Macromol. Symp. **2008**, 264, 168–175 DOI: 10.1002/masy.200850427

Physical and Electrical Properties of Chlorophyllin/Carboxymethyl Chitin and Chlorophyllin/Carboxymethyl Chitosan Blend Films

Tuspon Thanpitcha,[1] *Anuvat Sirivat,*[1] *Alexander M. Jamieson,*[2] *Ratana Rujiravanit*[*1]

Summary: Blend films consisting of chlorophyllin dispersed in carboxymethyl chitin (CM-chitin) and carboxymethyl chitosan (CM-chitosan) were prepared by solution casting and characterized for their physical and electrical properties. Homogeneous films were obtained having chlorophyllin content up to 50 wt% in the CM-chitin matrix and 30 wt% in the CM-chitosan matrix. Agglomeration of chlorophyllin particles in the chlorophyllin/CM-chitosan blend was observed, when chlorophyllin content reached 50 wt%. The electrical conductivity of CM-chitosan was higher than that of CM-chitin by approximately 2 orders of magnitude due to the higher content of amino polar groups. The electrical conductivity increased with increasing chlorophyllin content. The addition of metal ion salts improved the electrical conductivity of the blend films.

Keywords: blends; chlorophyllin; electrical conductivity; polyelectrolytes; water-soluble polymers

Introduction

Chlorophyllin, a water soluble derivative of chlorophyll, has been found to possess semi-conducting properties, due to its structure, which has a conjugated double bond system chelated around a central molecular Cu.[1] The conductivity derives mainly from electronic conduction owing to the delocalization of electrons. In addition to chelation of metal ions, the heterocyclic structure of chlorophyllin exhibits many advantageous properties, including high stability against thermal and chemical decomposition, and intense absorption in the visible spectrum. Moreover, chlorophyllin has a wide range of potential applications such as in solar cells,[2–4] in mutagenic and carcinogen inhibition,[5] and as an anti-oxidant,[6] or an opto-electronic transducer.[7] However, chlorophyllin itself is difficult to process into objects having high mechanical strength, especially in the form of a thin film. Therefore, achievement of the above-mentioned applications has been limited. It follows that attempts to solve these drawbacks are of interest. A polymer blend or polymer composite might be an effective method to improve the processibility and mechanical properties while retaining the inherent properties of the chlorophyllin.

Carboxymethyl chitin (CM-chitin) and carboxymethyl chitosan (CM-chitosan) are anionic polyelectrolyte materials, obtained by the carboxymethylation reaction of chitin and chitosan with monochloroacetic acid. This leads to the creation of new materials which are soluble in water. CM-chitin and CM-chitosan can properly serve as the

---

[1] The Petroleum and Petrochemical College, Chulalongkorn University, Bangkok 10330, Thailand
Fax; (+66) 221 84459;
E-mail: ratana.r@chula.ac.th

[2] Department of Macromolecular Science, Case Western Reserve University, Ohio, USA

polymer matrix in the polymer blend system due to their ability to form a film with high mechanical strength. Furthermore, since their structures also contain a large number of charged groups, they are expected to possess semi-conducting properties, via an ionic conduction mechanism. Shriver *et al.* (1983) reported that ionic conduction occurs in polyelectrolyte materials via dissociation of electrostatic bonds between counterions and the polyelectrolyte chain, and formation of new bonds with other groups. This causes the motion of ions along the polymer chain resulting in the electrical conduction.[8] Moreover, they also found that the ionic conduction not only depended on the mobility of the ions but also on the polymer structure, e.g. amorphous materials provide higher conductivity than crystalline materials.[9] Therefore, we may expect that CM-chitin and CM-chitosan might be good candidates for use in applications such as electronic devices. Other advantages of CM-chitin and CM-chitosan, e.g. their biodegradability, non-toxicity, biocompatibility, and water solubility, have been exploited in the past.[10] CM-chitin and CM-chitosan have received attention in the field of electronic devices, and more recently in biomedical and pharmaceutical applications, such as drug controlled release,[11] wound healing,[12] antimicrobials,[13] food products, and cosmetics.[14]

The objectives of the present work are to prepare and characterize blend films, consisting of chlorophyllin dispersed in CM-chitin and CM-chitosan, and to investigate their overall electronic and ionic conductivity. Moreover, the effect of blend composition and the type of metal ion salt on the electrical conductivity of the blend films are explored.

