International Journal of Technology (2010) 1: 65-73
ISSN 2086-9614

PREPARATION AND CHARACTERIZATION OF CHITOSAN/MONTMORILLONITE (MMT) NANOCOMPOSITE SYSTEMS

Hery Haerudin[1], Andika W. Pramono[2], Dona Sulistia Kusuma[1*], Aisyiyah Jenie[1],
Nicolas H. Voelcker[3], Christopher Gibson[3]

[1]*Research Center for Chemistry, Indonesian Institute of Sciences,*
Kawasan PUSPIPTEK, Serpong, Tangerang 15314, Indonesia
[2]*Research Center for Metallurgy, Indonesian Institute of Sciences,*
Kawasan PUSPIPTEK, Serpong, Tangerang 15314, Indonesia
[3]*School of Chemistry, Physics and Earth Sciences, Flinders University of South Australia,*
GPO Box 2100, Bedford Park, South Australia, 5042, Australia

(Received: June 2010 / Revised: June 2010 / Accepted: July 2010)

ABSTRACT

A natural- based nanocomposite film consisting of chitosan, montmorillonite (MMT) and cashew nut shell liquid (CNSL) was synthesized. The nanocomposite was prepared by mixing a suspension of clay particles (filler, MMT) with a solution containing chitosan as the macroscopic polymer matrix. In this study, it was proposed that non-ionic long-chain alkyl molecules with possible interactions with the amine group of chitosan could be used as a plasticizer. As a natu-ral source for these compounds, an extract of CNSL was used. A series of chitosan/MMT com-posite samples containing two different clay contents and a sample with an additional CNSL were prepared. FTIR spectroscopy of the nanocomposite films indicated that, by addition of CNSL, amide groups of the chitosan are probably less attached and have more space for vibra-tion. CNSL seems to provide intermolecular spaces between the chitosan molecules. Atomic force microscopy (AFM) analysis showed that the composite contained particles measuring 100 nm or less, which confirmed that the nanocomposite had been successfully produced by this method. Addition of CNSL as plasticizer improved the tensile strength by 10% and the elastic modulus by almost 18%. Cell growth was observed on all the nanocomposite samples studied.

Keywords: Chitosan; CNSL; Montmorillonite (MMT); Nanocomposite

1. INTRODUCTION

The use of biopolymers as components of composites for packaging materials is a very popular topic for current studies. Studies regarding the improvement of composite properties such as brittleness, low heat distortion temperature, gas permeability, etc. for a wide range of applications are very much of interest. Additionally, the development of biobased nanocomposites are carried out with the intention of providing physical protection for food, improving food integri-ty, and preventing contamination from microbes and fungi (Rhim et al., 2006). The use of bio-based nanocomposites for packaging material has been reviewed (Akbari et al., 2007). Chitosan as a biopolymer is also widely studied for development of a new composite material with montmorillonite (MMT) clay.

---

[*] Corresponding author's email: d_sulistia@yahoo.com, Tel. +62-21-7560929, Fax. +62-21-7650549

Various experiments including the modification of MMT properties by converting the MMT into organo-montmorillonite (OMMT) prior to the nanocomposite preparation (Wang et al., 2008), as well as the variation of MMT concentration and dimensions to enhance the properties of the chitosan/MMT composite were studied (Tang et al., 2009). Thorough studies on the interaction of small molecules such as glycerol, as a plasticizer, with chitosan in chitosan film were also conducted (Garrido et al., 2007). Regarding the limited water- resistant and mechanical properties of original biopolymers, studies on the improvement of those properties by including hydrophobic material into the biopolymer composite (Liu et al., 2007), or by the addition of other small molecules to enhance hydrophobicity (Rivero et al., 2009) or plasticity (Suyatma, et al., 2005) were carried out. Since application of the chitosan composite as a packaging material for food is strongly considered, especially due to its antimicrobial activity, most studies of the chitosan/clay nanocomposite have also included antimicrobial activity testing (Dutta et al., 2009).

