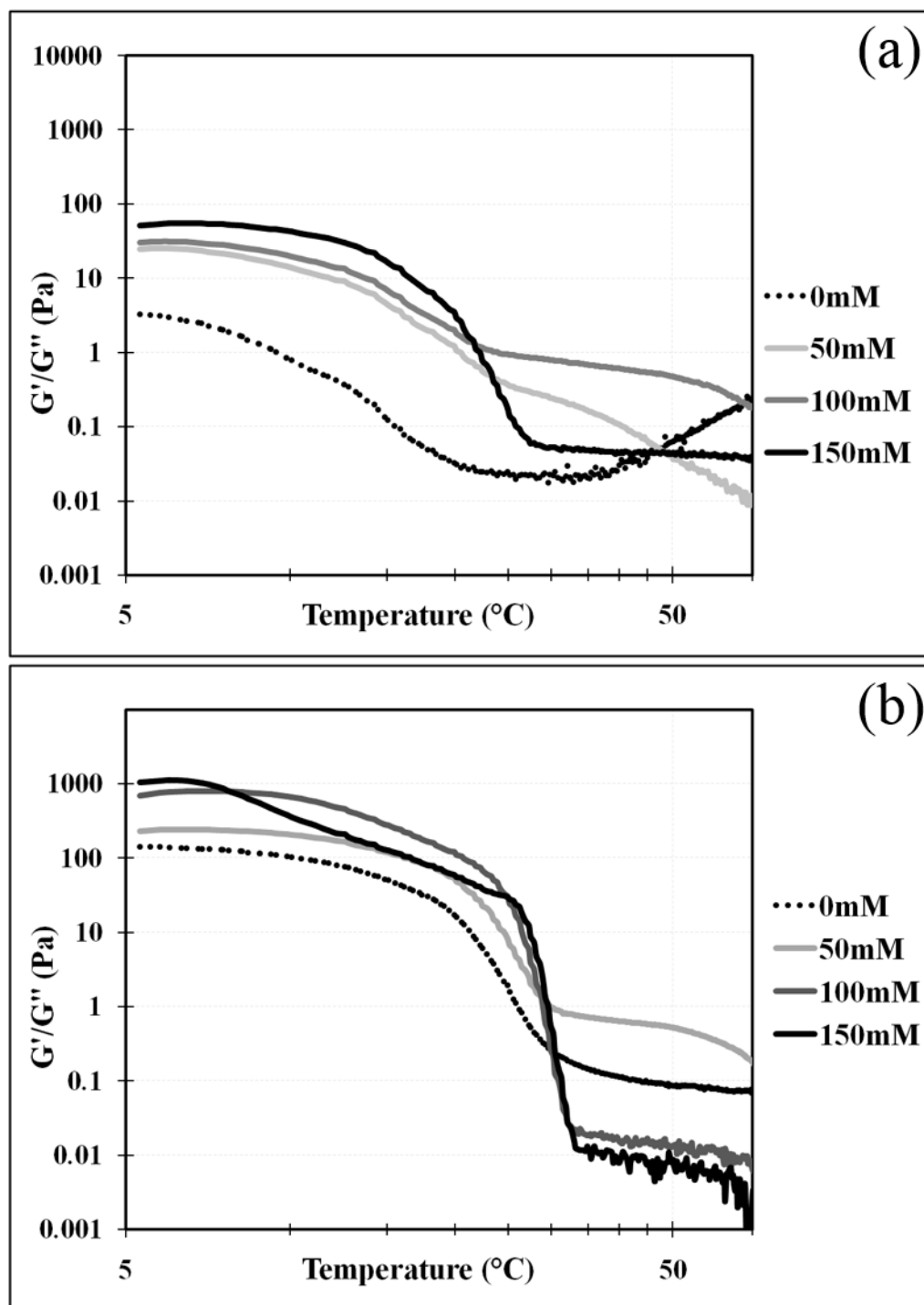


Figure 23. Comparison of the elastic moduli (G') for 0, 50, 100 and 150 mM urea of (a) 1.5% and (b) 2.0% Na kappa-carrageenan in the temperature range 5 – 70 $^{\circ}\text{C}$.

