Effects of salts on the sol-gel transition and gel network structure

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EFFECTS OF SALTS ON THE SOL-GEL TRANSITION AND GEL NETWORK STRUCTURE

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2005

Effects of Salts on the Sol-Gel Transition and Gel Network Structure

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Abstract

In this study, the effects of various salt-out salts, salt-in salts and salt mixtures on the sol-gel transition of aqueous methylcellulose (MC) solutions have been studied systematically by means of micro differential scanning calorimetry (micro DSC), rheometry and UV/Vis spectroscopy.

It was found that the heating process was endothermic resulting in gelation while the cooling (when degelation occurred) was exothermic for both MC solutions with and without salts. The addition of salts did not change the patterns of gelation and degelation behaviors of MC. However, the salts could shift the sol-gel transition and the gel-sol transition to lower or higher temperatures from a salt-free MC solution, depending on the salt type and salt concentration. In addition, each salt was found to have its own concentration limit for producing a stable aqueous solution of MC at a given concentration of MC, which was able to relate to the anionic charge density of the salt. The “salt-out strength”, defined as the salt effect per mol anion, was obtained for each anion studied. Cations were proved to have weaker effects than anions. The thermodynamic mechanisms involved in the salt-out and salt-in gelation behaviors were discussed.

The salt mixture was found to have a combined effect from the salt-out and salt-in salts in the mixture, and the salt effect was dependent on the water hydration abilities of the component ions and ion concentration. The sol-gel transition temperature nicely followed a linear rule of mixing.

Rheological measurements were conducted for MC solutions with the typical salt-out and salt-in salts. Upon heating, the gel network structure was formed as evidenced by the growth of the elastic modulus and the appearance of a rubbery plateau. The gel network became stronger with adding of a salt. The salt effects were in good agreement with thermodynamic results. The curves' continuity and pattern indicated that the viscoelastic behaviors involved in all solutions were similar.

Turbidity of MC solutions with or without salt was measured by UV/Vis spectroscopy during a thermal cycle. The clouding behavior gave another supportive evidence for the MC sol-gel transition, as well as the salt effects on it, and the mechanisms proposed can be further understood from optical point of view.

The morphological image of MC gels was characterized by SEM after freeze-drying. Fibrous and mesh-like structure was observed. The technique used to obtain the gel morphology was discussed and proved to be an effective tool to study gel network structure.

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Chapter 1 Introduction

1.1 Overview

Salt effects on aqueous solution systems have been extensively studied in recent years and different mechanisms were proposed based on the systems studied. Most of the studies about this topic focus on the “Hofmeister series” [Hofmeister, 1888], an order of ions ranked in terms of how strongly they affect the hydrophobicity of the solution. It plays a significant role in a wide range of biological and physicochemical phenomena, such as the cloud point of polymer and surfactant solutions, the action of ions in ion-channels of biological membranes, and the surface tension of electrolyte solutions. [Erbil and Sarac, 2002; Amiya and Tanaka, 1987]

According to the theory of Hofmeister Series, ions can be classified as either kosmotropes (structure-makers) or chaotropes (structure-breakers), referring to the ability of an ion to stabilize or weaken (or destroy) the water structure, respectively. In the typical Hofmeister order $\text{SO}_4^{2-} > \text{F}^- > \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \text{I}^- > \text{SCN}^-$, ions on the left, called kosmotropes, are proved to increase hydrophobicity in an aqueous solution system (or called salt-out effect), whereas ions on the right tend to make the solution more hydrophilic (salt-in effect correspondingly). Since anions are found to have much stronger effects than cations, the anion type tends to determine whether a salt behaves like salt-out or salt-in. The salt-out strength of different salts is mostly dependent on the charge density of the individual anion. With regard to a salt mixture, its effect is expected to be a combination from individual anions, which deliver the salt-out or salt-in effect independently.

Hydrophobically modified cellulose derivatives like methylcellulose (MC) undergo the sol-gel transition in water upon heating. The gelation is due to the hydrophobic association of hydrophobic groups or segments when water becomes a poorer solvent for them at higher temperatures. MC is one of typical cellulose derivatives that can form a thermoreversible hydrogel in water upon heating and dissolve while cooling.

Hydrogels are sensitive to different environmental conditions, such as temperature, solvent, co-existing solutes including ions, pH, electrical or magnetic field, etc. Among these conditions, effects of salts or ions on the sol-gel transition of hydrogels are of great interest and importance because salts are commonly present in many systems such as biological systems. Recent technical advances have led to the development of micro-calorimeters such as isothermal titration calorimeters (ITC) and micro differential scanning calorimeters (DSC), which can detect small amounts of heat (a few micro-calories) absorbed or released under an isothermal titration condition or during a well-controlled heating or cooling process (micro DSC). The thermodynamic results obtained from DSC will aid the understanding of the salt effects on hydrophobic association/dissociation and the kinetic factors affecting the formation of junctions for gel network structure.

As the most direct and convincing way to study gelation, rheometry is employed to monitor the mechanical properties during the formation of a three-dimensional gel network corresponding to the growth of clusters or aggregates due to the change of temperatures or solvent systems. The dynamic viscoelastic properties such as storage modulus G' and loss modulus G'' on heating and cooling are measured, which are related to the gel developing process, such as the gelling point, weak gel structure and

matured gel network. The presence of salts may affect the viscoelastic performance of the solution, the sol-gel transition temperature, and eventually the mechanical strength of the gel. The viscoelastic properties can be correlated with the thermal properties obtained from the microthermal analysis to provide a better understanding of salt effects on the gelation and degelation.

Further more; UV/Vis is employed as supporting evidence to observe the clouding behavior during the gelation and degelation processes. The critical transition temperature, defined as clouding point during heating, is obtained and proved to be correlated with the sol-gel transition temperature defined through previous studies on thermal and viscoelastic behaviors. Again, the clouding points for MC solutions with salt-out salts, salt-in salts and salt mixtures show the similar changing trend as a function of salt concentration when compared with calorimetric and rheometric measurements. The salt effect mechanisms proposed before can be used to explain the turbidity behavior observed.

Finally, Freeze-Drying (FD) and Scanning Electron Microscopy (SEM) have been developed as image processing techniques to analyze the gel physical network morphology of MC hydrogels formed by heating MC solution above the lower critical solution temperature (LCST). FD is an important method used to prepare a dried gel representing the real network structure in the wet state. Water content, water expelling rate from the gel and drying condition (on the plate or in the cylinder) are critical factors to determine the pore size and fiber structures after the drying process. Therefore, in this research, the microscopic characterization, cross-link density, composition and homogeneity of MC hydrogels are investigated at different polymer

concentrations and drying conditions, and the image analysis is conducted through SEM observation.

1.2 Scope and Objectives

In this work salts are added into a MC solution to study their influence on the sol-gel transition and network structure, and the corresponding mechanisms. Micro DSC is employed to determine the thermodynamic properties of MC solutions with or without salts during the hydrophobic association of MC upon heating or the dissociation of MC upon cooling. Rheometry is used to determine the viscoelastic properties during heating and cooling. UV/Vis is used to observe the change in turbidity during the thermal process. The correlation between the thermal, rheological and clouding behavior will be investigated.

Various salts including salts from two categories (salt-out and salt-in) with different concentrations, salt mixtures consisting of a salt-out salt and a salt-in salt are added into MC solutions to study their effects on gelation, such as critical transition temperature, gel strength etc. We aim to develop a better understanding of salt-accelerated and salt-delayed sol-gel transition behaviors of MC solutions from a molecular origin.

As part of the effort to study the gel physical network structure, SEM is employed to characterize the microscopic image of cryogel prepared by freeze-drying. The purpose of this research is to figure out the true three-dimensional network structure of MC hydrogels, and to demonstrate the relationship between water content and the pore

size of gels. We hope to develop a new method for the study of morphological structure in other gel systems.

1.3 Outline of the Dissertation

There are totally eight chapters in this report. Literature Review and Experimental section are presented in Chapters 2 and 3 respectively. The results and discussion are delivered in the following four chapters. They focus on effects of salts on thermodynamic, rheological and turbidity behavior, and morphology and image study respectively. The salt effects on the sol-gel transition and gel network structure and the corresponding mechanisms are discussed systematically in this report. Chapter 8 summarizes this project and provides some recommendations for future work.

Chapter 2 Literature Review

This chapter consists of three main parts. In the first part, the salt effects on the water structure are discussed from the molecular level, which provides the background for salt effects on the sol-gel transition of MC in aqueous solutions studied in the later part. The second part focuses on the formation of hydrogels, especially the thermo-sensitive and thermoreversible physical gels formed from MC aqueous solutions, and the gelation mechanism. The third is about the techniques used in this work to study gels by characterizing the sol-gel transition in terms of thermodynamic, rheological properties, as well as turbidity etc. The morphology study shows direct images of a network microstructure, which is another effective tool to investigate gel and gel network.

2.1 Salt Effects on Water Structure

2.1.1 Hydrogen Bonding in Water

Hydrogen bonding is a strong dipole-dipole interaction that occurs between hydrogen atom bonded to small strongly electronegative atom (O, N, or F) and other such electronegative atoms. Pauling [1939] used the term “hydrogen bond” for the first time in 1939. In the format of $X - H \cdots A$, where X and A represent electronegative atoms, he assumed that only if X and A were very electronegative, would the shielding of H and in turn the electrostatic attraction between H and A be sufficiently high to term the interaction as a hydrogen bond. Table 2.1 has therefore compared electronegativities of some elements, implying that, to form a hydrogen bond, X and A would be restricted to

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halide elements (F, Cl, Br and I), O and N. In water the hydrogen atom is covalently attached to the oxygen atom of a water molecule (covalent bond, about 492 KJ mol^{-1} [Ruscic et al., 20021). The hydrogen atom has an additional attraction (i.e. the hydrogen bond, about 23.3 KJ mol^{-1}) to a neighboring oxygen atom of another water molecule, which is far greater than a van der Waals interaction (about 1.3 KJ mol^{-1}). This hydrogen bonding between water molecules is figured as $\text{HO} - \text{H} \cdots \text{OH}_2$, in which both X and A are O. When A is replaced by other electronegative elements, the strength of hydrogen bond will depend on the electronegativity of A. A higher electronegativity results in a stronger hydrogen bond. It is obvious that among all elements, F can build the strongest hydrogen bond with water molecules.

Table 2.1: Electronegativity of elements. It is quoted from Desiraju, G. R.; Steiner, T. [2001]. *The WeakHydrogen Bond*, Oxford University Press, p 3. The original data were given by Pauling L. [1939]

H 2.1				
Li 1.0	C 2.5	N 3.0	O 3.5	F 4.0
Na 0.9	Si 1.8	P 2.1	S 2.5	Cl 3.0
K 0.8	Ge 1.8	As 2.0	Se 2.4	Br 2.8
Rb 0.8	Sn 1.8	Sb 1.9	Te 2.1	I 2.5

Other discussions on the strength of hydrogen bonding focus on the bond length and angle. Generally speaking, the bond angle has less contribution than bond length [Rao, 1972], and the dependency of bond strength on bond length is an exponential decay with distance [Espinosa et al., 1998]. Some researchers consider that the hydrogen bond will

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be broken if the bond length is greater than 3.1 Å [Khan, 2000]. There is a trade-off between the covalent and hydrogen bonding strengths: in a typical hydrogen-bonded chain $\text{O} - \text{H} \cdots \text{O} - \text{H} \cdots \text{O}$, a stronger $\text{H} \cdots \text{O}$ bond results in a weaker $\text{O}-\text{H}$ covalent bond and a shorter $\text{O} \cdots \text{O}$ distance. The weakening of the $\text{O} - \text{H}$ covalent bond gives rise to a good indicator of hydrogen bonding energy. Therefore, when discussing the strength of hydrogen bonding, one has to not only consider the electronegativity of a given atom, but also the covalent bond of cooperative hydrogen, the intermolecular distance, and the orientation and positions of the other bonded and non-bonded atoms, etc.

The hydrogen bonding patterns are random in liquid water, but the hydrogen bonded water molecules may be cooperative as a tetrahedral network, meaning that one water molecule is an acceptor for one hydrogen bond, and a donor for the other. Water molecules surrounded by hydrogen bonds tend to clump together, forming stable clusters for both statistical and energetic reasons [Stanley and Teixeira, 1980]. In salt solutions, however, the chain-balance can be broken. Strongly hydrated ions considerably increase the difference between the hydrogen bond donating and accepting capacity of the linked water molecules, resulting in the breakdown of the tetrahedral network. Cations, for instance, may induce strong cooperative hydrogen-bonding around them due to the polarization of water $\text{O} - \text{H}$ by cation-lone pair interactions ($\text{Cation}^+ \cdots \text{O}-\text{H} \cdots \text{O}-\text{H}$) [Luck et al., 1997]. Total hydrogen bonding around ions may be disrupted as if the electron pair acceptance increases in water around cations so the electron pair donating capability of these water molecules is reduced. In the case of anions, opposite effects happen, i.e. the electron pair donating ability increases while the accepting capability decreases in water

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around anions. These changes in the relative hydration ability of salt solutions are responsible for the so-called salt-out and salt-in behaviors of hydrophilic polymer gels [Muta et al., 2003]. Figure 2.1 illustrates the influence of cation and anion on water hydrogen bonding chains.

Figure 2.1: Interactions of anion and cation with water molecules.

Anions hydrate more strongly than cations for the same ionic radius because water hydrogen atoms can approach to anions more closely than the water oxygen atoms to cations, giving rise to a greater electrostatic potential in the interaction between anion and water. Also, breaking of such hydrogen bonds is relatively slow due to the difficulty in finding a new hydrogen-bonding partner [Kropnam and Bakker, 2001].

2.1.2 The Hofmeister Series

The Hofmeister series [Hofmeister, 1888], is originated from the ranking of various ions toward their ability to precipitate a mixture of hen egg white proteins.

Anions: $\text{SCN}^- > \text{NO}_3^- > \text{Cl}^- > \text{citrate} > \text{acetate}^- > \text{SO}_4^{2-}$

Cations: $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{Na}^+ = \text{K}^+ > \text{NH}_4^+ > \text{N}(\text{CH}_3)_4^+$

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They have been shown to have a much more general utility including the structuring or denaturation of biological macromolecules. For example, the Hofmeister series are usually adopted to compare the ability of ions to stabilize the structure of proteins. However, the relative positions (mostly corresponding to the degree of strong hydration as shown below) in the series should be used as only an indicative because they may vary with types of proteins, pH, temperature, etc. The relative order of cations may reverse with different anions under some circumstances due to ion pair effects as shown in the following figure.

Figure 2.2: Ability to stabilize proteins and degree of hydration in Hofmeister Series. Ions on the left show great ability to stabilize proteins, while ions on the right destabilize proteins.

Ions may destroy a natural hydrogen-bonded network of water, showing effects similar to increasing temperature or pressure. Ions, which exhibit weaker interactions with water than water itself (or weakly hydrated), are known as structure-breakers or chaotropes, whereas ions having the opposite effect are known as structure-makers or kosmotropes, i.e. exhibiting strong interactions with water molecules. “Salt-out” and “Salt-in” are alternative terms used for “kosmotrope” and “chaotrope” respectively, referring to the correlative property of reducing or increasing solubility of a solute in water. The former

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tends to decrease the solubility of a solute in water, while the latter can increase the solubility of non-polar solutes.

Leontidis [2002] in his paper correlated the kosmotropic and chaotropic effects observed with the ionic properties such as ionic radius, charge density, free energy and entropy of hydration, as well as polarizability, etc. In his opinion, kosmotropic ions are usually small with relatively high charge density. They bind larger water clusters more strongly but lose their water of hydration with a great difficulty. On the other hand, chaotropic ions are large with low charge density and a loose hydration sheath, and may serve themselves as solvating species in the solution.

Hribar [2002] also pointed out that smaller ions were bound more closely to water molecules than larger ions. Small ions possessing high charge density are strongly hydrated, with small or negative entropies of hydration, creating some local order and higher local density. They bind larger water clusters more strongly. On the other hand, large single charged ions such as I^- or Cs^+ , with a smaller charge density, bind water loosely and therefore cause less restriction of water conformation. Such large ions are able to sit comfortably within a water network structure, acting like hydrophobic molecules, and being pushed on by strong water-water interactions. Their interactions with other atoms are not only dependant on charge but also on van der Waals forces. Recently, Ninfam [1997] also concluded that dispersion forces are involved in most Hofmeister effects reported. The dispersion force, which is defined (together with dipole-dipole force and hydrogen bonding force) as one of the attractive forces between neutral

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molecules, is grouped together with the dipole-dipole force and termed as van der Waals force. The existence of dispersion force between the large ions and surrounding water molecules shows that the interaction in between is much weaker than that of small ions.

2.1.3 Salt Effects on Aqueous Solutions

Salt effects on solution systems have been extensively studied in recent years, especially on non-ionic surfactants and polymer solutions. Different mechanisms were proposed based on the compounds studied and measurements conducted.