Experimental Part

Preparation of CM-Chitin and CM-Chitosan

CM-chitin and CM-chitosan, with degree of substitution (%DS) equal to 43% and 62%, respectively, were prepared by reaction of chitin and chitosan, with degree of deacetylation (%DD) equal to 20% and 80%, respectively, with monochloroacetic acid under basic conditions, according to the method described by Wongpanit *et al.*[15] In a typical procedure, CM–chitin was prepared by suspending 5 g of chitin powder in 100 g of 42% w/w NaOH. The suspension was stored under reduced pressure for 30 min. Then 160 g of crushed ice was added to the suspension and the mixture was stirred at below 5 °C for 30 min. A pre-cooled solution at a temperature below 5 °C, containing 27 g monochloroacetic acid in 70 ml of 14% w/w NaOH, was slowly added to the mixture with vigorous stirring. The reaction was maintained at 0–5 °C for 30 min. After settling at room temperature overnight, the mixture was neutralized with glacial acetic acid, and subsequently dialyzed in running water, followed by dialysis with distilled water for 1 day. The dialysate was centrifuged at 10,000 rpm for 10 min to remove insoluble material. A white solid was recovered from the supernatant by adding it drop-wise into acetone. The product was washed with ethanol, filtered, and dried in a vacuum at room temperature.

CM-chitosan was prepared by mixing chitosan powder (10 g), NaOH (13.5 g), water (20 ml), and isopropanol (80 ml); the suspension was stirred at 50 °C for 1 h. 15 g of monochloroacetic acid in 20 ml isopropanol was slowly added to the mixture over a period of 30 min. The reaction was maintained at 50 °C for 4 h. 70% ethanol was used to stop the reaction. The solid product was filtered and dried under reduced pressure at room temperature. The dried product was purified by dissolving in distilled water. The CM-chitosan solution was centrifuged at 10,000 rpm for 10 min, followed by dialysis in distilled water until becoming neutral.

Preparation of Chlorophyllin/CM-Chitin and Chlorophyllin/CM-Chitosan Blend Films

Chlorophyllin was dissolved in distilled water to obtain a 1 wt% solution. 1 wt% CM-chitin and CM-chitosan solutions were obtained by dissolving in distilled water with vigorous stirring. 0.005 wt% Glutaraldehyde was added to the CM-chitin and CM-chitosan solutions. Depending on the desired amount of chlorophyllin in the blend film product, an appropriate amount of chlorophyllin solution was mixed with CM-chitin or CM-chitosan solution. The mixture was stirred for 12 h at room temperature. The resulting solution was cast onto a stainless steel mold and dried at 55 °C for 12 h. Finally, the film was removed from the mold and stored in a desiccator prior to use.

Characterization Methods

FTIR spectra of the samples were recorded using a Thermo Nicolet Nexus 670. The surface and cross-sectional morphologies were investigated using a scanning electron microscope (JOEL, model JSM-5800LV). Differential scanning calorimetries were recorded using a Mettler-Toledo DSC 822. X-ray diffraction (Rigaku, model D/MAX-2000) was used to characterize the crystalline structure of products. The electrical conductivity of the films was measured at 25 °C using a custom-made two-point probe with an electrometer/ high resistance meter (Keithley, model 7517A).

