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Critical Exponents of Thermal Phase Transitions of κ-Carrageenan in Various Salt Solutions

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Summary: The steady state fluorescence (SSF) technique was employed to study the phase transitions of κ-carrageenan in NaCl and KCl solutions. Pyranine was used as a fluorescence probe for monitoring these transitions. Scattered light, I_{sc}, and fluorescence intensity, I, was monitored against temperature to determine transition temperatures and exponents. It was observed that transition temperatures are strongly correlated with the NaCl and KCl contents. The weight-average degree of polymerization, DP_w and gel fraction G, exponents (γ and β) were measured and found to be in accord with the classical Flory-Stockmayer model.

Keywords: carrageenan; critical exponents; fluorescence; gelation; percolation

Introduction

Carrageenans are water-soluble sulfated anionic galactans, extracted from species of marine red algae. κ-carrageenan forms gels through specific interactions with metal ions.[1] Biocompatibility of these gels makes them valuable in a number of applications. For example they are used in dairy and household products such as toothpastes and lotions. In food applications, carrageenans are used for gelation, thickeninig and stabilization.[2] They are also investigated for pharmaceutical and environmental applications due to their ability to immobilize micro-organisms.[3]

In κ-carrageenan gels ionic interaction between OSO_3^- groups and K^+ ions together with intra and interchain hydrogen bonds give rise to helical structures. κ-carrageenan assumes a random coil conformation in the sol state; low temperature induce twisting of anhydrogalactose sequences into double helices. Gelation of κ-carrageenan has been investigated by a variety of techniques such as small angle neutron scattering,[4] rheology,[5–7] light scattering,[5] photon transmission,[8] small angle x-ray scattering.[9,10]

There are extensive studies on the effect of salts on macroscopic properties of the κ-carrageenan.[11–13] In terms of the effect on gelation, monovalent alkali metal ions are classified into two groups. One group includes cations such as potassium, rubidium and cesium, which strongly promote gelation of κ-carrageenan. The other group includes cations such as sodium and lithium, which scarcely promote the gelation. The strength of the promoting effects of divalent cations such as calcium, magnesium and strontium is intermediate.[14]

In this work, the thermal phase transitions of κ-carrageenan in NaCl and KCl solutions were studied using the steady state fluorescence (SSF) technique. Pyranine (P) (a derivative of pyrene molecule) was introduced as fluorescence probe. It was observed that during the sol-gel transition of carrageenan, pyranine intensity, I, showed a continuous increase. Scattered light intensity, I_{sc}, was also monitored to detect the changes of turbidity during the sol-gel phase transition. However, on reheating during the gel-sol transition, I and I_{sc} intensities decreased. The necessary correction for the pyranine intensity was made to produce the real

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sol-gel and gel-sol transition curves. Sol-gel and gel-sol transition temperatures were determined for each curve of samples in various NaCl and KCl solutions. It was observed that T_{sg} and T_{gs} values increased by increasing NaCl and KCl content. The measured weight-average degree of polymerization exponents, γ and gel fraction exponents, β, were found to be in accord with the classical Flory – Stockmayer model in thermal phase transitions. This theory predicts that helices and double helices should form the Cayley tree structure in the gel network.

Theoretical Considerations

The mechanism of the sol-gel transition has been described in terms of the percolation theory by a number of authors.[15,16] According to this theory, in the sol state molecules of the solute join into small aggregates, called clusters, which grow in size during gelation. The sol-gel phase transition from sol state to the gel state occurs when small clusters link together and form a single giant cluster, which occupies most of the volume. The moment at which the giant cluster just starts to appear, indicates the gel point, $p = p_c$, where the conversion factor p is the fraction of the bonds which have been formed between the molecules. Therefore, the system is called a gel for p above p_c, a sol for p below p_c. In the gel state, the number of finite clusters decreases during the gelation process, whereas the size of the giant cluster grows until all molecules are involved in its network. For the critical exponents near the sol-gel phase transition, classical theories like those of Flory–Stockmayer predict one set of exponents, whereas scaling theories based on lattice percolation predict different exponents. The two groups of theories differ in their treatment of intramolecular loops, space dimensionality, and excluded volume effects.