It is known that the properties of the polymer/clay composite are strongly affected by the addition of surfactant(s) into the composite. On those systems, the typical molecules are long-chain alkyl quaternary ammonium chlorides (Xie et al., 2003), which are easily incorporated into the clay structure. Large molecule additions, except the use of long-chain alkyl quaternary amines or polyglycol (Bajdik et al., 2009), to manipulate the composite properties are seldom studied. In the present study, it was proposed that non-ionic long-chain alkyl molecules with possible interactions with the amine group of chitosan could be used as a plasticizer. As a natural source for these compounds, an extract of cashew nut shell (cashew nut shell liquid-CNSL) was used. A series of chitosan/MMT composite samples containing two different clay contents and a sample with an additional CNSL were prepared. CNSL is known as a source for cardol, carda-nol, and anacardic acid, and is a phenolic compound with a long-chain alkyl group. A typical solvent-extracted CNSL contains anacardic acid (60-65%), cardol (15-20%), cardanol (10%) and traces of methylcardol (Kumar et al., 2002). Addition of CNSL as a source of relatively large molecules into the composite was intended also to improve the hydrophobicity as well as the mechanical properties of resulting composites.

2. EXPERIMENTAL

2.1. Materials

Montmorillonite (MMT) employed in this study was K10 montmorillonite from Sigma-Aldrich. It is well known as having a CEC of 70-100 meq/100 g. Chitosan purchased from Sigma-Aldrich (Cat. No. C3646) had a degree of deacetylation of 85% minimum. Commercial cetyltrimethyl ammonium bromide (CTAB) from E-Merck (Cat. No. 1.02342) was employed for this study. Cashew Nut Shell Liquid was extracted from cashew nut shells originating from Sumba Island, Indonesia. Prior to its use for composite preparation, chitosan was pre-treated in a basic solution of 10% NaOH. The extraction procedure for CNSL was as follows. Fresh redistilled n-hexane was added to the cashew nut shell in a round glass flask. Extraction was carried out by refluxing 100 g cashew nut shell in 250 ml of n-hexane for 2 hours; this procedure was followed by filtration and evaporation of n-hexane.

2.2. Preparation of na-montmorillonite(Na-MMT) and organo-montmorillonite (OMMT)

The OMMT was prepared by cation exchange between Na-MMT galleries and CTAB in an aqueous solution. MMT (2 g) was dispersed using a stirrer in distilled water to obtain clay suspension, and allowed to stand for 24 hours after vigorous stirring for 30 minutes. CTAB (2 g) was dissolved in distilled water, and then dropped slowly into the MMT suspension at 85°C under stirring. After stirring for 4 hours, the product was washed several times with distilled water and filtered to ensure the complete removal of bromide ions, which were detected with $AgNO_3$ until no AgBr precipitate was found. The product was dried at 85°C to yield OMMT.

2.3. Preparation of chitosan/montmorillonite composite

The chitosan solution was prepared using a 1% acetic acid solution. 4 g chitosan was dissolved in 100 ml acetic acid solution (1%) and stirred for 4 hours to obtain a homogeneous mixture. A 2% clay suspension in acetic acid solution (1%) was also prepared. The suspension was added into the resulting gel such that a 1% and 3% MMT content in the mixture was obtained. The mixture was stirred at 50°C for 2 days to obtain a homogeneous chitosan/MMT suspension. This procedure was carried out using K10 montmorillonite (Sigma-Aldrich), Na-exchanged K10 and CTAB exchanged K10. For samples containing CNSL, a solution of 25 mg CNSL in 100 ml ethanol was prepared separately. The CNSL solution was added accordingly into the chitosan/MMT mixture after a rigorous 2 days of mixing, and then a final 1% CNSL content was obtained.

2.4. Characterization

2.4.1. Infrared (IR) spectroscopy

All IR spectra were obtained using a Nicolet Avatar 370MCT (Thermo Electron Corporation, USA) equipped with a standard transmission accessory. Spectra of the composite films were recorded and analyzed using OMNIC version 7.0 software, in the range of 650-4000 cm^{-1} at a resolution of 1 cm^{-1}; the background was taken using an original chitosan powder. IR of the composite film was performed in transmission mode with crushed powders in KBr discs in the range of 650–4000 cm^{-1} at a resolution of 1 cm^{-1}.