Results and Discussion

FT-IR spectra

FT-IR spectra were used to characterize the chemical structure of pure chlorophyllin, CM-chitin, CM-chitosan and their blend films (data not shown). Peaks characteristic of CM-chitin were observed at 1657 cm^{-1}, 1563 cm^{-1} and 1413 cm^{-1}, assigned to C=O stretching, and asymmetric and symmetric stretching of the carboxylate anion, respectively.[15] Peaks characteristic of CM-chitosan appeared at 1602 cm^{-1}, 1413 cm^{-1}, assigned to asymmetric and symmetric stretching of the carboxylate anion, respectively.[16,17] Peaks characteristic of chlorophyllin were observed at 2924 cm^{-1}, 1632 cm^{-1}, 1600 cm^{-1}, 1567 cm^{-1} and 1398 cm^{-1}, corresponding to C-H stretching (conjugated bond), aldehyde carbonyl stretching, a combination of C=C and C=N stretching, and asymmetric and symmetric stretching of the carboxylate anion, respectively. The FTIR spectrum of chlorophyllin was consistent with the previous work of Farag.[18] For both chlorophyllin/CM-chitin and chlorophyllin/CM-chitosan blend films, peaks characteristic of chlorophyllin were observed in all spectra. No new peaks were observed, suggesting that no new chemical bonds are formed. Moreover, the characteristic peaks of chlorophyllin became increasingly dominant with increasing chlorophyllin content.

Morphology

The visible characteristics of both chlorophyllin/CM-chitin and chlorophyllin/CM-chitosan blend films are a homogeneous dark green color, the color of pure chlorophyllin. The surface and cross-sectional morphology of pure materials and blend films were investigated by SEM. CM-chitin film shows a smooth surface while a slightly rough surface can be observed when blending chlorophyllin with a CM-chitin matrix. In addition, the cross-sectional fracture surfaces of pure CM-chitin and the blend films appear homogeneous, indicating a uniform dispersion of chlorophyllin in the matrix, as shown in Figure 1. However, for chlorophyllin/CM-chitosan blend films, while the fracture surfaces appear homogeneous at chlorophyllin contents up to 30 wt%, as seen in Figure 2a, 2b, and 2c, clear evidence of inhomogeneity can be observed at 50 wt% chlorophyllin content (Figure 2d). This is presumably due to the agglomeration of chlorophyllin particles at high chlorophyllin content. This finding can be compared with our previous work, which investigated blend films of polyaniline and chitosan; agglomeration of polyaniline was observed in the blend film with 50 wt% polyaniline content. The latter agglomeration presumably reflects a repulsive interaction (incompatibility) between polyaniline and chitosan.[19] In the present case, the