It is found that the solubility of a non-ionic solute containing ether groups decreases with increasing temperature or with addition of appropriate salts [Karlstrom, et al. 1990]. Changing of cloud points can be observed as a result. Some researchers attribute it to dehydration. Karlstrom [1990] concluded that salts with kosmotropic anions tended to compete for water with the ether groups of PEO-based surfactants, thus leading to lower cloud points. This effect has been documented for several years in the study of PEO and non-ionic cellulose ether (EHEC) solutions which contain the structural entity $-\text{CH}_2-\text{OCH}_2\text{CH}_2\text{O}-\text{CH}_2-$ [Karlstrom, et al., 1990 and Weckstrom, 1985]. Depression of cloud points for these solutions follows the expected Hofmeister sequence, while large chaotropic ions may actually induce the increasing of cloud point temperature. Alexandridis and Holzwarth [1997] also reported that the cloud points of PEO-PPO-PEO amphiphilic copolymer systems were affected by Hofmeister anions in the same way as that of other non-ionic systems. This phenomenon is again observed in methylcellulose (MC) aqueous system with addition of various salts as studied in this project.

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Kabalnov et al. [1995] used salt adsorption/depletion to explain the trends of depressing cloud points in his investigation on oil-water-surfactant systems. He proposed that the salt effects were due to the salt adsorption/depletion in the bicontinuous micro-emulsion phase. The salt-out salts are shown to desorb water, leading to dehydration of the monolayer, whereas the salt-in salts to absorb at the interfacial monolayer. He even measured the adsorption of salts in the non-ionic surfactant monolayers of bicontinuous microemulsions.

Another mechanism proposed by Muta et al. [2003] involves the electron-pair acceptance/donation (EPA/EPD) ability of water. The hydration of an ion changes the charge density on the hydrogen and oxygen atoms of water, resulting in the change of EPA/EPD ability in water, and then causes the stabilization or destabilization of the solution system. His explanation is constructive, since for physical hydrogels hydrogen bonding is considered as one of the main forces for the establishment or destruction of a solubilisation balance.

Collins [1997], on the other hand, presented that the ion effects on water structure are caused by a competition between ion-water interaction and water-water interaction. The former is dominated by the charge density of ions, while the latter is by hydrogen bonds. Collins also explained that anions were stronger than cations at water structuring because of the asymmetry of charge in a water molecule. His mechanism would be useful for us to explain the salt effects on the thermal dynamic behavior of MC hydrogel.

Among the researches of salt effects on solution systems, there are few works found on studies of effects of salts on the sol-gel transition of MC aqueous solutions—the subject of our study. Hirrien et al. [1998] reported that the gelation shifted to lower temperature by adding some chloride salts into a MC solution. Kundu et al. [2001] also found that addition of salts like NaCl, $(\text{NH}_4)_2\text{SO}_4$ and Na_2CO_3 lowered the gelation temperatures of MC solutions due to the mechanism of dehydration. However, no reports have been found on studies of salt-in behavior of MC gelation. These are subjects to be conducted in this project.

2.2 Formation of Hydrogels and Mechanisms

As well known, it is difficult to give a precise definition of a gel. The general picture of a gel is that it is made of a solution or of a colloidal suspension which, though containing a considerable amount of solvent, shows the properties of a soft viscoelastic solid rather than a viscous liquid [Clark A. H., 1996].

Hydrogels, one important member of gels, are three-dimensional, hydrophilic and polymeric networks capable of imbibing large amounts of water. Different types of linear, long-chain polymers which can form hydrogels have been recognized over decades. Our study will focus on thermo-sensitive and reversible hydrogels prepared from aqueous polymer solutions.

2.2.1 Physical Hydrogels

2.2.1.1 General Observation of Physical Hydrogels

Physical gelation for hydrogels, which is associated with phase transition, usually takes place in response to infinitesimal changes in temperature, solvent composition, pH, ionic composition or small electric fields [Amiya and Tanaka, 1987]. Upon the changes of environmental conditions, an aqueous polymer solution (sol state) is transformed into a gel, a uniform non-flowing viscoelastic solid on an experimental time scale, while maintaining the integrity [Jeong et al., 2002]. The transition of polymer solutions into gels (gelation) is considered as a result of phase transition by some researchers. According to Yuris [1988], gelation of a polymer solution can be characterized as a process involving a continuous but dramatic increase in viscosity accompanied by a gradual enhancement of elastic properties. Gelation results in “freezing” of the whole system, causing the formation of a polymer structural network which encompasses the whole bulk of the system and retains the solvent.

Influence of temperature

For a thermo-sensitive polymer solution with a “lower critical solution temperature” (LCST), the phase separation happens when the solution temperature is raised to the LCST [Shalaby, 1991]. By heating the solution to above LCST, a thermoreversible gel is formed as a result of phase separation and the establishment of the physical network structure.

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Lipatov [1948] suggested that upon changing of temperature the interactions among polymer groups lead to the formation of intermolecular links in polymer solutions, so that the solubility of polymer in water becomes poorer. The specific influence of polymer solubility on the ability of the polymer to gel is illustrated by the fact that gelation can be observed during either a temperature increasing or decreasing process, depending on the LCST or UCST (upper critical solution temperature).

Critical concentration

Besides LCST or UCST, another important parameter is the critical concentration, at which the sol-gel transition takes place for a given polymer-solvent system. The existence of this critical concentration for gelation is associated with the critical number of inter-molecular bonds for percolation [Yuris, 1998]. Hence the critical concentration, at which gelation begins, corresponds to the beginning of the preferential formation of intermolecular bonds with decreasing solubility.

Effects of additives

Gelation in the same way as a structure formation may be caused by the introduction of additives into polymer solutions due to the decrease of polymer solubility in a given solvent [Yuris, 1988]. The external components may cause either chemical reaction or physical change, resulting in the formation of intermolecular bonds between the polymer molecules. More often, the solubilizing ability of the solvent can easily be reduced by small amounts of additives leading to gelation.

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Influence of chain flexibility

Finally, one should note gelation may depend on the flexibility of macromolecules. The conformation of a polymer chain in solution is affected by the flexibility of the chain and its interaction with the solvent. For flexible molecules, viscosity of a dilute solution in a poor solvent will be lower than that in a good solvent [Tager, 1958]. For rigid molecules, the viscosity of a polymer solution in a good solvent will be lower than that of a poor solvent. It is mainly because that the critical concentration for gelation for rigid polymers should be lower than for flexible polymers [Clark, 1996]. Thus, the structure of the macromolecule determines its ability to form a gel.

Above all, gelation occurs when the polymer has a limited solubility in the solvent. Hence all factors determining the polymer solubility tend to affect the process of gelation. Gelation is the formation of a continuous three-dimensional network of macromolecules, while the precipitation should not occur. The introduction of additives may promote the formation of intermolecular bonds to assist gel formation. Also the ability for a polymer to form a gel is closely linked with the chain structure of polymer molecules.

2.2.1.2 Mechanisms of Physical Gelation

Among the recent studies on the properties of thermoreversible hydrogels, different mechanisms were proposed on the basis of polymers from which gels were formed.

Two-stage transition

It is widely accepted that the gel formation process or transition from a solution to a gel can be divided into two stages. The first stage is characterized by an appreciable increase

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in the viscosity of the system, but in general the system retains the inherent properties of liquid solutions. The second stage begins from the moment of the emergence of elasticity, i.e. the beginning of the emergence of the properties of a solid. Subsequently the elasticity gradually increases and reaches a limiting value determined by the gel structure and the gel formation conditions. Gel formation can be characterized by the subsequent formation of a continuous three-dimensional structure at the end of the second stage.

The physical transition from a solution to a gel can be considered by analogy with a process of the cross-linking of linear macromolecules. Flory and Stockmayer [1953] proposed that during macromolecular inter-cross-linking, the viscosity increases gradually and then the connection of all molecules into a continuous three-dimensional structure occurs at or beyond the gel-point. This moment corresponds to the emergence of elastic properties. The position of the gel-point is determined by the presence of a critical number of intermolecular bonds for the formation of the three-dimensional structure. Beyond the gel-point, a further increase in the number of intermolecular bonds in the system takes place, leading to strengthening of the network. This Flory-Stockmayer theory can be used for description of gel-formation processes in the solutions.

Aggregates and hydrogen bonding

It is obvious that during the gel formation from dilute solutions, the appearance of stable clusters or molecular aggregates is necessary. Their existence is corroborated by determining the turbidity of the solution during gelation. Light-scattering measurement is also an effective way which shows that the size of aggregates and number of molecules in

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the aggregates increase continuously with increasing solution concentration. These aggregates do not disintegrate with gentle heating of the solution. The change in the size and number of molecular aggregates levels off much earlier than the appearance of the gel network as evidenced by light-scattering. This provides a basis for the conclusion that the formation of aggregates takes place in the initial stage of gel-formation, followed by the linking of aggregates into a continuous three-dimensional structure. The aggregates that appear at high dilution are stable.

The hydrogen bonds between gelatin molecules are considered as one of the main causes for the formation of aggregates and the chains are united by hydrogen bonds into a network [Yuris, 1988]. On the basis of this concept one may conclude that gelation lies in the formation of strong intermolecular bonds of both localized and non-localized chains. In this instance the interaction of individual molecular chains rather than existing ones in a concentrated solution of molecular aggregates, plays a significant role in the formation of a three-dimensional network.

Phase separation

Another important explanation proposed is the phenomenon of phase separation during the sol-gel transition. Tanigami [1989], for example, concluded from his study on poly (organophosphazene), that the phase separation in the aqueous system is attributed to the balance of the hydrophilic and hydrophobic groups in the polymer and the gelation is noted to occur by hydrogen bonding and the side groups. Such phase separation is also an entropy-driven process, where the release of structured bound-water from the

hydrophobic groups along the polymer's main chain is the major contributing thermodynamic force.

2.2.2 Hydrogels from MC Aqueous Solutions

2.2.2.1 Methyl Cellulose (MC)

Natural cellulose is made of repeat units of monomer glucose, as shown in Figure 2.3 for its chemical formula, where three Rs are all H [Yoshihito et al., 2001]. Cellulose is insoluble in water. It forms crystals where strong intra-molecular and intra-strand hydrogen bonds hold the network flat. This tendency to form crystals utilizing extensive intra- and inter-molecular hydrogen bonding makes it completely insoluble in normal aqueous solutions.

Figure 2.3: Chemical Formula of Cellulose and Methylcellulose.

Figure 2.4 displays the molecular chain of cellulose with hydrogen bonds. It is thought that water molecules promote the formation of the natural cellulose crystals by helping to align the chains through hydrogen-bonded bridging.

Figure 2.4: Intra- and Inter-molecular Hydrogen Bonds of Cellulose Chain.

When a certain fraction of hydroxyl groups is substituted by hydrophobic groups such as methyl groups (some Rs in Figure 2.3 are represented by CH_3), intermolecular hydrogen bonds are cancelled to result in water soluble cellulose [Guenet, 1992]. This modification is expressed as the degree of substitution (DS), which is defined by the number of methoxyl groups divided by the number of glucose units [Kabayashi et al., 1999]. The resultant derivatives are called hydrophobically modified cellulose or water-soluble cellulose. However, a complete substitution of all hydroxyl groups in cellulose with hydrophobic groups (ie. DS is 3) makes resultant cellulose derivatives water-insoluble again. An optimum level of substitution is required for appropriate water solubility, which is usually between 1.4 and 2.0. In water, there exist three possible intermolecular interactions among cellulose chains or between cellulose and water: first, hydrogen bonding between unmodified hydroxyl groups on cellulose chains; second, hydrogen bonding between the hydroxyl groups of cellulose and water molecules; third,

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hydrophobic association between hydrophobic groups (i.e. methyl groups for MC) on cellulose chains which are introduced in the hydrophobic modification process.

Commercial methylcellulose is produced when cellulose is exposed to aqueous sodium hydroxide and methyl chloride under mechanical mixing, where the methylation occurs more rapidly in NaOH-rich and/or higher temperature regions [Yoshihito et al., 2001].

2.2.2.2 Gelation from MC Aqueous Solutions

MC dissolves in water at low temperatures. However, when a MC aqueous solution is heated, it becomes turbid and finally a gel is formed at certain temperature. Such gels are typical thermo-sensitive and reversible hydrogels with lower critical solution temperature as discussed above.

According to Kabayashi et al. [1999], at low temperatures, water molecules are presumed to form “cage-like” structures to surround the hydrophobic methoxyl groups, causing the MC to become water-soluble. Upon heating, these structures distort and break, leading to the exposure of the hydrophobic regions, and inducing the formation of aggregates. When an optimum balance between the hydrophilic and hydrophobic moieties is reduced, it undergoes a sol-to-gel transition in water. As the strength of the hydrogen bonding or hydrophobic association is dependent on temperature, the formation of micelle structures or networks is also temperature-dependent. This sol-gel transition temperature is determined by the hydrophobic substitution of cellulose for the hydroxyl groups. Water becomes a poorer solvent with increasing temperature and polymer-polymer interactions

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become dominant at high temperatures, resulting in formation of a gel [Bekturov and Bimendina, 1981]. For MC with an appropriate DS, it is reported that thermal gelation can take place in the temperature range from about 50 to 70 °C.

The gelation of MC has been studied extensively. Overall, the experimental evidence favors a two-stage process, in which heating in the low temperature regime increases the inter-chain clustering due to hydrophobic association, and the actual gelation at higher temperature competes with liquid-liquid phase separation [Tanaka et al., 1995]. On the other hand, hydrophobically modified cellulose is thermoreversible in hydrophobic association. This means that the associative aggregates or junctions formed at elevated temperatures can return to a liquid state again upon cooling through the dissociation of hydrophobic groups. The formation of hydrogen bonding and/or hydrophobic association on heating or cooling may easily cause a cellulose solution to have a complicated microstructure that varies with environmental and processing conditions.

2.3 Techniques to Study Gelation of MC

Over the decades, many techniques have been developed to investigate the gel formation of MC, including small-angle neutron scattering (SANS) [Kabayashi et al., 1999], dynamic light-scattering (DLS) [Kabayashi et al., 1999], infrared (IR) and Raman spectroscopy, optical rotatory dispersion (ORD), X-ray diffraction (XRD), nuclear magnetic resonance (NMR) [Piculell and Nilsson, 1989; Zhang and Furo, 1993], ATR/FT-IR spectroscopy [Muta et al., 2002], UV/VIS [Bischel and Roggendorf, 2002; Zheng et al., 2004], rheometry [Kabayashi et al., 1999 and Li et al., 2002] and differential

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scanning calorimeter (DSC), etc. The most recently developed technique includes Scanning Electron Microscopy (SEM) [Jang and Park, 2002; Kim et al., 2003], Atomic Force Microscopy (AFM) [Kobiki and Suzuki, 1999] or Transmission Electron Microscopy (TEM) [Liu et al., 1996], which can be used to study morphology of gel network structures. The detailed introductions regarding four techniques employed in this study, i.e. DSC, rheology, Uv/vis and SEM, are given in the following sections.

2.3.1 DSC and Thermodynamic Properties

Calorimetry is a thermal detection method for a chemical or physical process where heat is either generated or absorbed. This method permits the study of molecular interactions and conformational energetics for many natural materials. Thus, DSC has been widely employed for determination of thermodynamic parameters associated with processes which are initiated by either an increase in temperature (up-scans) or a decrease in temperature (down-scans). The high sensitivity and accuracy of this measurement result in high quality thermodynamic data and detailed information at the molecular level. In such cases, it is possible to relate the molecular changes, which occur as a function of temperature, to the energetics of the system [Leharne, 1998]. Therefore, it is a precise instrument used to study a wide range of thermal transitions especially in biological systems [Cooper and Johnson, 1994].

The VP-DSC used in this research is an ultra-sensitive differential scanning micro-calorimeter that uses a cell feedback network to differentially measure heat produced or absorbed between the sample and reference cell which has effective volumes of

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approximately 0.5ml. The raw output conventionally shows power as a function of time. To extract data, which contain thermodynamic significance, the axes of the trace output are transformed to molar excess heat capacity (C_p) versus temperature. This is automatically conducted by the software system. The up-scan information provides great insight into the conformational energetics of polymers. By varying solvent conditions the effect of additives which either stabilize or destabilize polymers can also be evaluated. On the other hand, with the down scan mode, substantial information may be obtained about the reversibility of thermal processes. A suitable baseline is fitted to the data and subsequently subtracted. So that the enthalpy change (ΔH), considered as the total energy during the process may be calculated using the equation, which is also the integrated curve area:

$$\Delta H = \int_{T_1}^{T_2} C_p dT$$

At the same time, the entropy change (ΔS) can be directly calculated through:

$$\Delta S = \int_{T_1}^{T_2} (C_p / T) dT$$

Li et al. [2002] observed that upon heating of a MC aqueous solution, there is an endothermic peak. The peak temperature (T_m), which is defined as the sol-gel transition temperature, is independent of polymer concentration. However, the total energy defined by the endothermic peak area is found to be a linear function of polymer concentration. On the other hand, when sample is cooled subsequently, a broad exothermic peak appears, with its peak height and broadness increased with polymer concentration.

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Through the micro-DSC, thermo-dynamic properties of MC solutions with/without salts, such as heat capacity (C_p), peak temperature during gelation (T_m), enthalpy change (ΔH) and entropy change (ΔS), during the formation of gels can be detected and compared to work out with a reasonable mechanism fitting well to the thermal behaviors.

2.3.2 Rheology and Viscoelasticity

One of the essential features of gelation is the approach of an aggregating system to a critical “gel point” where a percolating network first comes into existence [Clark, 1996]. It is the temperature at which solid-like elastic characteristic starts to develop rapidly, and the sol-gel transition takes place. Since this is primarily a mechanical event, mechanical measurement is essential for gel study. Using a rheological method, viscoelasticity in this region can be examined, and the formation of a three-dimensional gel network can be rheologically monitored. Rheology of a gel is correlated to the network structure and cross-link density. The rheological method is the most direct and reliable way for determination of the sol-gel transition and characterization of mechanical properties of three-dimensional gels, while other techniques such as spectroscopic and scattering methods are unable to do.