Tapping mode AFM was performed on a Multimode Nanoscope IV microscope (Veeco Corporation, USA). Commercial Si cantilevers (FESP, Veeco Corporation, USA) were used for all experiments. The images were processed and analyzed using the Nanoscope 5.31r1 software (Veeco Corporation, USA).

2.4.2. Differential scanning calorimetry (DSC)

In the present work, DSC 2920 (TA Instruments) was used for the measurement. The instrumental cell constant and temperature calibration were performed using indium. Chitosan/MMT samples were analyzed under continuous heating conditions (10°C min^{-1}) between room temperature and 200°C. 8-10 ± 0.1 mg of sample was weighed into a standard aluminium pan and placed onto the DSC cell platform.

2.4.3. Mechanical properties

The mechanical properties of chitosan/ MMT nanocomposites were tested using a Universal Testing Machine, Orientec Co. Ltd., Model UCT, 5-T. The specimens were prepared according to ISO 527-2. All specimens were tested using a crosshead speed of 1 mm/min.

2.4.4. Cell growth test

1 cm^2 membranes were transferred to wells of a 12 well Iwaki tissue culture plate (Cat. No. 3815-012). Membranes were seeded with HEK 293 cells at density 10^5 cells/cm^2 ($3.85x10^5$ cells per well in a 2 mL volume) in DMEM (Invitrogen) +10% FCS (JRH) + 2 mM L-glutamine + 100 U/ mL penicillin 100ug/mL streptomycin (Invitrogen). HEK 293 cells were grown in the presence of the membranes for 20 hours at 37C 5% CO_2. At 20 hours media was removed and replaced with 2 ng/mL Hoescht 33342 (Sigma) stain in complete media and incubated at 37°C 5% CO_2 for 0.5 hours. Staining was visualized under appropriate fluorescent excitation/emission conditions for Hoescht stain. The photographs were taken with a 20xobjective on a Leica laborluxD fluorescent scope.

3. RESULTS AND DISCUSSION

Table 1 as shown below lists the samples used for the studies. Each sample consists of 3% clay or its modified one. CNSL was added as a solution in ethanol; 1% w/w of the chitosan was used for film preparation.

Table 1 List of samples tested for the studies

Clay type		CNSL Addition
K10	Untreated	No
K10-CTAB	Organic clay	No
K10-CN	Untreated	Yes
K10-CTAB-CN	Organic clay	Yes

