



CLAY – LOADED CHITOSAN-BASED BIOCOMPATIBLE COMPOSITE FILMS IN DENTISTRY

Balaram Naik	SDM College of Dental Sciences, Dharwad, India 580 002
Tanvi Dugge*	SDM College of Dental Sciences, Dharwad, India 580 002*Corresponding Author
V. Ranijikanth	Green Nanotechnology Laboratory, College of Agriculture, Dharwad, India 580 005
K. Raghava Reddy	School of Chemical and Biomolecular Engineering University of Sydney, NSW 2006, Australia
Nikolaos Soldatos	Department of Periodontics and Dental Hygiene, School of Dentistry, University of Texas Health Science Centre at Houston, United States of America 77054
Shyam S. Shukla	Department of Chemistry and Biochemistry, Lamar University, Beaumont, Texas, United States of America, 77710
Mallikarjuna Nadagouda	Department of Mechanical and Materials Engineering, Wright Strate University, Dayton, Ohio 45324, United States
Tejraj M. Aminabhavi	Department of Chemistry and Biochemistry, Lamar University, Beaumont, Texas, United States of America, 77710
Amit Pachlag	SDM College of Dental Sciences, Dharwad, India 580 002

ABSTRACT Chitosan-derived nanocomposites loaded with montmorillonite clay, designated as CS/MMT, were prepared by physical mixing of chitosan polymer and sodium montmorillonite (MMT) particles. The main objective of this work was to obtain transparent films that may have useful applications as dental materials in dental surgery. The polymer thin films were prepared by loading varying concentrations of clay particles and their physico-mechanical as well as antibacterial properties were measured.

Materials and Methods: Chitosan, MMT clay, acetic acid glacial, distilled water are used as received. Composite membranes of pure chitosan (CS) and CS/MMT were prepared. Chitosan solution was prepared by dissolving CS in acetic acid. MMT clay particles were then dispersed in water in a beaker and added to the previously prepared CS solution. Uniform thickness films or membranes were casted on a clean flat glass plate, dried and peeled off to remove the films formed. Pristine CS film was prepared in the same manner except that clay filler particles were not added. The films thus prepared were all characterized by SEM, XRD, and UTM to understand their properties.

Results: Tensile strength, yield strength, modulus of elasticity increased significantly with increasing concentration of the clay particles. The addition of silver nitrate nanoparticles showed significantly higher antimicrobial activity compared to pure chitosan film.

Conclusions: The results suggest a potential application of nanocomposites developed based on natural biopolymers that are biodegradable and biocompatible materials having good antimicrobial activity.

KEYWORDS : chitosan, FTIR, montmorillonite, nanocomposites, X-ray diffraction

1. INTRODUCTION

The cationic biopolymer chitosan, being a high molecular weight polysaccharide, is composed mainly of b-(1,4)-linked 2-deoxy-2-amino-D-glucopyranose units, which can be intercalated in layered silicates through cationic exchange and hydrogen bonding processes. The resulting layer in nanocomposites represents interesting structural and functional properties. From the view point of applications, the layered nanocomposites of a layered silicate and a natural biopolymer chitosan also provides attractive functional properties in dental fields. Chitosan is nontoxic and exhibits high mechanical strength, hydrophilic character, and good adhesion to tissue. It is usually used as a food additive, a supporting material for chromatography and a chelating polymer for heavy metals removal [1–4]. In bio-related applications chitosan has been used for immobilization of biomolecules on solid substrates [5] and as an anticoagulant or a wound-healing accelerator due to its biocompatibility [6]. Chitosan is also employed as a coagulant in water treatment processes to remove colloidal particles, similar to other polysaccharides, due to its well-known aggregation effect with organic matters [7].

Among the many applications, chitosan-based antimicrobial films and coatings have been attracting much attention from food industries [8–10]. Biopolymer-based films and coatings are intended to function as barriers against moisture, oxygen, flavor, aroma, and oil, thereby improving food quality and enhancing the shelf life of food products. Furthermore, biopolymer films are excellent vehicles for incorporating a wide variety of additives, such as antioxidants,

antifungal agents, antimicrobials, colors and other nutrients. One of the promising ways to modify biopolymer properties such as inherent water sensitivity, relatively low stiffness and strength and low thermal stability, is to make hybrid films with biopolymers that can be further loaded with nanosized clays to form nanocomposite films. Such nanocomposite films consisting of inorganic nanolayers of layered silicates (e.g., montmorillonite) and organic polymers have recently evoked intense research interests in materials science area [11–15].