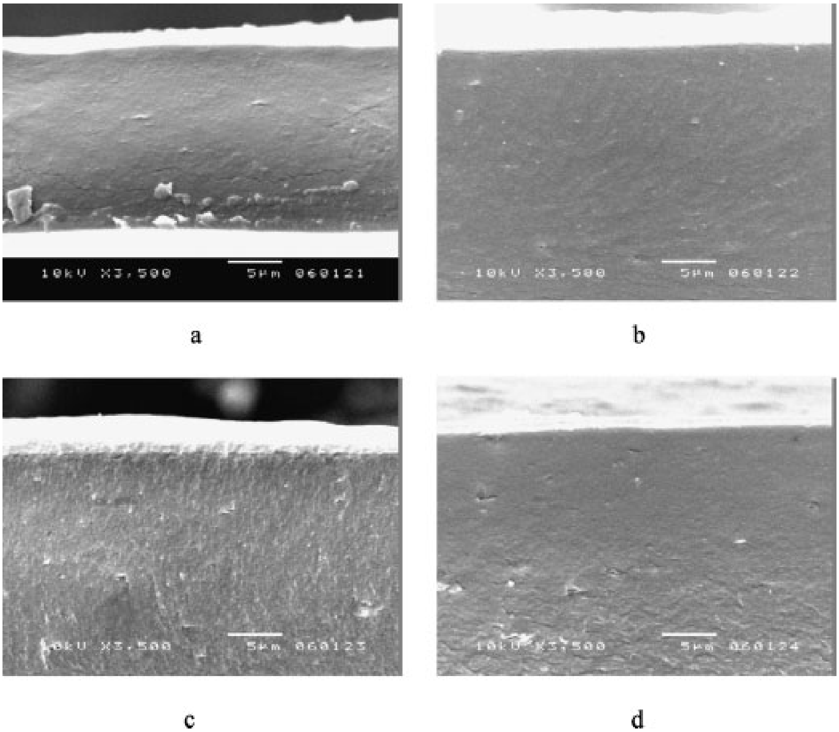

Figure 1.
Cross-sectional morphology of chlorophyllin/CM-chitin blend films: a) CM-chitin; b) 10 wt% chlorophyllin content; c) 30 wt% chlorophyllin content; d) 50 wt% chlorophyllin content.

agglomeration likely reflects an incompatibility between CM-chitosan and chlorophyllin.

Differential Scanning Calorimetry (DSC)

DSC thermograms of chlorophyllin, CM-chitin, CM-chitosan, and the blend films are shown in Figure 3 and Figure 4. Chlorophyllin shows two endothermic peaks at around 170 °C and 280 °C followed by a broad exothermic peak at around 440 °C, which correspond, respectively, to the evaporation of water, the elimination of Cu ion from the chlorophyllin structure, and the degradation of the chlorophyllin structure. Pure CM-chitin exhibits an endothermic peak at approximately 150 °C and an exothermic peak at 283 °C. These peaks correspond to the loss of water and the degradation of the CM-chitin structure, respectively. For the chlorophyllin/CM-chitin blend films, the degradation temperature of CM-chitin was found to increase to 290 °C with chlorophyllin content increasing up to 50 wt%. This finding suggests that chlorophyllin can form an intermolecular interaction with the CM-chitin structure, resulting in an enhancement of the degradation temperature of the CM-chitin matrix.[19] Similar to CM-chitin, pure CM-chitosan exhibits an endothermic peak at around 150 °C and an exothermic peak at 280 °C, corresponding to the loss of water and the degradation of CM-chitosan structure, respectively. However, the degradation temperature of CM-chitosan gradually decreases from 280 °C to 272 °C when the chlorophyllin content is increased to 50 wt%. This finding may reflect the incompatibility between the chlorophyllin and the CM-chitosan. The phase-separated domains of high electron density chlorophyllin may enhance heat