3.4.1.2 Effect of guanidinium chloride:

The changes in the elastic moduli (G') as a function of frequency are compared for 1.5% sodium kappa-carrageenan (NaKC) at 5, 25 and 45 °C in the presence of 0, 50, 100 and 150 mM guanidinium chloride in Fig 24. The G' at 5 °C and 1 Hz for pure NaKC is 2.7 Pa (Fig. 24a), and the addition of salt increases it substantially to 178.5, 256.1, and 166.4 Pa for 50, 100 and 150 mM, respectively. There are wave-like undulations at lower frequencies in 150 mM NaKC sample. Similar trend prevails at 25 °C too (Fig. 24b). As expected at 45 °C the G' reduces substantially to 0.02, 0.1, 0.0 and 0.01 Pa (Fig. 24c), for same salt combination, respectively. Increasing the polysaccharide concentration to 2.0% raises the G' to 110.2, 172.6, 1622.9 and 5594.6 Pa (Fig. 22a) for same salt combination, respectively, at 5 °C. These values suggest substantial increase consequent to the addition of 100 and 150 mM salt amounts. At 25°C these amounts have reduced to 0.79, 28.0, 167.9, and 2003.8 Pa demonstrating the role of temperature, as well as the effect of guanidinium ions, especially in yielding higher elastic moduli. The observed undulations in the elastic moduli could possibly be attributed to the start-up or transient effects wherein the viscoelastic materials take longer durations to attain steady state under certain shear conditions. This phenomenon appears to be quite novel, and further studies involving a variety of polysaccharide and salt concentrations need to be examined to understand the origin and structural implications.

The temperature ramp data are also interesting. For example, at 10 °C, the G' values for the 1.5% solutions are 0.8, 160.5, 14419.4 and 3410 Pa (Fig. 24a) for 0, 50, 100 and 150 mM of salt, respectively. On increasing the polysaccharide concentration to 2% (w/w),

they further increase to 103.5, 173.7, 2661.8, and 269 Pa (Fig. 8b), in the same salt order. While the reduction in the elastic moduli at higher salt amount suggests the denaturing effect of guanidinium ions, the enhanced values at the lower amounts certainly indicate their participation in network formation.