The polymer/clay nanocomposites comprise of an organic/inorganic hybrid polymer matrix containing platelet-shaped clay particles that have the particle sizes in the order of a few nanometers extending up to several hundred nanometers long. Partly because of their high aspect ratio and high surface area, the clay particles, if properly dispersed in the polymer matrix at a loading level of 1–5 wt%, impart unique combinations of physical and chemical properties; these become attractive films for dental research/clinical work. Examples of such property enhancements include decreased permeability of gases and liquids, better resistance to solvents, increased thermal stability, and improved mechanical properties. Moreover, biodegradability is also retained. The overall objective of this work is to prepare intercalative chitosan–clay nanocomposites (films) of different chitosan loading concentrations for applications in dental work.

2. Experimental

2.1. Material and Methods

Chitosan low molecular weight was purchased from Sigma-Aldrich

(St. Louis, USA). MMT clay, acetic acid glacial, all purchased from HIMEDIA lab (Mumbai, India). Distilled water was prepared in lab.

2.2. Membrane fabrication

Composite films of pureCS and CS/MMT (3, 5, and 7 wt.%) were prepared by solution casting method and then incorporated with MMT clay particles in 3, 5 and 7 wt. % with respect to the weight of the polymer. A 4 g of CS was dissolved in 80 mL of 1% acetic acid with constant stirring at room temperature. Separately, particles were weighed and dispersed in 20 mL of water by sonication for 120 min and were added to the previously prepared CS solution, stirred for a further period of 24 h. Uniform thickness films were then casted by pouring the solution on a dust-free glass plate. These films were dried and peeled off from the glass plate. Pristine CS film was prepared in the same manner except that clay filler particles were not added. Film thicknesses as measured by a micrometer screw gauge were around 50 ± 1.0 mm. The membranes were fully characterized by SEM, XRD and UTM to understand the physical properties.

2.3. Membrane characterizations

Scanning electron microscopy (SEM)

Scanning electron microscopy micrographs of pristine chitosan and chitosan-MMT loaded films were obtained using low and high resolution (Mag 300X 5kV) to understand the uniform distribution of MMT nanoparticles throughout the film. SEM (Jeol JSM-6360) was done on thin films equipped with energy dispersive analysis of x-ray (EDAX). Since the film was non-conductive, gold coating was necessary before subjecting to SEM experiments.

Universal Testing Machine

Tensile strength and elongation at break of the pristine CS, CS-MMT (3, 5) and CS-MMT (7%) filled matrix films were measured using the Universal testing machine (UTM) (Star Testing Systems, Model STS 248, Load cell 1000 N Max capacity). Test specimens were prepared in the form of dumbbell shapes as per ASTM D-882-95a standards. A 25 mm gauge length (Grip to Grip distance) is maintained. Samples were pulled up to break at 50 mm/min cross head speed. Yield strength, tensile strength and elongation data were recorded in the software. Modulus of elasticity was calculated using the software by collecting stress/strain data in the linear portion of stress vs. strain curve. For all measurements, 20 mm film width was considered. Elongation at break was measured on 25 mm gauge length.

3. Results and discussion

3.1. Morphological properties MMT/CS composite films

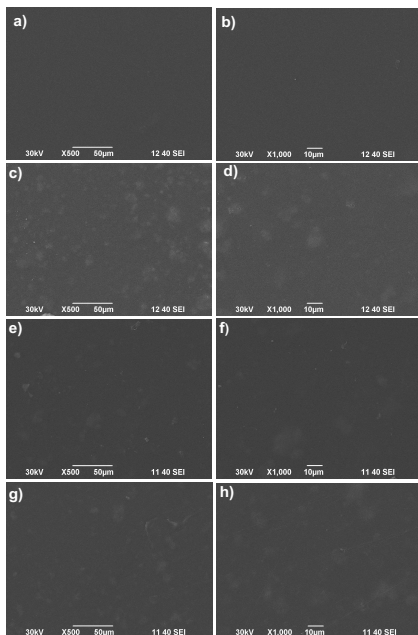


Figure 1. Low and high magnification images of (a and b) for pure CS, (c and d) for 3 wt% MMT/CS, (e and f) for 5 wt% MMT/CS, and (g and h) for 7 wt% MMT/CS composite films.

