Poly(vinyl alcohol)/chitosan/clay nano-composite films

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Abstract: The present attempt is intended to prepare nano-composites of polyvinyl alcohol (PVA) blended chitosan and nano-clay montmorillonite (MMT) particles. PVA/chitosan films containing nano-clays particles were prepared by coating method and their physical and mechanical properties were studied by tensile tests, X-ray diffraction (XRD) and scanning electron microscopy (SEM). The XRD results confirmed that the property improvements were related to the MMT exfoliation and good interaction between PVA/chitosan blend and MMT. Mechanical properties of the films were influenced significantly by the nano-clay particles. [Hamid Mahdavi , Hamid Mirzadeh , Mohammad Jalal Zohuriaan-Mehr , Fateme Talebnezhad . **Poly(vinyl alcohol)/chitosan/clay nano-composite films.** *J Am Sci* 2013;9(8):203-214]. (ISSN: 1545-1003). http://www.jofamericanscience.org.30

Key words: polyvinyl alcohol, chitosan, nano-composite, nano-clay, montmorillonite

2.⏺.Introduction

Chitosan is an amine group containing polysaccharide with different molecular weights. As shown in Figure 1, free amine and hydroxyl groups give unique properties to this biopolymer and due this reason, it has wide applications in various industries such as agriculture, waste water treatment, food, pharmacy, cosmetics and medicine [1].
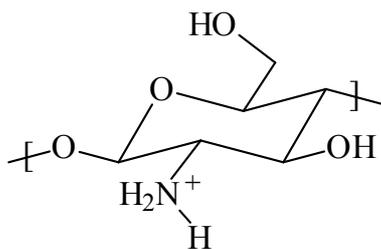
Figure 1. Repeating unit of the chemical structure of the biopolymer chitosan; pH<7.

This substance is antibacterial and antifungal; it can prevent from bacteria growth which causes infection. Antimicrobial properties of chitosan involve a wide range of microorganisms as fungi, bacteria and viruses. This property of chitosan depends on chitosan type, degree of polymerization, acetylation, nutrient substrate composition, environmental conditions such as water activity and/or moisture content of substrate. Antibacterial properties of chitosan are different from its derivatives. Antimicrobial effect of chitosan is faster on fungi and algae, in comparison with most bacteria [1].

Hence, utilizing this substance as new dressing is important in medicine, so that many researches, either academic or in industry have focused on it. This substance is usually used in form of gel and film for treating wounds and burns, but one of the main problems of this substance as a dressing is high flow in the gel phase and weak mechanical properties in the film phase. Therefore, various methods are provided to overcome the shortcoming of chitosan based polymeric films, such as combining with other polymers like PVA, cellulose and poly(ethylene oxide) (PEO), synthesis of chitosan copolymers, and applying physical and mechanical crosslinking agents [2, 3].

Owing the synthetic difficulties (e.g., residual chemicals and their subsequent side effects), combining with other materials is usually a preferable

approach favoring processability, safety and economic issues [2, 4].

PVA is a semi crystalline, water soluble polymer. This polymer is resistant against chemicals and heat. It has a good conductivity, biological compatibility with human tissues and biodegradability. Combination of PVA and chitosan was prepared to provide new materials with biological functions and unique physical and mechanical properties [2, 5].

Montmorillonite (MMT) is the most common natural layered silicate which is applied due to its low cost, availability, environment compatibility, capability to improve mechanical strength to decrease water vapor permeability, and to progress the polymer film efficiency as dressings. High water absorption of this silicate is related to its high surface area and cation-exchange capacity [6]. Although there are several literatures about combination of chitosan/PVA [5, 7, 8], there are no certain and valid reports about the effect of nano-clays on mechanical and antibacterial properties of chitosan/PVA bio-composite as a dressing. In this work, effect of nano-clay was investigated on physical and mechanical properties of the nano-composite films.

2. Experimental

2.1. Materials

Chitosan (molecular weight 29000 g/mol, degree of deacetylation 83% from fluka), Polyvinyl alcohol (PVA) with high molecular weight (85000-146000 Dalton) and hydrolysis degree of 99% were supplied by Aldrich. Montmorillonite (MMT) was purchased from Japanese Kunipia co. Chemicals were used as received.

2.2 Nano-composite preparation

Traditionally, nano-composite formulations have been developed by simple experimental method as one variable factor at a time. This method is time consuming and requires a lot of materials. Moreover, it may be difficult to evolve an ideal formulation using this classical technique, since the combined effects of

independent variables are not considered. It is therefore, essential to understand the complexity of the nano-composite formulations by using the established statistical tools such as factorial designs. The factorial design experiments were performed in random order and then various formulations of the composite were developed.

The designed matrix from a single replicate of 33 experiments along with the formulation codes are given in Table 1. The variables (factors) studied were chitosan concentration, PVA concentration and MMT ratio.