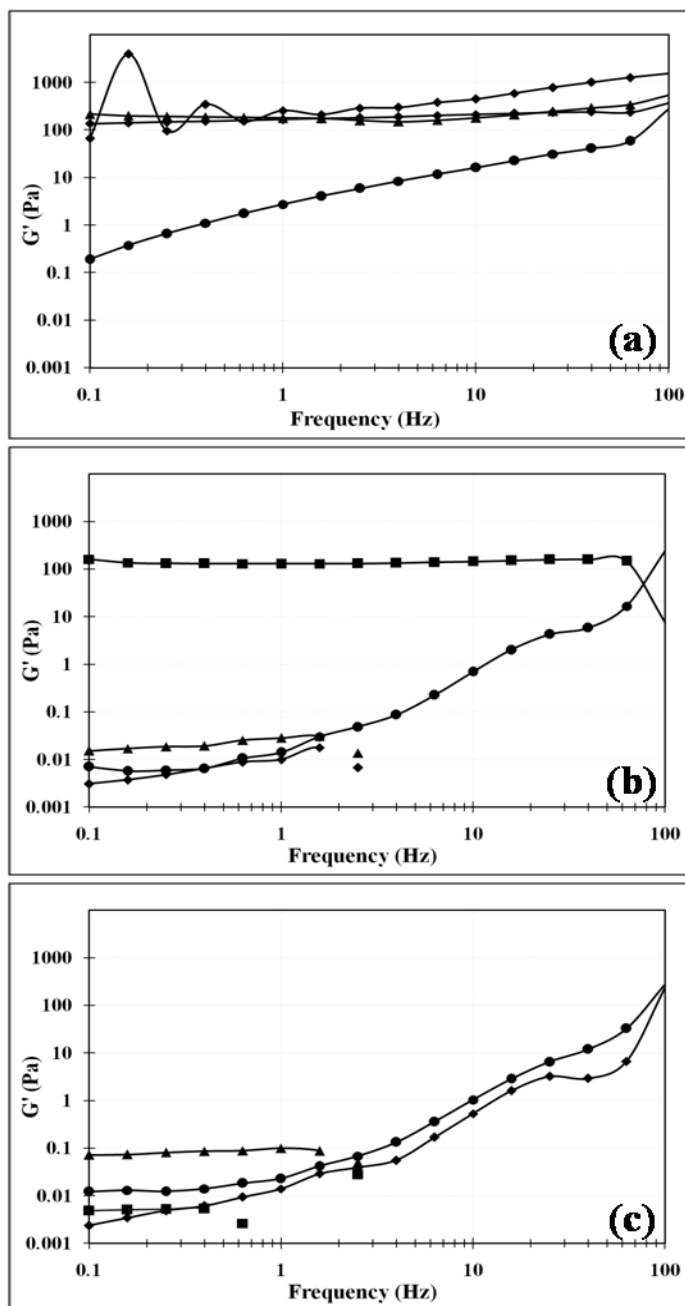


Figure 24. Comparison of the elastic moduli (G') for 1.5% Na kappa-carrageenan in the frequency range 0.1 – 100 Hz in the presence of (●) 0, (▲) 50, (■) 100, and 150 (◆) mM guanidinium chloride at (a) 5, (b) 25 and (c) 45 °C.

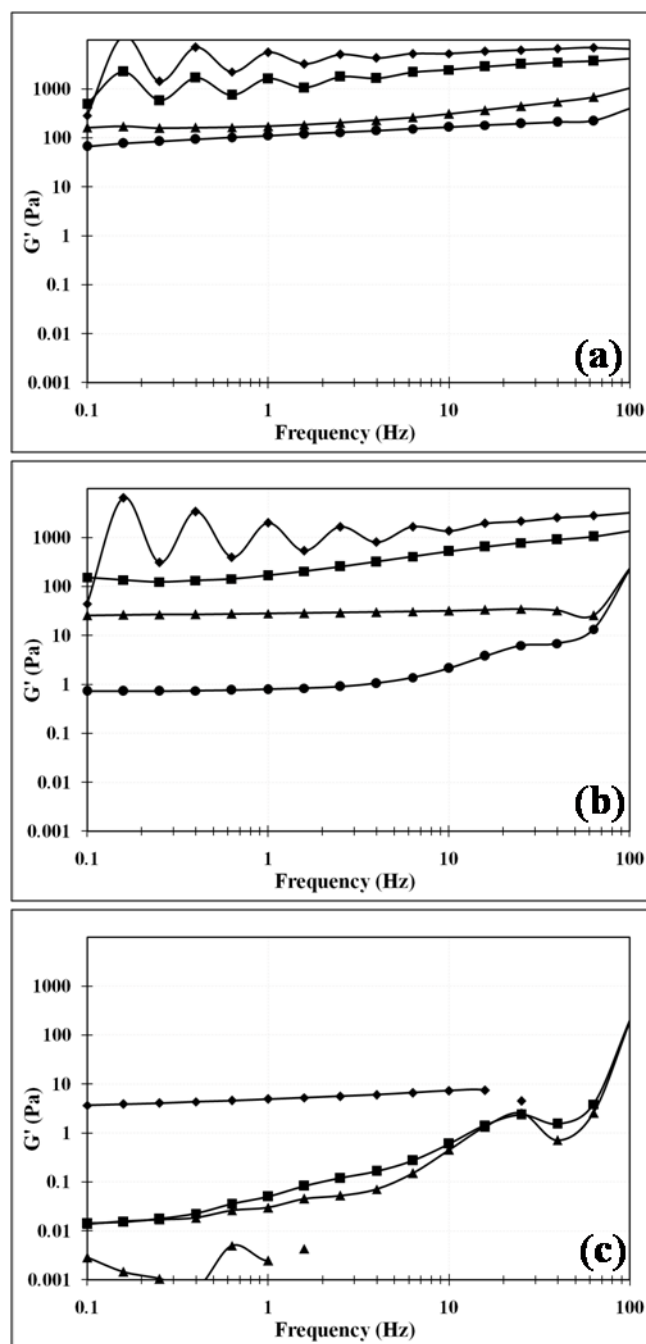


Figure 25. Comparison of the elastic moduli (G') for 1.5% Na kappa-carrageenan in the frequency range 0.1 – 100 Hz in the presence of (●) 0, (▲) 50, (■) 100, and 150 (◆) mM guanidinium chloride at (a) 5, (b) 25 and (c) 45 °C.

