

Chelation of Chitosan Derivatives with Zinc Ions.

II. Association Complexes of Zn^{2+} onto *O,N*-Carboxymethyl Chitosan

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ABSTRACT: The chelation of zinc ions onto *O,N*-carboxymethyl chitosan (ONCMCh) was characterized using a Fourier transform infrared (FTIR) spectrophotometer and a scanning electron microscope (SEM). From the FTIR spectra, little change in the absorption intensities and frequencies at $3300\text{--}3600\text{ cm}^{-1}$ of Zn^{2+} —ONCMCh chelated specimens suggested that —OH and —NH₂ groups were not participating in the chelation reaction. The absence of absorption bands at $1755\text{--}1700\text{ cm}^{-1}$ suggested that the carboxyl group C=O was not ionized, and the ionized C=O bands were observed at $1400\text{--}1600\text{ cm}^{-1}$ for chelated specimens. Thus, the chelation sites took place at the carboxyl group rather than at the —OH and NH₂ groups. It also confirmed that water-insoluble chelates, which were formed through the Zn—O and Zn—N bonds, presented a tetrahedral structure. The water-soluble complexes where zinc ions connected with oxygen of C=O and water molecules were only due to electron attraction. Formation of different microstructures on the surfaces, as revealed by SEM, provided evidence to distinguish different chelating mechanisms between water-soluble and water-insoluble complexes. © 2000 John Wiley & Sons, Inc. *J Appl Polym Sci* 79: 1476–1485, 2001

Key words: carbonyl; amine; microstructure; morphology; FTIR; SEM

INTRODUCTION

The phenomenon of complex formation between metal ions and carbohydrates is not new. After the first article on an adduct of D-glucose with sodium chloride was published in 1825,¹ complex compounds attracted as much attention for their tremendous importance in solving the problem of chemical bonding as for their own unique properties and practical applications.

Much of today's research has further indicated that the capture of metal ions by forming complexes with chitosan and its derivatives has been

used in the sequestration or removal of metal ions, solvent extraction, dyeing, catalysis, water treatment, and many other industrial processes.² It has also been realized, in recent years, that the mechanism of complex formation of metals with chitosan is manifold and probably dominated by different processes taking place simultaneously, such as adsorption, ion-exchange and chelation, under different conditions. Butelman³ patented the preparation of complexes of iron and other metals with sulfonated derivatives of chitosan. Chiessi et al.⁴ reported different mechanisms for the formation of copper complexes with chitosan ligands attaching to pentanedioic acid, propanoic acid, and the deoxylactyl moiety. In a subsequent study,⁵ these same authors indicated that the coordination of the polymeric ligands to Fe^{3+} and

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Cu^{2+} ions involved the donor nitrogen atom of the uncharged amines. The additional charge interactions with OH groups at the side chain were responsible for a decrease in symmetry in metal-chitosan complexes. Recently, Japanese scientists demonstrated similar complex formation of crosslinked Cu^{2+} -chitosan and carboxymethylated chitosan with palladium and platinum.⁶ However, the site of the complexation between carboxymethyl chitin and Cu^{2+} was a cavity between the carboxyl groups attaching to the carbon C3 of the neighboring residue.⁷

Zn^{2+} ions, which have received the least attention, have been found to form a unique acetate complex. The acetate group and the zinc atom to be chelated were interacted with two water molecules to form a $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ complex.^{8,9} In addition to the bridging and bidentate behavior, a unidentate attachment of the acetate group was observed in the chemistry of zinc.¹⁰

In a previous article, we reported the preparation of chelates of *O,N*-carboxymethyl chitosan (ONCMCh) with Zn^{2+} ions and the kinetic parameters influencing the complexation yields of water-insoluble and water-soluble products.¹¹ While the formation of water-soluble products was occasioned by the electrostatic-attraction mechanism, the water-insoluble products were predominantly formed by chelation of Zn^{2+} with ONCMCh. The objective of this investigation was to elucidate the formation of coordinate linkages and the surface microstructure when Zn^{2+} ions were complexing with ONCMCh under specific conditions. Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) were used.

EXPERIMENTAL

Preparation of Chelated Compounds

The chelated compounds of ONCMCh for this study were prepared by the Hayes' method which was described in a previous article.^{11,13} In short, chitosan sodium salt reacted with monochloroacetic acid at 25 and 60°C for 1 and 3 h, respectively, to prepare *O*-substituted and *N*-substituted CMChs. In the present experiment, sodium monochloroacetate was used to displace its acid form. Water-soluble and water-insoluble complexes were generated by this method.