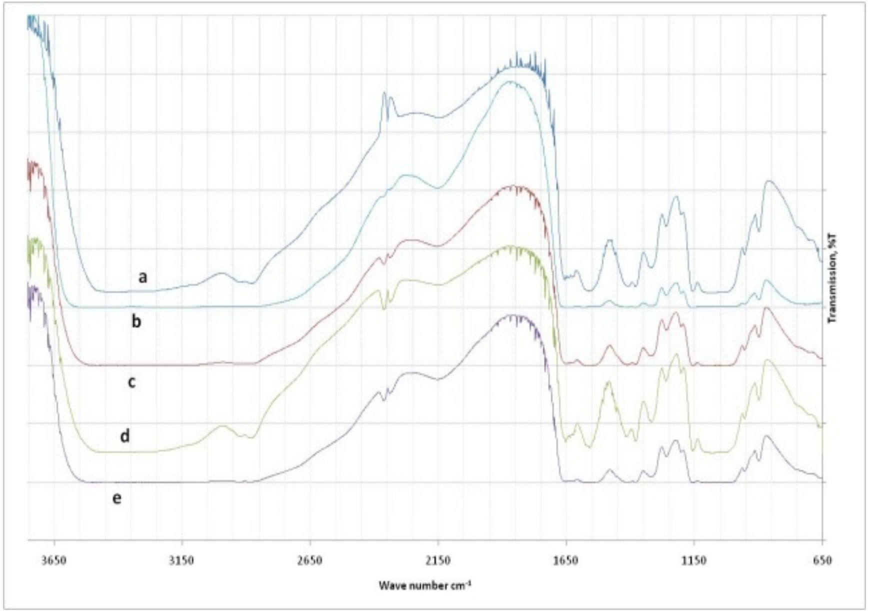
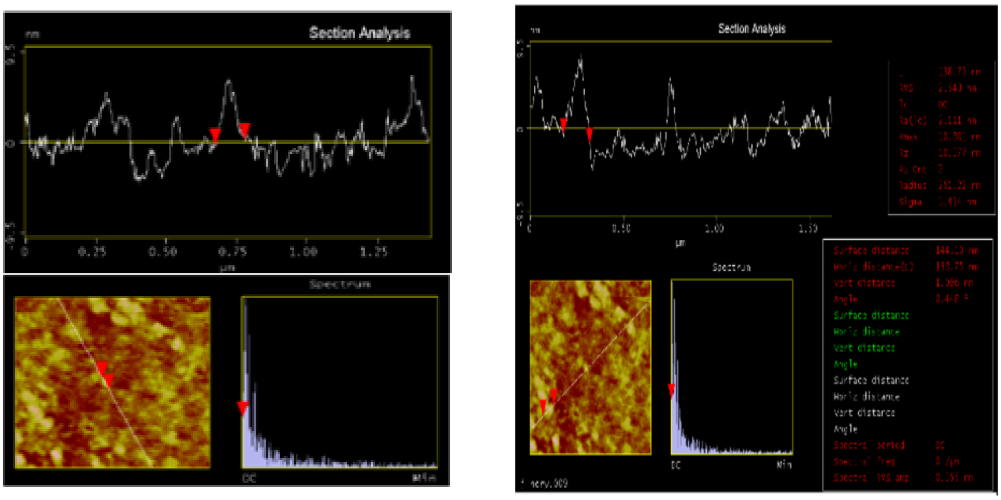


Figure 1 Transmission FTIR spectra of (a) pure chitosan (CS) film; (b) CS/3% K10; (c) CS/3% K10-CN; (d) CS/3% K10-CTAB; and (e) CS/3% K10-CTAB-CN composites

Generally, the amide bands are shifted to the higher wave number by addition of the CNSL. By addition of CNSL, amide groups of the chitosan are probably less attached and have more space for vibration. CNSL seems to provide intermolecular spaces between the chitosan molecules. This should create a higher plasticity in the resulting material. It should be noted that the C-O stretching band around 1160 cm^{-1} remained undisturbed by the addition of CNSL.

It should be noted that, at the pre-processing stage, raw data sets are passed through a filter that generates output in the form of more data which can be further used for actual comparative analysis between species. It is evident from Figure 2 that the dataset contains characters other than pure nucleotide bases. These illegal characters are removed by application of a cleansing filter. At first instance it is worth noting that analysis should be made with original data values; any garbage collection may lead to detritions of results.



(a) (b)

Figure 2 Morphology analysis of longitudinal section of CS/K10-CTAB using AFM; (a) Particle horizontal size of 103 nm (b) Particle horizontal size of 139 nm

3.1. Infrared spectroscopy

FTIR analyses of the composite films were performed to study the structural changes caused by modification of the clay component prior to the preparation of the composite. The IR spectra were taken by transmission as well as the DRIFT method. The spectrum of chitosan is characterized by broad and intense bands at 3450-3200 cm^{-1} (hydrogen bonded OH stretching overlapped with N-H stretching bands); C-H stretching band at 2783 cm^{-1}. The N-H deformation band of chitosan was found at 1559 cm^{-1}. The strong bands at 1656 cm^{-1} and 1599 cm^{-1} are ascribed to the amide I (C=O stretching) and amide II (N-H bending modes) of the chitosan.