Table 1. Formulations of films

formulations	Chitosan composition ratio (w/v)	PVA composition ratio (w/v)	MMT composition ratio (w/wchitosan)
F_1	6	0	1.5
F_2	0	5	1.5
F_3	6	5	1.5
F_4	0	2.5	0
F_5	6	2.5	0
F_6	0	2.5	3
F_7	6	2.5	3
F_8	3	0	0
F_9	3	5	0
F_{10}	3	0	3
F_{11}	3	5	3
F_{12}	3	2.5	1.5

Chitosan solutions in samples F5, F8 and F9 (3 and 6 % v/w) were dissolved in an aqueous solution of acetic acid (4.5 % v/v) with different concentrations at room temperature and the obtained solution was stirred for 4 hours.

MMT was firstly added to distilled water to prepare 1.5% and 3% of the clay suspension. The suspension was stirred at 60 °C for 6 hours and chitosan was added concurrently. PVA transparent solutions (2.5 and 5% w/v) were prepared in water at 120 °C while stirring for 20 min. The chitosan, nano-clay and PVA solutions of different proportions were then mixed to obtain the mixtures with weight ratios of PVA/chitosan/nano-clay, and the resultant mixtures were stirred for 5 h.

Then it was casted upon a glass plate in an atmosphere without dust and at room temperature. The glass plates were left to dry at 30 °C for 24 hours. Obtained films were separated from glass plates after drying. After performing the required experiments, appropriate formulations were selected to investigate antibacterial properties.

2.3. Characterization

X-ray diffraction (XRD) instrument (Siemens, D500, Germany) was applied using Ni-filtered Cu-kα radiation with diffraction angle (2θ) from 5 to 40 degree, scan rate of 1.2°/min and step size of 0.02.

Mechanical properties including tensile strength and elongation at break which are measured by Santam Tensile Machine (SMT-20, Iran) according to ASTM D822. The obtained films were cut into rectangular specimens (50×25 mm) for mechanical tests. Film thickness was measured in some parts by a digital micrometer. Specimens were hold carefully between vertical clamps. After that, clamps moved with the rate of 20 mm/min until the specimen was broken at room temperature. Regarded to the recorded profiles, tensile strength (σ) and elongation at break (ε) could be calculated:

$$\sigma = \frac{F_{max}}{A} \left(N/m^2 \right) \qquad (1)$$

$$\varepsilon = \frac{L_R}{L_o} \times 100 \ (\%) \qquad (2)$$

Where F_{max} (N) was the maximum force, A (m^2) was cross section and LR was the sample's length at break and L0 was the initial specimen's length (30 mm). Each experiment was performed 3 times.

Swellings of specimens in double distilled water (DDW) were measured by weighing water content increment. After weighing the specimen in dry phase, it was immersed in de-ionized water for 24 hours. The swollen specimens were weighed specified intervals

after smooth surface drying out of the DDW by absorbent paper and degree of swelling was measured as follows:

$$Swelling\% = \frac{Ws - Wd}{W_d} \times 100 \qquad (3)$$

Where Ws was swollen specimen's weight and Wd was the dried sample's weight. Water vapor transmission rate (WVTR) of samples was measured according to European Drug Monographs [9]. For this measurement, patch of samples were cut into 40 mm diameter and were placed as a cap on top of a bottle with 35 mm diameter containing 25 ml water. Then the bottle was kept in a chamber at 35 °C and moisture content of 35% for 24 hours. The system weight loss was considered as an indicator of WVTR. WVTR was measured for each specimen by the following equation:

$$WVTR(g/m^2.h) = \frac{W_i - W_f}{24 \, A} \times 10^6 \qquad (4)$$

Where A was surface area of the mouth of bottle (mm^2), W_i and W_f were weights of system (bottle and cap) before and after keeping in chamber.

To estimate surface energy of nano-composites at ambient temperature, a Kruess–G10 contact angle measurement apparatus (Germany), was used. All measurements were performed at similar times and 6 times were repeated for each sample.

3. Results and discussion

3.1. X-ray diffraction

Typically, The determination of an exfoliated nano-composite has been based on XRD and TEM results[10]. Due to ease of usage and availability, XRD is mostly used for nano-composites structure characterization. According to the position, shape and intensity of main reflections from dispersed silicate

layers, characterization of nano-composites structure is possible [11].

Figure 2 shows XRD results of various formulations. In MMT nano-particles, $2\theta=9.54°$ (i.e., interlayer spacing 11.641 Å), whereas the peak at 25° could show clay impurities [12]. According to the Bragg equation ($d= n\lambda/2\sin\theta$), the interlayer spacing for pristine MMT was calculated to be 11.641 Å. X-ray diffractogram of PVA (sample F4) showed a sharp peak at $2\theta=24.66°$ (i.e., d-spacing of 4.53316 Å) which showed a crystalline domains for PVA. Chitosan film (sample F8) featured a weak peak at $2\theta=24.88°$ (due to d-spacing 4.4937 Å) which is representative of chitosan semi-crystalline structure [13, 14]. By combining PVA with chitosan without clay (formulations F5 and F9; Table 1), intensity of PVA peak reduced [15]. It is obvious from the diffractograms of F4 and F5, and also F8 and F9 that the peak intensity of PVA/chitosan blends increased with PVA content [16, 17]. It could be deduced that strong interactions existed between PVA and chitosan [17]. PVA peak was broadened and its intensity decreased while chitosan content increased in PVA/chitosan blend. In addition, no peaks were observed in the range of 3–9° due to lack of clay in the formulations [18].