FTIR Spectroscopy

The infrared spectra of water-insoluble and water-soluble compounds of ONCMCh- Zn^{2+} were

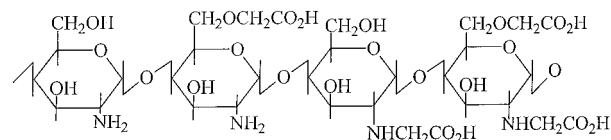


Figure 1 Chemical structure of ONCMCh with DS < 1.0.

obtained from a Nicolet 20 DX FTIR spectrophotometer. Pressed pellets were prepared by grinding the powder specimens with IR-grade KBr in an agate mortar.

SEM

An SEM study was performed with a JSM-IC 848 scanning electron microscope. The specimens were sputter-coated with gold prior to examination.

RESULTS AND DISCUSSION

CMCh is a carboxymethyl ether of polyglucosamine which has unique properties and has been used for pharmaceutical application.¹² The degree of substitution of carboxymethyl groups per glucosamine monomer unit in the chitosan used was determined to be less than 1.0. Therefore, the principal functional groups existing in ONCMCh are hydroxyl, carboxyl, and amine groups. The general structure of ONCMCh is shown in Figure 1. Obviously, these functional groups are all metal-sensitive and capable of formation of complexes with Zn^{2+} ions. The modes of bonding of Zn^{2+} to ONCMCh ligand was determined by FTIR spectroscopy.

FTIR Spectra

Generally, it is difficult to use infrared spectroscopy to elucidate fully the structure of complexes. However, it can provide some useful information on the functional groups of the ligand chelated with metal ions, especially to the metal complexes of carboxylic acids.^{14,15} This is usually performed by examination of the carbon-oxygen stretching frequencies and their related values to the nature of the carboxylate coordination. Because of the low symmetry of carboxylate ions, $\text{R}-\text{COO}^-$, different types of carboxylate coordinations cannot be distinguished on the basis of the number of infrared active vibrations.¹⁶ The FTIR spectra of

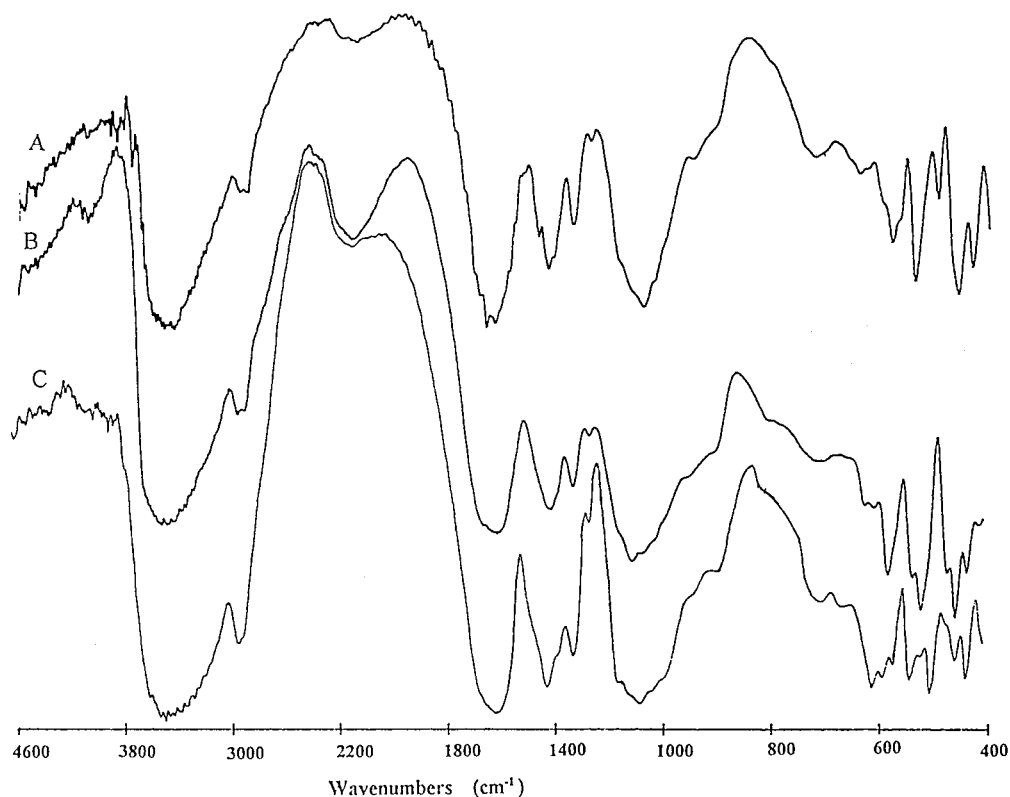


Figure 2 FTIR spectra of ONCMCh and its water-insoluble complexes. Spectrum A: 2.0 g ONCMCh, 0.005M Zn^{2+} , 50°C, 2 h; spectrum B: 2.0 g ONCMCh, 0.005M Zn^{2+} , 50°C, 4 h; spectrum C: ONCMCh (control).