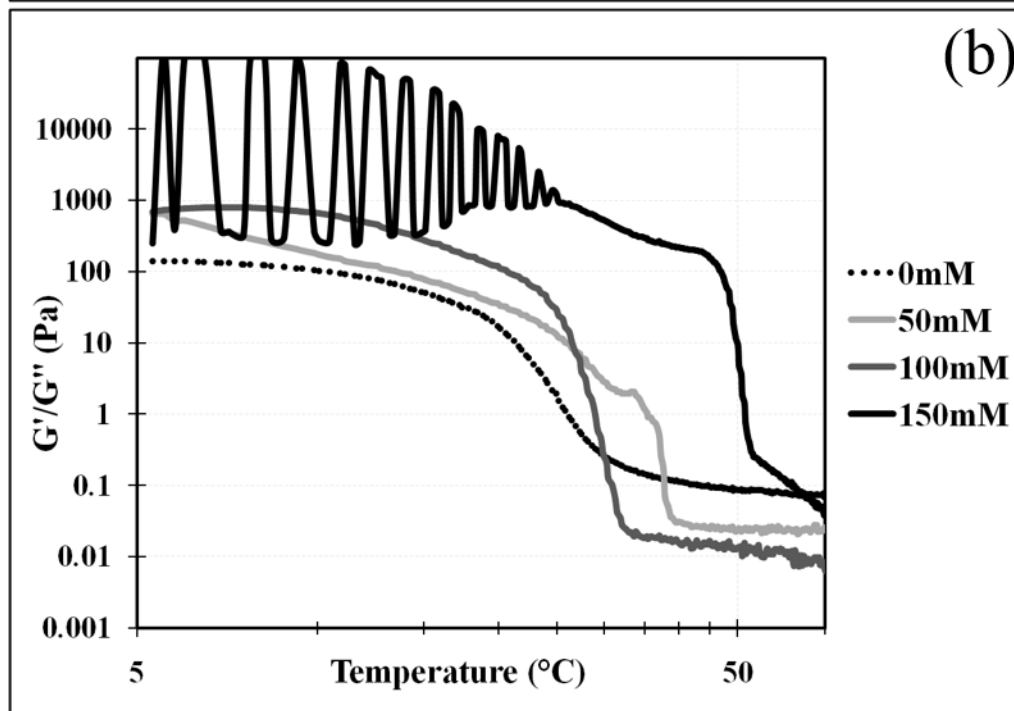
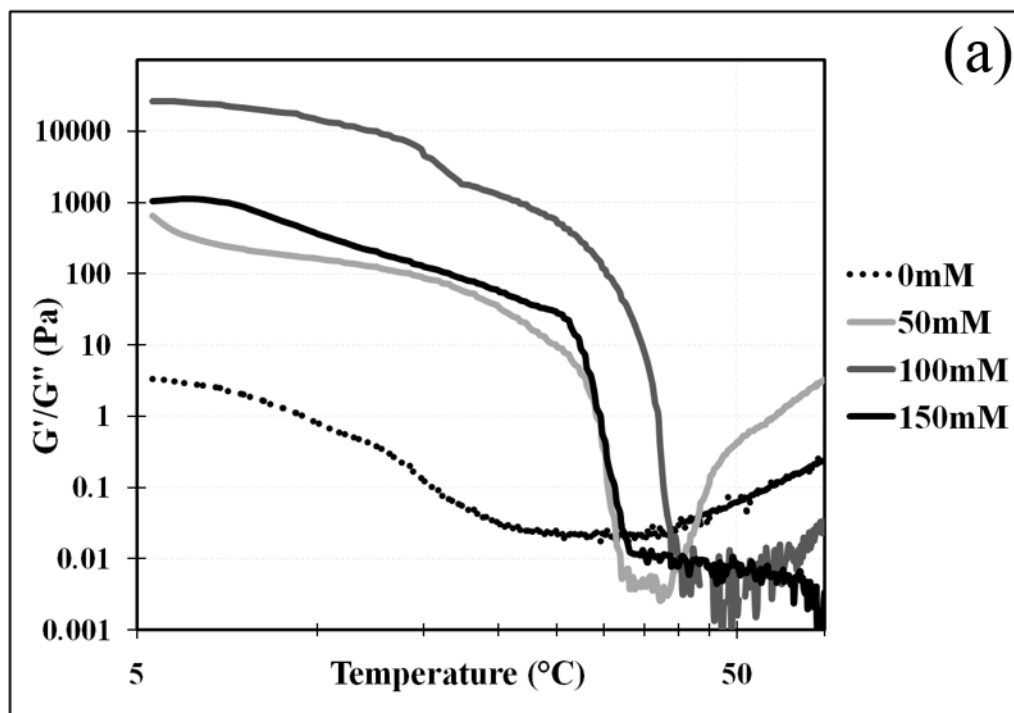


Figure 26. Comparison of the elastic moduli (G') for 0, 50 and 100 mM guanidinium chloride at (a) 1.5 and (b) 2.0% Na kappa-carrageenan in the temperature range 5 – 70 °C.