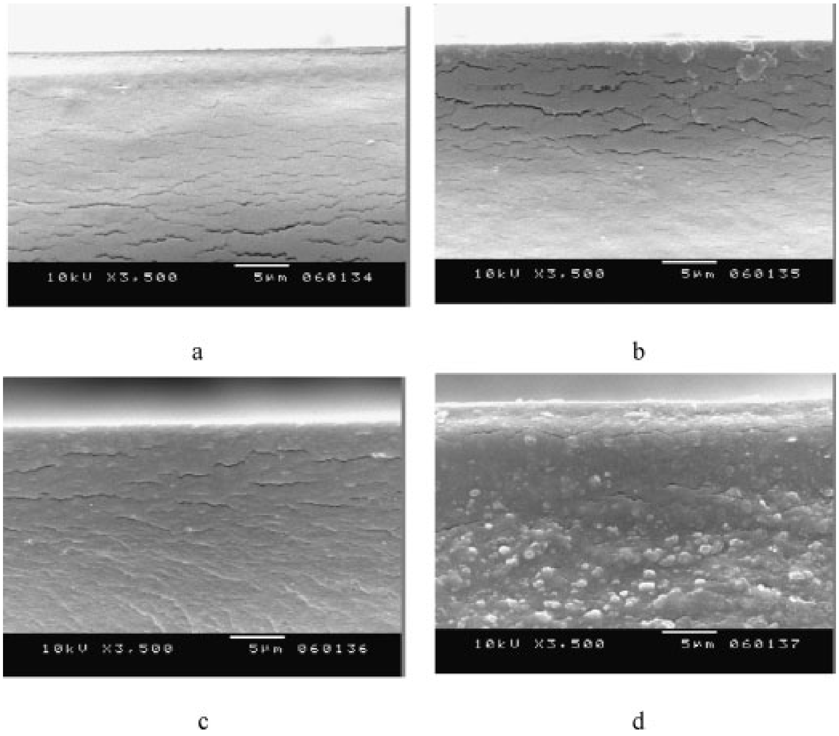

Figure 2.
Cross-sectional morphology of chlorophyllin/CM-chitosan blend films: a) CM-chitosan; b) 10 wt% chlorophyllin content; c) 30 wt% chlorophyllin content; d) 50 wt% chlorophyllin content.

transfer to the CM-chitosan, facilitating its thermal decomposition.

Wide-Angle X-ray Diffraction (WAXD)

Wide-angle x-ray diffraction (WAXD) patterns of chlorophyllin, CM-chitin, CM-chitosan, and the blend films are shown in Figure 5 and Figure 6. The diffraction pattern of chlorophyllin shows a small peak near 10 ° and a broad peak in the range $2\theta = 15\text{–}25°$. This result suggests that the structure of chlorophyllin is amorphous.

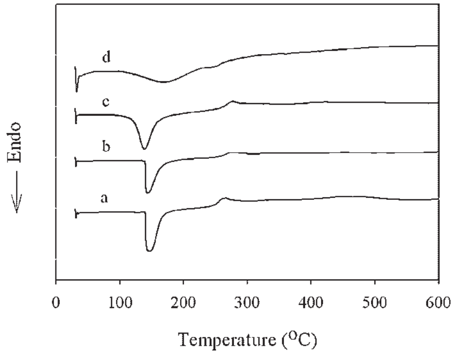

Figure 3.
DSC thermograms of chlorophyllin/CM-chitin blend films: a) CM-chitin; b) 10 wt% chlorophyllin content; c) 50 wt% chlorophyllin content; and d) chlorophyllin.

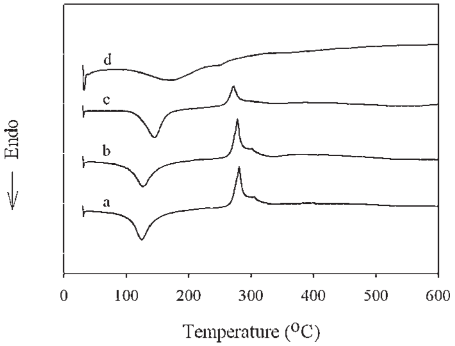

Figure 4.
DSC thermograms of chlorophyllin/CM-chitosan blend films: a) CM-chitosan; b) 10 wt% chlorophyllin content; c) 50 wt% chlorophyllin content; and d) chlorophyllin.

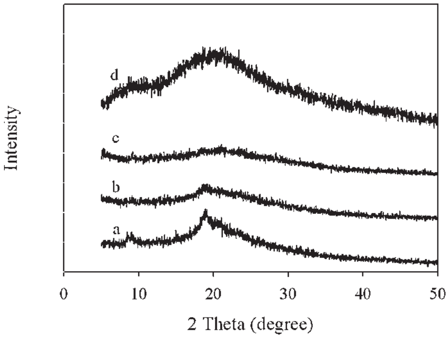

Figure 5.
XRD diffraction patterns of chlorophyllin/CM-chitin blend films: a) CM-chitin; b) 10 wt% chlorophyllin content; c) 50 wt% chlorophyllin content; and d) chlorophyllin.

In contrast, the diffraction pattern of CM-chitin shows a sharp peaks at $2\theta = 9^\circ$ and at $2\theta = 18^\circ$, indicating the semi-crystalline structure of CM-chitin. For the blends, the diffraction peak at $2\theta = 9^\circ$ vanishes at all compositions while the peak at $2\theta = 18^\circ$ decreases with increasing chlorophyllin content.[20] This implies that the chlorophyllin forms a strong interaction with the CM-chitin that disrupts the CM-chitin crystal structure. This results in the dramatic decrease in degree of crystallinity of the CM-chitin. In the case of pure CM-chitosan, a broad peak centered at $2\theta = 20^\circ$ was observed, indicating an amorphous structure. The diffraction pattern of the chlorophyllin/CM-chitosan appears to be the sum of the individual component diffractograms, consistent with the SEM result which shows a phase-separated morphology.