In the polymer nano-composites, exerted MMT was expected to show a peak in the range of 3 to 9°if no interaction was occurred. Nevertheless, in exfoliated samples, silicate layers dispersed homogenously and showed no separate peaks in this range [18]. As it was observed in Figure 2, after mixing clay with chitosan and/or PVA, MMT peaks disappeared in XRD spectra of PVA/MMT samples, which showed that the clay was dispersed in the polymer matrix, and successfully exfoliated by both polymer chains [19]. The crystalline peaks got stronger and sharper after using MMT which is representative of higher crystallites or denser packing in main chain in comparison with the neat PVA. This result corresponded to the reports in literatures [20,16]. They clearly showed that chitosan and PVA chains might insert the space layers of nano-clays, layers dispersed in the matrix and proper exfoliation obtained [17].
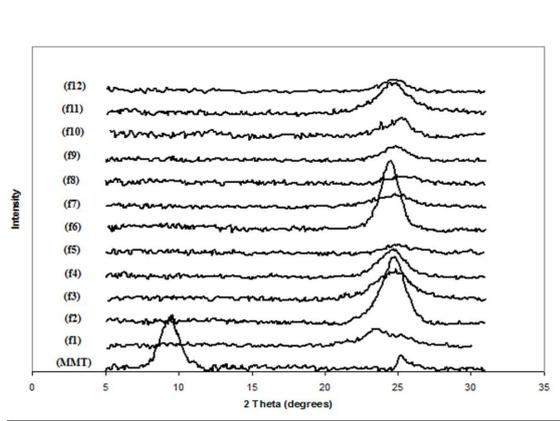
Figure 2. Chitosan, PVA, MMT and nano-composites XRD spectra.

3.2. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) has many advantages in morphology investigations. SEM images of various formulations are displayed in Figure 3. Pure PVA films (Fig. 3a) showed smooth surfaces with a homogenous matrix. Addition of MMT to PVA showed homogenous dispersion and led to un-homogenous films with rough surfaces(Fig. 3b) [8]. The existed pores on pure chitosan film surface could be attributed to water vaporization after the film coating (Fig. 3c). Homogenous dispersion of clay in chitosan matrix, also led to rough and un-homogenous surface, as the PVA/clay films(Fig. 3d), whereas chitosan/PVA films showed homogenous and smooth surfaces which is representative of high immiscibility and similar genus of PVA and chitosan

