Addition of Nanocopper to Organoclay to Improve Permeability and Antibacterial Activity of Polypropylene Nanocomposite Films

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Abstract: - Effect of organoclay with copper nanoparticles (OBEN-CuNP) on barrier properties and antibacterial activity of polypropylene nanocomposite blown film was investigated. Water vapor permeability and oxygen permeability of blown film samples were studied in accordance to ASTM E398 and ASTM D3985, respectively. Antibacterial activity was studied by the agar diffusion test. Organoclay caused flow path of water vapor to be more tactoids and thus reduced water vapor flow rate as compared to homogeneous PP matrix. Adding OBEN-CuNP into PP films increased water vapor permeability due to hydrophilicity of PVP coated on CuNP. Oxygen permeability of the PP nanocomposite films increased with increasing CuNP content. From the agar diffusion tests, it is found that there were inhibition zones surrounding OBEN-CuNP pastes. However, although the clear zone was not observed when using film samples, it was obvious that the microbial cannot grow on the films and it suggested that copper ions from the film samples diffused into agar very slowly.

Key-Words: - Antibacterial activity; Copper nanoparticles; Water vapor permeability; Oxygen permeability; Polypropylene nanocomposite films

1 Introduction
PP/clay nanocomposites can be prepared through the melt blending with modified clay and with functionalized oligomers such as maleic anhydride grafted propylene (PP-g-MA) as compatibilizers. The structural characterization of PP/organoclay hybrids has yielded relevant information about the influence of the composition (i.e., clay and compatibilizer contents) and the processing conditions on the morphological features that determine the barrier properties, such as the degree of intercalation of the polymer chains in the silicate galleries, the extent of exfoliation of clay lamellae, and the generated crystalline structures. Recent studies on the transport properties of PP/organoclay nanocomposites for various organic vapors have reported. Villaluenga et al. [1] reported that an appreciable reduction in gas permeability with the addition of both the unmodified and modified nanoclay compared to the unfilled PP film was achieved. For helium, diffusion rather than solubility was responsible for the reduction in the permeability, in contrast, for nitrogen and oxygen, both diffusivity and solubility were reduced by the presence of fillers. This reduction may be attributed to the tortuous path for diffusing gas molecules and the reduced molecular mobility of polymer chains due to the presence of filler particles. Mirzadeh et al. [2] reported the decrease of oxygen permeability coefficient in PP/Clay nanocomposite films attributed to the quantity of organoclay and compatibilizer and their influence on morphology and orientation of silicate layers.

In contrast, Dumont et al. [3] reported that the unmodified PP exhibited fairly better barrier performance than its mixtures with PP-g-MA and/or OMMT for water vapor, toluene and methanol. The only improvement of barrier properties in their work was obtained for the helium barrier properties. They explained the negative effect on barrier properties of PP film by the interactions occurring between the diffusing molecules and the inherently hydrophilic clay or the quaternary ammonium salt used as a surfactant in the organically modified silicate layers.

For the application of food packaging, good gas barrier property may not be always needed. It is dependent on the nature of the fresh foods, e.g. vegetables, fruits, meats, etc. For fruits or vegetables, the mechanism of ripening influences the storage condition to extend the shelf life. Many
fruits need good transport of water to prevent the growth of fungi. Moreover, to ensure that the spoilage caused by microbial attack should be minimized, the versatile antimicrobial agents like metal nanoparticles e.g. nanosilver should be employed. In this work, we introduced nanocopper to function as antibacterial agent. Nanocopper is from the same group of elements as silver but rather easy to prepare and its precursor is not expensive. Coyle et al. [4] showed that both copper and silver complexes with 1,10-phenanthroline, the antifungal compound, could perform as highly active antifungal agents. Jaiswal et al. [5] found that silver, zinc and copper were good antibacterial agents.

Several test methods have been developed to determine the efficacy of antimicrobial film packaging. The tests to evaluate the antibacterial properties generally fall into two categories: agar diffusion test (qualitative method) and dynamic shake test (quantitative method). The bacterial species Escherichia coli (Gram negative), Staphylococcus aureus (Gram positive) and Klebsiella pneumoniae (Gram negative) are used in most test methods. The agar diffusion tests are only qualitative, but are simple to perform and are most suitable when a large number of samples have to be screened for the presence of antimicrobial activity. In this method, the agar surface is inoculated by making a parallel streak, and then the sample is pressed onto the inoculated plate. The method is used for obtaining an estimate of activity, in that the growth of the inoculum organism decreases from one end of each streak to the other and from one streak to the next resulting in increasing degrees of sensitivity. After incubation at 37 ± 2 °C for 18–24 hours, a clear area of interrupted growth (clear zone) underneath and along the sides of the test material indicates antibacterial activity of the specimen. The difference in zones of inhibition does not necessarily mean that a specimen is more biocidal or less. The zone of inhibition depends on the migratory property of the antibacterial agent to diffuse into the agar; hence, it does not depend only on the strength of the biocidal agent.

Bentonite organoclay and copper nanoparticles were added into polypropylene films for barrier property and antimicrobial activity, respectively. Fabrication of nanocomposite films was performed via a water-cooled blown film extrusion typically used to produce clear films for food packaging. In this chapter, effect of CuNP content (5, 10, 15 and 20 wt% of total filler) on barrier properties and antibacterial activity of PP nanocomposite blown films were investigated.