Today, the preferred method for studying complete passage of a system through the gel point is dynamic mechanical spectroscopy [Clark, 1987 and Ferry, 1980]. In this technique the sample is enclosed between parallel plates, controlled to some suitable temperature, and then subjected to a small oscillatory disturbance (for example a simple shear strain). The relationship between this input strain and the corresponding oscillating

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stress response is then established in terms of amplitudes as the storage (G') and the loss (G'') components of the dynamic shear modulus, or the elastic and viscous components, respectively. For a simple liquid, both G' and G'' are small, and G'' exceeds G' . G' measures the elastic solid character. When under some given conditions, transformation to solid from liquid occurs, the loss and storage components crossover, and G' , in particular, increases rapidly (Figure 2.5). By the time that the gel has matured, G' is usually large, and at least one order of magnitude greater than G'' .

Figure 2.5: Typical curve of G' versus time for a gelling system. Note the tendency for modulus to level off at long times.

The gel point can be identified by the described rheological behavior. For some studies on polymer systems, the gel point is usually characterized by the crossover point ($\tan \delta = G''/G' = 1$) of elastic (G') and loss (G'') moduli, [Tung and Dynes, 1982] though there are other definitions [Clark, 1995]. Surapanani et al. [1994] (study on borosilicate gels prepared from metal alkoxides), Chambon and Winter [1987] (study on polyurethanes),

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for example, showed that this is not a reliable criterion for the determination of the gel point in all network polymer systems, as the time of the crossover depends on measuring frequency and gelation does not necessarily occur when $G''=G'$. Chambon and Winter [1987] reported that a general method to detect the gel point is the observation of the frequency independence of the tangent of loss angle ($\tan \delta$).

Subsequent measurement of G' versus frequency can then be used to explore linear viscoelastic character and a demonstrable independence of this quantity in relation to frequency (together with G' always much less than G'') is often taken as a criterion for true gel formation. Further experiments can be performed including temperature sweep (the dynamic modulus responds to heating and cooling), strain sweep (modulus is a function of increased strain), etc. So far, only linear mechanical behavior has been considered. The dynamic mechanical approach is aimed at measuring undisturbed properties of the gelling system relating closely to the network which is being formed.

The temperature effect on gelation of MC aqueous solutions may be studied with a dynamic temperature ramp test. Since an aqueous MC solution does not gel at the storage temperature ($\sim 10^\circ\text{C}$) or room temperature (25°C), the gelation is promoted at an elevated temperature. The dynamic storage modulus (G') and loss modulus (G'') are examined as a function of temperature at a low frequency and a low heating rate. Our previous study found that when the temperature is low ($<15^\circ\text{C}$), the MC solution exhibits the liquid-like behavior, i.e. $G'' > G'$ which is the typical characteristic of a liquid state; G' increases with increasing temperature gradually, followed by a sharp increase around the gel point,

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until it reaches a G' plateau. The elastic behavior indicates the formation of a gel network after the critical gelation temperature. This process is also considered by Yoshihito et al. [2001] as the loss of the fluidity of sol and the appearance of elasticity.

2.3.3 UV-Visible Spectroscopy and Turbidity

As observed by various researchers [Kabalov, 1995; Karlstom, 1990; Alexandridis and Holzwarth, 1997], gels are formed with the appearance of clouding point, i.e. the turbidity of the gels is found to increase as the gelling systems undergo the sol-gel transition. Jonsson et al. [1998] defined the clouding point as the temperature at which phase separation occurs for 1 % polymer solution. Therefore, measurement of clouding points and turbidity can be used to interpret the macromolecular phase transition from hydrophilic to a hydrophobic structure of the polymers. [Erbil and Sarac, 2002] In other words, macromolecular phase separation, which occurs as a result of certain conditions such as temperature, addition of composition, pH, solution concentration etc., can be characterized in terms of the turbidity measurement. In their study, Egermayer et al. [2004] called the cloud point the (concentration dependent) phase separation temperature.

The clouding point and turbidity are also considered to be related to the size and shape of clusters or aggregates in the system. Hence, Erbil and Sarac [2002] used the spectroscopic measurement to study the development of aggregates in the system. Boschel and Roggendorf [2002] in their study of water-based sodium borosilicate sol-gel characterization described that the materials became turbid during gelation. The main reason for the decrease of optical transmission is the formation of gel structures in the

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colloidal range which lead to light scattering, and the formation of a net-like structure during gelation is therefore observed via optical microscopy. Chen et al. [1998] also correlated the gel transmission with the gelation process. According to them, gel forming resulted in lower optical transmission, since the helix domains in the gelled state had spatial variations and dynamic concentration fluctuations which led to strong light scattering. Zheng et al. [2004] explained that if the sample was a strong scatter, which meant that the particle size was bigger than wavelength of the light, intraparticle interference occurred between light waves scattered from the same particle, and the intensity of the light diminished as a result.

UV-visible spectroscopy and optical microscopy are used to investigate the clouding behavior and homogeneity of the solution or the decrease of transmission due to development of turbidity during gelation or phase separation. Turbidity is defined as the reduction in fractional light intensity per unit penetration length in the sample, i.e. $\text{Turbidity} = - (1/I) (\delta I / \delta x)$, with I the light intensity. Since the transmitted light intensity I_t and the incident intensity I_o are related to the sample turbidity Tur and thickness L (assuming no absorption by the chromophores):

$$I_t / I = e^{-\text{Tur} L}$$

Turbidity can be determined from the ratio of the transmitted light intensity I_t to the incident intensity I . In this work, transmission is monitored by UV-visible spectrometer in the form of absorbance A under visible light (i.g. 400-700 nm wavelength).

$$A = \log (I_t / I_o)$$

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It is subsequently converted to transmittance T according to the Beer's Law.

$$A = \log (1/T)$$

Through the UV spectroscopic measurement, absorbance/transmittance by incident light (with certain wavelength) is obtained as a function of temperature in order to record the change in clouding behavior during the thermal cycle when gelation occurs.

The determination of cloud point is distinguishing for different researchers. Zheng et al. [2004] in their work on sol-gel transition of methylcellulose defined the temperature at which 50% of the overall transmittance in the sol-gel transition process was reached as the cloud point. Egermayer et al. [2004] described that for his sample of modified Ethyl(hydroxyethyl) cellulose solution a region of nearly temperature independent turbidity was found at low temperatures. At high temperatures, the turbidity increased gradually with a kink in the plot compared with the low temperatures. The intersection of two straight lines drawn through points above and below the kink was defined as the cloud point.

2.3.4 Morphology Study and Freeze-drying

Besides techniques based on thermal, rheological and turbidity properties of gels, another valuable tool to study gels is direct microstructure analysis, i.e. using microscopy to observe the gel network image. The direct SEM, AFM, or TEM imaging provides a three-dimensional topographical contrast of polymer hydrogels, [Kim et al., 2003] and the different microstructures of the gel in relation to polymer concentration, as well as different composition contents. Correlation can be observed between the microstructure

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from microscopy and rheological properties. Mathieu et al. [1997] obtained a complex porous structure of resorcinol-formaldehyde gels from nanometer (using TEM) to micrometer (with SEM) length scale. Kato et al. [2003] found the honeycomb structure of gel (Poly-NIPAAm gel) was formed by hydrophobic bonds between polymer chains. Water in the gel was expelled through the honeycomb pores. Kim et al. [2002] also observed the honeycomb structures in the carbopol gel systems using cryo-SEM analysis, while the fibrous three dimensional gel network became irregular at the lower level of polymer content. As the amount of polymer was increased, strings of fibrous network were thicker. Therefore, the nature of the different microstructures of the gel in relation to polymer concentration could be well elucidated through cryo-SEM observation.

However, it is impossible to investigate the structure of the wet gels directly by conventional electron microscopy because of the requirement of high vacuum during measurement. [Boschel and Roggendorf, 2002] Hydrogel samples have to be dried before the microscopy measurement; otherwise, damage will be caused to the instruments. Some researchers used supercritical drying in which the gel structure can be best kept. [Tamon et al., 2000] These gels are called aerogels, which are characterized by an important and continuous porosity, a high surface area and a microstructure formed by interconnected colloidal-like particles or polymeric chains. [Mathieu et al., 1997] Nevertheless, the cost of supercritical drying is extremely expensive. Freeze drying is also available to prepare mesoporous gel, during which the water is removed from frozen material directly through sublimation. In some occasions, the freeze drying is used as an effective method to obtain dry substance without destroying the structure, which means that when water is added, the new solution will exhibit the same characteristic of the original sample. The shrinkage

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of gel is proved to be much smaller than that of hot-air drying, and the deformation to gel structure such as warping and cracking can be avoided.[Brinker and Scherer, 1990] The dry gel is called cryogel. The morphology of polyvinyl chloride (PVC) has been investigated by Yang and Geil [1983] from SEM observation of a freeze-fracture gel, showing a fibrous network. Mathieu et al. [1997] in the study on resorcinol-formaldehyde gel processed by freeze-drying got a brown monolithic structure with a very large and complex porous distribution.

Another important topic is to ensure that the cryogel morphology represents the real gel structure. Scherer [1993] examined the process of freezing a liquid in the pores of a gel. He showed that nucleation within the gel occurred mainly by a heterogeneous mechanism. He analyzed the stress exerted on the network by a growing crystal and gave criterion for fracture of the network. He also stated that if crystals nucleate internally and the network is strong enough to withstand the stress exerted by the crystals, then a gel could be freeze-dried without shrinkage or damage. Hong and Chen [1998] in their work on freeze-dried PVC gels concluded that no remarkable shrinkage (less than 1 %) of the gel volume occurred after freeze-drying, thus, the network structure of freeze-dried gel may be considered to be the same as that of wet gel. Freeze-drying is therefore regarded as a reliable method to prepare dried gel for morphological observation.

Freeze-drying is to eliminate the solidified solvent by low-pressure sublimation. It consists of three steps: (i) freezing: the gel is frozen so that the water present in the material is converted to ice; (ii) sublimation: the solidified solvent is removed from the

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gel by direct conversion from solid to vapor under a reduced pressure; (iii) desorption: the residual solvent adsorbed on the solid is converted to vapor and removed from the material under very low pressure. In order to produce sublimation at the interface, the pressure in the freeze-dryer must be lower than the vapor pressure of the solidified solvent at the considered temperature.

Chapter 3 Materials and Experiments

3.1 Materials

A cellulose derivative, methylcellulose (MC), with the trade name of SM4000, made by etherification of pulp cellulose (pulp), is kindly provided by Shinetsu Chemical Co. Ltd., Japan. The manufacture's specifications indicate that the methylcellulose has an average molecular weight of 310, 000 and an average degree of substitution (DS) of 1.8 determined by light scattering. The viscosity range was reported by manufacturer to be 4.54 Pa s at 20°C for a 2 wt % aqueous solution. The material was used as received without further purification.

Prior to use, they were vacuum-dried and kept in a desiccator at room temperature. The reason of vacuum-dry is because the polymer tends to absorb moisture even kept under dry conditions. Considering the low concentration of solutions used in the test (the appropriate concentrations of MC aqueous solutions suitable for DSC test range from 0.3 to about 2.5 wt %), small moisture content will cause big errors. To vacuum-dry, put SM4000 into a bottle until half full, and cover a piece of aluminum foil on the bottle with some small holes on it, then put it in the vacuum oven at 55°C for 24 hours.

There are totally nine salts with analytical grade used in this study as additives in MC aqueous solutions. They were purchased mainly from Sino Chemical Co. (Pte) Ltd, and used as received. After opening, salts are kept in a desiccator to prevent moisture. More information about the salts and the concentrations of the tested samples are listed in Table 3.1.

Table 3.1: Description of salts used and the concentrations of tested samples for each salt.

Salts	Source	Description	Max. conc. (M)	Tested samples (M)
NaCl	Sino Chemical Co. (Pte) Ltd	GR for analysis ACS, ISO	1	0.2 0.4 0.6 0.8
NaBr		Extra pure DAB, Ph Eur		
NaI		GR for analysis		
NaNO ₃		GR for analysis ACS, ISO		
Na ₂ SO ₄		GR for analysis ACS, ISO	0.1	0.02, 0.05, 0.08
Na ₃ PO ₄		GR for analysis ACS	0.05	0.01, 0.02, 0.04
KCl		GR for analysis ACS, ISO	1	0.2 0.4 0.6 0.8
KI		GR for analysis ISO		
NaSCN	Sigma-Aldrich Pte Ltd	Min 98% ACS reagent		

Samples were prepared in mol/l, or M. Max. Conc. represents the maximum concentration of salt in a 0.03mM MC aqueous solution. GR is the abbreviation for graphite, meaning the composite material is made from graphite fiber. ISO stands for International Organization for Standardization. ACS is for American Chemical Standard. DAB and Ph Eur represent the European Directorate for the quality of chemicals and Pharmaceutical Quality Standard respectively.

3.2 Sample Preparation

In this work, the MC concentration was fixed to be 0.03 mM (approximately 0.93 wt %) and the salt concentration was varied in order for us to compare the effects of different salts on the sol-gel transition of MC. At the same time, from previous experiments, 0.3–2.5 wt % is considered as the appropriate concentration range to get stable aqueous MC solutions for micro DSC tests. 0.93 wt % is right within this range. The reason to use molarity to represent polymer and salt concentrations instead of weight percentage which is widely used by other researchers [Li et al., 2002; Zheng et al., 2004; Kundu, 2001] is that we intend to compare salt-out strength of individual ions and propose the mechanism from a molecular level. This sample preparation procedure also makes the mixed-salt experiments feasible and analyzable.

To make a sample, firstly a pure 0.03 mM MC solution of was prepared by dispersing the weighed MC powder in hot water (about 70°C) for complete swelling of polymer, after that it was kept in a refrigerator for at least 24h. A uniform and transparent solution was then obtained. The weighed salt was added to the transparent MC solution. After well shaking, it was transferred to the refrigerator and kept below 10°C before measurements. For each type of salt, three or four samples with increasing salt contents in MC solutions (with the same concentration of 0.03mM) were prepared for the micro DSC, rheology and turbidity measurements (as shown in Table 3.1). All samples were prepared with deionized water from the Millipore water purifier.

In the subsequent mixed salts tests, two salts (NaCl and NaI) were chosen as components to make the salt mixture since they show typical characteristic of salt-out and salt-in in the single salt experiments. The total salt content in each solution was fixed to be 0.4 M, and the ratio of NaCl and NaI in the salt mixture was varied. Firstly, samples with 0.4 M NaCl and 0.4 M NaI in 0.03 mM MC aqueous solutions were made (named as sample {a} and {b} respectively); then 15 ml of {a} and 5 ml of {b} were measured with pipette and mixed to get a sample of NaCl:NaI=3:1. To make sure it was homogeneous, the sample was stirred on a magnetic hotplate with stir bars for more than half an hour before transferred to the refrigerator. Varied amounts of {a} and {b} were mixed to get other mixed-salt samples, as shown in Table 3.2.

Table 3.2: Preparation of mixed-salt samples. {a} is the sample with 0.4 M NaCl in 0.03 mM MC aqueous solution; (b) is that of 0.4 M NaI in 0.03 mM MC solution.

Molar Ratio of NaCl : NaI		
3:1	1:1	1:3
15 ml {a}	10 ml {a}	5 ml {a}
+	+	+
5 ml {b}	10 ml {b}	15 ml {b}

3.3 Experiments

3.3.1 Microthermal Measurements

A micro differential scanning calorimeter (VP-DSC microcalorimeter, Microcal Inc.) is used to determine the thermal properties of salted MC solutions. According to Stephen [1998], the thermal properties such as transition temperatures, enthalpy changes and entropy changes can be obtained from DSC measurements. When scanning up in temperature, DSC continuously measures the excess heat capacity (ΔC_p) as a function of temperature. This information provides great insight into the conformational energetics of polymers in solutions. This heat capacity is later converted to relative heat capacity by a baseline subtraction. By varying solvent condition the effect of additives which either stabilize or destabilize polymers can also be evaluated. In the down scan mode, subsequent information may be obtained about the energy exchange between the sample and the heater during cooling, as well as the reversibility of the whole thermal process.

Here every test included heating the sample from 10°C to 85°C, and then cooling it from 85°C back to 10°C with a temperature-scanning rate of 1°C/min. The slow heating (or cooling) rate employed allows efficient heat transfer and detecting. Li [2002] has discussed the effect of heating (cooling) rate and found that the whole pattern of the endothermic peak did not change significantly with the heating rate, but the transition temperature reduced at a lower rate, implying that the molecular association of MC is a rate-dependent process. Plotnikov et al. [1997] also concluded that the highest sensitivity was obtained with slow measuring times or low heating rate. Using a fast measuring time, a sharp peak was observed, while a much broader transition was expected with slower measuring times. A slow heating or cooling rate

of $1^{\circ}\text{C}/\text{min}$ is employed for all samples to explore the details of the thermal induced association behavior of the cellulose polymer in water. The slow scan mode is of the concern of high sensitivity (to resolve the small signal changes), and more importantly it is suitable for the slow hydrophobic association procedure from relatively stiff cellulose chains.