due to formation of hydrogen bonds and inter molecular interactions of these polymers (Fig. 3e)[16]. Exfoliated structure was observed in XRD results. These results were corroborated by SEM results. Homogenous dispersion of nano-particles and homogenous matrix in SEM images were evidences for exfoliated structure [9]. This observation corresponded to Rhim et al reports [21].
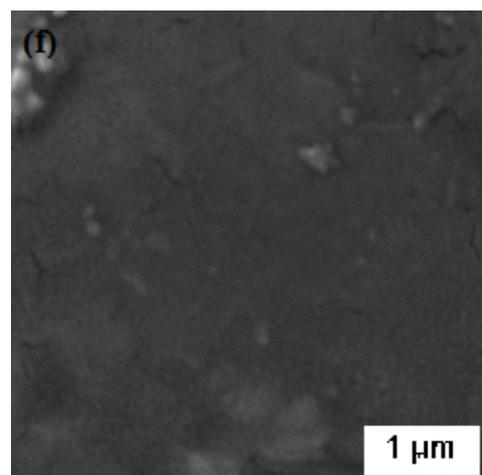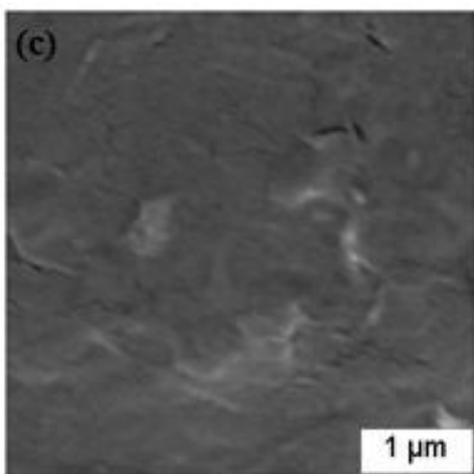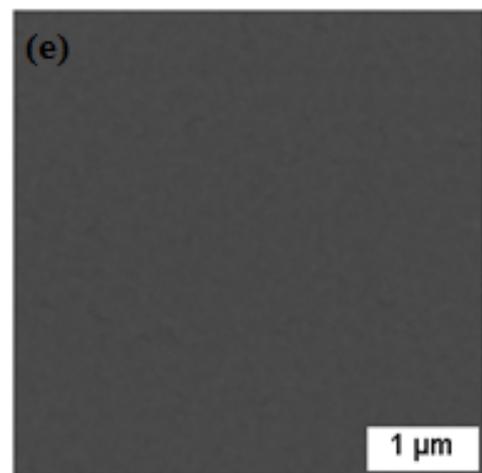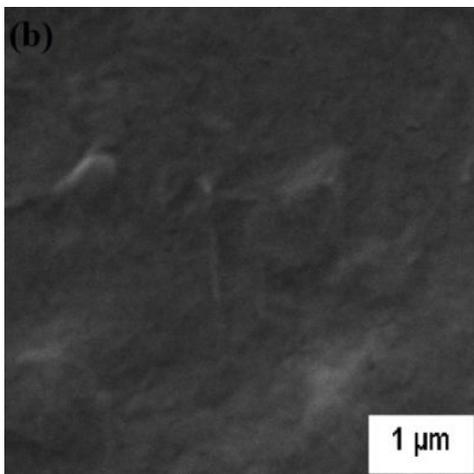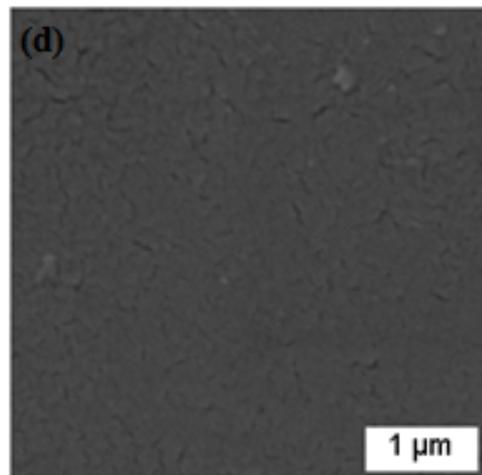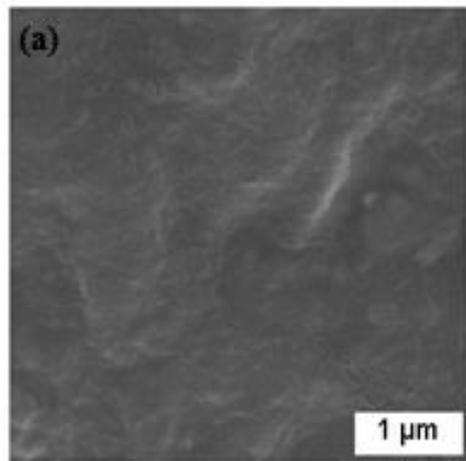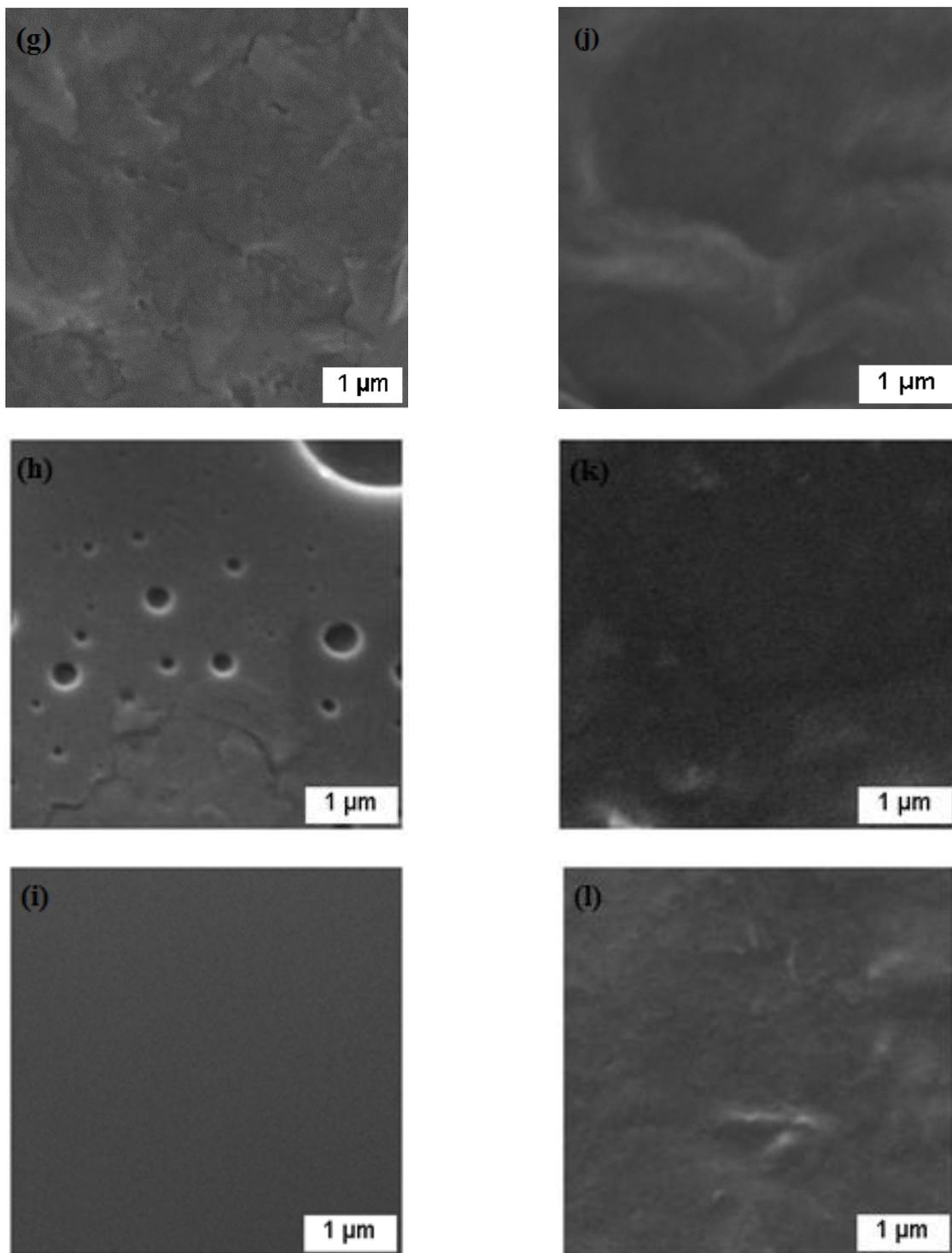
Figure 3. SEM images of (a) f1, (b) f2, (c) f3, (d) f4, (e) f5, (f) f6, (g) f7, (h) f8, (i) f9, (j) f10, (k) f11, (l) f12