3.2. Atomic force microscopy (AFM)

AFM analysis was conducted to determine the nanoscale structure of the composite synthesized. As shown in Figure 4, the MMT particle forming the composite is characterized by lengths of 103 nm (Fig. 4a) and 139 (Fig. 4b). However, the surface height is less than 9.5 nm. This can be explained as follows. The MMT particles forming the composite only consist of a few layers, resulting in MMT particles with thicknesses of less than 10 nm. Although the length of the particle is more than 100 nm, the formation of particles of less than 10 nm indicates the presence of exfoliation from the MMT particles. Since the MMT are used as fillers, then most likely the composite synthesized was in the form of *ordered intercalated nanocomposite*.

3.3. Differential scanning calorimetry

As shown by the thermogram (Figure 3), glass transition in DSC was not observed, indicating that amorphous structure does not exist. No thermoset cure temperature was observed from 25 to 250°C. Most probably no crosslinking, formation of new bonds occurred in the material at that temperature range. DSC thermograms show only that water released from the film at similar temperature range as on the original chitosan when the formula of composite film was containing CTAB. It could be explained that the preparation of organo-montmorillonite (OMMT) removed most of the trapped water molecules from the MMT, such that less water existed in the MMT as well as in the resulting composite film. It was expected that the hydrophobicity would be increased by the addition of CNSL, which means, it was also expected that water molecules would be released at a much lower temperature from the film containing CNSL in the formulation.

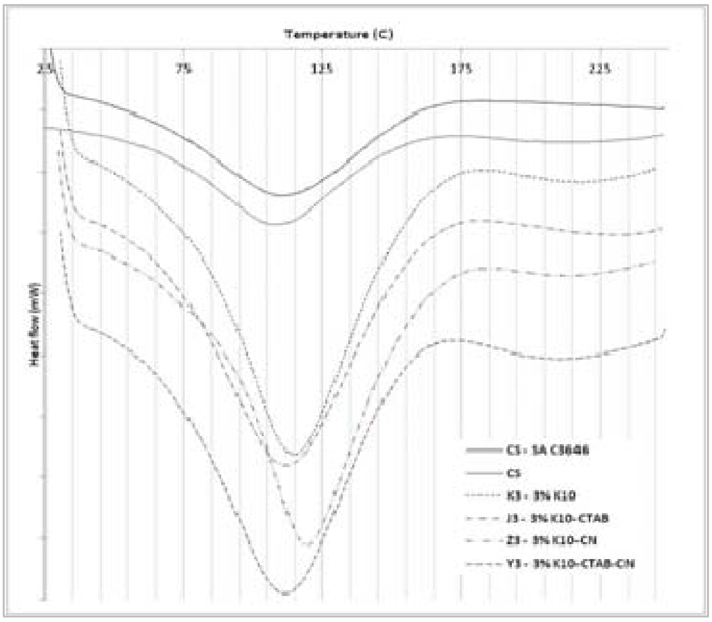


Figure 3 DSC thermogram of samples. From above downwards: (a) chitosan (CS) powder (C3636, Sigma-Aldrich); (b) CS film; (c) CS/3%-K10; (d) CS/3% K10-CTAB; (e) CS/K10-CN and (f) CS/K10-CTAB-CN

The thermograms show that there was no effect for OMMT, but on composites containing plain unmodified MMT (c and e), water molecules were released at a higher temperature, and CNSL addition to the formula seemed to result in stronger water retention in the film. The CNSL component molecules retained the trapped water molecules on montmorillonite.