Historically the exact solution of the sol-gel transition was given first by Flory and Stockmayer on a special lattice called Bethe lattice where the closed loops were ignored. The critical exponents for the weight average degree of polymerization, DP_w and the gel fraction, G, are equal to unity independent of the dimensionality in the Flory–Stockmayer model which is also called classical theory or kinetic theory.[17,18]

The dependence of the gel fraction G and the weight average degree of polymerization, DP_w on the fraction of bonds, p is given by the following power law

$$G \propto (p - p_c)^{\beta}, \quad p \to p_c^{+} \tag{1}$$

$$DP_w \propto (p_c - p)^{-\gamma}, \quad p \to p_c^{-} \tag{2}$$

where β and γ are the critical exponents.

In this work, it can be argued that the total fluorescence intensity from the bonded pyranines follows the weight-average degree of polymerization and the growing gel fraction below and above the gel point, respectively. This proportionality can be easily shown for site percolation as follows. The probability that a site belongs to a cluster of size s is given by $n_s\, s$, where n_s is the number of *s-cluster* (number of clusters including s sites) per lattice site. The probability that an arbitrary site belongs to any cluster is p, this is simply the probability of arbitrary occupation of site. Thus, the probability $w_s = \frac{n_s \cdot s}{\sum_s n_s \cdot s}$ is the cluster to which an arbitrary occupied site belongs contains exactly s site, and thus the average cluster size, S is calculated by the following relation

$$S = \sum_s w_s \cdot s = \frac{\sum n_s \cdot s^2}{\sum n_s \cdot s} \tag{3}$$

Definition of the average cluster size is the same for all dimensions, although n_s cannot be calculated exactly in higher dimensions. This definition is also true for the bond percolation.

Now, to show that below p_c, pyranine intensity is proportional to S, let N_p be the number of pyranine molecules and N_m that of the other molecules in the lattice. Thus, the total lattice site, N is equal to $N_p + N_m$. The probability, p_p that an arbitrary site is a

pyranine molecule is N_p/N. The probability, P_y, that an arbitrary site is both a pyranine and belongs to the *s-cluster* can be calculated as a product of w_s and p_p as follow

$$P_y = p_p w_s = \frac{p_p n_s s}{\sum n_s s} \quad (4)$$

Thus the total number of pyranine molecules in the clusters including s sites will be P_ys. The total fluorescence intensity, I, which is proportional to the total number of pyranines trapped in the finite clusters, can be calculated as a summation over all s-clusters

$$I \sim \sum_s P_y s = \sum_s \frac{p_p n_s s}{\sum n_s s} s = \frac{\sum p_p n_s s^2}{\sum n_s s} \quad (5)$$

where p_p can be taken out of the summation since the concentration of the pyranine is fixed for our work,

$$I \sim p_p \frac{\sum n_s s^2}{\sum n_s s} = p_p S \quad (6)$$

Thus, the last expression shows that the total normalized fluorescent intensity, I is proportional to the average cluster size, S. Note that the proportionality factor, p_p is simply the concentration of the pyranine molecules in the sample cell (or the number of pyranine molecules in the lattice). The intensity I will be linearly proportional to the average cluster size, provided that the pyranine concentration is not high enough to quench the fluorescence intensity by the reabsorption mechanism and no other parameter like viscosity influencing the fluorescence intensity in addition to the concentration of pyranine.