The respective salt solution (no MC) with the same salt concentration was used in the reference cell. For example, when the sample of 0.2 M NaCl in the 0.03 mM MC solution is injected in the sample cell, the reference cell will be filled with the 0.2 M NaCl aqueous solution. For one series of tests (i.e. same type of salt, various salt contents) we always started from the lowest salt concentration in order to minimize the concentration effect of the former test on the later one. After one series of tests were completed, both the sample and reference cells were thoroughly cleaned with a water baseline test. To do the cleaning, the sample cell was first rinsed with a Sodium Dodecylsulfate (SDS) solution for 20 minutes, so as to remove the viscous MC solutions easier. After that, both sample and reference cells were cleaned with a continuous flow of deionized water through a thin plastic tube until a no-contamination condition was reached, when the water baseline test showed a straight line. The next test could then begin.

One of the major limitations of this micro DSC is that a highly viscous liquid can not be injected into the sample cell. Therefore the MC concentration should be carefully chosen with consideration of salt effects on the viscosity of MC solutions.

Another issue needed to mention is degassing of sample solutions before injecting them into the sample cell. Since the effective volume of cell is just 0.5 ml, even one bubble will cause big error in taking up the effective volume. If the sample is injected directly after taken out from refrigerator without any degassing procedure, bubbles will emerge while the cell temperature is increased, resulting in some irregular peaks in the DSC curve. To prevent such a problem, the sample should be placed at room temperature for at least 30 minutes to resume pressure balance with the atmosphere.

3.3.2 Rheological Measurements

Rheological measurements were performed using an oscillatory plate rheometer (ARES 100FRTN1, Rheometric Scientific). The rheometer is equipped with two sensitive force transducers for torque measurements ranging from 0.004 to 100 g cm. there are two parallel plates with one plate stationary and the other rotating at certain rate assuming no slip at these surfaces and neglecting inertial force. There are two diameters of plates to be used, i.e. 25 and 50 cm for relatively high viscosity and low viscosity solutions respectively.

Since it is easier to load and unload viscous or soft samples with the parallel plate geometry than with cone and plate or other geometries, parallel plates are usually preferred for measuring viscoelastic functions such as shear storage modulus G' and loss modulus G'' , or the compliance J as a function of time, temperature, or angular frequency. In the linear viscoelastic region, they are independent of the strain.

To prevent dehydration during rheological measurements, a thin layer of low-viscosity silicon oil was placed on the periphery surface of the solution held between

the plates. All the dynamic viscoelastic measurements (i. e. temperature sweep) were carried out from 20 to 80 °C at an angular frequency of 1 rad/s and appropriate shear strains to ensure the linearity of viscoelasticity. The heating rate was 1 °C/min, the same as that of the DSC test's, so that the temperature induced association behaviors would be comparable between rheological and thermal measurements. The gap between two plates was kept at about 2 mm for all tests to avoid its influence on shear rate and edge effects etc.

In the dynamic temperature ramp sweep test, the setting of the strain is very important. The strain should be low enough to ensure the linear mechanical behavior (within the linear range, the modulus is independent of the strain). On the other hand, for the low viscous solutions at low temperatures (low value of G' and G''), smaller strains are unable to cause detectable torque for accurate measurement of G' and G'' . The strain had to be increased to increase the torque sensitivity during the test. Strain sweep experiments were conducted at 25 °C and 70 °C respectively. Both G' and G'' were found to be independent of the strain up to 70% at angular frequency of 1 rad/s, implying that any strain below 70 % was within a linear viscoelasticity range. Thus 50% strain was employed at low temperature (or sol state) for all tests. However such strain might be too high for gels at high temperatures, affecting the accurate reading of G . Thus the setting strain was reduced to 20 % in the gel state.

3.3.3 Optical Measurement

An Agilent 8453 ultraviolet-visible spectroscopy system equipped with a temperature controller was used for the turbidity measurements. Samples were placed in the plastic cell with dimensions of 1 x 1 x 5 cm³ for optical transmission measurements,

and deionized water was used as a standard. The cell was covered by a plastic cap to prevent evaporation. Thermal denaturation test was then carried out and the absorbance at 500 nm (visible range) was detected when the sample solution in cell was first heated from 20 °C to around 80 °C and subsequently cooled back to 20 °C at a rate of 1 °C /min. In addition to the temperature sensor, a thermo-couple was placed inside the sample cell for temperature measurement. The absorbance data were later converted to transmittance according to Beer's Law.

Solution homogeneity can be a problem, especially for viscous solutions. It is possible that due to convection induced gradients, rapid absorbance changes might give irreproducible data and affect the sensibility of the measurements. To minimize convection effects, longer holding time (in this work, 1 minute) was set, which means that once the desired temperature was reached, an additional holding time of 1 minute was applied, prior to light beam inserting and absorbance recording. Using a thermo stable cell holder and/or a stirring module are also recommendable.

3.3.4 Freeze-drying and Morphological measurement

To obtain the cryogels for image observation, the MC solution in disk shape plate was first kept in water bath at 70 °C for half an hour to get the stable and firm hydrogel. It was then immersed in liquid nitrogen (-110 °C) bath for pre-freezing before the frozen material was transferred to the freeze-dryer (Christ Freeze Dryer, ALPHA 2), where water in the gel sublimed directly to vapor under reduced pressures, skipping the liquid state. In order to promote sublimation at the interface, the pressure in the freeze dryer must be lower than the vapor pressure of the solidified solvent at the considered temperature. The technical specifications of the ice condenser of freeze dryer are

therefore important in determining the evacuation capability and efficiency. The ice condenser temperature determines the water vapor partial pressure in the system. The lower the ice condenser temperature, the more effectively the product can be dried in the final drying phase. Thus for an ice condenser temperature of approximately $-40\text{ }^{\circ}\text{C}$ it is possible to achieve a pressure of 0.1 mbar during final drying phase, however for a temperature of $-70\text{ }^{\circ}\text{C}$ it will be 0.002 mbar. The drying is considered to be finished when the freeze-dried gel weight becomes constant. For a MC solution of about 5ml, approximate 10 hours are needed for drying under vacuum.

After obtaining the dry cryogel, fractions of sample were transferred into a sputter coater (Polaron SC 7640, Emitech). Gold was sputtered on the specimen surface differentially for 300 seconds. The specimen was then examined by Scanning Electron Microscopy (JEOL JSM-5600LV) at 20 kV and about $80\text{ }\mu\text{A}$.

Chapter 4: Salt Effects on Thermodynamic Properties

In this chapter, we will discuss the thermodynamic results of MC aqueous solutions added with different salts and salt mixtures. The DSC curves will show significant changes in thermal properties of MC solutions when salts are added. Some salts would make the sol-gel transition occur at lower temperatures, whereas others shift the sol-gel transition temperatures to higher temperatures. These phenomena are termed salt-out and salt-in behaviors. The salts are accordingly classified as salt-out salts and salt-in salts, as stated by Hofmeister [1888], Daugherty et al. [2001] and Barbara et al. [2002]. In this chapter, salt effects on thermal properties of MC aqueous solutions are studied systematically. For the single-salt series, the influence of salt concentration, salt-out and salt-in phenomena of anions, as well as cations effects on the sol-gel transition are discussed in detail. On the other hand, a salt mixture containing a salt-out and a salt-in salt is added into the MC solution to study its influence on the thermodynamic and properties during gelation and degelation.

4.1 Effects of Single Salts

4.1.1 Influence of Salt Concentration

When one salt is added into a MC aqueous solution, the thermodynamic properties during gelation and degelation will be changed correspondently. Various salts show significantly different effects depending mostly on the salt type, which decides whether the salt assists or suppresses the MC sol-gel transition. And for the same salt, the degree of effect is a function of the salt concentration. A typical example for the calorimetric thermal behavior of a 0.03 mM MC solution with a salt-out salt (NaCl) is shown in Figure 4.1.

Figure 4.1: Relative thermal capacity C_p as a function of temperature for 0.03mM methylcellulose aqueous solutions in the presence of various NaCl concentrations (mol/l): (top) in the heating process and (bottom) in the cooling process. The heating and cooling rates were 1°C/min.

As mentioned in Table 3.1, the suitable salt concentration of NaCl in 0.03 mM MC solution ranges from 0.1 to 1 M. When the NaCl concentration is more than 1 M, the MC solution is either too viscous to be injected into the sample cell or not transparent even kept in a refrigerator for more than 24 hours. Therefore the four samples with

0.2, 0.4, 0.6 and 0.8 M NaCl were tested. A sharp endothermic peak is observed for each sample in the heating process, whereas cooling is exothermic with a wide shoulder before the deepest valley. The curve's continuity and pattern show that the thermal behaviors involved in all the solutions are similar. However, with adding of the salt, the peak shifts to the left (i.e. lower temperature), and the higher the NaCl concentration is, the more the peak shifts. This indicates that the addition of the salt assists the sol-gel transition to occur at a lower temperature.

Table 4.1: Peak temperatures (T_m) during heating and cooling, enthalpy change (ΔH) and entropy change (ΔS) on heating for various NaCl concentrations (M) in 0.03mM MC solutions.

	NaCl Concentration (M)				
	0	0.2	0.4	0.6	0.8
T_m on heating (°C)	61.1	55.3	50.1	46.8	43.1
T_m on cooling (°C)	31.9	28.2	24.2	21.7	18.7
ΔH ($\times 10^3$ KJ/Mol)	3.67	4.85	6.05	6.96	7.87
ΔS (KJ/Mol K)	12.84	15.71	18.51	21.33	24.74

The peak temperatures (T_m) during heating and cooling are obtained from the DSC curves (Figure 4.1) and listed in Table 4.1. As can be seen, the peak temperature (T_m) for the pure MC solution in the heating process, which is defined as the sol-gel transition temperature [Li et al., 2002], is 61.1°C, while for a 0.8 M NaCl sample, the sol-gel transition shifts to 43.1°C. At the same time, when cooling the broad exothermic peak appears at 31.9°C for the salt-free sample, whereas 28.2°C for the 0.2 M NaCl solution, and 18.7°C for the 0.8 M NaCl one.

It is extensively accepted that the formation of cage-like structures for MC can explain the water solubility of MC at low temperatures. Haque et al. [1993], Sarkar [1995], and Kabayashi et al. [1999], for example, all supported this idea in their research. Water molecules form hydrogen bonds along MC chains and/or the cage-like structures surrounding the methyl groups. Heating of a MC solution will cause the destruction of the cage structures of water or hydrogen bonds, and the exposure of hydrophobic regions of MC, leading to the formation of hydrophobic aggregations of MC. As temperature increases, the number and size of hydrophobic aggregates increase. When the number and size have reached certain critical level, a solid-like gel will eventually begin to form [Li et al. 2002]. The smooth peak for the heating curve is the result of gelation.

The addition of a salt will affect the structure of water [Barbara et al., 2002], which is mainly due to the interactions between ions and water molecules. NaCl, which is categorized as a salt-out salt, exhibits stronger interaction(s) with water than those hydrogen bonds between water molecules. As a result of its strong hydration in water, some of original hydrogen bonding network (including cage-like structures) formed by water molecules is destroyed by the salt and this effect is similar to increasing temperature.

In other words, ions tend to induce the restructuring of water. In MC solutions, they will compete with MC chains for the water molecules, and they succeed in attracting more water molecules surrounding them due to their stronger hydration abilities than neighboring macromolecules. This competition forces the MC chains to lose water molecules, consequently causes the decrease of MC solubility in water. As a result, at

the same temperature, there are more hydrophobic aggregates of MC in a salted MC solution than a salt-free MC solution. Thus, upon heating it will be easier for a salted MC solution to accumulate and meet the requirement for the critical number of hydrophobic aggregates to form a gel, so that the sol-gel transition occurs at lower temperature, or it is better to say that the salt accelerates the formation of a MC gel.

The increased salt content results in less free water molecules available around MC chains, and stronger hydrophobic environment for MC. Therefore, the gelation shifts further to the left with increasing concentration of NaCl, as shown in Figure 4.1.

In the cooling process, it is noted that the exothermic peak, with a shoulder at the high temperature side, is much broader than its respective endothermic peak in the heating process. The difference in the peak temperature between heating and cooling is significant. This hysteresis is considered to be caused by the two-step dissociation or degelation of MC. For the samples with various salt contents, the exothermic curves appear similarly, but they shift to lower temperatures with increasing salt content. Therefore we assume that the appearance of the shoulder in the cooling thermogram is independent of salt. In other words, the presence of salts does not affect the mechanism of degelation or disassociation of MC.

Another remarkable feature in Figure 4.1 is that the peak area becomes bigger with increasing salt content. This indicates that the enthalpy change ΔH increases with the addition of salt, which can be obtained by integration of the relative heat capacity (C_p) versus temperature (T). At the same time the entropy change ΔS can be calculated.

The results of ΔH and ΔS in the heating process are given in Table 4.1. They are plotted in Figure 4.2 as a function of NaCl concentration. It is observed that both enthalpy change and entropy change are the linear functions of salt concentration. These results imply that the total enthalpy change or entropy change involved in the sol-gel transition is determined by the total content of the solute (i.e. MC) and co-existing solutes (i.e. salts). The destruction of the hydrogen bonds between MC and water, the formation of hydrophobic aggregations of MC, and the constitution of a gel network structure are considered as the major attributions to the detectable endothermic heat in the heating process. In the presence of a salt, more energy is consumed to break not only the hydrogen bonds (including cage structures) between the MC chains and water molecules, but also the interactions between ions and water. As a result, the enthalpy change is positive and increases with the addition of salt.

Figure 4.2: Enthalpy change (ΔH) and entropy change (ΔS) during gelation as a function of NaCl concentration (M). The MC concentration was fixed as 0.03mM.

With regard to the entropy change, according to the Gibbs equation ($\Delta G = \Delta H - T\Delta S$), since $\Delta H > 0$, ΔS must be positive, in order to keep a negative value of the free energy change ΔG . The gelation involves the destruction of hydrogen bonds between MC chains and water as well as the interactions between ions and water molecules, which are relatively ordered at low temperatures. As temperature increases, the bonds between ions and water molecules are broken, thus both ions and water become random and less ordered. So, these "free" ions would contribute more positive entropy change to the gelation process. That is why a MC solution with salt has higher positive entropy change than a pure MC solution, and why ΔS is a linear function of salt content. In the presence of salt as a reverse process, dissociation has negative ΔS with a similar trend to that in the salt-free MC solution.

Similar to NaCl, for eight other salts, each has a group of continuously varying DSC curves and corresponding peak temperatures, enthalpy changes and entropy changes when the calorimetric tests were conducted upon the MC solutions with changing salt content. Addition of a salt shifts the gelation and degelation curves continuously to higher or lower temperature ranges, depending on the salt type and the salt concentration. Figure 4.3 presents the heating curves of various Na_2SO_4 and NaBr in 0.03 mM MC solutions as an evidence of the similarity and difference among salts. It is obvious that both NaBr and Na_2SO_4 show the salt-out effects on gelation. The only difference is the shift distance of the curve by the same salt content. NaBr shifts curves much less than Na_2SO_4 . For instance, 0.4 M NaBr reduces the sol-gel transition temperature by 5.3 °C, whereas 0.08 M Na_2SO_4 can shift the curve to 11.2 °C lower. The salt-out effects that they show are significantly different. All these original thermal data were obtained for the further analysis.

(a)

Figure 4.3: DSC heating curves for various (a) Na_2SO_4 and (b) NaBr in 0.03mM MC solutions.

4.1.2 Effects of Anions and Salt-out Strength

When the cation is the same, variation of anions allows us to study effects of anions. Sodium halide salts studied (NaCl, NaBr, NaI) show the great difference in their effects on the sol-gel transition of MC. The heating curves with 0.8 M of NaCl, NaBr and NaI added into 0.03 mM MC solutions, together with the salt-free 0.03 mM MC solution are plotted in Figure 4.4 with their respective peak temperatures indicated. NaCl and NaBr curves stay to the left of the salt-free sample, while NaI is located at the right.

Figure 4.4: Relative thermal capacity C_p for 0.03mM MC solutions each containing 0.8 mol/l NaCl, 0.8 mol/l NaBr and 0.8 mol/l NaI, as well as the non-salt MC solution in a heating process at 1°C/min. The peak temperature for each sol-gel transition is indicated.

It is shown that at the same salt concentration of 0.8 M, NaCl causes the peak temperature to shift by 18.0°C, and NaBr by 9.0°C, but NaI, in contrast, increases the peak temperature by 6.1°C. NaI exhibits the symptom of a salt-in behavior that delays

the occurrence of the sol-gel transition with a different mechanism from the salt-out one. The salt effects in Figure 4.4 follow the Hofmeister sequence: $F^- > Cl^- > Br^- > I^-$.

It is generally accepted that in a solution a salt affects a solute like MC through the interaction between ions and water but not through the interaction between ions and the solute. In the case of MC, interruption of the hydrogen-bonded network between MC chains and water in the solution by the ions leads to the change of MC solubility in water. Therefore, effects of salts depend on the structure-making ability of individual ions and the strength of the interaction with water molecules.

It is well known that solvation of a solute in water by hydrogen bonding is important. In a MC aqueous solution containing a salt, water molecules solvate the anions of the salt by forming hydrogen bonds with them, which are much stronger than hydrogen bonds formed between water molecules because the water molecules are more attractive by the negative charges of ions. For these four halide ions, as each of them contains the same charge of -1, the inherent electronegativities of the corresponding halogen atoms are not important to the solvation. Instead, the most important factor affecting the solvation strength is the ionic radius of each halide ion.