3.5 Thermal behavior

DSC thermograms for urea and guanidinium NaKC are reported in in Figs. 27, 28, and 29.

The starting 1.5 and 2% solutions without added salt did not yield any melting peaks.

3.5.1.1 Effect of urea:

The addition of urea produces small peaks with mainly low enthalpies (Fig. 27 and 28).

In 1.5% NaKC with 50 mM salt, the onset and melting peak temperatures are 32.4 and 38.4 °C, respectively. The melting enthalpy is quite low as 0.02 J/g (Table 4). With an

increase of salt to 100 mM the melting peak is observed at more or less the same

temperature but is quite broad with an enthalpy of 0.10 J/g (fig. 27b). At 150 mM of urea,

the melting peak temperature drops to 34.6 °C possibly due to reduced gel-strength. At 2%

NaKC with 50 mM salt, the onset and peak temperatures are 28.5 and 38.0 °C,

respectively, suggesting peak broadening. As observed with 1.5% solutions, even here

the onset and melting temperatures remain similar to 100 mM salt and further increase in

urea does not substantially alter these temperatures presumably due to the dominant

effect of polysaccharide concentration.

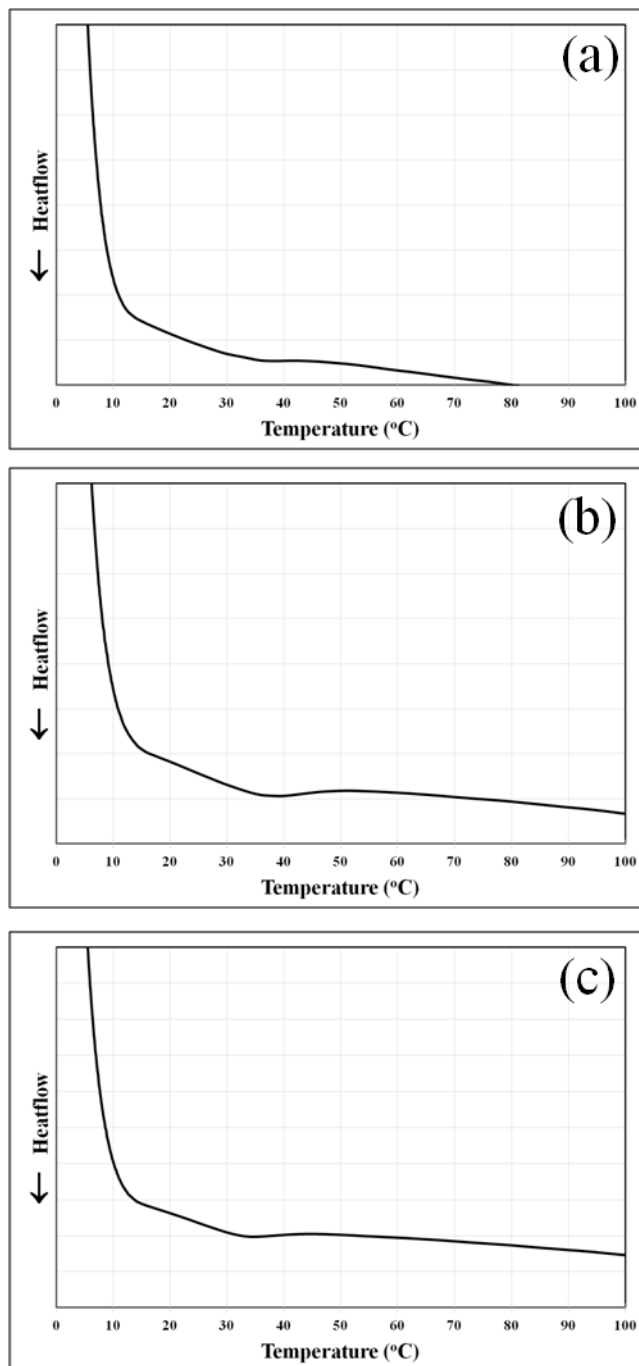


Figure 27. DSC thermograms of (a) 1.5% NaKC 50 mM urea, (b) 1.5% NaKC + 100 mM urea, (c) 1.5% NaKC + 150mM urea.