Electrical Property

Effect of Blend Composition

Comparisons of the electrical conductivities of chlorophyllin/CM-chitin, and chlorophyllin/CM-chitosan blend films are shown in Figure 7. Pure CM-chitin exhibits an electrical conductivity of 4.65×10^{-10} S/cm. For the blend films, as the chlorophyllin content increases from 10 to 50 wt%, the electrical conductivity increases from 1.33×10^{-9} S/cm to 9.47×10^{-9} S/cm. The enhancement of electrical conductivity is evidently due to the addition of chlorophyllin, whose structure features an extensive π-conjugation system. The conduction mechanism of chlorophyllin is mainly electronic conduction produced via the delocalization of electrons around the conjugation system.[21] In contrast, pure CM-chitosan film shows a higher electrical conductivity (ca. 1.06×10^{-8} S/cm), by approximately 2 orders of magnitude, relative to that of pure CM-chitin. CM-chitosan has a higher ionic conductivity due to the increased amino and

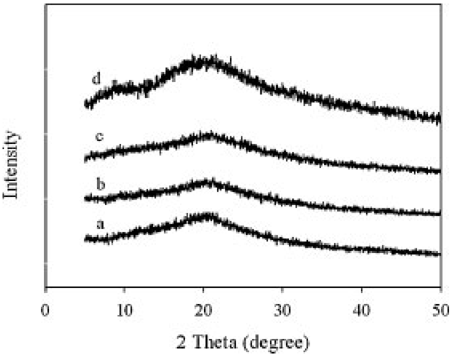

Figure 6.
XRD diffraction patterns of chlorophyllin/CM-chitosan blend films: a) CM-chitosan; b) 10 wt% chlorophyllin content; c) 50 wt% chlorophyllin content; and d) chlorophyllin.

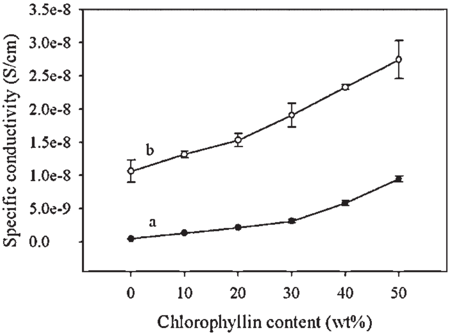

Figure 7.
The electrical conductivity of the blend films as a function of chlorophyllin content: a) chlorophyllin/CM-chitin and b) chlorophyllin/CM-chitosan.

Table 1.
The electrical conductivity of chlophyllin/CM-chitin and chlorophyllin/CM-chitosan blend films as a function of types of metal ion salt.

Types of metal ion salt[b)]	Specific conductivity of chlorophyllin/CM-chitin[a)] (S/cm)	Specific conductivity of chlorophyllin/CM-chitosan[a)] (S/cm)
No salt added	$5.84 \times 10^{-9} \pm 3.80 \times 10^{-10}$	$2.33 \times 10^{-8} \pm 3.65 \times 10^{-10}$
LiCl	$2.26 \times 10^{-8} \pm 2.28 \times 10^{-9}$	$1.26 \times 10^{-7} \pm 5.72 \times 10^{-9}$
NaCl	$2.93 \times 10^{-8} \pm 1.71 \times 10^{-9}$	$1.61 \times 10^{-7} \pm 4.69 \times 10^{-9}$
$CaCl_2$	$8.20 \times 10^{-9} \pm 4.51 \times 10^{-10}$	$6.53 \times 10^{-8} \pm 3.15 \times 10^{-9}$
$AlCl_3$	$1.11 \times 10^{-8} \pm 1.05 \times 10^{-9}$	$1.10 \times 10^{-7} \pm 9.35 \times 10^{-10}$
$FeCl_3$	$9.52 \times 10^{-9} \pm 7.65 \times 10^{-10}$	$1.11 \times 10^{-7} \pm 2.30 \times 10^{-9}$

a) The blend films with 40 wt% chlorophyllin content were used;.
b) The added metal ion salt was 0.1 mole%/g. of CM-chitin or CM-chitosan.