The SEM micrographs (Fig. 1 a and b) of pure CS are confirmative of

the absence of the MMT particles in the matrix. Figure 1 c and d indicates the uniform dispersion of MMT particles. As the % of MMT loading increased, the aggregated particles are observed as shown in Fig. 1 e and f as well as Fig. 1 g and h. It can be realized that addition of higher amount of MMT in the CS matrix has not shown great differences between the morphological set of the composite films.

3.2. Particle size analysis

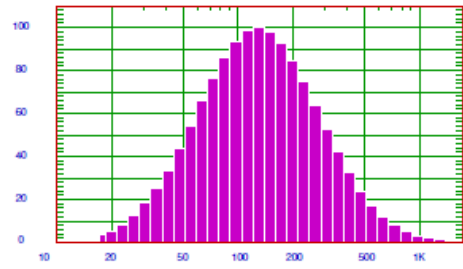


Figure 2. Particle size analysis of MMT nanoparticles.

Particle size analysis of MMT (Fig. 2) was measured using (NICOMP UAS) particle size analyzer by taking 400 mg of MMT clay sample in 10 ml water and was subjected to sonification up to 30 min to avoid the agglomeration of particles during the measurements. Particle size curve shows a bell shape curve representing the Gaussian distribution and the mean particle size was measured to be 120 nm.

3.3. Structural properties of MMT/CS composite films

Figure 3 shows the XRD graphs (measured on Philips PW1729) of pure chitosan, different weight % clay loaded composite films. In Fig. 3a, for pure chitosan, the observed angles, $2\theta = 10^\circ, 15^\circ, 22^\circ$ suggest the amorphous nature of chitosan. As the concentration of MMT increased, the crystalline nature of CS also increased in CS-MMT composite films. Thus, clay dispersion within CS matrix could change the morphology of the film after loading with MMT. According to the XRD diffraction pattern, increase in the basal spacing observed suggests the intercalation of chitosan molecules between the silicate layers of MMT, but as the concentration of MMT increased the crystalline nature of CS also increases as evident in CS-MMT composite films. In any case, the original crystallinity of chitosan is maintained ever after addition of MMT since around $2\theta = 220$, the peak intensity is still maintained. The thermal stability of chitosan was remarkably improved in the interlayer space due to strong electrostatic interactions of the cationic chitosan molecules with anionic silicate layers. Due to polycationic nature of chitosan at low acidic medium, intercalation of chitosan molecules in MMT layers takes place mainly via cationic exchange reaction.

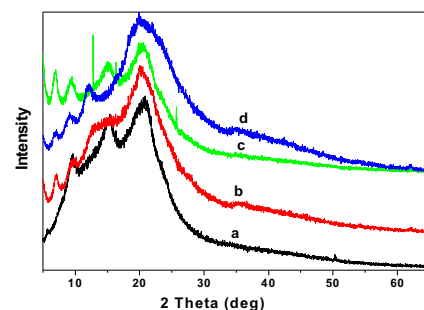


Figure 3. XRD pattern of (a) pure CS, (b) 3% MMT/CS, (c) 5% MMT/CS, and (d) 7% MMT/CS composite films

3.4. FTIR spectra of MMT/CS composite membranes

FTIR measurements (Nicolet) were performed to identify possible interactions between CS and MMT particles. Figure 4 shows the spectra MMT/CS composite films where we can observe a bond at 3400 cm^{-1} indicating N-H stretching vibration of NH_2 group. The peak at 2800 cm^{-1} shows C-H stretching vibrations of CH_2 groups, the peaks at 1650 and 1500 cm^{-1} indicate N-H bending and that at 1350 cm^{-1} is due to C-H bending. The intense band at 1033 cm^{-1} is assigned to C-O stretching.