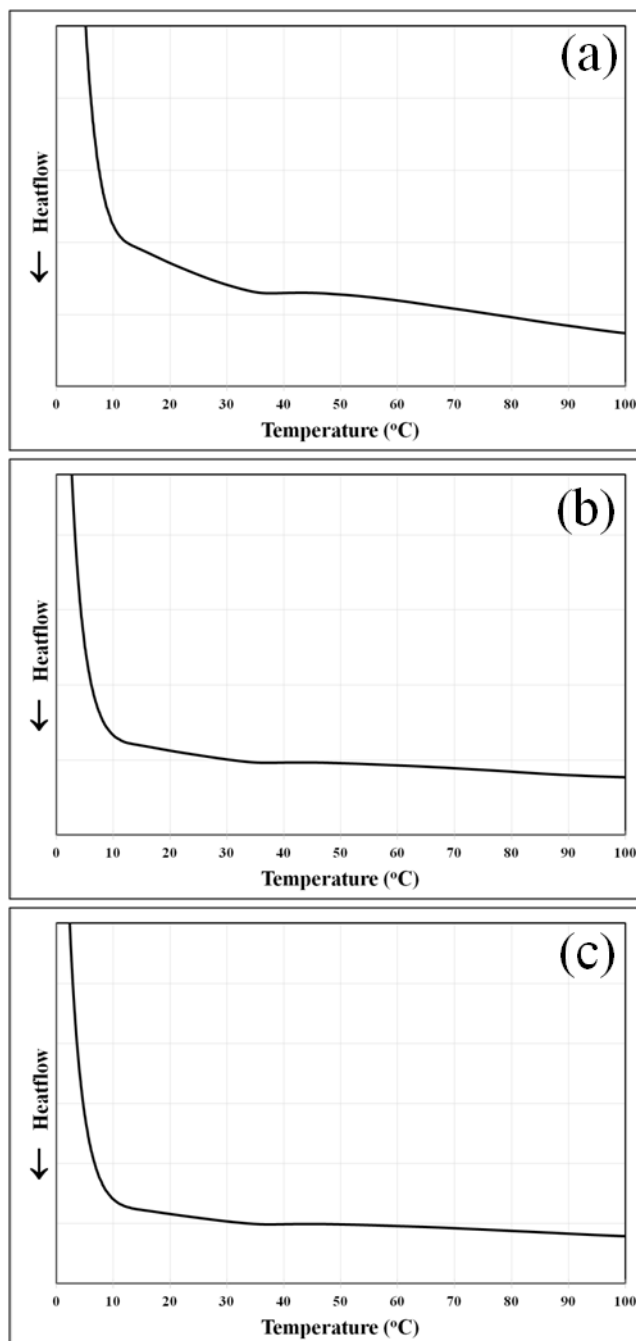


Figure 28. DSC thermograms of (a) 2% NaKC 50 mM urea, (b) 2% NaKC + 100 mM urea, (c) 2% NaKC + 150mM urea.

3.5.1.2 Effect of guanidine chloride (GCl):

The addition of guanidine chloride produces small peaks with low enthalpies (Fig. 29 and 30). In 1.5% NaKC with 50 mM GCl, the onset and melting peak temperatures are 25.2 and 32.4 °C, respectively. The melting enthalpy is as low as 0.01 J/g (Table 4). At 100 mM GCl the melting peak temperature rises to 36.1 °C, but the melting peak is very broad and hence the calculated enthalpy is quite unreliable. At 150 mM GCl, the melting peak temperature further rises to 40.7 °C indicating favorable association between guanidinium ions and kappa-carrageenan chains in yielding increased gel strength. The melting behavior of 2.0 % solutions is similar to 1.5% solutions excepting that the observed peaks are narrow.