ONCMCh and its chelates with Zn^{2+} are shown in Figures 2 and 3 for water-insoluble and water-soluble compounds, respectively. The assignment of the principal bands for these specimens is shown in Table I. It must be pointed out that spectra A and B were obtained from the water-insoluble chelated products.

FTIR Analysis of Water-Insoluble Product

OH and NH Stretching Vibrations

For the unchelated, control ONCMCh, FTIR spectra showed a broad band at 3353–3500 cm^{-1} , which corresponds to the amine ($-NH_2$) and hydroxyl ($-OH$) groups. The stretching vibrations of OH in carboxylic salts are found at 3500–3300 cm^{-1} , and its asymmetric and symmetric modes appeared near 3500 and 3300 cm^{-1} . The amine NH stretching band is usually found in the 3130–3030- cm^{-1} region.¹⁷ The shape of the OH absorption band and its relative intensity can sometimes be used to give an indication of the type of structure present. Since there is a good deal of over-

lapping between the OH and NH vibrations in this region, making the differentiation is not always possible. Based on these assignments, the bands at 3300–3600 cm^{-1} with a small shoulder should be associated mainly with NH_2 and OH groups. The presence of both groups with little change in the shape and frequencies indicates neither the $-OH$ nor the $-NH_2$ group are involved in complexation.^{18,19} On the other hand, when comparing the bands appearing at 1326 and 1261 cm^{-1} in Figure 2, no major change and shifting were observed from those bands among the complexes A, B, and control C specimens. Both 1326 and 1261 cm^{-1} represented the OH deformation vibrations.^{20,21} Thus, this is a strong indication that OH groups in ONCMCh are not involved in metal complexation.

The band around 2900 cm^{-1} is usually assigned to $-CH$ and double-bond vibrations. However, the following observations were made:

1. Two characteristic bands in the 3000–2000- cm^{-1} region are usually shown by

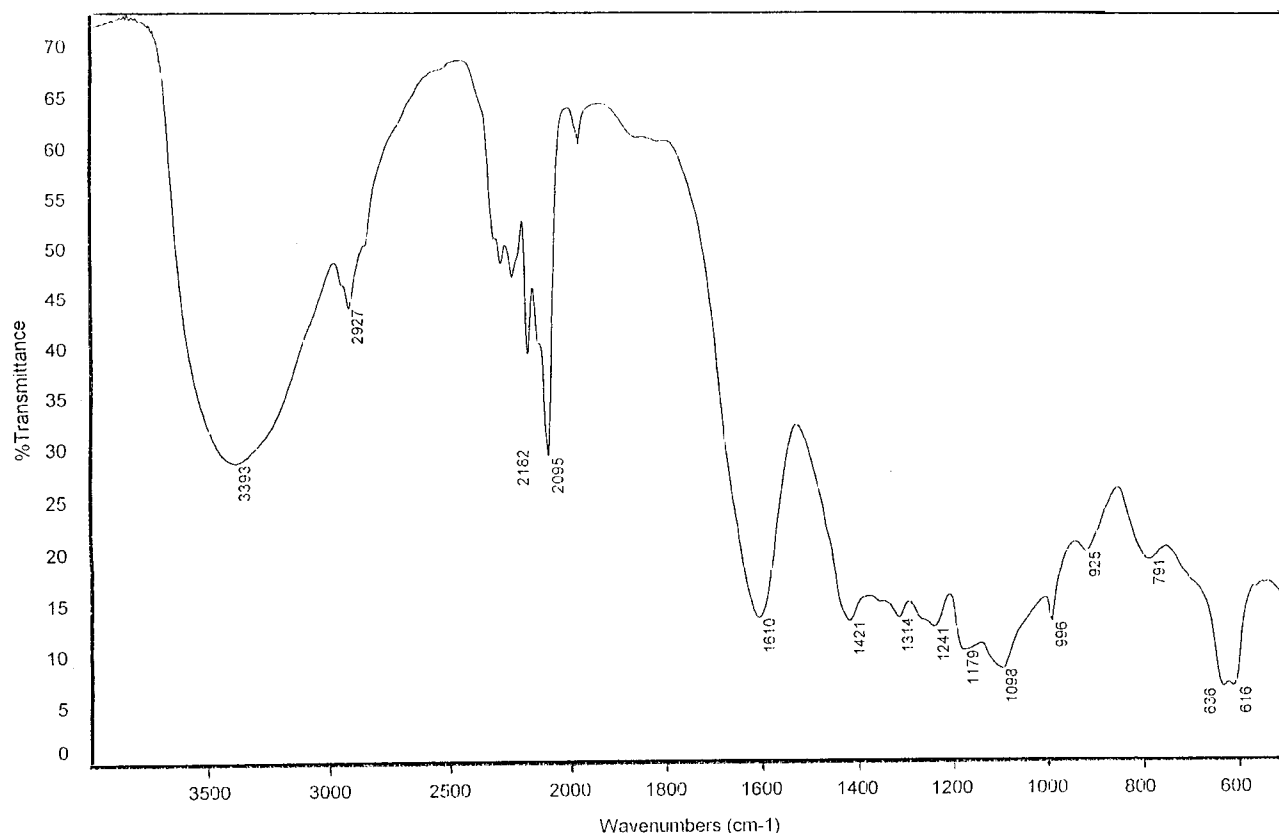


Figure 3 FTIR spectrum of water-soluble complex of ONCMCh.

amino acids. As can be seen in Figure 2, two bands near the 2900- and 2140- cm^{-1} regions are shown for the free ligand and its complexes. Structurally, the ONCMCh can be taken into account as a *N*-chitosan-substituted amino acid.²²

- For *N*-substituted amino acids, a stretching vibration near the 2900- cm^{-1} region

must be shown by the charged $=\text{N}^+\text{H}-$ or the $-\text{N}^+\text{H}_2-$ groups.^{23,24}

Accordingly, we assigned the band at 2918 cm^{-1} to the charged NH. Since under an acid condition, $-\text{NH} + \text{H}^+ \rightarrow \text{NH}_2^+$, and, hence, the secondary amine ions involved in complexation with Zn^{2+} . This was confirmed by the emer-

Table I Major FTIR Bands and Their Assignments for ONCMCh and Its Complexes

| Bands | ONCMCh-Zn ²⁺ Spectrum A | ONCMCh-Zn ²⁺ Spectrum B | ONCMCh Spectrum C |
|---|---------------------------------------|---------------------------------------|----------------------|
| $\nu(\text{OH}, \text{NH}_2, \text{NH})$ | 3550, 3435 | 3500, 3344 | 3550-3350 |
| $\nu(\text{NH}_2^+)$ | 2943, 2890 | 2943, 2885 | 2918 |
| $\nu_{\text{as}}(\text{CO}_2^-)$ | 1655, 1614 | 1614 | 1614 |
| $\nu_{\text{sy}}(\text{CO}_2^-)$ | 1458, 1417 | 1417 | 1417 |
| $\nu(\text{C}-\text{O}, \text{C}-\text{N})$ | 1056 | 1113 | 1070 |
| $\nu(\text{Zn}-\text{N})$ | 564, 531 | 575, 528, 511 | |
| $\nu(\text{Zn}-\text{O})$ | 490, 457, 424 | 466, 446, 430 | |

Reaction conditions: spectrum A: 2.0 g ONCMCh, 200 cc 0.005M Zn^{2+} , 50°C, 2 h; spectrum B: 2.0 g ONCMCh, 200 cc 0.005M Zn^{2+} , 50°C, 4 h; spectrum C: ONCMCh (control).

gences of band splitting and shifting in frequencies.

Another characteristic band in this region is near 2140 cm^{-1} . Liefer and Lippincott²⁵ identified this band as a combinatory band associated with the COO^- group having a zwitterion structure.

COOH Stretching Vibrations

In general, the un-ionized and uncoordinated carboxylic acid $\text{C}=\text{O}$ stretching band occurs at $1750\text{--}1700\text{ cm}^{-1}$, whereas the ionized and coordinated CO_2 stretching band occurs at $1650\text{--}1590\text{ cm}^{-1}$.²⁶ In this study, no absorption band in the region of $1750\text{--}1700\text{ cm}^{-1}$ was observed, and the presence of strong bands at 1614 and 1417 cm^{-1} were observed in control specimens, indicating that the carboxyl groups in ONCMCh have a high degree of an ionic characteristic. Furthermore, the 1614 and 1417 cm^{-1} bands also correspond to the asymmetric and symmetrical stretchings of the carboxylate anion, respectively.