A small ion, because its charge is more concentrated, should be more strongly solvated than a larger one. According to Israelachvili [1991], the ionic radii of F^- , Cl^- , Br^- and I^- are 1.36, 1.81, 1.95 and $2.16 \text{ \AA}$ respectively. Therefore, the solvation strength by water for the halide ions follows the order of $F^- > Cl^- > Br^- > I^-$. This explains why the halide ions affected the sol-gel transition of MC differently as shown in Figure 4.4.

When the anions have different charges, both ionic charge and size have to be taken into account. It is interesting to know from our experiments that the hydration ability is strongly affected by the ion's charge value. During the sample preparation, we first noted that each salt used shows an individual concentration limitation with the same content of MC in solution. If the added salt is higher than its concentration limit, the solution will precipitate, or a gel may be formed at room temperature. The salt concentration limits are found to be approximately dependent on the anionic charges. For example, for the single-charged anions (NO_3^- , SCN^- , Cl^- , Br^- and I^-), the salt concentration limits are about 1 M, while for the double-charged ions (SO_4^{2-}) they fall below 0.1 M. When it comes to the triple-charged phosphate ion, the concentration limit is further reduced to be as low as about 0.05 M. Thus, it is concluded that the higher the anionic charge, the stronger the salt-out effect, which results in lowering the necessary concentration of the salt that brings MC to the same sol-gel transition temperature.

Bockris [1998] also reported that the extent of ionic hydration depended upon the valence of the ion. So, triple-charged phosphate is expected to be more effective than the double-charged sulfate and single-charged nitrate in accelerating gelation, i.e. $\text{PO}_4^{3-} > \text{SO}_4^{2-} > \text{NO}_3^- > \text{SCN}^-$, which still follows the Hofmeister series as well. Together with the factor of ion size, the so-called "charge density" is considered as the main decisive factor for the ability of water-structure-making by ions. The charge densities of related anions are listed in Table 4.2 as follows.

Table 4.2: Charge densities of selected anions.

Charge density (-mC/m ²)						
F ⁻	SO ₄ ²⁻	Cl ⁻	Br ⁻	NO ₃ ⁻	I ⁻	SCN ⁻
720	481	389	331	397	263	281

Data are obtained from Babu and Lim [1999] and Marcus [1991].

The most concern here is not the absolute values and the unit, but the relative positions in the sequence. It has been clearly indicated that the charge density follows $\text{SO}_4^{2-} > \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \text{I}^- > \text{SCN}^-$, which is entirely the same as the Hofmeister sequence. This can be excellently used to explain their abilities to modify the water structure and their influence to a MC gelation process.

A summary about effects of various anions on transition temperature, enthalpy change is given in Figure 4.5. The approximately linear dependences of T_m and ΔH on salt concentration are observed for all the concentration ranges studied. As shown in Figure 4.5 (a), the effects of salts on the transition temperature are dramatically different, resulting in the different slopes for each line. The higher the salt effect, the steeper the trend line is. In addition, a line with a positive slope means that the salt is a salt-in salt (i.e. NaI, NaSCN), while the down-going line indicates the salting-out trend. We define the effect per mole of the added ion as “salt-out strength” as given in Table 4.3.

Figure 4.5: Salt effects on thermodynamic properties during gelation as a function of salt concentration: (a) peak temperature and (b) enthalpy change (ΔH). The MC concentration was 0.03mM.

Here the “salt-out effect” is represented by the difference between the individual peak temperature (T_m) and that of the salt-free MC solution (T_o) during the heating process, and the “salt-out strength” is the salt-out effect caused by adding 1M of anion into a 0.03mM MC solution.

Table 4.3: Salt-out strength ($^{\circ}\text{C}/\text{M}$) for various anions that is defined by the decrease of the transition temperature ($T_o - T_m$) in the heating process per mole of a sodium salt.

	Salt Content (M)	T_m ($^{\circ}\text{C}$)	$(T_o - T_m)/\text{salt cont.}$	Average ($^{\circ}\text{C}/\text{M}$)
PO_4^{3-}	0.01	58.2	297	287.3
	0.02	54.8	315	
	0.04	51.1	250	
SO_4^{2-}	0.02	56.9	210	156.7
	0.05	55.1	120	
	0.08	49.9	140	
Cl^-	0.2	55.3	29	25.7
	0.4	50.1	27.5	
	0.6	46.8	23.8	
	0.8	43.1	22.5	
Br^-	0.2	58.1	15.0	13.0
	0.4	55.8	13.3	
	0.6	53.6	12.5	
	0.8	52.2	11.1	
NO_3^-	0.2	59.6	7.5	8.2
	0.4	57.6	8.8	
	0.6	56.3	8.0	
	0.8	54.5	8.3	

T_m is the sol-gel transition temperature for each salt-containing MC aqueous solution (MC = 0.03mM), and T_o (= 61.1 $^{\circ}\text{C}$) is the sol-gel transition temperature for the 0.03mM MC solution without salts.

The results given in Table 4.3 suggest that the ability for an anion to decrease the transition temperature of MC solution, or the ability to control the transition behavior, is closely related to the charge density of the anion, which determines the value of “salt-out strength”. The sequence of “salt-out strength” in Table 4.3 is also correlated to the charge density of ions presented in Table 4.2. Ions possessing higher charge

density can bind water molecules more strongly, while a larger ionic radius (lower charge density) may mean that the charge is more diffuse (i.e. spread out), therefore less water is attracted.

The valence seems to be more effective than ion size. Leontidis [2002] concluded from his research that the charge is a primary ionic property. For ions with the same charge, their size, e.g. the Pauling radius, is the next most fundamental factor. Our results have supported this statement. It is obvious that for the single-charged ions, their salt-out strengths differ within tens depending on the ionic size (i.e. NO_3^- : 8.3; Br^- : 13.0; Cl^- : 25.7); while increasing in valence causes the salt-out strength to vary in centuplicate, for example SO_4^{2-} is 156.7, while PO_4^{3-} 's salt-out strength jumps to 287.3.

4.1.3 Salt-in Phenomenon

Although many salts (Figure 4.5 and Table 4.3) assist in the formation of gels, some salts (e.g. NaI, NaSCN) on the other hand can increase the transition temperature, which is similar to suppressing the gel formation as shown in Figure 4.5. The details of the thermal behavior for the MC solutions containing NaI are given in Figure 4.6.

It is observed that in the presence of I^- , the sol-gel transition peak occurs beyond 61.1°C , the peak temperature for the MC solution without salts. At the same time, the endothermic heat increases with increasing salt concentration. The higher level of ΔH for these MC/salt solutions can be explained by the same mechanism as salt-out salts, which is attributed to the destruction of interactions between ions and water molecules. However, the adverse mechanism is needed to understand the increasing peak

temperature with adding I^- or SCN^- . According to Hofmeister [1888], both I^- and SCN^- are classified as Chaotropes or salt-in ions, which are weakly hydrated in water. These big single-charged ions, acting like hydrophobic molecules, exhibit weaker interactions with water than water itself. They are expected to have strong dispersion forces with other solutes in the system (here the MC chains) [Ninfam, 1997]. Because of their low charge density, they have weak electric fields and a loose hydration sheath, whose water can be easily removed. It can be anticipated that these large ions with certain polarizabilities may easily lose their attracted water and consequently increase the solubility of MC in water. From above discussion, it is confirmed that adding chaotropes such as I^- and SCN^- makes the MC solutions more stable.

Figure 4.6: Relative thermal capacity C_p for 0.03mM MC solutions in the presence of various NaI concentrations (mol).

Song et al. demonstrated in 1991 from the experiment with the NMR Spectroscopy that I^- and SCN^- had the higher binding affinity with poly (vinylpyrrolidone) in the aqueous solution. From this point of view, if adding a salt can create a more stable environment, the solubility of a polymer in water will be enhanced. Thus the salt-in behavior may be explained. However, considering the cage structure proposed previously for the gelation process of MC solutions, these big weakly hydrated ions are not likely to approach MC chains, letting alone a “binding affinity”. More possibly, they can sit comfortably between MC chains, repulsing water molecules to the macromolecules.

Alexandridis [1995] attributed the salt-in behavior to two possible mechanisms: one is indirect where salt-in ions facilitate the hydration of non-polar solutes; and the other is more direct, whereby they are assumed to have almost no effect on the water structure, but replace some of the water molecules in the hydration shell of the solutes. The former explanation seems unsuitable to our system, as it is difficult to use this proposed facilitation to explain the salt-out phenomenon; the latter is recommendable to our study.

Adding salt-in ions into a MC aqueous system to some extent is equal to adding another solvent or replacing water. So that MC chains are dispersed in the solution resulting in fewer chances of interconnection of MC chains. Therefore, on heating it is more difficult for MC to form hydrophobic aggregates at the same temperature. To reach the critical requirement for intermolecular hydrophobic association, more energy is needed, leading to a higher sol-gel transition temperature. This delayed gelling process is described as a salt-suppressed sol-gel transition.

4.1.4 Effects of Cations

Cations were reported to have less effects on water structure than anions [Barbara et al., 2002; Byeongmoon et al., 2002; Kabalnov et al., 1995; Muta et al., 2002 and Strodoubtev et al., 1995], which has been once again proved by our experiments. The DSC heating curves for 0.8 M NaCl, KCl, NaI and KI in the 0.03 mM MC solution, together with the salt-free MC solution are compared in Figure 4.7.

Figure 4.7: DSC heating curves for a 0.03mM MC aqueous solution and 0.03mM MC solutions each containing 0.8 M of a salt. The salts used were NaCl, KCl, NaI, and KI.

It is shown that the 0.8 M NaCl curve appears very closely to the KCl one, so do the NaI and KI curves. The two groups, belonging to salt-out and salt-in respectively, have dramatically different effects on the gelation. One reduces the transition temperature, while the other makes it happen at higher temperature. The transition

peak temperatures during the heating process for various salt contents are plotted in Figure 4.8.

Figure 4.8: The sol-gel transition temperature (i.e. the peak temperature in a DSC heating curve) as a function of salt concentration. The four salts, NaCl, KCl, NaI, and KI, were used.

The nearly linear dependence of the transition temperature on the salt concentration is observed for each salt. The up-going or down-going trend of the line represents the outcome of salt effects. Both Figures 4.7 and 4.8 indicate that variation of anion type, rather than cation, is a more effective means for modulating thermal properties of a MC aqueous solution.

Florin [1984] argued that the different effects between anions and cations are because the ion size and polarizability vary more with anions than cations. Collin [1997]

explained that anions are stronger than cations at water ordering because of the asymmetry of charge in a water molecule: the negative end of water's dipole is nearer to the center of the water molecule than the positive end. Therefore, anions would show stronger electrostatic potential at the surface of a water molecule than cations do. Our results are partially in agreement with his opinion. To find out an appropriate explanation for why cations have much weaker effect than anions from a molecular origin, a molecular schematic description is given in Figure 2.1 of Chapter 2, reproduced here for quick reference.

Figure 2.1: Interactions of cation and anion with water molecules.

It is obvious that the binding between the cation and the oxygen atom of water is not considered as a hydrogen bond, but its lone electron pair may disrupt hydrogen bonds between water molecules, resulting in the rearrangement of water hydrogen bond's donating and accepting capacity. Anions, however, can bind to the hydrogen atom of water to form hydrogen bonds with H atoms. Due to the bigger size of oxygen atom than the hydrogen atom, the water hydrogen atom can approach more closely to an anion than the water oxygen atom to a cation, leading to a stronger bonding between an anion and a water molecule than that between a cation and a water molecule. Thus, it can be inferred that the sol-gel transition of MC in water is more sensitive to the variation of anions than that of cations. When the anion is the same, salts with

different cations exhibit similar effects. When the anion is varied, the effects of salts are significantly changed. This is why cations have weaker effects on the water structure than anions, and further on the thermal properties of MC solutions.

4.2 Effects of a Salt Mixture on Thermal Behavior

In the previous section, we focus on effects of a single salt on the MC sol-gel transition. The influencing factors such as salt concentration, types of anion and cation were investigated systematically by means of micro DSC. However, salts are usually present in practical studies such as biological systems as a multi-salted system, implying that effects of salts on conformation and activities of solutions should be considered as the co-effects from component salts. So far, no reports have been found on effects of a salt mixture on hydrogels. In the following experiments, a salt mixture which contains a salt-out and a salt-in salt is added into the MC solution to further observe its influence on the thermodynamic properties during gelation and degelation.

The mechanism proposed about the effects of single salts makes it clear that salts affect the gelation or degelation of MC in solution through a water-restructuring process rather than direct interactions with MC chains. Therefore, the tendency and degree of salt effects will depend on the salt-out (or salt-in) strength of respective ions and their concentrations. When a salt-out salt and a salt-in salt coexist in a MC solution, if there are no interactions between the salt-out and salt-in salts to affect the water structuring, the coexisting ions in the system are supposed to deliver their influences independently, and the salt effects will be observed as a combination of contributions from component ions. So that gelation is controllable through adjusting the composition of a salt mixture. This hypothesis will be examined in the following.

4.2.1 Design of Salt Mixtures

As concluded previously, the anions are more effective in modulating thermal properties of MC aqueous solutions than cations. In fact, the anions are considered the key factor to decide whether a salt tends to assist or suppress the gelation process. NaCl and NaI, which are both single-charged, and classified as typical salt-out and salt-in salts respectively, are chosen as components to prepare the salt mixture.

The mixed-salt samples with different molar ratios of NaCl to NaI were prepared by ensuring that the total salt molarity in MC solutions was the same. For example, the MC solution might contain either 0.4 M NaCl, or 0.3 M NaCl and 0.1 M NaI etc. This is to ensure that the different effects are purely due to varying salt ratios instead of the difference in the salt concentration, since the salt effects were observed to be a linear function of the salt concentration according to the results of single-salt experiment. [Xu et al., 2004] The compositions of the five tested MC solutions in the presence of salts or salt mixtures are listed in Table 4.4.

Table 4.4 Compositions of Tested MC Solutions with NaCl-NaI Salt Mixtures.

Samples Compositions	0.4 M NaCl	Molar ratio of NaCl : NaI			0.4 M NaI
		3 : 1	1 : 1	1 : 3	
NaCl (M)	0.4	0.3	0.2	0.1	--
NaI (M)	--	0.1	0.2	0.3	0.4
MC	0.03mM				

Again, salt solutions with the same molar ratios of componential salts were used in the reference cell during the calorimetric running. Other experimental parameters were remained the same as those of single salt and salt-free MC solutions, i.e. the

samples were heated from 10 to 85 °C and then cooled subsequently back to 10 °C with the same heating and cooling rate of 1 °C/min.

4.2.2 Thermal Results of Salt Mixtures in MC solutions

The DSC heating and cooling curves for MC solutions with the NaCl-NaI salt mixtures of different salt ratios are presented in Figure 4.9. The total salt concentration was 0.4 M for every solution sample. As shown in Figure 4.9, the curve of pure salt-out sample (i.e. 0.4 M NaCl) appears on the leftmost side with the lowest transition temperature. With the increase of NaI proportion in the salt mixture, the curve shifts smoothly to higher temperatures with a reduced peak height, until ending at the pure salt-in one (i.e. 0.4 M NaI). It is clear that the greatest salt-out effects appear in the MC solution with 100% NaCl, as it shows the lowest sol-gel transition temperature. The salt-out strength of the salt mixture then decreases gradually with reducing NaCl and increasing NaI ratio, following the sequence of 3:1, 1:1 and 1:3. The peak temperature of the NaCl:NaI=1:3 sample is 61.5 °C, which is very close to that (61.1 °C) of the salt-free MC solution, indicating that the salt mixture made up of 1 mol of NaCl and 3 mol of NaI has nearly no effect on the gelation temperature. This is like an acid-base neutralization process where at the equivalence point the acid has just been completely neutralized by the base. However, although the transition peak temperatures are similar between the salt-free solution and the 1:3 salted solutions, the different peak heights indicate the difference in the total endothermic heat. This is attributed to the fact that in the heating process the extra energy has to be used to destroy the interactions between the salt ions and water molecules. After the DSC curve passes over the salt-free MC solution one, it starts to show the salt-in effects.

Figure 4.9 Relative thermal capacity C_p as a function of temperature during a heating process at 1°C/min for 0.03mM MC solutions in the presence of salt mixtures with various NaCl versus NaI ratios. The star line represents the salt-free 0.03mM MC solution.

All these DSC curves show a similar shape or pattern, and they shift to left or right along the temperature axis depending on the salt type and concentration. These results strongly suggest that the gelation mechanisms involved in all MC solutions are similar. As the MC concentration and the total salt concentration in all solutions are the same (i.e. 0.03mM of MC and 0.4 M of salts), the differences in transition temperature and enthalpy change are only due to the different salt composition. Therefore, the gelation of MC should be adjustable with a designed salt mixture.

According to the mechanism proposed, in the MC solution with mixed NaCl and NaI, Cl^- in the solution tries to destroy the cage-like structures between MC chains and water molecules, causing a higher hydrophobicity of MC in water. I^- , on the other hand, tends to disperse the aggregates, preventing MC chains from interconnection, so as to increase the solubility as well as the stability of MC in water. Since both Cl^- and I^- affect the gelation of MC through interactions with water molecules, their influences are inferable to be independent with each other. Thus, the solubility of MC in water with the salt mixture is modified by the combination effect of salt-out and salt-in when a new balance between hydrophilic and hydrophobic effects is established; a new sol-gel transition temperature upon heating will be resulted.