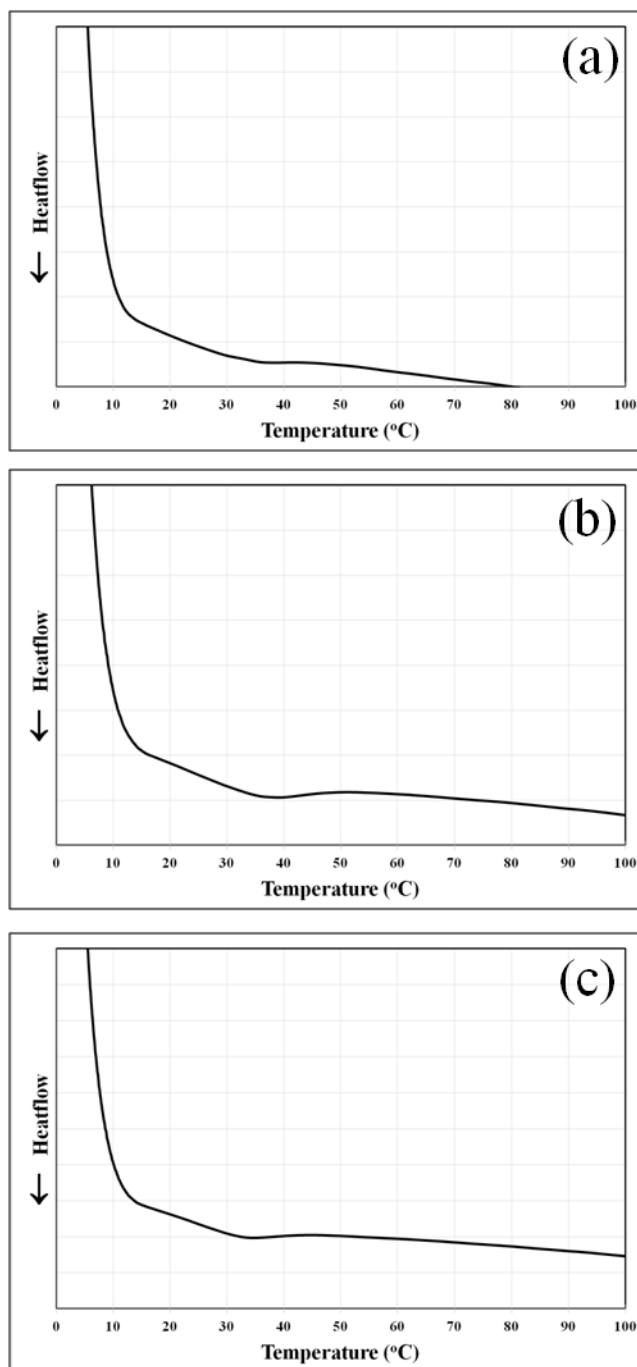


Figure 29. DSC thermograms of (a) 1.5% NaKC 50 mM guanidine chloride, (b) 1.5% NaKC + 100 mM guanidine chloride, (c) 1.5% NaKC + 150mM guanidinium chloride.