In comparison of the IR spectra of the ONCMCh- Zn^{2+} complexes in A, B, and free ligand C, the absence of bands about $1750\text{--}1700\text{ cm}^{-1}$ in all specimens suggested that the carboxyl group $\text{C}=\text{O}$ was un-ionized. The ionized carbonyl frequencies in chelating samples remaining at the usual positions near 1600 and 1400 cm^{-1} were observed. This suggests that the chelation sites of ONCMCh with Zn^{2+} occurred in the ionized moiety of the carboxyl groups. This is plausible because the FTIR spectra exhibit essentially the same resonance ionic bonds in the carboxylate ion and a degree of equivalence between the two oxygen atoms of the carboxyl group to form ionic $\text{C}-\text{Zn}^{2+}$ bonds in complexes. The oxygen-metal bonds in these complexes must be essentially ionic in nature.^{27,28}

Spectrum A also revealed that the chelate exhibits two extra bands at 1655 and 1458 cm^{-1} . This asymmetric characteristic of carboxyl groups probably is associated with the nonionic form of carboxyl in an alternate complex which is produced in the early period of chelation (i.e., 2-h reaction). When the reaction was prolonged to 4 h (spectrum B), these two bands disappeared and only the ionic characteristic bands at 1614 and 1417 cm^{-1} remained. This distinct change can be due to the unstable complex structures being formed at the early stage of the reaction. As the reaction was extended, the complex structures were gradually destroyed or converted into a resonance ionic carboxyl in the complexes.

Other Characteristic Bands

The other characteristic bands may also be used to identify ONCMCh:

1. The bands near 1070 cm^{-1} , which appeared in the free ligand (spectrum C), are usually attributed to $\text{C}-\text{O}$ and the $\text{C}-\text{N}$ stretching vibrations.^{21,29} These characteristic bands change in their intensities and frequencies in the spectra of Zn-ONCMCh complexes, indicating that $\text{C}-\text{O}$ and $\text{C}-\text{N}$ groups are involved in the coordination.³⁰
2. The glucose ring vibrations are strongly coupled to the changes in the electron distribution caused by the ionization and complexation of the moiety in the free ligand. Hence, the bands between 800 and 500 cm^{-1} , due to the skeletal deformations of $\text{C}-\text{C}-\text{C}$, $\text{C}-\text{O}-\text{C}$, or $\text{C}-\text{C}-\text{N}$ groups of the free ligand,^{31,32} showed considerable alterations upon chelation with zinc ions (Fig. 2).
3. The band assignments to the $\text{M}-\text{N}$ stretching vibrations of the amine complexes have always been controversial. However, the new bands observed in the ranges of $511\text{--}580$ and $424\text{--}490\text{ cm}^{-1}$, which were not found in the spectra of the free ligand, are possibly attributed to $\text{M}-\text{N}$ and $\text{M}-\text{O}$ vibrations modes.^{33,34}

From these observations, it is quite clear that zinc ions formed complexes with the nitrogen and oxygen atoms in ONCMCh. The FTIR analyses clearly demonstrated the participation of $-\text{COOH}$ and $-\text{NH}$ groups in the water-soluble products in the chelation reaction.

Based on these findings coupled with earlier works on amino acid and other carboxylic acids which display a tetrahedral coordination with Zn^{2+} , plausible complex mechanisms for the water-insoluble products are proposed as follows (Fig. 4): Complex A is the complex formed at the early stage of reaction. It is readily converted into complex B as the reaction prolonged from 2 to 4 h. Complex C is the most unstable one because of the exclusion of COOH groups between the neighboring glucose rings.

FTIR Analysis of Water-Soluble Product

For water-soluble products, the FTIR spectrum is completely different from that of water-insoluble ones. These are the observations:

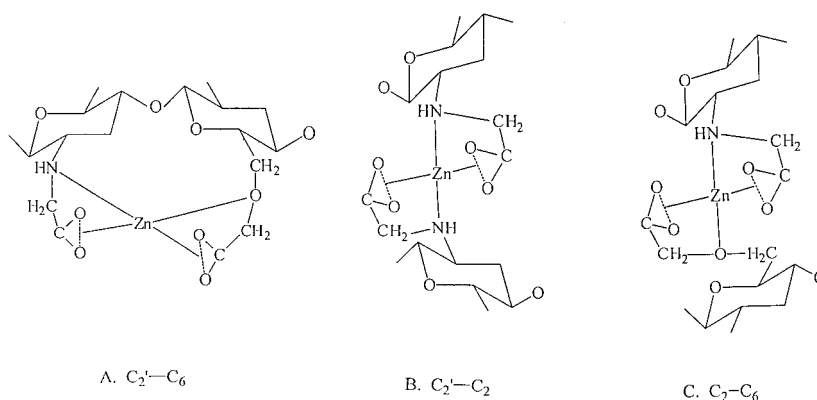


Figure 4 Plausible mechanisms for formation of water-insoluble chelates.