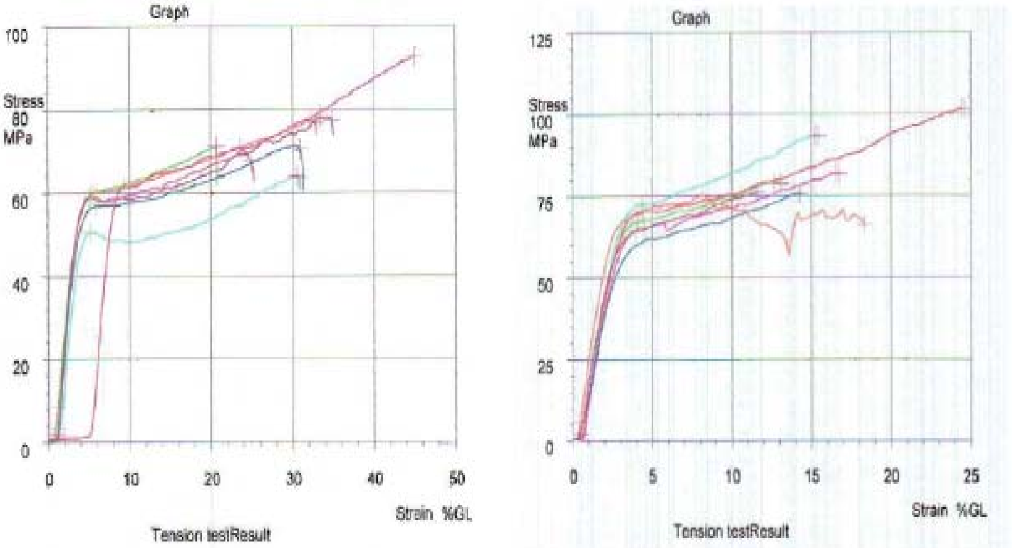


Figure 4 Tension test graphic results: (a) CS/K10-CTAB (b) CS/K10-CTAB-CN

3.4. Mechanical properties

From the measurements shown in Table 2, it is observable that the addition of CNSL increases the modulus elasticity, yield strength, tensile strength, as well as the rupture of the composite.

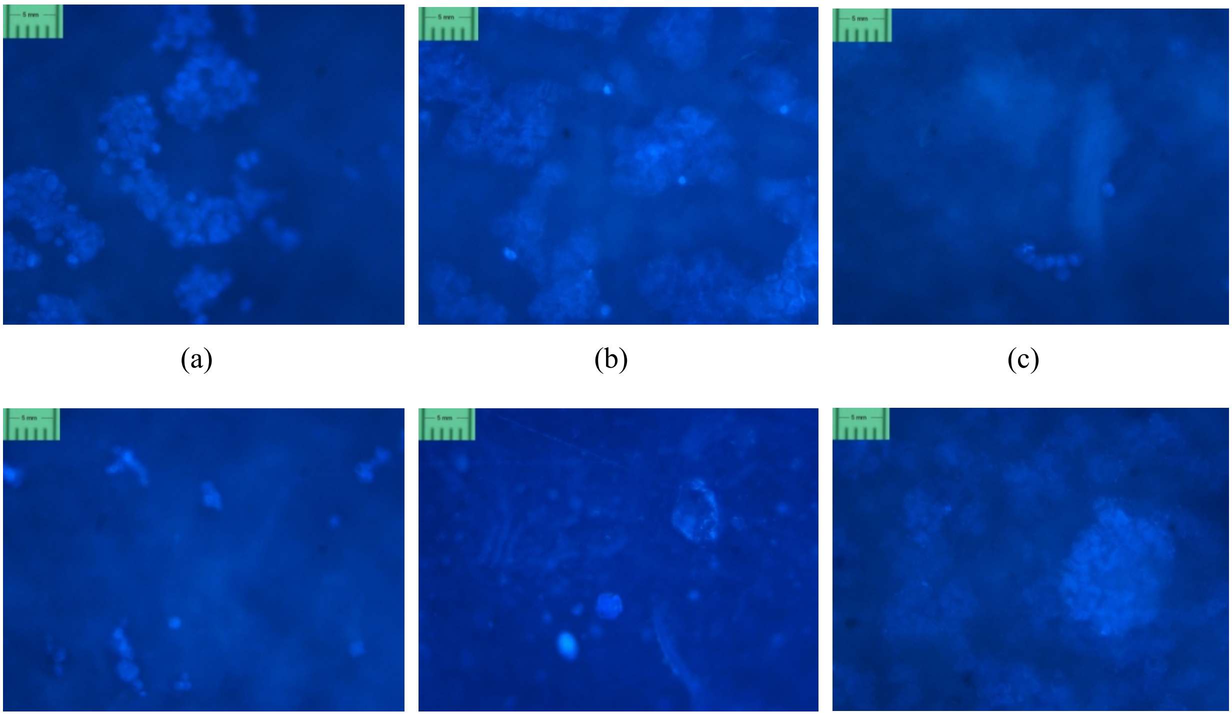
The tensile strength is improved by 10% and the elastic modulus by almost 18%.