3.3. Mechanical properties

The thickness of prepared films was measured using a micrometer (Mitutoyo, Kanagawa, Japan) at three different places and mean values were calculated.

Tensile test shows film strength and elasticity which are determined according to strength at break. A clear advantage of nano-composites in comparison with common composites was improvement of strength by adding fillers. These results are mainly due to high surface area of fillers which create interactions with matrix in nano scale [12]. Mechanical properties were measured and summarized in table 2. Measurements were performed in the environment and at 25 °C.

As table 2 shows, strengths of samples no. 4 and 8 were 42.3 and 9.8 MPa, respectively which changed to 16.4 and 26.9 MPa for sample no. 5 and 9 after mixing two polymers. Tensile strength increased with the content of PVA due to the increase of crystalline phase [5]. Chitosan has shown high elasticity (65.2), so increasing chitosan content resulted in more flexibility [22,5].

In general, mixing MMT and PVA resulted in diffusion of polymer chains in to organophillic silicate layers and strong interfacial interactions. Hence, it was expected that nano-composites tolerated more external load in comparison with pure PVA [23,24]. When 3% MMT was added to PVA (f6), the strength changed to 50.7 MPa which showed a 20% increment in comparison with pure PVA. In nano-composites in which chitosan was the matrix phase, strength increased with the content of MMT in comparison with pure chitosan.

When 3% MMT was added to chitosan (f10) strength changed to 15.1 MPa which is approximately 54% higher than pure chitosan. Significant increment in tensile strength by adding MMT could be attributed to formation of exfoliated structure, homogenous dispersion of MMT in chitosan matrix and also strong interactions between chitosan and MMT due to formation of hydrogen bonds during manufacturing and drying processes [9,3,25]. The primary hydrogen bonds were formed between hydroxyl and amine groups in chitosan molecules and clay hydroxyl groups and finally mixing these two bonds with hydroxyl groups of PVA. Figure 4 shows these

interactions schematically. Elasticity decreased in composites by the increase of MMT. This remarkable elongation at break might be due to chain confinements [26].

The above results are also true in composites with PVA/chitosan matrix. Results showed that although nano-clays showed synergistic effect in tensile strength, they caused large reduction in polymer flexibilities.

Table 2. Tensile strength and elasticity in various formulations

formulation	Thickness (mm)	Tensile strength (MPa)	Elongation (%)
F1	0.13± 0.01	14.4±1.0	14.3±0.9
F2	0.11± 0.05	62.2±3.2	1.5±0.4
F3	0.15± 0.06	35.7±1.5	5.7±0.5
F4	0.10± 0.07	42.3±2.3	3.4±0.8
F5	0.11± 0.01	16.4±1.1	46.9±1.3
F6	0.12± 0.06	50.7±2.2	1.2±0.2
F7	0.11± 0.07	20.6±0.9	1.5±0.3
F8	0.11± 0.08	9.8±1.2	65.2±1.5
F9	0.12± 0.07	26.9±0.9	23.1±0.9
F10	0.12± 0.07	15.1±1.1	7.0±0.7
F11	0.10± 0.09	31.7±0.9	4.46±0.5
F12	0. 9± 0.08	14.1±0.8	15.0±0.4