The peak temperatures during heating of MC solutions with the salt mixtures, together with the two respective single salt ones are compared in Table 4.5. And these temperatures are plotted in Figure 4.10 versus the fraction of NaI in the salt mixture.

Table 4.5 Peak Temperatures of micro DSC Heating Curves for 0.03mM MC Solutions with the Addition of NaCl-NaI Salt Mixtures in Different Ratios. The Total Salt Concentration in every Sample is 0.4 M.^a

	Pure NaCl	3:1	1:1	1:3	Pure NaI
Peak Temp in DSC (°C)	50.1	54.4	56.9	61.5	64.8
Theoretical Peak Temp* (°C)	50.1	53.8	57.5	61.1	64.8

*Calculated peak temperature according to the linear rule of mixing

^a The total salt concentration in every sample was 0.4 M

Figure 4.10 Peak temperatures during gelation of 0.03mM MC solutions added with NaCl-NaI salt mixtures as a function of NaI fraction. The total salt concentration in each sample is 0.4 M. The best fitting function of the data is indicated.

From the results, a linear relationship is found to exist between the peak temperature and the NaI fraction. A linear rule of mixing is then simply obtained:

$$T_p = m_1 T_{p1} + m_2 T_{p2}$$

where T_p , T_{p1} , and T_{p2} are the peak temperatures for the MC solutions with a salt mixture, NaCl, and NaI, respectively, and m_1 represents the molar fraction of each salt component in the salt mixture. At the total salt concentration of 0.4 M (i.e. Figure

4.10), T_{p1} and T_{p2} are 50.1 °C (NaCl) and 64.8 °C (NaI), respectively. When 50.1 and 64.8 are used as T_{p1} and T_{p2} , respectively, to calculate T_p using the above equation, the theoretical values of T_p (as shown in Table 4.5) are obtained, and they are plotted as the solid straight line in Figure 4.10. The excellent consistence between the experimental data and the solid line indicates that a linear rule of mixing is valid for the MC solutions mixed with the salt mixtures. This linear rule of mixing also proves our hypothesis that the effects of NaCl and NaI on the sol-gel transition of MC are independent. Any gelation temperature can be exactly predicted using the above equation without doing real experiments. Within the concentration limit above which the salt or salt mixture may cause precipitation of MC, we can reasonably consider that the linear rule of mixing will be valid at any other total salt concentrations.

Thus, the gelation of MC becomes controllable by using a tailored salt mixture of NaCl and NaI. Similarly, when other salts are used as components in a salt mixture and added into a MC solution, the modified gelation temperature of MC would depend on the total water hydration abilities of the component ions and the individual ion concentration. In other words, each components of the salt mixture would contribute its own part in affecting the sol-gel transition, and each contribution would be independent of the other. However, the above hypothesis for other salt pairs (not the pair of NaCl and NaI) would not be valid if there are strong interactions between the salt-out salt and the salt-in salt used.

4.3 Summary

In this chapter, the salt effects on the thermoreversible sol-gel transition behavior of methylcellulose aqueous solutions were investigated systematically by means of micro DSC. The influencing factors such as single salt concentration, type of anion and cation, as well as components in a salt mixture were studied in detail and the mechanisms were proposed. It was found that the sol-gel transition temperature, enthalpy change and entropy change varied significantly with salt type and concentration. The anions were more effective in modulating thermal properties of MC solutions than cations. Some salts (e.g. NaCl, Na₂SO₄) assist in the sol-gel transition while others (e.g. KI, NaSCN) tend to suppress the formation of the gel. Our results are in good agreement with the sequence of the Hofmeister series. It was also found that addition of these salts to a MC solution, no matter a single salt or a salt mixture, did not change the pattern or the mechanism of MC gelation, but the significant shift of the gelation temperature is observed as a function of salt concentration and salt composition. The effect was considered to be due to the hydration of ions in the aqueous solution. The strength of interactions between ions and water molecules determined the salt effects. The “salt-out strength” was defined and obtained for individual anions, which could be correlated with the ion charge density. As for a salt mixture, the gelation temperature was observed to be a linear function of salt ratio when the salt concentration in all samples is fixed. The effects of salts in the mixture were independent with each other, and a linear rule of mixing was found for the effect of the salt mixture. Therefore, the gelation of MC is controllable by a salt mixture consisting of a salt-out salt and a salt-in salt.

Chapter 5: Salt Effects on Rheological Properties

5.1 Rheological Properties during the Sol-gel Transition

In this chapter, rheological tests are used as a supplementary way to study the sol-gel transition behavior of MC solutions, besides thermodynamic analysis using micro DSC, to further prove if the endothermic and exothermic peaks are correlated with the gelation and degelation of MC respectively. The formation and deformation of the three-dimensional gel network are monitored by means of rheology at the same heating and cooling rates in the similar range of temperature. The dynamic viscoelastic properties such as G' and G'' on heating and cooling are measured, which are expected to correlate with the thermodynamic behavior detected by DSC. The dynamic mechanical measurements will provide a better understanding of association and dissociation of MC in solutions upon the change of temperature and at the same time demonstrate the occurrence of gelation upon heating and that of degelation upon cooling from the mechanical point of view.

5.1.1 Determination of Strain Range for Linear Viscoelasticity

To ensure the correlation with thermal dynamic tests, dynamic temperature sweep tests were conducted using the oscillatory rheometer in a similar range of temperature to that of DSC measurements at the same heating rate (1 °C/min). During each experiment the sample was heated from 25 °C to 80 °C at a given small strain, the storage and loss moduli (G' and G'') were measured as a function of temperature. Since the dynamic mechanical approach is aimed to measure undisturbed gelling properties, G' and G'' should be measured within a linear response range where there is no any structure breakage during the measurement, and the material functions are

only functions of the angular frequency [Kim et al., 2003]. Thus, theoretically speaking the applied strain should be as low as possible to ensure the linearity of viscoelasticity. According to the linear scaling law [Wineman and Rajagopal, 2000], in the experiment of the stress response to an applied strain, one material has a characteristic linear region starting from 0 to an upper critical strain. Within this linear region, the modulus is independent of the strain, or the stress is the linear function of strain. We can also define linear viscoelasticity for a given material using a range of stress in which strain varies linearly with stress [Macosko, 1994].

For the same methylcellulose SM 4000, Li [2002] used a low shear strain of 5% for the aqueous solutions with the concentrations ranging from 1 to 2.5 wt%. However, this shear strain is too low for the 0.03 mM MC solution system (about 0.93 wt %) in this study. Gels were classified into two categories by Li [2002]: weak gels (for concentration ≤ 1 wt %) and strong gels (for $c > 1$ wt %). Therefore, the MC solutions used in this work (about 0.93 wt %) are supposed to form weak gels. Due to the low concentration of MC and then low viscosity of solutions at low temperatures, the shear torque is too small to be accurately measured by the transducer when the same shear strain (5%) is employed. From this point of view, the applied strain has to be increased so as to ensure readable and accurate torque.

The preliminary measurement of G' and G'' versus strain (strain sweep test) was carried out to explore the linear viscoelastic character of this solution. Two tests were conducted on 0.03 mM MC solutions at 25 °C and 70 °C respectively. The experimental results show that the moduli are independent of the strain up to 60 %, as illustrated in Figure 5.1. It can be concluded that the shear deformation up to 60 % is

still within the linear region of the MC solution. Thus, in this study, a strain of 50 % was applied to samples at low temperatures when they appeared liquid-like with low moduli. However, the strain was reduced to 20 % after the gel had been formed to avoid overloading or incorrect reading of moduli.

Figure 5.1: G' and G'' of 0.03 mM MC solution are independent of strain up to 60 % at 25 °C.

5.1.2 Rheological Behavior of MC Solutions on Heating and Cooling

An example is given in Figure 5.2 for a 0.03 mM MC solution without salts, where the heating and cooling rates were about 1 °C/min and an angular frequency of 1 rad/s was used. As shown in Figure 5.2, in the heating process from 15 to about 78 °C, there are three distinct regions. The first region is before the crossover of storage modulus G' and loss modulus G'' , where both G' and G'' are small and G' is below G'' , which shows the common viscous behavior of a liquid. In the second region, G' crosses over G'' with increasing temperature, indicating that the elastic component is becoming dominant over the viscous component. G' keeps a gradual rising trend until

about 58 °C, and at the same time G'' also increases slowly with temperature, but always falls below G' .

Figure 5.2: Storage modulus G' and loss modulus G'' as a function of temperature in a heating and a cooling processes for a 0.03 mM MC solution. A shear strain of 50 % was applied in the sol state and adjusted to 20 % after the gel formation. The heating and cooling rates were about 1 °C/min and the angular frequency of 1 rad/s was used.

Since the shear modulus represents interaction between associated clusters or aggregations in the gelling system, and is considered to be proportional to the cross-link density [Carnali and Naser, 1992], the steadily increasing elastic modulus implies an increase in elasticity of the network or cross-link density. Because G' is always higher (no more than one order of magnitude) than G'' , it is assumed that an elastic structure of MC is being formed in water with elevated temperatures. However, on the basis of the micro-calorimetric results shown in Chapter 4, if the hydrophobic association is considered as the only driving force for the formation of a gel or microgel, the second region would not be responsible for gelation because there is no

endothermic peak in this range. There might be some weak hydrophobic association during this region, but these hydrophobic aggregates are not strong enough to build a three-dimensional network structure.

In the last region, G' rises rapidly after 58 °C by more than 10 times of magnitude over a very narrow temperature range, passing the transition temperature of 61.1 °C defined in Chapter 4, and eventually reaches the plateau at about 70 °C. G'' also shows the sharp increase simultaneously, except that it is much weaker than G' . The sharp increase in elastic modulus, as well as the remarkable difference between G' and G'' indicate a significant enhancement in the cross-link density, meaning that the gel has matured beyond that temperature.

Traditionally, the crossover of G' and G'' is used as an indication of the sol-gel transition point [Yoshihito et al., 2001 and Ferry, 1980]. This method is simple and convenient, but the gel point defined by Ferry is usually dependent on frequency used in the measurement. In our system it does not seem reasonable to employ the traditional definition (i.e. crossover of G' and G'') for the sol-gel transition in MC aqueous solutions. First of all, after the crossover in the second region the elastic modulus G' is below ten pascals, which are too low to behave like a complete gel; secondly the crossover point of G' and G'' , in Figure 5.2 is far below the onset temperature observed in the DSC curve. The most significant finding here is that there is an abrupt increase of G' in the vicinity of 61.1 °C, which is correlated excellently with the endothermic peak observed by the micro DSC measurements. Therefore, it would be more appropriate to define the temperature at which an abrupt increase of G' occurs as the sol-gel transition point rather than the crossover point of G' and G'' ,

Chapter 5: Salt effects on rheological properties

and consider the gradual increase range as an accumulating stage (energy as well as elastic strength) to form a gel.

It is considered that water molecules form cage-like structures to surround the hydrophobic groups of MC, so that MC shows good water solubility at low temperatures. These hydrogen bonds and / or cage-like structures around MC chains will be destroyed while heating, leading to exposure of hydrophobic regions, and consequently to the formation of MC hydrophobic aggregates. Such destruction reflects directly in the rheological properties. For example, the steady increase in elastic modulus is corresponding to the growth of clusters or aggregates in semidilute solution, since clusters or aggregates, acting as fillers, tend to increase the viscosity of the solution, which is reflected directly by the G' .

Starting from the plateau at the high temperatures, the cooling process was conducted at a similar cooling rate of 1 °C/min. In contrast to the sharp increase of G' in the heating process in the temperature range from 58 to 70 °C, the gradual decrease in G' with temperature in the early cooling process shows an outstanding deviation from the heating curve. This clearly indicates that the thermally induced hydrophobic dissociation is not an exact reversal of the hydrophobic association occurring in the heating process. Interestingly, the crossover of G' and G'' once again happens after the rapid drop from the plateau, which is near to the peak exothermic temperature in the DSC cooling curve as can be found in Figure 4.1. As discussed in Chapter 4, there is a significant difference between the heating and cooling peak temperatures, which is considered to be caused by the two-step dissociation or degelation of MC [Li et al., 2002]. At the early stage of cooling, G' maintains high value, indicating that although

there is dissociation in the gel, the gel network structure is still strong with the certain level of elasticity, and disassociation is a slow process. When the temperature decreases to be below 40 °C, G' begins to drop dramatically, implying that the network has reached the verge of the overall disruption in the gel network.

Figure 5.3: Schematic MC sol-gel transition displayed by the storage modulus G' in a heating and cooling circle:

1. Liquid viscoelastic behavior
2. Weak and elastic microgel structure
3. Formation of gel network
4. Gradual weakening of gel network
5. Disruption of whole gel structure

Before a MC gel reaches the verge of the disruption of the overall gel network, the network structure has to be gradually weakened through the cooling process. The weakening of the network can be achieved by decreasing the hydrophobic association strength through reducing temperature. The sharp drop in G' after 40 °C can be considered as the massive destruction of the whole gel network. After that all the

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hydrophobic junctions are disintegrated, and MC chains become free again. As a result, the system returns to the original liquid state with low G' and G'' values, and G' is once again lower than G'' after a complete thermoreversible circle.

The schematic illustration of gelation and degelation process reflecting in elastic modulus is given at Figure 5.3 for a better understanding.

5.2 Effects of Salts on Rheological Properties

Similarly to the results of thermodynamic properties in micro DSC, the salt effects on rheology depend on the salt type and salt concentration in the MC solution. Having known the micro-thermal results and the conclusions from Chapter 4, the rheological measurements are able to be designed in a much more direct and focusing way. In this study, one typical salt-out salt (NaCl) and salt-in salt (NaI) were chosen and added into MC aqueous solutions with various salt concentrations in order to observe their effects on the sol-gel transition. Furthermore, a salt mixture consisting of a salt-out salt (NaCl) and a salt-in salt (NaI) was added into a 0.03 mM MC aqueous solution, and the effects on the gelation from samples with various salt ratios were compared when the total salt concentration was fixed.

5.2.1 Salt-out Effects

The temperature dependence of the storage modulus G' during the heating process for MC solutions with various NaCl concentrations is displayed in Figure 5.4. As can be clearly seen from this figure, G' is a monotonically increasing function of temperature. In the presence of NaCl, the G' curves follow the same trace of the salt-free MC solution as described in the previous section except that they shift to the lower

temperatures as NaCl increases. The rheological results indicate that NaCl favors the sol-gel transition of MC. The speedily increase in G' (here defined as the sol-gel transition temperature) for the salt-free MC solution occurs at 64.1 °C. And for the sample with 0.2 M NaCl in MC solution, it happens at 58.2 °C, or 5.9 °C earlier, whereas at 44.7 °C (9.4 °C earlier) for 0.8 M NaCl sample. NaCl has shifted the gel forming temperature to lower, and more salt causes greater effects, which is consistent with the observation of thermal behavior in Figure 4.1. For all samples with or without NaCl, the G' curves follow the same shape, implying that the addition of salt does not change the patterns of gelation during the thermal process, and viscoelastic behavior involved is similar as a result.

Figure 5.4: Storage modulus G' as a function of temperature during heating process on 0.03 mM MC solutions with various NaCl concentrations. The arrows indicate the temperatures of sharp increasing points. Strain 50 % was used at low temperatures, and it was reduced to 20 % after gels had formed.

Addition of salt-out ions has similar effects as increasing temperature since they both cause the loss of water along MC chains. As described before, NaCl is categorized as a salt-out salt, or kosmotropes, or water-structure-makers, mainly due to the strong hydration of Cl^- in water. Cl^- tends to reduce the solubility of other solutes (i.e. MC) in water by taking water molecules away from MC chains, and making stronger interactions with water. The existence of Cl^- destroys the hydrogen-bonding balance by competing with MC molecules for the water molecules, and successfully attracting more water molecules round them due to the strong hydration ability of Cl^- . As a result, at the same temperature, there are more hydrophobic aggregates in the MC solution with Cl^- , which contribute to the higher elastic modulus. Thus an easier networking condition is created in the presence of a salt-out salt, and a lower critical temperature is the subsequent result. It is noted that the sharply increasing regions of G' almost coincide with their respective endothermic peaks in DSC curves, demonstrating a similar transformation behavior involved during heating, although different properties, i.e. mechanical properties in the former and thermodynamic properties in the latter, are measured. If the endothermic heat emerging during the heating process is considered to be attributed mainly to the constitution of gel network structure, then the rheological result reflects the process when the system changes from a viscous solution to an elastic gel.

Another important feature in Figure 5.4 is that the plateaus of the G' curves after gelation tends to enhance with increasing salt concentration, indicating that the gel elastic strength becomes higher after adding more salt. This can be considered by the fact that the presence of the salt enhances the hydrophobicity of MC to result in a stronger hydrophobic association and further a stronger gel network. Although the

adding of salt does not change the patterns of gelation and degelation, the increase in the G' plateau indicates the increase in the cross-linking density of the MC gel, which would reflect that there is some interaction between salt and the hydrophobic association. Ions might not have much direct contribution to the gel strength. They may, however, influence the gel strength by increasing the hydrophobic association strength. From the molecular point of view, since the degree of substitution (DS) of SM 4000 used in this study is 1.8, one glucose unit should have averagely 1.8 methoxyls, although some hydrophobic segments might be tri-substituted, some are di- and mono-substituted. For a salt-free MC solution the aggregation is mainly attributed to the most hydrophobic segments, e.g. tri-substituted, upon heating. The addition of salts creates a more hydrophobic environment, thus, those less hydrophobic groups which do not take part in the gelation in the former case might be cross-linked, contributing to a higher cross-linking density and a stronger gel network. At the same time, less hydrophilic segments result in stronger cross-linking points, and thus the higher elastic modulus.