Experimental Part

κ-carrageenan (Sigma) and pyranine were used for gel preparation by dissolving them in hot distilled water (pH 6.5) with KCl and NaCl solution. Pyranine concentration was taken as 4×10^{-4} M for all samples. Mainly two different set of experiments were carried out: The first set samples were prepared with constant carrageenan content (2 wt.%) in various KCl contents. These samples were named as C2K0 (no KCl), C2K02 (0.2 wt.%), C2K04 (0.4 wt.%) and C2K08 (0.8 wt.%). The second set samples were prepared with various NaCl contents from 0.4 to 1.0 (wt.%). These samples were named as C2Na04, C2Na06, C2Na08 and C2Na1, respectively. The compositions and symbols of the studied gels with various KCl and NaCl solutions are listed in Table 1 and 2.

Table 1.
The compositions, symbols, sol-gel and gel-sol transition temperatures and critical exponents for the gels in various KCl solutions.

Gel	KCl content (wt. %)	Sol – gel transition			Gel – sol transition		
		T_{sg}(°C)	β	γ	T_{gs}(°C)	β	γ
C2K0	0	39.1	1.01	0.76	55.6	0.95	0.55
C2K02	0.2	43.9	0.93	0.60	60.7	0.93	0.54
C2K04	0.4	49.7	0.85	0.64	65.9	1.03	0.65
C2K08	0.8	60.9	1.01	0.31	76.2	0.93	0.20

Table 2.
The compositions, symbols, sol-gel and gel-sol transition temperatures and critical exponents for the gels in various NaCl solutions.

Gel	NaCl content (wt. %)	Sol – gel transition			Gel – sol transition		
		T_{sg}(°C)	β	γ	T_{gs}(°C)	β	γ
C2Na04	0.4	37.9	0.75	0.81	54.5	0.95	0.79
C2Na06	0.6	40.1	0.92	0.85	58.5	0.98	0.82
C2Na08	0.8	42.0	1.13	1.27	59.0	1.07	1.05
C2Na1	1.0	44.6	0.91	1.19	62.3	0.95	0.88

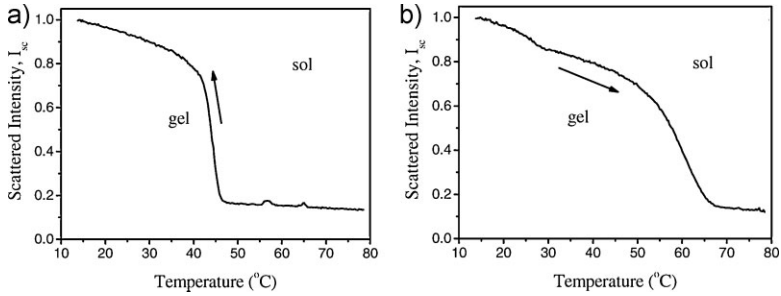

Figure 1.
Temperature variation of scattered ligt intensities for C2K02 sample during (a) sol-gel transition and (b) gel-sol transition.

The fluorescence intensity measurements were carried out using a Perkin Elmer spectrometer Model LS-50, equipped with temperature control. The carrageenan sol at 80 °C was transferred into the glass cell and left to cool to room temperature. All measurements were made at the front face position; slit widths were kept at 5 nm. *P* was excited at 360 nm and emission was detected at 515 nm in *in situ* experiments. Variations in the scattered and fluorescence emission intensity of pyranine were monitored as a function of temperature.

Results and Discussion

Figure 1a and 1b show the temperature variation of scattered light intensity, I_{sc} for C2K02 gel in the sol-gel and gel-sol transition, respectively. It can be seen that I_{sc} increased dramatically upon cooling the carrageenan samples in both cases indicating that the gel turbidity increased considerably.

Fluorescence intensity, *I*, shown in Figure 2a and 2b was also measured. It increased continuously upon cooling predicting that pyranine molecules are trapped in the gelling environment which prevents its quenching. However, I_{sc} decreased and *I* increased upon heating the carrageenan gels.