Chitosan

Nano-clay

Chitosan + Nano-clay

Polyvinyl alcohol

Chitosan + Nano-clay + Polyvinyl alcohol
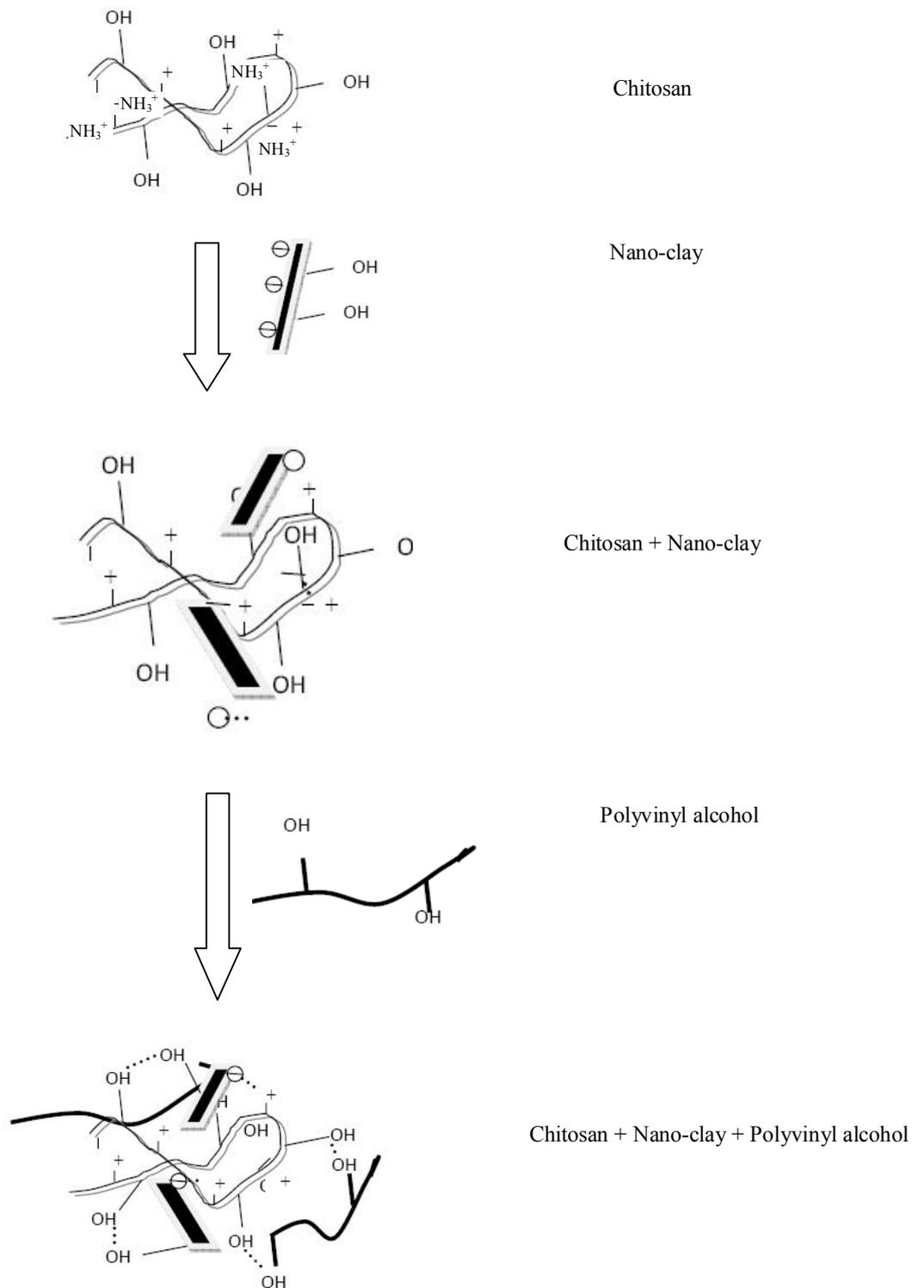
Figure 4. Schematic of polymer/polymer reaction with nano-clay

3.4. Degree of swelling

In this work, water uptake was measured as an important characterization in dressings. This feature shows capacity of a dressing in absorbing exudates from wounds. As it was seen in figure 5, water uptake in pure chitosan film (f8) was higher than pure PVA films (f4), because of PVA higher MW and its higher hydrolysis degree than chitosan. The applied chitosan had a low MW and this resulted in easier diffusion of water molecules in chitosan chains. Thus, addition of chitosan to PVA led to increment of water uptake. As seen in literatures, addition of MMT to PVA matrix reduced nano-composite swelling [24]. This was due to polar- polar interactions between PVA and silicate layers. This factor confined polymer chain movements, limited water molecules dispersion and therefore water uptake decreased [27]. This deduction is true in formulations in which PVA was the predominant matrix.

Addition of clay to chitosan led to increase in water uptake in comparison with pure chitosan. As reported by Kampeerapappun Piyaporn et.al [27], content of chitosan has a great influence on water uptake than MMT, and this is because of hydrophobic acetyl groups in chitosan molecule. This could be seen in formulations in which chitosan is the predominant matrix.
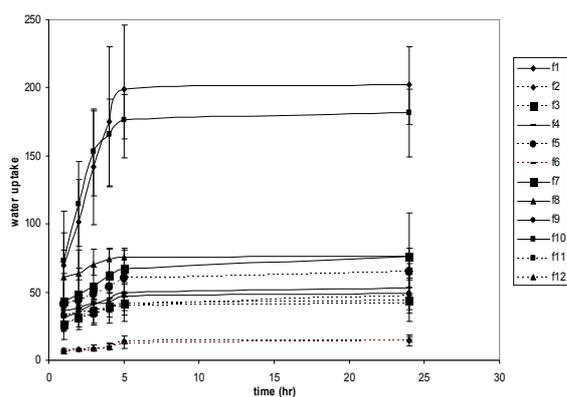
Figure 5.Water uptake of nano-composites.

3.5. Water Vapor Transmission Rate

WVTR is a factor which shows the transmission rate of water through a substance in human body. The high values of WVTR indicates that the drying process is fast, in turn, there is much tendency to make the skin dry. On the other hand, low values of WVTR indicate the accumulation of exudates at skin surface, which would result in delayed healing process and hence an increase in bacterial growth risk.