1. A broad and strong band appearing at $\sim 3400\text{ cm}^{-1}$ in the spectrum of the Zn complex is related to the vibration of the bound water molecule. This band is absent from the control spectrum. The presence of the water molecule is confirmed by the observation of H_2O bending modes in the region of $1610\text{--}500\text{ cm}^{-1}$. The appearance of the bands at 1340 and 1240 cm^{-1} associated with OH in-plane and 990 and 925 cm^{-1} due to OH out-of-plane deformation vibrations suggest that the OH groups in ONCMCh did not participate in the complex formation with the zinc ions.
2. The presence of C—N characteristic bands near $2000\text{--}2300\text{ cm}^{-1}$ suggests that neither NH nor NH_2 is involved in the complex formation.¹⁷
3. Like the water-insoluble products, the bands at 1610 and 1412 cm^{-1} are attributed to the bound form of carboxylic groups in the complex. It is in its ionized COO^- form.
4. A medium intensity of the Zn—O absorption band at near 630 cm^{-1} and no band appearing at about 500 cm^{-1} imply that complexation took place by metal—oxygen binding only.⁹

According to the above observations, the plausible complex structures for water-soluble products are proposed as in Figure 5.

SEM Observations for Microstructural Formation

Despite the recent interest in chitosan—metal complexes, there is very little information on the morphological structures of chelated chitosan and

its derivatives. The evolution of formation of microstructures between the water-soluble and water-insoluble products produced by chelation was studied by SEM.

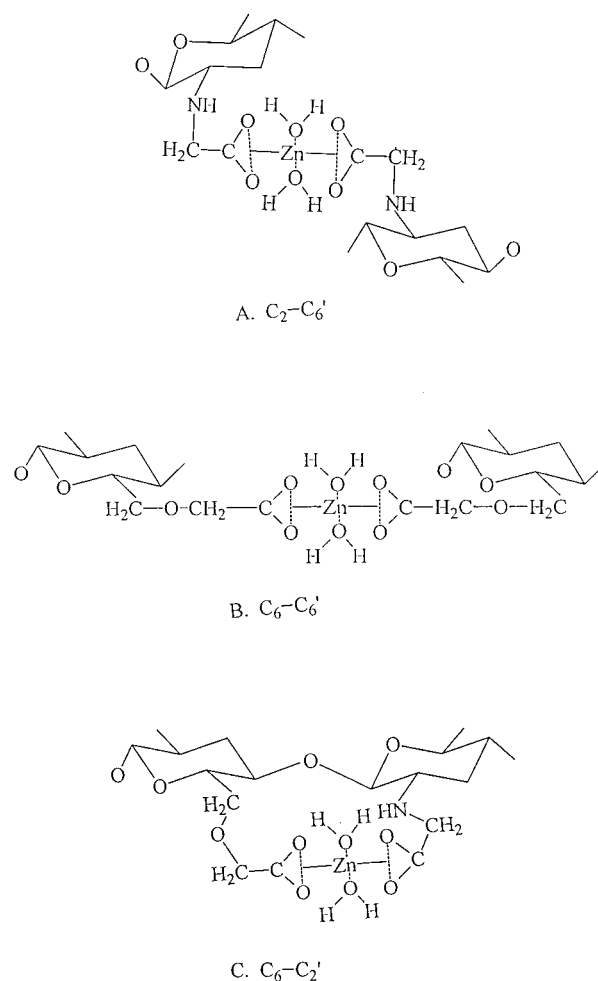


Figure 5 Plausible mechanisms for formation of water-soluble chelates.