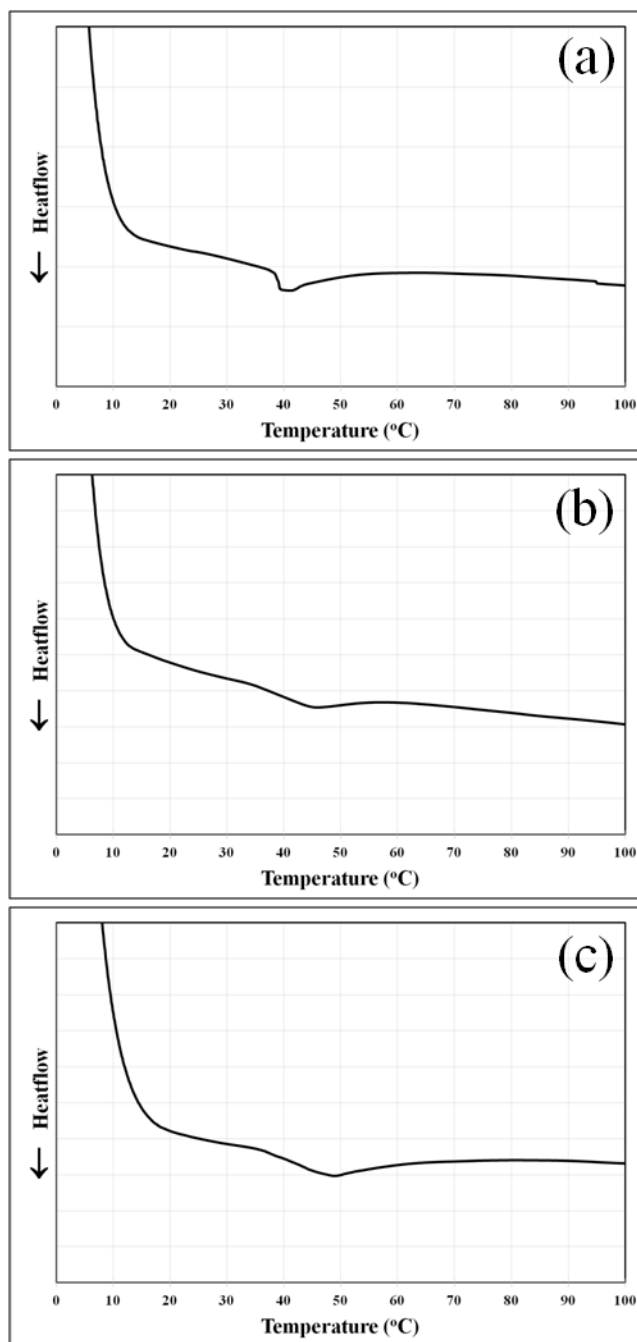


Figure 30. DSC thermograms of (a) 2.0% NaKC 50 mM guanidine chloride, (b) 2.0% NaKC + 100 mM guanidine chloride, (c) 2% NaKC + 150mM guanidinium chloride.

Table 4. DSC analysis of 1.5 and 2% sodium kappa-carrageenan (NaKC) solutions in the presence of urea (U) and guanidinium chloride (GCl).

NaKC (% w/w)	Salt & amount(mM)	Onset temperature (°C)	Peak temperature (°C)	Enthalpy (J/g)
1.5	GCl 50	32.4	38.4	0.02
	GCl 100	30.0	38.8	0.10
	GCl 150	24.4	34.6	0.16
	U 50	25.2	32.4	0.06
	U 100	Unclear	36.1	Unclear
	U 150	Unclear	40.7	Unclear
2.0	GCl 50	39.8	41.4	0.15
	GCl 100	37.1	49.0	0.34
	GCl 150	37.0	49.1	0.38
	U 50	28.5	38.0	0.08
	U 100	29.0	35.9	0.07
	U 150	31.8	37.1	0.02

3.5.2 Results and Discussion

With increasing concentrations of guanidinium chloride, there is only a small decline in the mean onset and peak temperatures, while in 2% solutions with higher salts some increase in the peak temperature is noticed (Fig. 29 and 30). This suggests minimal or no disruption in the carrageenan network and is in agreement with the literature observations that suggest the reduction in the denature efficiency of guanidinium chloride in the presence of salt bridges (Lim, Rosgen, & Englander, 2009; J. S. Smith & Scholtz, 1996).

On the other hand, the increase in the melting peak temperature indicates the participation of guanidinium ions in network formation. Such behavior has been attributed to possible hydrogen bonding of guanidinium ions with water molecules (Mason et al., 2004) and carbonyl groups (Makhatadze & Privalov, 1992), but further research is required to understand its interactions with carrageenan chains.

In the case of urea:NaKC, the melting peaks are at much lower temperatures compared to the GCl:NaKC suggesting a reduction in gel strength (Figs. 27 and 28).

3.6 Conclusions

Viscoelastic measurements have shown that urea and guanidinium chloride do not have the same denaturant effect on kappa-carrageenan as observed in the case of proteins. Guanidinium addition greatly increases the gel strength that could as well be due to the formation of rigid aggregates that indeed leads to stress dissipation problems. The addition of urea results in a slight increase in gel-strength, but is reduced upon heat treatment, thus could be amenable for producing oriented fibers for X-ray analysis towards understanding their role in the junction zone formation, if any.

CHAPTER 4. RECOMMENDATIONS FOR FUTURE WORK

4.1 Exact mechanism of sodium iodide influencing the gel network formation of sodium kappa-carrageenan

In the presence of sodium iodide (Chapter 2) an increase in the sodium-kappa carrageenan T_g (gel-to-sol transition) has been observed along with high melting peak temperatures, and well-oriented and crystalline X-ray fiber diffraction patterns, suggest favorable participation of iodide ions in the kappa-carrageenan network formation. However, the precise knowledge about the role of iodide ions in controlling the secondary and tertiary of kappa-carrageenan could only be realized upon solving the three-dimensional structure. In this regard, a focused study on the effect of sodium iodide on the three-dimensional structure of kappa-carrageenan could provide valuable insights.

4.2 Study of the stress dissipation rates with guanidinium chloride

The high elastic moduli (G') values (Chapter 3) observed in the presence of guanidinium chloride do suggest the possible applications of kappa-carrageenan in the lubricant industry. However, the possible stress dissipation errors with wave-like elastic behavior are quite interesting. A systematic study needs to be carried out focusing on stress dissipation times as well as the stability of gels at various temperature/pressure conditions for comprehensive understanding of this novel phenomenon.

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