It can be postulated that during gelation (cooling process) helices and double helices are formed through the association of carrageenan molecules. Then they aggregate to higher-order assemblies and create an infinite network. On reheating, the initial double helix aggregates are destroyed and then the double helices are decomposed to carrageenan molecules, which results in the destruction of the gel

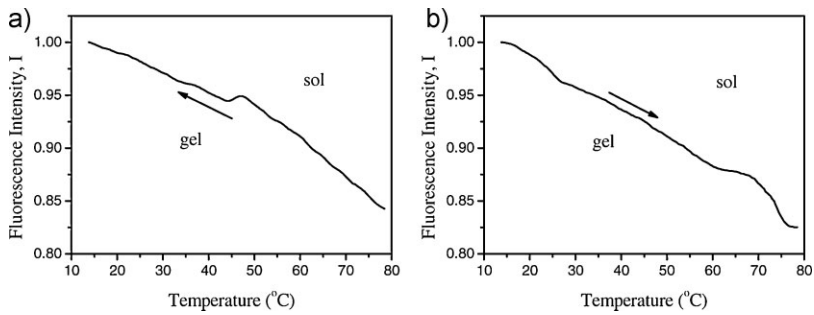

Figure 2.
Temperature variation of fluorescence intensities for C2K02 sample in (a) sol-gel transition and (b) gel-sol transition.

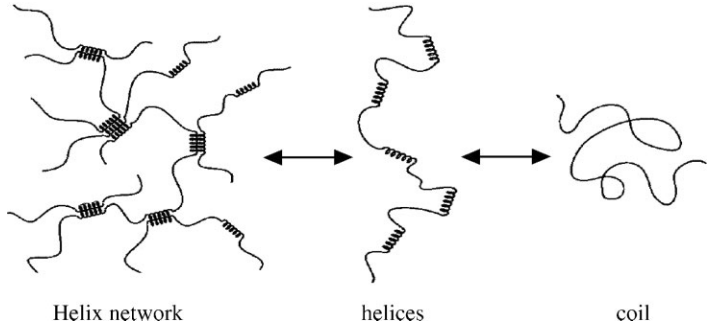

Figure 3.
Sol-gel and gel-sol transition and the structure of the helix network.

structure. A representation of the above process is shown in Figure 3.

In order to elaborate the above results, intensity I has to be corrected dividing it by transmitted light intensity. Since the shape of the excitation light has the character of I_{tr}, the observed fluorescence intensity is the convolution of I_{tr} with the desired fluorescence intensity, I_c. Figure 4a and 4b present the corrected fluorescence intensities, $I_{c,}$ for C2K02 sample in the sol-gel and gel-sol transition, respectively.

The peak positions of the first derivatives (dI_c/dT) of these sigmoidal curves can produce the sol-gel and gel-sol transition temperatures (T_{sg} and T_{gs}), see Figure 5.

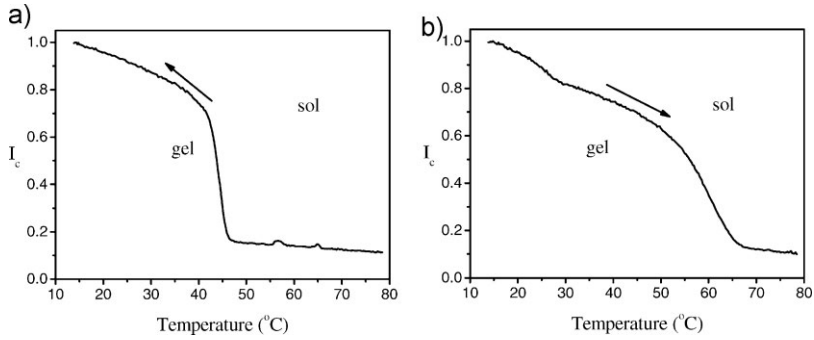

Figure 4.
Temperature variation of corrected fluorescence intensities for C2K02 sample in (a) sol-gel transition and (b) gel-sol transition.