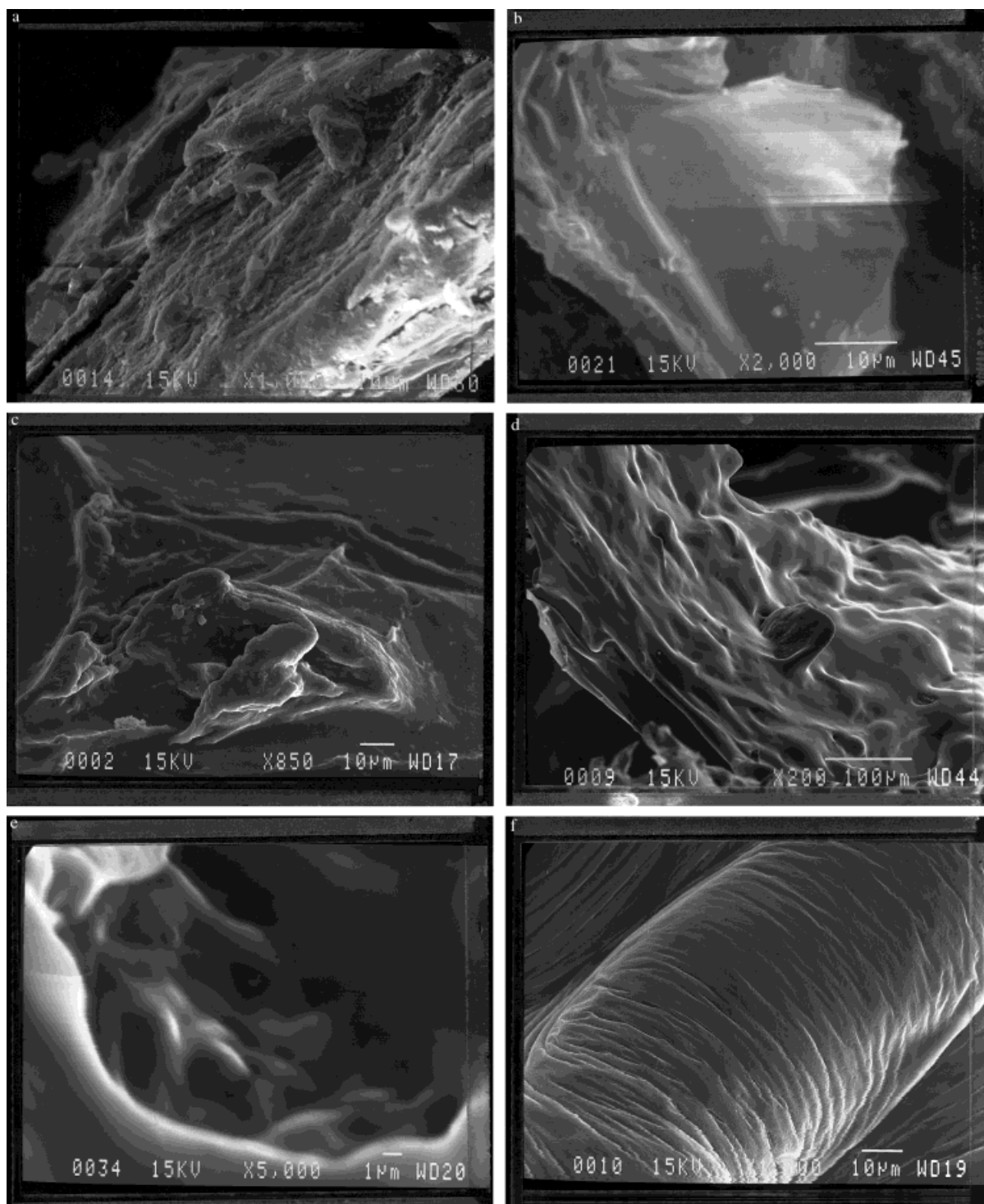


Figure 6 SEM micrographs of ONCMCh chelated with zinc ions.

The scanning electron micrographs of unchelated, control ONCMCh showed either a rough surface with a few undulations [Fig. 6(a)] or a somewhat smooth surface with few wrinkles [Fig. 6(b)]. These microstructures are believed to be the

result of a carboxymethylation reaction. This microstructure showed a drastic change after ONCMCh was complexed with Zn. For example, the Zn-ONCMCh, water-insoluble complex with a zinc content of 3.8% showed an opening and