Table 2 Tension test results for nanocomposite samples

Sample code		Yield strength [Mpa]	Tensile strength [Mpa]	Rupture [Mpa]	Elastic Modulus [Mpa]
CS/K10-CTAB	avg	59.4	76.9	75.9	2470
	std. dev.	1.5	8.4	9	220
CS/K10-CTAB-CN	avg	66.8	84.6	84.5	2910
	std. dev.	3.9	10.6	10.7	250

3.4. Cell growth test

It was difficult to differentiate the living cell from the film, since the chitosan film gives a strong background fluorescence using this particular method. Despite this problem, it could be concluded that cell growth was observed on all the samples studied.



(a) (b) (c)

(d) (e) (f)

Figure 5 Cell growth test on chitosan film; (a) CS (Chitosan film): ++ (b) Z3 (3% K10-CN): + (c) J3 (3% K10-CTAB): No cells (d) K3 (3% K10): + (e) Y3 (3% K10-CTAB-CN): + (f) J3 (3% K10-CTAB): ++

4. CONCLUSION

A natural- based nanocomposite film consisting of montmorillonite, chitosan and cashew nut shell liquid (CNSL) has been successfully synthesized. It has been proven that CNSL seems to provide intermolecular spaces between the chitosan molecules. AFM analysis showed that the composite contained particles measuring 100 nm or less, which confirmed that nanocomposite had been successfully produced by this method. The preparation of organo-montmorillonite removed most of the trapped water molecules from the montmorillonite, such that less water existed in the montmorillonite as well as in the resulting composite film, as shown by the DSC analysis. However, the addition of CNSL component molecules retained the trapped water molecules on montmorillonite. The addition of CNSL as a plasticizer improved the tensile strength by 10% and the elastic modulus by almost 18%. Cell growth was observed on all the nanocomposite samples studied.

5. ACKNOWLEDGEMENT

The present work was co-sponsored by the Endeavour Program of the Australian Government and was presented at the 11th International Conference on Quality in Research.

6. REFERENCES

Akbari, Z., Ghomashchi, T. & Moghadam, S., 2007. Improvement in Food Packaging Industry with Biobased Nanocomposites, *International Journal of Food Engineering*, vol. 3: Iss. 4, Article 3, DOI: 10.2202/1556-3758.1120.

Bae, S.B., Kim, C.K., Kim, K. & Chung, I.J., 2008. The effect of organic modifiers with different chain lengths on the dispersion of clay layers in HTPB (hydroxyl terminated polybutadiene), *European Polymer Journal*, vol. 44, pp.3385-3392.

Bajdik, J., Marciello, M., Caramellab, C., Domjánc, A., Süveghd, K., Mareke, T. & Pintye-Hódi, K., 2009. Evaluation of surface and microstructure of differently plasticized chitosan films, *J. Pharm. Biomed. Analysis*, vol. 49, pp.655-659.

Caner, C., Vergano, P.J. & Wiles, J.L. 1998. Chitosan Film Mechanical and Permeation Properties as Affected by Acid, Plasticizer, and Storage, *J. Food Sci.*, vol.63, no.6, pp.1049-1053.

Costache, M.C., Heidecker, M.J., Manias, E. & Wilkie, C.A., 2006. Preparation and characterization of poly(ethylene terephthalate)/clay nanocomposites by melt blending using thermally stable surfactants, *Polym. Adv. Technol.*, vol. 17, pp.764-771.

Dutta, P.K., Tripathi, S., Mehrotra, G.K. & Dutta, J., 2009. Perspectives for chitosan based antimicrobial films in food applications, *Food Chemistry*, 114, pp.1173-1182.