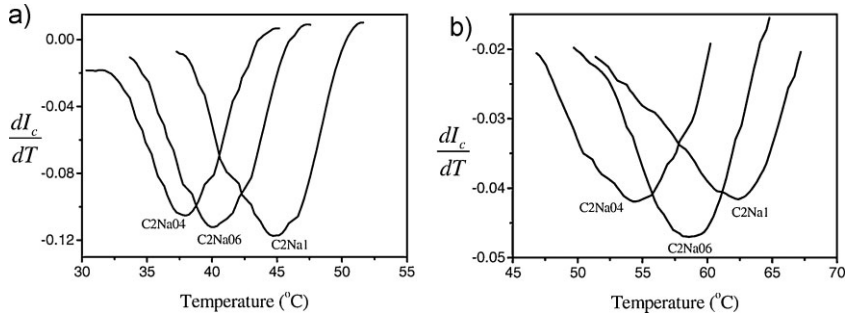

Figure 5.
The first derivative of I_c curves, dI_c/dT versus temperature, T for (a) sol-gel transition and (b) gel-sol transition.

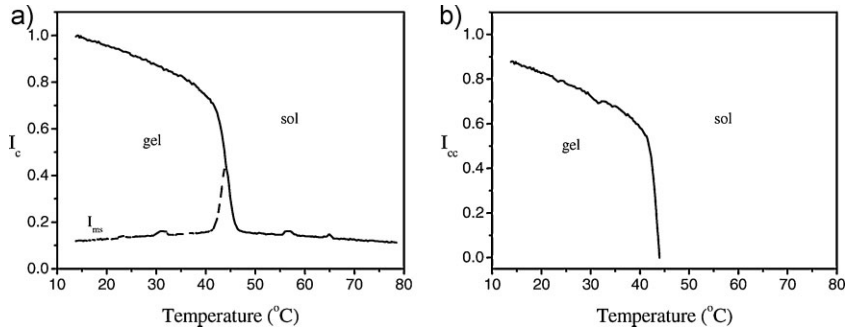

Figure 6.
(a) Subtraction of the mirror symmetry of I_c above T_{sg} from the curve below T_{sg}, (b) The resulting I_{cc} curve represents the pyranines embedded solely in the growing gel fraction.

These temperatures are listed in Table 1 and 2 for all gel samples. It can be observed that T_{sg} values are much lower than T_{gs} values for all carrageenan samples. In the case of cation content, both T_{sg} and T_{gs} values are higher than the gel containing no external cation. Thus, in the presence of cations, stronger gels can be formed, which require higher temperatures due to the cation attraction.

The gelation theory often makes the assumption that the conversion factor p determines the behavior of the gelation process, in the mean time p may depend on temperature. Therefore, above the gel point i.e. for $T > T_{sg}$ the fluorescence intensity, I_c measures the weight average degree of polymerization (or average cluster size) in the sol-gel transition path. However for $T < T_{sg}$ the intensity, I_c measures the gel fraction G, the fraction of the monomers that belong to the macroscopic network. Then Equation 1 and 2 can be written in the following form

$$I_{cc} = (I_c - I_{ms}) \propto G \propto (T_{sg} - T)^{\beta}, \quad T \to T_{sg}^{-} \tag{7}$$

$$I_c \propto DP_w \propto (T - T_{sg})^{-\gamma}, \; T \to T_{sg}^{+} \tag{8}$$

In Equation 7 I_{cc} solely originates from the pyranines embedded in the growing gel fraction, G i.e. I_{cc} is produced by subtracting the mirror symmetry (I_{ms}) of I_c intensity belonging to pyranines embedded in the small clusters from the total I_c intensity. Figure 6a and 6b illustrate the above procedure. Here the mirror symmetry I_{ms} of the I_c curve above T_{sg} is subtracted from the curve below T_{sg} as shown in Figure 6a. The resulting curve is given in Figure 6b.

The double logarithmic plots of the data are presented in Figure 7a and 7b for C2K02 sample. The critical exponents β and γ were produced by fitting the data to the double logarithmic form of Equation 7 and 8. They are listed in Table 1 and 2.