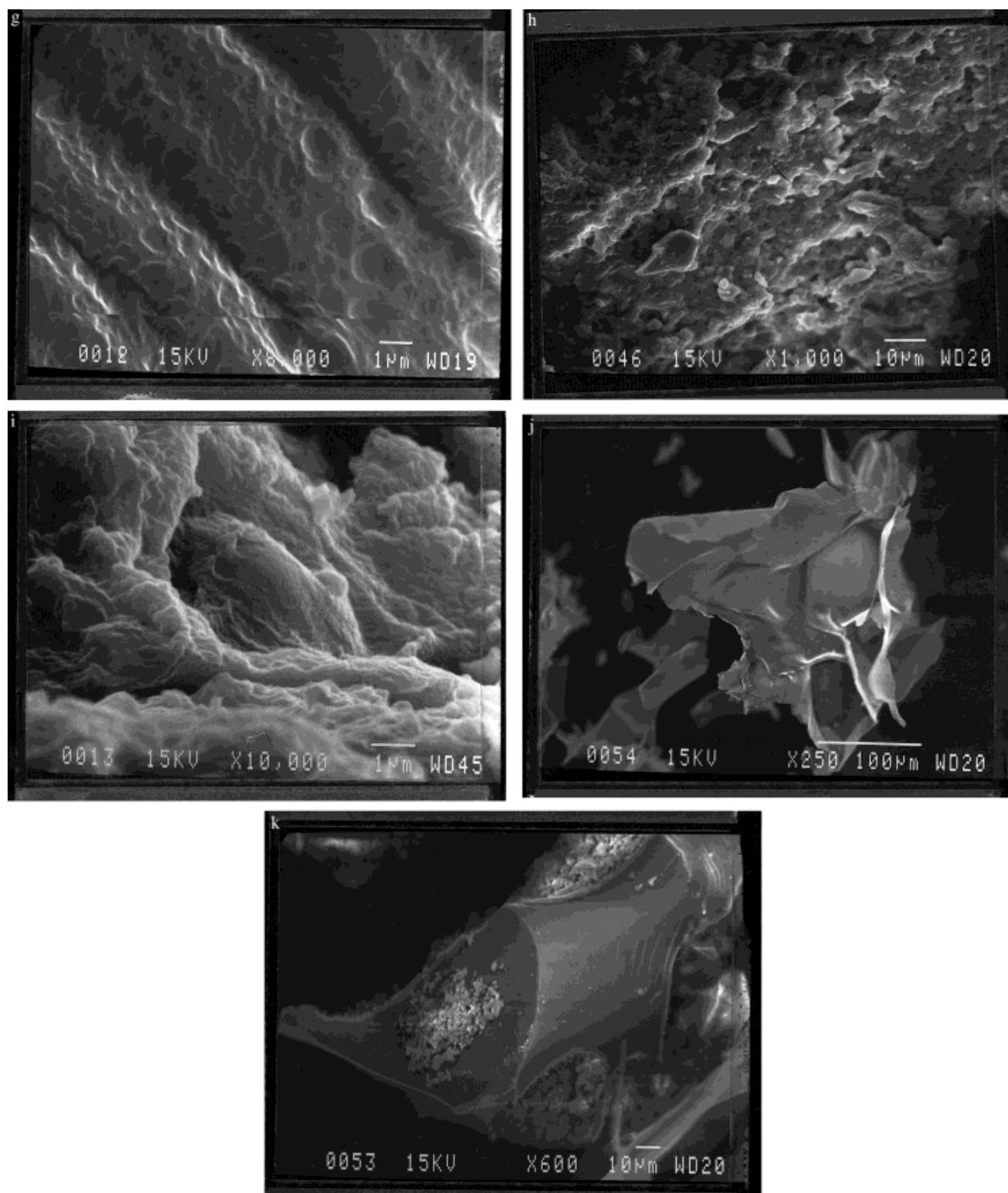


Figure 6 (Continued from the previous page)

somewhat folding microstructure appearing along with some granules located around the “open-mouth” areas [Fig. 6(c)]. When the zinc content reached 5.0%, an entirely different morphological structure emerged. The surface of the chelated polymer was coated completely with molten

undulations [Fig. 6(d)]. With closer examination, it was found that most of the granules were distributed underneath the polymeric surface [Fig. 6(e)]. This observation provides evidence that Zn-chelated ONCMCh was located underneath the polymer surface. This phenomenon became dis-

cernible when the Zn content increased to 7.0%, as shown in Figure 6(f). The chelated polymer is aligned in a uniform and regular way to form many ridges. It is interesting to note, by closer examination, that these ridges consist of many granules aligned in the same direction underneath the polymer surface [Fig. 6(g)].

When the Zn content continuously increased to 11%, those ridges underneath the chelated polymer expanded in volume and size, eventually sprouted out of the surface. As a result, a coarse surface with different shapes and sizes of granules can be seen [Fig. 6(h)]. At this stage, of course, these granules were still embedded completely in the ligand polymer with striated microstructures as shown in Figure 6(i).

For a water-soluble product, it is interesting to find a totally different surface microstructure from a water-insoluble one, that is, at a low Zn content of ~ 3%, the Zn ions appeared to be non-uniformly deposited over the polymer [Fig. 6(j)]. In other words, the zinc was only locally deposited over the surface. This could be recognized remarkably by increasing the zinc content to 5.8%. The Zn ions were highly centered on some parts of the polymer surface as shown in Figure 6(k).

The formation of different microstructures observed by SEM between water-insoluble and water-soluble products can be attributed to the formation of complexes by different mechanisms, that is, for a water-insoluble product, zinc ions were usually chelated and involved in the ligand polymer. For the water-soluble product, zinc ions are only deposited over the surface of the ligand polymer by an electron attraction.

CONCLUSIONS

From the analyses of the FTIR spectra and observations of the SEM micrographs, the following conclusions are drawn:

1. ONCMCh is a highly effective chelating ligand with zinc ions. The chelation through the carboxyl group is more probable than it is through the hydroxyl groups.
2. Water-insoluble chelates which were formed through the Zn—O and Zn—N bonds appear to establish tetrahedral complexes.
3. For water-soluble complexes, zinc ions only connected with the oxygen of the carboxyl and water molecules by electron attraction.

4. The formation of different microstructures on the surface was determined by SEM. It provided evidence to distinguish the different complex mechanisms between water-insoluble and water-soluble products.

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