Gabrovskaa, K., Georgievaa, A., Godjevargovaa, T., Stoilovab, O. & Manolova, N., 2007. Poly(acrylonitrile) chitosan composite membranes for urease immobilization, *J. Biotech.*, vol. 129, no. 4, pp.674-680, May 2007.

Kumar, P.P., Paramashivappa, R., Vithayathil, P.J., Subba Rao, P.V. & Rao, A.S., 2002. Process for Isolation of Cardanol from Technical Cashew (Anacardium occidentale L.) Nut Shell Liquid, *J Agric. Food Chem.*, vol. 50, pp. 4705-708.

Liu, Y.L., Yua, C.H., Leea, K.R. & Lai, J.Y., 2007. Chitosan/poly-(tetrafluoroethylene) composite membranes using in pervaporation dehydration processes, J. Membrane Sci., vol. 287, no. 2, pp. 230-236.

Luckachan, G.E. & Pillai, C.K.S., 2006. Chitosan/oligo L-lactide graft copolymers: Effect of hydrophobic side chains on the physico-chemical properties and biodegradability, *Carbohydrate Polymers*, vol. 64, no. 2, pp.254-266.

No, H.K., Meyers, S.P., Prinyawiwatakul, W. & Xu, Z., 2007. Applications of Chitosan for Improvement of Quality and Shelf Life of Foods: A Review, *J. Food Sci.*, vol. 72, no. 5, pp. 87-100.

Quijada-Garrido, I., Iglesias-González, V., Mazón-Arechederra, J.M. & Barrales-Rienda, J.M., 2007. The role played by the interactions of small molecules with chitosan and their transition temperatures. Glass-forming liquids: 1,2,3-Propantriol (glycerol), *Carbohydrate Polymers*, vol. 68, pp.173-186.

Rhim, J.W., Hong, S.I., Park, H.M. & Ng, P.K.W., 2006. Preparation and Characterization of Chitosan-Based Nanocomposite Films with Antimicrobial Activity, *J. Agric. Food Chem.*, vol. 54, no. 16, pp.5814-5822.

Rivero, S., García, M.A. & Pinotti, A., 2009. Composite and bi-layer films based on gelatin and chitosan, *J. Food Eng.*, vol. 90, no. 4, pp.531-539.

Ruiz-Hitzky, E., Darder, M. & Aranda, P., 2005. Functional biopolymer nanocomposites based on layered solids, *J. Mater. Chem.*, vol. 15, pp.3650-3662.

Silva, C.L., Pereira, J.C., Ramalho, A., Pais, A.A. & Sousa, J.J.S., 2008. Films based on chitosan polyelectrolyte complexes for skin drug delivery: Development and characterization, *J. Membrane Sci.*, vol. 320, pp.268-279.

Suyatma, N.E., Tighzert, L., Copinet, A. & Coma, V., 2005. Effects of Hydrophilic Plasticizers on Mechanical, Thermal, and Surface Properties of Chitosan Films, *J. Agric. Food Chem.*, vol. 53, pp. 3950-3957.

Tang, C., Chen, N., Zhang, Q., Wang, K., Fu, Q. & Zhang, X., 2009. Preparation and properties of chitosan nanocomposites with nanofillers of different dimensions, *Polym. Degrad. Stab.*, vol. 94, no. 1, pp.124-131.

Wang, X., Du, Y., Yang, J., Tang, Y. & Luo, J., 2008. Preparation, characterization, and antimicrobial activity of quaternized chitosan/organic montmorillonite nanocomposites", *J. Biomed. Mater. Res. A*, vol. 84, no. 2, pp.384-390.

Wang, X., Du, Y., Yang, J.H., Wang, X.H., Shi, X.W. & Hu, Y., 2006. Preparation, characterization and antimicrobial activity of chitosan/layered silicate nanocomposites, *Polymer*, vol. 47, pp.6738-6744.

Xie, W., Hwu, J.Y., Jiang, G.J., Buthelezi, T.M. & Pan, W.P., 2003. A Study of the Effect of Surfactants on the Properties of Polystyrene-Montmorillonite Nanocomposites, *Polym. Eng. Sci.*, vol. 43, no. 1, pp.214-222.