It is seen that the average γ and β values are very close to the values of classical Flory – Stockmayer model. From that one may conclude that I_c measures the average coil (small cluster) size above T_{sg} in sol – gel transition paths. I_{cc} detects helices and double helices (gel fraction) during sol – gel transition paths below T_{sg}. Since the formation of gel from the helices and double helices should obey the classical Bethe lattice, the connection of helices and double helices must be in the Cayley tree form.

Moreover, the critical exponents during gel-sol transition upon heating the gels can also be calculated. The gel-sol transition temperatures (T_{gs}) were determined by following the similar treatment used to obtain T_{gs} temperatures. The critical exponents β and γ were produced by fitting the data to the double logarithmic form of Equation 7 and 8 as shown in Figure 8a and 8b for C2Na1 sample. It should be noted that the values of the critical exponents for

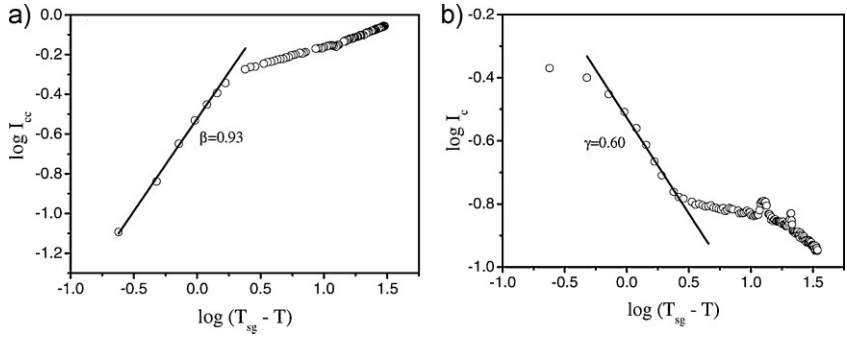

Figure 7.
log-log plots of the data for C2K02 sample and their fits to Equation 7 and 8.

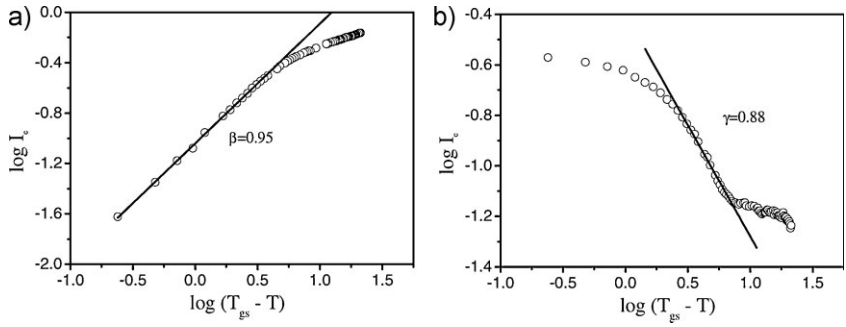

Figure 8.
log-log plots of the data for C2Na1 sample and their fits to Equation 7 and 8.

the sol-gel transition are in agreement with those for gel-sol transitions. All results are presented in Table 1 and 2.

Now one can compare these results with the results produced in similar systems. The sol-gel transition of the polysaccharide gellan gum has been investigated by the dynamic and static light scattering techniques.[19] The critical exponent, γ and ν corresponding to the cluster mass and correlation length were measured and found to be 1.66 and 0.88, respectively, which are in good agreement with the lattice percolation model. The critical exponents of the elastic modulus, t and correlation length, γ were measured near the sol gel transition temperature of agarose gel using a combination of rheolograph, differential scanning calorimetry and circular dichroism spectroscopy techniques; these were found to be 0.8 and 1.87, respectively.[20]

Conclusion

In summary, this paper has shown that the measured weight-average degree of polymerization exponents, γ and gel fraction exponents, β obeyed the classical Flory – Stockmayer model during the thermal phase transitions of carrageenan in various salts. It is important to note that all carrageenan systems belong to the same universality class, independent of the salt solution type. The sol-gel and gel-sol transition temperatures were determined, T_{sg} values being much lower than T_{gs} values.

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