carboxylate content in the CM-chitosan structure. For the chlorophyllin/CM-chitosan blend films, the electrical conductivity of the blend films increases from 1.32×10^{-8} S/cm to 2.74×10^{-8} S/cm when the chlorophyllin content is increased from 10 to 50 wt%.

Effect of Metal Ion Salt

Table 1 shows the variation in electrical conductivity of 40 wt% chlorophyllin loaded CM-chitin and CM-chitosan blend films doped with different types of metal ion salt (0.1 mole %/g. of CM-chitin or CM-chitosan). The data show that, in general, the addition of metal ion salt causes an increase in electrical conductivity of the blend films. This result is presumably due to association of the metal cation with polar or charged groups on the polymer, e.g. complexation between the cation and the lone pair electron of the amino or hydroxy group in the CM-chitin and CM-chitosan structures.[22] Such association results in the enhancement of ionic conductivity of the CM-chitin and the CM-chitosan matrix. A possible mechanism has been described by Shriver and coworkers, who reported that ion transport in polyelectrolyte materials proceeds via formation of a transition state, in which a metal ion, bound to a particular polar group, associates with a polar group on a new segment of polymer chain, before dissociating from the old site. This leads to the hopping of ions from one polymer chain to the next.[8] However, the nature of the metal ion salt, including the size and the valence of the metal ion, can also influence the electrical conductivity of the blend films.[23–25] The ionic conduction mechanism of polyelectrolyte materials is dependent on the mobility of the metal ion, the degree of crystallinity, and the interaction between the polymer and the salt.[8,26,27]

Thus, the electrical conductivity of the blend films can be varied by changing the type of metal ion salt. For example (Table 1), blends loaded with lithium and sodium ions, which are mono-valent, the electrical conductivity of the blend film exhibits higher values compared to films loaded with divalent and trivalent metal ions. This result can be explained on the basis that the interaction between the monovalent Li^+ and Na^+ with electronegative groups in CM-chitin and CM-chitosan structures is weaker than the corresponding interaction of multivalent ions like Ca^{2+}, Fe^{3+}, and Al^{3+}, which can even form physical cross-links between COO^- groups in CM-chitin and CM-chitosan matrixes. This results in a higher mobility of Li^+ and Na^+ in the matrixes and consequently a higher conductivity is obtained. In addition, the formation of physical cross-links by di- and tri-valence metal ions could be deduced from a significant increase in viscosity of the blend solution of CM-chitin and CM-chitosan.

Conclusion

Blend films of chlorophyllin/CM-chitin and chlorophyllin/CM-chitosan were successfully prepared by solution casting. Flexible, homogeneous films of chlorophyllin/CM-chitin were obtained with chlorophyllin content up to 50 wt%, whereas agglomeration of chlorophyllin was observed in chlorophyllin/CM-chitosan films having 50 wt% chlorophyllin content. The electrical conductivity of each blend increases with increasing chlorophyllin content. This is a direct result of the addition of chlorophyllin whose structure features a highly π-conjugated system. Moreover, the chlorophyllin/CM-chitosan films exhibit higher electrical conductivity than the chlorophyllin/CM-chitin films. This is the result of the higher polarity of the CM-chitosan structure relative to the CM-chitin structure. Finally, the electrical conductivity of the blend films can be improved by the addition of a metal ion salt. The electrical conductivity of the doped films depends strongly on the type of metal ion salt.