The correlation between the thermal dynamic results and viscoelasticity during the gelation of MC solutions with NaCl suggests that when NaCl is replaced by other salt-out salts, similar effects can be observed, i.e. the modulus curves will continuously move to lower temperatures while maintaining the same shape; more salt results in lower transition temperature; the sharp increase in G' passes over the peak temperatures in the DSC curve; the plateau elastic modulus to be reached increases with salt concentration. Furthermore, the salt effects on rheological properties are predicted to follow the Hofmeister sequence, which is $\text{PO}_4^{3-} > \text{SO}_4^{2-} > \text{Cl}^- > \text{Br}^- > \text{NO}_3^-$, depending on the anion's salt-out strength as listed in the previous chapter.

5.2.2 Salt-in Effects

NaI was chosen as a typical example of salt-in salts and the rheological measurements were conducted. G' during heating for the MC solutions with addition of 0.2 M, 0.4 M and 0.6 M NaI is presented in Figure 5.5 where the sharp increase G' are observed. The G' curve shifts to higher temperatures with increasing the salt content, indicating a clear salt-in effect.

Figure 5.5: Storage modulus G' as a function of temperature during a heating process at about 1°C for 0.03 mM MC solutions with various NaI concentrations. An angular frequency of 1 rad/s was applied.

It is evident that the rheological behavior is consistent with the thermal behavior discussed previously. According to thermal dynamic properties in DSC, the salt-in anion Γ^- can shift the endothermic peak to higher temperature, together with higher on-set and off-set temperatures. Therefore, the samples with high Γ^- concentrations (e.g. higher than 0.6 M) may exhibit the G' plateau at temperatures higher than 85°C .

However, the plate rheometer (ARES 100FTN1), which uses a water bath to control temperature, does not allow the working temperature to be 80 °C and above. So, when the heating process ends at 80 °C, the gelation is yet to be completed. As a result, the G' terminates before the full plateau is formed and the G' here is not high enough to represent the strength of the gel network. In this case, only the transition temperatures with various I^- concentrations can be obtained, and the strength of gels is not able to compare.

Nevertheless, the salt-in effects of I^- are clear and still significant. As indicated in Figure 5.5, the storage modulus–temperature pattern for 0.03 mM MC solutions with various NaI contents is seen to be consistent with that of samples with added NaCl, except that the sharp increase of G' occurs at higher temperatures, resulting in the higher sol-gel transition temperature. For a salt-free MC solution, the abrupt rising of G' occurs at about 64.1 °C; with 0.2 M NaI added, the G' curve shifts to the right side by 0.3 °C, and by 5.8 °C for 0.6 M one. It is clear that adding of NaI is prone to delay the sharp increase of elastic modulus, making the gelation slower and more difficult. What is more, the transition temperatures are dependent on the salt concentration, agreeing with the previous results from DSC curves.

I^- is classified as Chaotrope or a salt-in ion according to the Hofmeister theory, which can be weakly hydrated in water due to its relatively low charge density. Because I^- is large and has large polarizability, it may easily lose water molecules bonded, and it is certainly expected to have strong dispersion interaction with MC chains in the aqueous solution. Acting like hydrophobic molecules among MC chains and water, they disperse hydrophobic regions of MC from connecting with each other, so that the

MC solution is more stable at a given temperature, showing a higher solubility in water. It is inferable from Figure 5.5 that the MC solutions with NaI show more liquid-like behavior with lower elastic modulus than the salt-free one at the same temperature during the thermal circle. A higher temperature is required to reach the minimal elastic modulus for a solid-like gel to form.

Another important point to be mentioned is the weaker effects from NaI than NaCl on the sol-gel transition, when compared the salt-out effects caused by NaCl in Figure 5.4 and salt-in one from NaI in Figure 5.5. This can be easily estimated by the temperature shift caused by the same amount of salts. For example, the adding of 0.6 M NaCl causes a decrease of the MC sol-gel transition temperature by about 16 °C, while 0.6 M NaI increase the transition temperature by less than 7 °C. The reason for the weak salt-in effect would be attributed to the high temperature effect. As a salt-in salt enhances the sol-gel transition temperature with the mechanism of changing the interaction between MC chains and water molecules, the higher the temperature, the less salt effect is expected. This is because the temperature effect on water structures competes with the salt effect and the former becomes stronger with increasing temperature than the latter. When temperature approaches the boiling temperature of water, the water structures are mainly destroyed by heat so that the effects of salts will be negligible.

5.2.3 Effects of a Salt Mixture on Rheological Properties

The rheological results for the MC solutions with the salt mixtures are presented in Figure 5.6. All the G' curves follow a pattern similar to that of the salt-free solution or the single-salt-containing samples as shown in Figure 5.4 and 5.5.

Figure 5.6 Temperature dependence of storage modulus G' in the heating process at about $1\text{ }^{\circ}\text{C}/\text{min}$ for 0.03mM MC solutions in the presence of salt mixtures with various NaCl versus NaI ratios.

From the 0.4 M NaCl induced MC solution, the G' curve shifts nearly in parallel to the right side with decreasing NaCl and increasing NaI, until the 0.4 NaI one. The abrupt rising of G' appears within a similar narrow temperature range, which is corresponding to the sharp endothermic peak of DSC (see Figure 4.9), demonstrating the consistence between the thermal behavior and the viscoelastic one during the gelation of MC in a similar heating process. However, the transition temperature in G' is always slightly higher than the peak temperature in DSC. This difference would be discussed in details in the following part.

At the same time, as exhibited in Figure 5.6, the MC solution containing 0.4 M NaCl shows the highest G' plateau among all the curves. The G' plateau is slightly reduced

with decreasing NaCl and increasing NaI. In other words, for the formation of a three-dimensional gel network at a fixed total concentration of salt(s), NaCl results in the strongest gel, followed by NaCl-NaI mixtures and eventually by NaI. As discussed in the earlier part, the difference in the gel strength is attributed to the hydrophobicity of MC caused by the salt ions in water. The continuous change of the gel strength from NaCl to NaI through the NaCl-NaI mixtures, strongly suggests that the salt-out and salt-in components in the salt mixture deliver their influences independently, and the gel strength of MC with a salt mixture depends on the composition of the salt mixture and the total salt concentration, which can be excellently correlated with the thermal results in Figure 4.9.

5.3 Comparison of Rheological Properties with Thermal Properties

From the micro DSC curves of MC solutions with or without salts, the endothermic peaks during heating and the exothermic peaks during cooling are observed. In the similar temperature ranges, abrupt rising and decreasing of storage modulus G' are obtained. In this section, the results will be combined and the correlation between the thermal and rheological behavior will be discussed.

The relative heat capacity C_p in micro DSC and storage modulus G' are plotted together in Figure 5.7 as a function of temperature for heating and cooling respectively. Since different samples show the similar pattern during gelation and degelation except the occurrence of characteristic temperature points, only the figures for the MC solution without salt are presented here.

Figure 5.7 Combination of storage modulus G' and heat capacity C_p for a 0.03 mM MC solution during a heating and cooling process. The heating and cooling rates are 1 °C/min.

It is obvious that the dramatic change of storage modulus G' and relative C_p sit at a similar temperature, demonstrating the consistence between the thermal behavior and the viscoelastic one during the gelation of MC, although the exact value might not be the same. For example, in the heating part, G' rises abruptly at about 64.1 °C, whereas the endothermic peak of DSC occurs at 61.1 °C. The former is 3 °C higher than the latter. The similar difference can be found on other samples, as listed in Table 5.1.

Table 5.1: Comparison of DSC peak temperatures and storage modulus G' sharp increasing temperatures upon heating for 0.03 mM MC solution and solutions with various NaCl, NaI and NaCl-NaI mixtures.

	DSC Peak Temp (°C)	G' Sharp Increase Temp (°C)
0.03 mM MC solution	61.1	64.1
NaCl 0.2 M	55.3	58.2
NaCl 0.4 M	50.1	52.9
NaCl 0.6 M	46.8	47.8
NaCl 0.8 M	43.1	44.7
NaI 0.2 M	63.3	65.4
NaI 0.4 M	64.8	67.8
NaI 0.6 M	66.3	69.9
NaCl : NaI = 3:1^a	54.4	56.7
NaCl : NaI = 1:1^a	56.9	59.1
NaCl : NaI = 1:3^a	61.5	64.5

For all samples tested, characteristic temperatures of G' are always slightly higher than the peak temperatures of DSC curves, although both of them show the same continuous increasing or decreasing trends with the added salts. Because the heating and cooling rates in both experiments are the same (1 °C/min) and the same sample is used, the same transformation behavior should occur upon heating. Although the small difference in the transition temperature between DSC and rheological

measurements would be considered to be due to the different sensitivities in methodology between DSC and rheology, we explain it here in a different way.

As discussed earlier, heating a MC solution will cause the destruction of the hydrogen bonds or “cage-like” structures between the water molecules and MC chains, leading to formation of hydrophobic aggregates of MC, followed by the formation of a network structure. In such a procedure, DSC measures the endothermic energy used to destroy the cage-like structures for the formation of hydrophobic aggregates of MC, while the abrupt increase of G' reflects the connection of the hydrophobic aggregates into a gel network. In other words, the system needs to absorb enough energy first for breaking up of hydrogen bonds, and for the subsequent exposure of MC aggregates followed by the formation of a gel cross-linking structure. The heat absorption is reflecting in the DSC curve, which records the thermodynamic behavior with increasing temperature; while the subsequent result is the change in viscoelasticity or sharp rise in G' . Therefore, the endothermic peak emerges first, followed by the increase of modulus in the subsequent step. As a conclusion, the difference between the peak temperature in DSC and the transition temperature observed by rheology is reasonable and inevitable.

5.4 Summary

In this chapter, rheological measurements are conducted for the same samples used in micro DSC measurements in a similar thermal process. The thermal behavior observed by DSC is nicely supported by the rheological properties. Upon heating, the gradual increases of G' and G'' are observed followed by remarkably increasing within a narrow temperature range, which is similar to the endothermic peak range in

DSC curve. The sol-gel transition is confirmed to occur in this narrow temperature range by both DSC and rheological measurements. The effects of salt-out salts, salt-in salts, as well as salt mixtures are consistent with the thermodynamic results, which are dependent on the salt type and the salt concentration. The curves' continuity and pattern show that the viscoelastic behavior involved in all the solutions is similar. The gel strength indicated by the G' plateau is affected by the salt type and salt concentration. A higher content of salt-out salt results in a stronger network structure. When the salt concentration is the same, the gel strength depends on the water restructuring abilities of the composition salts in the solution. The sol-gel transition temperature reflecting in storage modulus is slightly higher than the peak temperature of DSC curve. A possible mechanism has been proposed.

Chapter 6 Turbidity Evidence for Gelation

In this chapter, the clouding behavior and homogeneity of MC solutions with and without salts were investigated systematically through the measurement of turbidity during a thermal cycle. Change in optical transmission observed by the UV/Vis spectrophotometer during heating was used to study the micro-phase transition from a clear solution to a hydrophobic aggregation structure as the system underwent the sol-gel transition. The clouding behavior observed was proved to be in full agreement with the rheological and thermal dynamic properties during MC gelation discussed in the previous chapters. Therefore, salt effects on the MC sol-gel transition and the mechanisms proposed can be well supported and further understood from the optical point of view.

6.1 Changes in Turbidity during MC Gelation

6.1.1 Clouding Behavior during Sol-gel Transition

Absorbance value of 0.03mM salt-free MC solution at the wavelength of 500 nm during heating was first measured using UV/Vis spectroscopy equipped with a Peltier temperature controller. It was then converted into light transmittance and plotted in Figure 6.1 as a function of temperature. As illustrated in Figure 6.1, at low temperatures, the solution shows the high transmittance (nearly 100 %), indicating a clear solution state, which is independent of temperature before the transition. However, the transparent solution becomes progressively turbid when it was heated to a critical temperature, followed by the sharp decrease in transmittance. The sample becomes completely opaque within a narrow temperature range, and the transmittance

drops to nearly zero after that, implying the strong light scattering at high temperatures.

Figure 6.1: Clouding behavior of 0.03 mM MC solution during the thermal circle with the heating and cooling rate of 1 °C/min. The light transmittance is detected at 500 nm.

Figure 6.2: Photos of 0.03 mM MC solution at three different temperatures during heating, representing (a) clear solution at 25 °C; (b) semi-gel status at 61 °C; (c) mature gel at 75 °C.

The corresponding photos of the sample at three typical temperatures can be found in Figure 6.2, representing the transparent solution at low temperature (25 °C), the semi-gel status at the critical transition temperature (61 °C), as well as the complete opaque

gel after gelation (75 °C). The photos clearly show that falling of optical transmission is a result of in the micro-phase transition for gelation instead of precipitation.

In the subsequent cooling process, the turbidity remains unchanged until another critical temperature, when the turbid gel starts to melt, and the transmittance recovers back to the high value within a narrow temperature range. From the fact that the sample turns into a homogeneous, transparent solution after completing a loop, one may come to the same conclusion that the MC gelation is thermo-reversible as described earlier. On the other hand, a similar hysteresis between heating and cooling as shown in DSC and rheological measurements also occurs in the turbidity observation as can be found in Figure 6.1.

The decrease in the light transmittance with increasing temperature is believed to be associated with the gelation process. Sloodmaekers et al. [1991] in their study of Carrageenan gel, and Nossal [1985] in his study of Polyacrylamide gels owed the decreasing light transmittance to the helix domains in the gelled state as compared to that of coils in the solution, which might have spatial variations and dynamic concentration fluctuation, resulting in strong light scattering. In our study of MC gelation, the light scattering is considered to be caused by the hydrophobic aggregates of MC appearing during gelation. As we know, at low temperatures, a MC solution shows the good solubility because of the hydrogen bonds between water molecules and the MC chains and/or the cage-like structures surrounding the methyl groups. The solution is clear, (see in Figure 6.2 a) corresponding to a 100 % transmittance value. Heating of a MC solution will cause the destruction of the hydrogen bonds, and the exposure of hydrophobic regions of MC, leading to the formation of hydrophobic aggregates of MC. As temperature increases, the number and size of hydrophobic

aggregates increase, resulting in the strong scattering of the incident light once the particle size is comparable with the wavelength of 500 nm. The formation of hydrophobic aggregates of MC is gradual and continuous. The phenomenon, called micro-phase separation, is different from the macromolecular phase separation. In a micro phase separation like MC, no precipitation is observed even after the clouding point when the sample is so turbid that nearly all incident light is scattered. As shown in Figure 6.2 c, the sample has been integrated into a cloudy and solidified gel network structure instead of precipitation or macro-phase separation when the temperature is high enough.

Figure 6.3: First derivative of absorbance at the wavelength of 500 nm for MC 0.03mM aqueous solution during heating and cooling. The peak temperatures (62.9 $^{\circ}\text{C}$ in heating and 35.2 $^{\circ}\text{C}$ in cooling) are defined as the clouding point for gelation and melting point for degelation, respectively.

6.1.2 Determination of the Clouding Point

Zheng et al. [2004] defined the temperature at which 50 % of the overall transmittance was reached as the cloud point during the sol-gel transition. Emergayer et al. [2004] obtained the cloud point from the intersection of two straight lines drawn through absorbance at low and high temperatures respectively, or the onset temperature at which the absorbance started to increase. In this study, a more reasonable method is adopted, which is to use the first derivative of absorbance to determine the critical transition temperature.

As shown in Figure 6.3 for the derivative of absorbance with respect to temperature (dA/dT) as a function of the temperature, there exhibits one peak each for both heating and cooling process, which are defined as the clouding point and the melting point during the sol-gel and the gel-sol transitions, correspondingly. Take heating for instance, the derivative of absorbance keeps positive, which can be explained by the increase of absorbance or light scattering due to appearance of hydrophobic region around the MC chains with increasing temperature. The derivative increases sharply after 55 °C, indicating that the hydrophobic aggregates, both in size and in number, are growing in an accelerated path until reaching the peak point. After the peak, the increase of absorbance slows down although it continues to be more turbid, since the derivative value maintains positive. It is obvious that the development of aggregates now levels off, and the number and the size of aggregates are great enough to be connected into an integrated network structure. As a result, more light is scattered until the transmittance falls to nearly zero when the gel is completely formed. Therefore, the temperature when the first derivative of absorbance reaches the peak is the critical point during the transition. It is defined as the clouding point, after which the network starts to form.

In the subsequent cooling curve, the melting point is obtained as 35.2 °C, the peak temperature of the respective derivative curve. It is more than 30 °C lower than the clouding point. The hysteresis is consistent with the previous DSC and rheological results, implying that the hydrophobic dissociation occurring in the cooling process is not an exact reversal of the hydrophobic association occurring in the heating process. And the cooling peak is broader than that of heating, indicating a slower degelation procedure than gelation. This is also correlated with difference between heating and cooling DSC curves, when heating shows a sharp endothermic peak, compared to the broad exothermic peak with a shoulder.