As the figure 6 shows, the water vapor transmission rate for the pure poly vinyl alcohol (f4) is higher in comparison with the pure chitosan (f8). This could be attributed to the hydrophobicity of the surface of chitosan film compared to poly vinyl alcohol, which is the result of the presence if hydrophobic Acetyl groups in chitosan structure which are deacylated inadequately. The significance of this result stems from the fact that the residues of acetyl groups play an important role in inhibiting water vapor transmission [28].

It is clear that in 5th formulation, the presence of chitosan decreases the water vapor transmission in comparison with poly vinyl alcohol, but in 9th sample this trend is not seen. In sample 9th the dominant matrix, in contrast to the sample 9th, is polyvinyl alcohol, and because of the lower molecular weight of chitosan compared to polyvinyl alcohol, chitosan functions as a plasticizer and will increase the distance between layers and also will enhance the movement of chains, which result in a significant increase in water vapor transmission rate.

In general, the rate of water vapor transmission increases by adding nano-clay particles into the polymer matrices [24]. The decrease in water vapor transmission rate in nano-composite films is due to the presence of highly dispersed layers of nano-particles in polymer matrix. Water vapors transmite through the film in order to find an indirect pass way through the polymer composite, therefore, the length of the pass way for water vapor to exit from the polymer film would be elongated.

In fact, Montmorillonite improves the barrier properties of polymer films to water vapor transmission [24,9].
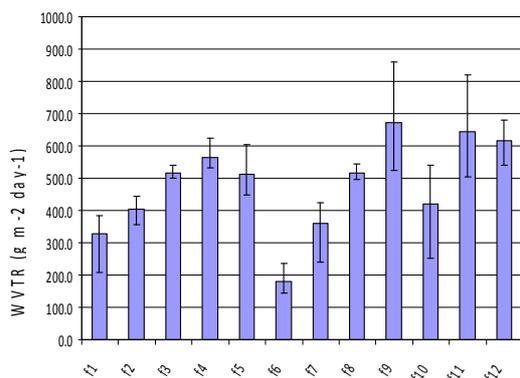
Figure 6. The water vapor transmission rate

3.6. Contact Angle Measurement

The contact angle test is used to obtain several properties such as surface homogeneity, hydrophilicity or hydrophobicity, and immigration of functional group to the surface.

The aim of this study is to consider the hydrophile or hydrophobe surface properties of nano-composite.

The contact angle measurement results for Poly vinyl alcohol (4th sample) and Chitosan (8th sample) with water droplet showed the value of 55.4 and 88.7 respectively. It can be clearly realized that polyvinyl alcohol is far more hydrophile than chitosan [29].

As it is observed, in composite films, by adding chitosan into poly vinyl alcohol matrix, the contact angle was increased, in turn, hydrophobicity has decreased.

By adding nano-clay particles into polymeric matrix, contact angle is decreased as a result of enhancement of hydrophilic property. This fact is related to the hydrophilic nature of nano-clay surface containing Na^+

and Ca^{2+} iones [21]. The same results are obtained in other samples, so these results would serve as a confinement for the results attained by water absorption methods.

Table 3. Measurement of contact angle for different nano-composite

formulation	cantact angle($^0\theta$)
F 1	86.1±2.45
F 2	54.0 ±0.85
F 3	70.7 ± 1.59
F 4	55.4 ±2.12
F 5	84.1±1.30
F 6	50.3± 0.76
F 7	80.4 ± 1.68
F 8	88.7±1.05
F 9	77.5 ± 2.81
F10	85.7±1.62
F11	73.0 ±2.03
F12	71.7±1.15

4. Conclusion

Chitosan based poly vinyl alcohol nano-clay composite films were produced successfully by a novel coating method by manual film applicator instrument. The product contained nano-clay particles has sufficiently good mechanical properties compared to composite films without nano clay. Composite films which were only contained 1.5 percent of nano-clay particles made a remarkable improvement in antibacterial activity properties. Simultaneously, nano-clay had a significant influence on WVTR of nano-composite films.

5. Acknowledgements

6. References

1. Jayakumar R, Prabaharan M, Sudheesh Kumar P, Nair S, Tamura H (2011) Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnology advances 29 (3):322-337.

2. Mirzadeh H, Mahdavi H (2010) Non drug based wound dressing polymer film and a method of producing the same. Publication number: 0218472 A1.

3. Abdollahi M, Rezaei M, Farzi G (2012) A novel active bionano-composite film incorporating rosemary essential oil and nano-clay into chitosan. Journal of Food Engineering 111(2):343-350.

4. Li JK, Wang N, Wu XS (1998) Poly (vinyl alcohol) nano-particles prepared by freezing–thawing process for protein/peptide drug delivery. Journal of controlled release 56 (1):117-126.

5. Mangala E, Kumar TS, Baskar S, Rao KP (2003) Development of chitosan/poly (vinyl alcohol) blend membranes as burn dressings. Trends in Biomaterials and Artificial Organs 17(1):34-40.

6. Sothornvit R, Rhim JW, Hong SI (2009) Effect of nano-clay type on the physical and antimicrobial properties of whey protein isolate/clay composite films. Journal of Food Engineering 91 (3):468-473.