Acknowledgements: The authors gratefully acknowledge Thailand Research Fund (TRF), Thailand and The Conductive and Electroactive Polymers Research Unit, Chulalongkorn University, Thailand for their financial support of this work. We also acknowledge Surapon Food Public Co. Ltd. for supplying the material for this work.

[1] D. D. Eley, R. S. Snart, Biochim, *Biophys. Acta* **1965**, *102*, 379.

[2] Y. Amao, Y. Yamada, K. Aoki, *J. Photochem. Photobiol. A* **2004**, *164*, 47.

[3] H. Spanggaard, F. C. Krebs, *Sol. Energy Mater. Sol. Cells* **2004**, *83*, 125.

[4] S. Hao, J. Wu, Y. Huang, J. Lin, *Sol. Energy* **2006**, *80*, 209.

[5] S. Arimoto-Kobayashi, N. Harada, R. Tokunaga, J. Odo, H. Hayatsu, *Mutation Res.* **1997**, *38*, 243.

[6] U. M. Lanfer-marquez, R. M. C. Barros, P. Sinnecker, *Food Res. Int.* **2005**, *38*, 885.

[7] S. Nespurek, J. Sworakowski, C. Combellas, G. Wang, M. Weiter, *Appl. Surf. Sci.* **2004**, *234*, 395.

[8] D. F. Shriver, R. Dupon, M. Stainer, *J. Power Sources* **1983**, *9*, 383.

[9] I. Pucic, A. Turkovic, *Solid State Ionics* **2005**, *176*, 1797.

[10] L. Zhao, L. Xu, H. Mitomo, F. Yoshii, *Carbohydr. Polym.* **2006**, *64*, 473.

[11] S. Tokura, Y. Miura, M. Johmen, N. Nishi, S. Nishimura, *J. Control. Release* **1994**, *28*, 235.

[12] X. Chen, Z. Wang, W. Liu, H. Park, *Biomaterials* **2002**, *23*, 4609.

[13] L. Zhao, H. Mitomo, M. Zhai, F. Yoshii, N. Nagasawa, T. Kume, *Carbohydr. Polym.* **2003**, *53*, 439.

[14] L. Chen, Y. Du, X. Zeng, *Carbohydr. Polym.* **2003**, *38*, 333.

[15] P. Wongpanit, N. Sanchavanakit, P. Pavasant, P. Supaphol, S. Tokura, R. Rujiravanit, *Macromol. Biosci.* **2005**, *5*, 1001.

[16] X. Chen, H. Park, *Carbohydr. Polym.* **2003**, *53*, 355.

[17] L. Fan, Y. Du, B. Zhang, J. Yang, J. Zhou, J. F. Kenedy, *Carbohydr. Polym.* **2006**, *66*, 149.

[18] A. A. M. Farag, *Spectrochim. Acta Part A* **2006**, *65*, 667.

[19] T. Thanpitcha, A. Sirivat, A. M. Jamieson, R. Rujiravanit, *Carbohydr. Polym.* **2006**, *64*, 560.

[20] N. A. Bakr, M. Ishras, *Polym. Test.* **2002**, *21*, 571.

[21] P. Chetri, N. N. Dass, N. S. Sarma, *Mater. Sci. Eng B* **2006**, *128*, 188.

[22] M. Z. A. Yahya, A. K. Arof, *Eur. Polym. J.* **2002**, *38*, 1191.

[23] K. Benmansour, K. Medjahed, L. Tenouga, A. Mansri, *Eur. Polym. J.* **2003**, *39*, 1443.

[24] I. F. Miller, *Energy* **1979**, *4*, 307.

[25] Y. Ikeda, M. Ikeda, F. Ito, *Carbohydr. Polym.* **2004**, *169*, 35.

[26] J. F. L. Nest, H. Cheradame, A. Gandini, *Solid State Ionics* **1988**, *28*, 1032.

[27] R. Tanaka, T. Fujita, H. Nishibayashi, S. Saito, *Solid State Ionics* **1993**, *60*, 119.