6.2 Salt Effects on Clouding Behavior

The effects of salt-out salts, salt-in salts and salt mixtures on turbidity of MC solutions during gelation and degelation have been investigated respectively by following the similar procedure as that of salt-free MC solutions by means of UV/Vis spectrometer. NaCl and NaI are the corresponding representatives of salt-out and salt-in salts respectively. Figure 6.4, Figure 6.5 and Figure 6.6 shows the light transmittance of MC aqueous solutions with various concentrations of NaCl, NaI and NaCl-NaI salt mixtures with different NaCl to NaI ratios during heating and cooling respectively. It is obvious that all transmittance curves follow similar pattern, implying the similar turbidity behavior involved in the thermal-induced sol-gel transition. However, NaCl shifts the curves to the left. The higher the salt concentration, the more the curve shifts. Within the transition temperature range, the MC solution added with NaCl shows the higher turbidity than the salt-free MC solution at the same temperature, or in other words, NaCl makes the turbidity appear at lower temperature.

On the other hand, NaI pushes the curves to the right, delaying the clouding behavior with increasing temperature. As for the salt mixtures in Figure 6.6, the transmittance curve falls between the two pure salt-out and salt-in curves with the same salt content, depending on the ratio of NaCl to NaI or the new balance of the salt-out and salt-in effect. From pure NaCl to pure NaI, the curves follow the trend of weakening salt-out and strengthening salt-in ability. In the following cooling curves, the sample becomes clear after the melting point with the reducing temperatures, remaining the similar delay from the clouding point during heating as what the thermal and rheological properties show during the thermal cycle. The position of the curve and the melting point depend on the salt-out strength of the salt mixture added, or the combined effect from Cl^- and I^- , since the Cl^- ions and I^- ions are proved to deliver their effects independently. The higher the salt-out strength or a higher ratio of NaCl results in a lower melting point.

The salt effects observed are completely supported by the mechanisms proposed earlier. NaCl tends to compete with MC chains for water molecules, resulting in breaking of the hydrogen bonds between the macromolecules and water, and in the decrease of MC solubility in water. Consequently, there are more hydrophobic aggregates in a salted MC solution than a salt-free one at the same temperature, leading to higher light scattering and turbidity. In other words, the clouding phenomena will appear at lower temperature for a NaCl-added MC solution. As for the NaI, a typical salt-in salt, it exhibits weaker interactions with water, acting like hydrophobic particles in the solution. So, the MC chains dispersed by big I^- ions in the solution, would have fewer chances of interconnection or association. At the same temperature, the solution is less cloudy because of fewer hydrophobic aggregates

existing in the system, leading to a higher transmittance than the salt free MC solution, and the delay of the clouding behavior.

Figure 6.4 Transmittance of MC solutions with different concentrations of salt-out salt (NaCl) during heating and cooling at the temperature changing rate of 1 °C/min. The absorbance is detected at 500 nm.

Figure 6.5 Transmittance of MC solutions with different concentrations of salt-in salt (NaI) during heating and cooling at the temperature changing rate of 1 °C/min. The absorbance is detected at 500 nm.

Figure 6.6 Transmittance of MC solutions with different ratios of NaCl-NaI mixtures during heating and cooling at the temperature changing rate of 1°C/min. The absorbance is detected at 500 nm.

6.3 Correlation with the Thermal and Rheological Properties

The calorimetric and rheometric results showing the salt-out and the salt-in sol-gel transitions of MC in aqueous solutions can be further supported optically. The critical temperatures in three measurements, i.e. the peak temperature in DSC heat capacity curve, the sharp increase temperature in elastic modulus, and the clouding point of light transmittance during heating, show the nice consistence with each other, although the values determined by the three methods may not necessarily be the exactly same because of the difference in methodologies and sensitivities for the three methods. As shown in Table 6.1, the clouding points, obtained as the temperatures at which the derivative of light absorbance from the incident light of 500 nm (dA/dT) hits the peak in the UV/Vis detection, are always slightly higher than the sol-gel transition temperatures determined by the DSC measurement, but close to those determined by rheological measurements.

Table 6.1: Comparison of DSC peak temperatures, storage modulus G' sharp increasing temperatures and clouding points upon heating for 0.03 mM MC solution and solutions with various NaCl, NaI and NaCl-NaI mixtures.

	DSC Peak Temp	G' Sharp Temp	Clouding Point
Salt-free solution	61.1	64.1	62.9
NaCl 0.2 M	55.3	58.2	56.0
NaCl 0.4 M	50.1	52.9	51.0
NaCl 0.6 M	46.8	47.8	49.0
NaCl 0.8 M	43.1	44.7	44.0
NaI 0.2 M	63.3	65.4	65.0
NaI 0.4 M	64.8	67.8	66.1
NaI 0.6 M	66.3	69.9	69.0

NaCl : NaI = 3:1^a	54.4	56.7	55.1
NaCl : NaI = 1:1^a	56.9	59.1	58.2
NaCl : NaI = 1:3^a	61.5	64.5	62.9

^a the total salt concentration is 0.4 M

From the clouding points for all salt-induced samples, the salt-out or salt-in effects caused by the adding salts are obvious and nicely correlated with the sol-gel transition temperature observed from the DSC and rheological results. With regard to the small difference between the individual critical temperatures, the explanation proposed in the previous chapter is also suitable and reasonable to the clouding behavior. Heat absorption occurs first to cause the destroying of the hydrogen bonds and the exposure of hydrophobic aggregates. The subsequent appearance of hydrophobic associations leads to the light scattering, and the turbidity, which can be detected by the optical measurement. Therefore, the turbidity should happen somewhat later after the heat absorbance. This is similar to the relationship between the internal change and the subsequent outcome. In conclusion, the turbidity result is in full agreement with the thermal and rheological behavior, and the mechanisms proposed previously are well supported by the clouding observation.

6.4 Summary

The optical transmittance of MC gels with and without salts was measured successfully as a function of temperature. The turbidity measurement is proved to be an effective tool to characterize the development of aggregates in the system and the formation of gel net work structure. The clouding point and melting point during the gelation and degelation are defined using the derivative of absorbance, which are in term consistent with the gelation temperatures detected by thermal and rheological methods respectively. Thus, the turbidity method applied here appears to offer a new,

precise and easy means to study the sol-gel transition in salt-induced MC gelation and related transitional properties, as well as the aggregation behavior of other gelling system, especially with the existence of other additives.

Chapter 7 Gel Morphology

After the calorimetric, rheological and turbidity studies on the effects of salt effects on the sol-gel transition of MC, Scanning Electron Microscopy (SEM) is employed in this chapter to directly observe the microscopic network structure of hydrogel after dried by freeze-drying (FD). FD is used as an image processing technique to maintain the cross-link structure of a gel formed at high temperature. At the same time, it is found that the drying may reduce some possible deformation to the gel morphology and micro-structure. The aim of the study is to investigate the microscopic morphology, cross-link density, and homogeneity of hydrogels formed from methylcellulose aqueous solutions with different polymer concentrations and with other additions. FD and SEM are used as an effective tool to obtain gel image in this study.

7.1 Drying on Dish

As described in Chapter 3, MC solutions were heated to a high temperature (70 °C) to get a stable solid gel, and then transferred to liquid nitrogen (about -110 °C) for pre-freezing followed by vacuum-drying at the same temperature. During the drying procedure, what container should be used to keep the sample is the first problem to be solved. According to Brinker and Scherer [1990], if the drying is in the form of a cylinder or sphere, the pressure gradients are shallower than in a plate, so the tensile stresses are smaller than in a plate. This reflects the fact that liquid flowing out of the cylinder passes through a volume of gel that increases with (radius)² [or (radius)³, for a sphere], so it has a smaller resistance than the case of liquid flowing in a straight line out of a plate. However, considering drying speed and efficiency, a plate seems a better choice than a cylinder.

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Therefore, in this work the sample is put either on a dish or in a tube during the whole procedure, and the subsequent results will be compared and discussed.

0.03 mM MC cryogel

0.05 mM MC cryogel

Figure 7.1 SEM images of MC cryogels with 0.03 mM and 0.05 mM MC at x150 and x400 magnification respectively. Specimens were obtained by freeze-drying on a dish, and observed through vertical section.

After freeze-drying, a white opaque fibrous cryogel with a high surface area and porosity is obtained. Since the dry sample is not as homogeneous as expected, both vertical and horizontal sections are sent to gold-sputtering and then SEM observation. As illustrated in Figure 7.1, on the vertical fractured surface, MC cryogels freeze-dried in the form of plate result in flakes with extremely high surface area. When the polymer concentration increases, flakes become thicker, denser and firmer. This can be related to the fact that

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higher concentration of MC solution leads to higher viscosity and greater gel strength evaluated by rheological measurement.

Figure 7.2 SEM images of MC cryogels with 0.03 mM and 0.05 mM MC at x 100 and x 600 magnification respectively. Specimens were obtained by freeze-drying on a dish. The observing surface is the horizontal section.

Figure 7.2, on the other hand, displays the SEM observation of the horizontally fractured surfaces of MC gels. Besides the section of flakes, there shows the micro-porous structure interconnecting the flakes to form the cross-linking. As observed in the vertical section in Figure 7.1, the flakes of 0.05 mM MC gel are denser with less space among them. Therefore, it exhibits less cross-linked and there is a smaller pore volume between two flakes as shown in the horizontally fractured surface in Figure 7.2. Compared with

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the sample at the concentration of 0.05 mM, where a complete homogeneous network structure could not be observed, the pore distribution in the gel with 0.03 mM MC is more regular between two flakes with the pore size of about 50 micros in diameter. However, generally speaking, the array and the scale of these pores are so irregular that in some area, fibers are just formed and entangled physically. There is not a homogeneous porous structure as expected. This may be due to the existence of a pressure gradient during water sublimation. Since the drying is conducted in the form of a plate with large surface area, the tension at the surface of the plate has inevitably caused certain deformation on the gel network structure. In other words, the heterogeneous distribution of fibrous structures obtained in Figure 7.1 and 7.2 might not represent the actual gel cross-linking structure in the wet state.

7.2 Drying in Tube

To minimize the possible tensile stress caused by pressure gradients, in the following experiments the sample is kept in a tube during the drying procedure, and new SEM microphotographs are obtained in Figure 7.3 with 400, 800 and 1600 magnifications correspondently. Instead of flake structures, the porous texture dried in the form of cylinder tends to form three-dimensional fibril-like network, although at low concentration, 0.03mM for example, the cell wall is too thin to develop full honeycomb cell structure, as displayed by Kim et al. [2003] the structure of Carbopol gel using cryo-SEM. In the case of the dry gel from 0.05 mM MC solution, it exhibits the mesh-like network with a high cross-link density. It is reasonable that the increase in the polymer concentration makes the hydrophobic aggregates associate more densely with each other,

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Figure 7.3 SEM images of MC cryogels with 0.03 mM and 0.05 mM MC at x 400, x 800 and x 1600 magnifications respectively. Specimens were obtained by freeze-drying in a tube.

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so that a network structure with smaller mesh size and a higher density is composed due to the increasing number of junction points. While at low polymer concentration, high water content results in big pores and small gel volumes, the weak microstructure can be easily destroyed when the specimen is fractured to make a cross section for gold sputtering. This can be well indicated in Figure 7.3 for the 0.03 mM MC gel at the magnification of 1600.

7.3 Discussion about Gel Imaging Technique

The greatest doubt for studying the gel morphology is that there is no certainty whether what is eventually seen has reflected the actual gel structure in the wet state. In this study, the dried gel is obtained using the freeze-drying method where the water is sublimated from the frozen gel under vacuum at freezing temperature. Hong and Chen [1998] in their study of polyvinyl chloride/dioxane gels assumed that the structure of freeze-dried gel was the same as that of wet gel, because no remarkable shrinkage (less than 1 %) of gel volume occurred after freeze-drying. However, from our experiments, it seems that other factors, such as drying form (in the tube or on the dish), drying speed (which depends much on the pressure of vacuum during water sublimation) and fracturing pattern will affect the morphology of dried gels.

From the experiments so far, the conclusion is, in order to minimize the shrinkage and deformation caused by freeze-drying, a sample should be put in the tube, and dried under high vacuum at possible higher temperature (but still in iced state) so as to speed up the drying process. At the same time, an advanced technique is needed to fracture the dried

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sample, and most ideally, the whole procedure, including freeze-drying, fracturing and SEM observation, is conducted under a cryo-system. In our preliminary tests, the result of SEM observation shows that the gel morphology consists of a fibril-like mesh structure and the mesh size decreases with increasing polymer concentration, which can be related to the increase in the aggregation degree and the number of junction points in the wet gel. It is shown that freeze-drying is an effective method to prepare dried gel network for morphology observation. But there are some problems that have not been solved in this work, e.g. the irregular porous array, which deserve to be further investigated.

7.4 Summary

The morphological image of MC gels can be characterized by SEM after freeze-drying. The fibrous structure and the pore size are affected by drying form and the polymer concentration. It is proved that the gel freeze-dried in a tube better reflects the real network in the wet state, where the mesh-like micro structure is observed. The higher polymer concentration results in smaller pores with higher density. The SEM observation reveals heterogeneities in pore array and distribution. This work has provided preliminary results for the morphological study of gels in the future.

Chapter 8 Conclusions and Recommendations on Future Work

8.1 Conclusions

The thermoreversible gelation of MC in various salt solutions was examined using DSC, rheometry, and turbidimetry. The influencing factors such as salt concentration, anion and cation were studied systematically. The possible mechanisms of salt effects on the MC sol-gel transition behavior were proposed from a molecular origin. The following conclusions can be drawn:

- The sol-gel transition temperature, enthalpy change and entropy change vary significantly with salt type, in which anions are more effective in modulating thermal properties of MC solutions than cations.
- Salts can be classified as salt-out and salt-in salts, primarily depending on their anions. Our results are in good agreement with the sequence of the Hofmeister series, $\text{PO}_4^{3-} > \text{SO}_4^{2-} > \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \text{I}^- > \text{SCN}^-$. Ions on the left show salt-out effects on MC gelation, which can reduce the sol-gel transition temperature and accelerate the gelling process; while I^- and SCN^- are typical examples as salt-in ions, which tend to delay the occurrence of MC gelation. The strength of interaction between ions and water molecules determines the salt effects.
- The “salt-out strength” is defined and obtained, which is found to be dependent on the charge density of the anion. The salt-out strengths of all anions tested are obtained and their effects on gelation are explained.

- Adding of the salts does not change the mechanism of gelation, but it is able to shift the gelation temperature, which is a linear function of salt concentration.
- When a salt mixture consisting of a salt-out and a salt-in salt is added into a MC solution, the pattern or mechanism of gelation of MC is not changed, but the gelation temperature is shifted to lower or higher temperatures according to the composition of the salt mixture. When the total salt concentration is fixed, the shift in the gelation temperature by the salt mixture is observed to be a linear function of salt ratio. The effects of salts in the mixture are independent of each other, and a linear rule of mixing is found for the effect of the salt mixture.
- In the rheological measurements, storage modulus G' and loss modulus G'' are measured to observe the development of MC gels with increasing temperature. The narrow temperature range where G' sharply increases is consistent with the peak region in the DSC curve, confirming the sol-gel transition on heating.
- The thermal behavior observed by micro DSC is nicely supported by the rheological properties. The gel strength indicated by the G' plateau is affected by the salt type and the salt concentration.
- The turbidity measurement gives another supportive evidence for the MC sol-gel transition, as well as the salt effects on it. The optical transmittance of the MC solution is found to decrease as the sample undergoes the sol-gel transition. The clouding point, defined as the temperature when the differential of absorbance with respect to temperature (dA/dT) comes to the peak, is obtained during the

heating, and the clouding point is corresponding to the sol-gel transition temperatures obtained in DSC and rheological measurement. The clouding points for MC aqueous solutions with added salt-out salts, salt-in salts and salt mixtures during the thermal cycles are obtained, and the same salt effects can be observed and explained with the same mechanism. Turbidity is proved to be a powerful tool for studying the MC sol-gel transition and the salt effects on the sol-gel transition of MC.

- The morphological image of MC gels can be characterized by SEM after freeze-drying. The fibrous structure and pore size are affected by drying forms and polymer concentration. It is proved that the gel freeze-dried in a tube better reflects the real network in the wet state, where the mesh-like micro structure is observed. The higher polymer concentration results in smaller pores with higher density. The SEM observation reveals heterogeneities in pore array and distribution, which is worth further investigation.

8.2 Recommendations for Future Work

In this study, we have focused on the effects from anions, which have been proved to be a decisive factor for the salt effects. Besides the potassium salt, which is proved to deliver a similar effect as sodium salt, more salts with various cations, especially cations with different charge number and ionic size (e.g. MgCl_2 , CaCl_2 , AlCl_3 , CrCl_3 etc.), can be used to further study the cationic effects. What is more, acid and alkali can be added individually into MC solutions to observe the pH influence. Besides salts, different surfactants are found to affect the gelation of MC extremely, which is to provide another direction in the study of MC gelation.

Furthermore, fitting of the curve is expected to be an effective way to study gelation and salt effects on gelation further, so that a model can be established to investigate the series of micro DSC curves systematically.

The preliminary study on gel morphological image has been conducted in this work. Two drying forms (i.e. plate and cylinder) have been used to detect the feasibility. Although the SEM morphological result is significant, the imaging and freeze-drying technique needs to be further optimized. For example, cryo-SEM and advanced gel cutting system might be employed to minimize the deformation during drying process. The influence of freeze-drying temperature and pressure also deserves further investigation. The FD and SEM morphological technique can be practiced in other gelling systems after improvement, including the salted MC hydrogels.

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