7. Bahrami SB, Kordestani SS, Mirzadeh H, Mansoori P (2003) Poly (vinyl alcohol)-chitosan blends: preparation, mechanical and physical properties. Iranian Polymer Journal 12:139-146.

8. Wang LC, Chen XG, Yu LJ, Li PW (2007) Controlled drug release through carboxymethyl-chitosan/poly (vinyl alcohol) blend films. Polymer Engineering & Science 47 (9):1373-1379.

9. Razzak MT, Darwis D, Sukirno Z.(2001) Irradiation of poly(vinylalcohol) and poly(vinyl pyrrolidone) belended hydrogel for wound dressing. Radiat Phys Chem 62:107–13.

9. Casariego A, Souza B, Cerqueira M, Teixeira J, Cruz L, Díaz R, Vicente A (2009) Chitosan/clay films' properties as affected by biopolymer and clay micro/nano-particles' concentrations. Food Hydrocolloids 23 (7):1895-1902.

10. Reddy B (2011) Advances in Diverse Industrial Applications of Nano-composites. InTech, DOI: 10.5772/1931.

11. Wang S, Shen L, Tong Y, Chen L, Phang I, Lim P, Liu T (2005) Biopolymer chitosan/montmorillonite nano-composites: preparation and characterization. Polymer Degradation and Stability 90 (1):123-131.

12. Cuppoletti J (2011) Nano-composites and Polymers with Analytical Methods. InTech. 33-50.

13. Dong Y, Ruan Y, Wang H, Zhao Y, Bi D (2004) Studies on glass transition temperature of chitosan with four techniques. Journal of applied polymer science 93 (4):1553-1558.

14. Bangyekan C, Aht-Ong D, Srikulkit K (2006) Preparation and properties evaluation of chitosan-coated cassava starch films. Carbohydrate Polymers 63 (1):61-71.

15. Yang JM, Su WY, Leu TL, Yang MC (2004) Evaluation of chitosan/PVA blended hydrogel membranes. Journal of membrane science 236 (1):39-51.

16. Zheng H, Du Y, Yu J, Huang R, Zhang L (2001) Preparation and characterization of chitosan/poly(vinyl alcohol) blend fibers. Journal of Applied Polymer Science 80 (13):2558-2565.

17. Kabiri Bamoradian K, Mirzadeh H, Zohuriaan-Mehr M.J. .Highly Rapid Preparation of a Bio-Modified Nano-clay With Chitosan. Iranian Polymer Journal 16(3); 147-151.

18. Park JH, Lee HW, Chae DK, Oh W, Yun JD, Deng Y, Yeum JH (2009) Electrospinning and characterization of poly(vinyl alcohol)/chitosan oligosaccharide/clay nano-composite nanofibers in aqueous solutions. Colloid & Polymer Science 287 (8):943-950.

19. Yeh JM, Yu MY, Liou SJ (2003) Dehydration of water–alcohol mixtures by vapor permeation through PVA/clay nano-composite membrane. Journal of Applied Polymer Science 89 (13):3632-3638.

20. Tang C, Chen N, Zhang Q, Wang K, Fu Q, Zhang X (2009) Preparation and properties of chitosan nano-composites with nanofillers of different dimensions. Polymer Degradation and Stability 94 (1):124-131.

21. Rhim JW, Hong SI, Park HM, Ng PKW (2006) Preparation and characterization of chitosan-based nano-composite films with antimicrobial activity. Journal of agricultural and food chemistry 54 (16):5814-5822.

22. Parida UK, Nayak AK, Binhani BK, Nayak P (2011) Synthesis and Characterization of Chitosan-Polyvinyl Alcohol Blended with Cloisite 30B for Controlled Release of the Anticancer Drug Curcumin. Journal of Biomaterials and Nanobiotechnology 2 (4):414-425.

23. Kokabi M, Sirousazar M, Hassan ZM (2007) PVA–clay nano-composite hydrogels for wound dressing. European polymer journal 43 (3):773-781.

24. Xu Y, Ren X, Hanna MA (2005) Chitosan/clay nano-composite film preparation and characterization. Journal of applied polymer science 99 (4):1684-1691

25. Silva SML, Braga CRC, Fook MVL, Raposo CMO, Carvalho LH, Canedo EL Application of Infrared Spectroscopy to Analysis of Chitosan/Clay Nano-composites. 43-62.

26. Sanglimsuwan A, Seeponkai N, Wootthikanokkhan J (2011) Effects of Concentration of Organically Modified Nano-clay on Properties of Sulfonated Poly (vinyl alcohol) Nano-composite Membranes. International Journal of Electrochemistry doi:10.4061/2011/785282.

27. Kampeerapappun P, Aht-ong D, Pentrakoon D, Srikulkit K (2007) Preparation of cassava starch/montmorillonite composite film. Carbohydrate Polymers 67 (2):155-163.

28. Koyano T, Koshizaki N, Umehara H, Nagura M, Minoura N (2000) Surface states of PVA/chitosan blended hydrogels. Polymer 41 (12):4461-4465.

29. El-Hefian EA, Nasef MM, Yahaya AH (2010) Preparation and Characterization of Chitosan/Polyvinyl Alcohol Blends-A Rheological Study. Journal of Chemistry 7 (S1):S349-S357.

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