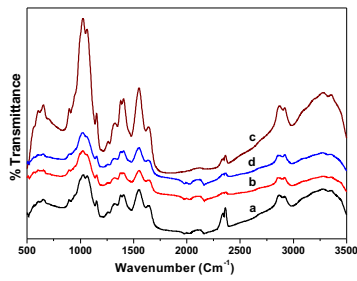


Figure 4. FTIR spectra of (a) pure CS, (b) 3%MMT/CS, (c) 5wt% MMT/CS, and (d) 7wt% MMT/CS composite membranes.

3.5. Swelling studies

The swelling studies were performed gravimetrically on all the films in water. To do this, dry weight of the circularly cut (diameter = 2.5 cm) pristine CS, CS/MMT-5 and CS/MMT-7 films were taken on a microbalance sensitive to ±0.01 mg, then samples were kept inside the specially designed airtight test bottles containing 20 cm³ test media at room temperature. Dry membranes were equilibrated by soaking in water in a sealed vessel at 300C for 24 h. Swollen films were weighed immediately after carefully blotting with a filter paper and all weights were measure using a digital microbalance. The % sorption was calculated as:

$$\% \text{ Sorption} = \left(\frac{W_w - W_0}{W_0} \right) \times 100$$

where W_w and W_0 are weights (g) of the sorbed and dry films, respectively.

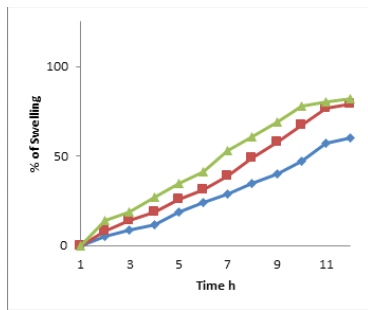


Figure 5. The swelling studies of CS composites with different wt% of MMT.

Result of swelling presented in Figure 5 for pure chitosan , 5 wt % and 7 wt% CS loaded clay particles. Swelling was dependent on the extent of filler loading. With increase of loading of filler MMT the swelling also increased.

3.5. The mechanical properties MMT/CS composite films

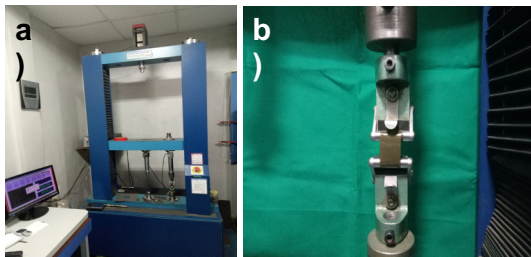


Table 1: The mechanical properties of MMT/CS composite membranes.

Films	Yield Strength (MPa)	Tensile Strength (MPa)	Elongation at Break (%)	Modulus of Elasticity (MPa)
3 wt% MMT/CS	41.64	43.76	12.4	533
5 wt% MMT/CS	46.61	51.09	15.6	668

7 wt% MMT/CS	51.59	58.46	18.8	803
--------------	-------	-------	------	-----

Accuracy of the machine: ±1%

The mechanical property measurements of MMT-CS composite films (see actual photograph and Table 1 data) using UTM and all collected data of tensile strength, yield strength, modulus of elasticity and elongation at break point of the composites increased significantly with increase in clay concentration

3.5. Table 2: Microbial Limit Test Films

The antimicrobial activity of pure chitosan, chitosan-based nanocomposites was tested quantitatively by the minimum inhibition concentration (MIC) measurement. Two representative food pathogenic bacteria including Staphylococcus aureus (Gram+/KCCM11764) and Escherichia coli (Gram-/KCCM 11600) were used for testing the antimicrobial activity. The cells of S. aureus and E. coli were cultivated on nutrient agar (peptone5g/L, beef extract3g/L, agar 20 g/L, pH 7.0) and incubated at 37°C for 2 days. The test samples were systematically diluted from 0.2 % (w/v) to 0.0125% (w/v) to determine the MIC values

Table 2 summarizes typical antimicrobial test results of pure chitosan and chitosan

	STAP HYLO COCO US	AURE US	GRA M	POSITI VE	ESCH ERIC HIA COLI	GRA M	NEGAT IVE	
Con %	Chitos an	3 wt % MMT/CS	5 wt % MMT/CS	7 wt % MMT/C S	Chitos an	3 wt % MMT/CS	5 wt % MMT/C S	7 wt % MMT/CS
0.2	X	X	X	X	X	X	X	X
0.1	O	X	X	X	X	X	X	X
0.05	O	X	X	X	O	X	X	X
0.25	O	O	X	X	O	X	X	X
0.01 25	O	O	O	X	O	O	O	O

montmorillonite nanocomposite films. Generally all composite materials, including controlled chitosan and chitosan nanocomposites exhibit antimicrobial activity. Interestingly, chitosan–montmorillonite nanocomposites show significantly higher antimicrobial activity against S. aureus and E. coli than pure chitosan and MMT. In particular, Gram-negative bacteria are more susceptible to the antimicrobial effects of chitosan-based materials as the Gram positives, presumably due to their thinner murine wall, may allow more rapid absorption of the ions into the cell [11].

It is well known that chitosan itself has antimicrobial activity due to its cationic property. Therefore, the increased antimicrobial activity of the nanocomposites is contradictory, because the positive charge of chitosan molecules is neutralized via an electrostatic interaction with anionic silicate layers. Thus the nanocomposites exhibit the synergic effect because the chitosan molecules are evenly distributed through the inorganic matrix .The resulting nanocomposites show a somewhat enhanced antimicrobial activity. In addition, a combined effect of chitosan and organic acid (i.e. acetic acid) may be increased the antimicrobial activity

Conclusions

- The intercalation of cationic biopolymer chitosan into Na+–montmorillonite through a cationic exchange process provides nanocomposites with enhanced thermal stability and antimicrobial activity.
- Thermal insulation effect of inorganic silicate layers and a strong electrostatic interaction between chitosan molecules and silicate layers are mainly responsible for the increased thermal stability.
- The highly dispersed chitosan molecules in the nanocomposites through layer-by-layer stacking structure play also an important role in enhancing the antimicrobial activity.
- These results suggest a potential application of chitosan–clay nanocomposites in the development of natural biopolymer-based biodegradable materials with antimicrobial activity.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. J. W. Park, M.-O. Park, K.K. Park, Bull. Kor. Chem. Soc. 5(1984)108–112.
2. C. Peniche-Covas, L. W. Alvarez, W. Arguelles -Monal, J. Appl. Polym. Sci. 46(1992) 1147–1150.
3. E. Guibal, C. Milot, O. Eterradosi, C. Gauffier, A. Domard, Int. J. Biol. Macromol. 24(1999)49–59.
4. T. Tianwei, H. Xiaojing, D. Weixia, J. Chem Technol, Biotechnol, 76(2001)191–195.
5. X. Wei, J. Cruz, W. Goroski, Anal. Chem. 74(2002)5039–5046.
6. L.L. Balassa, J.F. Prudden, Proceedings of the First International Conference on Chitin /chitosan, MIT, Boston, 1978, pp.335
7. J.R. Pan, C. Huang, S. Chen, Y.-C. Chung, Colloids Surf. A 147 (1999)359–364.
8. J. H. Han, Food Technol. 54(2000)56–65.
9. D. S. Cha, M. S. Chinnan, Crit. Rev. Food Sci. Nutr. 44 (2004)223–237.
10. A. Cagri, Z. Ustunol, E.T. Ryser, J. Food Prot. 67(2004)833–848.
11. J. W. Rhim, S. I. Hong, H. M. Park, P. K. W. Ng, J. Agric. Food Chem. 54(2006) 5814–5822.
12. E. Ruiz-Hitzky, M. Darder, P. Arnada, J. Mater. Chem. 15(2005)3650–3662.
13. M. Darder, M. Colilla, E. Ruiz-Hitzky, Chem. Mater. 15 (2003)3774–3780.
14. G. Lagaly, Appl. Clay Sci. 15 (1999)1–9.
15. E. P. Giannelis, Adv. Mater. 8 (1996)29–35.
16. Raziye Salehia, Mokhtar Aramia, Niyaz Mohammad Mahmoodib, Hajir Bahramia, Shooka Khorramfara Novel biocompatible composite (Chitosan–zinc oxide nano particle): Preparation, characterization and dye adsorption properties Colloids and Surfaces B: Bio interfaces 80 (2010) 86–93.
17. C.K.S. Pillai, Willi Paul, Chandra P. Sharma Chitin and chitosan polymers: Chemistry, solubility and fiber formation Progress in Polymer Science 34 (2009) 641–678