2 Experimental

2.1 Materials
Polypropylene homopolymer (PP 1102K) pellets with MFI of 4 g/10 min (190 °C, 2.16 kg) was purchased from IRPC Co. Ltd., Thailand. Additives such as slip and antiblock agents were not added. Bentonite organoclay (OBTN) was prepared in our laboratory by using sodium activated bentonite (kindly supplied by Thai Nippon Co., Ltd., Thailand) and distearoylethyl hydroxyethylmonium methosulfate and ceteryl alcohol. Surlyn® P350, sodium neutralized ethylene-methacrylic acid ionomer, with MFI of 4.5 g/10min (190 °C, 2.16 kg) was purchased from Dupont™, USA. Polyvinylpyrrolidone (PVP, MW 40000) and copper (II) nitrate (Cu(II)NO₃·5H₂O) was purchased from Sigma-Aldrich, Germany. L-ascorbic acid was purchased from Ajax Finechem, Australia.

2.2 Preparation of OBEN/CuNP masterbatch
The masterbatch of OBEN/CuNP was prepared by mixing dried OBEN/CuNP mixture with Surlyn® P350 in a ratio of 1:2 by weight, in a Haake Rheomex PTW-16 co-rotating twin-screw extruder with D = 16 mm and L/D = 25. The operating temperature of extruder was set at 180 °C with a screw speed of 70 rpm. Extrudate was pelletized for further mixed with PP pellets in a ratio of PP:OBEN/CuNP = 99:1 wt%.

2.3 Preparation of neat PP and PP nanocomposite blown films
Neat PP and PP nanocomposite blown film samples were fabricated using a water-quenched blown film extruder (PP50, Thailand). Temperature profiles were 210, 220, 220, and 210 °C from feed zone to die. Screw speed was kept constant at 80 rpm. Bubble forming ring of 23 cm in water bath was used, which final width of blown film was about 22-23 cm. Nip-roll speed and pull-out speed were adjusted to produce blown films with final thickness of 40-50 μm.

2.4 Permeability of neat PP and PP nanocomposite blown films
Vapor Permeation Tester Model L80-4000, LYSSY was used to determine water vapor permeability of neat PP and PP nanocomposite blown films. Water vapor permeation experiments were investigated.
following procedure described in ASTM E398. The test was performed at 38°C with water vapor pressure of 49.7 mmHg. The blown films were cut into circular shape with 15 cm in diameter. The thickness of films was measured using the peacock digital thickness gauge model PDN 12N by reading 15 points at random position over test area.

Oxygen Permeation Analyzer Model 8000, Illinois Instrument Inc., was used to determine oxygen permeability of neat PP and PP nanocomposite blown films. Gas permeation experiments were investigated following procedure described in ASTM D3985. The test was carried out at 23°C with oxygen flow rate of 40 cm³/min. The blown films were cut into circular shape with 15 cm in diameter. The thickness of films was measured by using the peacock digital thickness gauge model PDN 12N by reading 15 points at random position over test area.

2.5 Antimicrobial activity of PP and PP nanocomposite films
Bacterial sensitivity to antibiotics was carried out using the agar diffusion test. Prior to mixing, sample powder and distilled water were autoclaved at 120 °C. Paste of OBEN and OBEN-CuNP were prepared by mixing 0.2 gram of sample powder in 50 ml distilled water. The Escherichia coli (E.coli) suspension (100 μl of 10⁴ – 10⁵ CFU ml⁻¹) was applied uniformly on the surface of nutrient agar plate, and then five holes were cut on the agar plate for placing the powder paste. Distilled water was put into the center hole as the control reference. Each sample (OBEN, OBEN-CuNP5, OBEN-CuNP10, OBEN-CuNP15, and OBEN-CuNP20) were put into two holes in the plates. The plates were incubated at 35 °C for 24 h, after which photo of the inhibition zone (clear zone) surrounding the paste was taken for comparison. The same procedure was also performed using small pieces of neat PP and PP composite blown films as the antibiotics.

3 Results and Discussion

3.1 Water vapor permeability and oxygen permeability of neat PP and PP nanocomposite blown films
Table 1 presents water vapor permeability (WVP) of neat PP and PP nanocomposite blown films and graphically presented in Fig.1. Water vapor permeability of neat PP film is 5.44 x 10⁻¹³ g/m.s.Pa.

Table 1 Water vapor transmission rate and water vapor permeability of neat PP and PP nanocomposite blown films

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Film thickness (µm)</th>
<th>Water Vapor Transmission rate (g/m²•day)</th>
<th>Water Vapor Permeability (g/m•s•Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat PP</td>
<td>50</td>
<td>5.61</td>
<td>5.44 x 10⁻¹³</td>
</tr>
<tr>
<td>PP/Surlyn</td>
<td>38</td>
<td>6.41</td>
<td>4.73 x 10⁻¹³</td>
</tr>
<tr>
<td>PP/OBEN</td>
<td>33</td>
<td>7.75</td>
<td>4.96 x 10⁻¹³</td>
</tr>
<tr>
<td>PP/OBEN-Cu5</td>
<td>47</td>
<td>5.83</td>
<td>5.32 x 10⁻¹³</td>
</tr>
<tr>
<td>PP/OBEN-Cu10</td>
<td>43</td>
<td>6.56</td>
<td>5.48 x 10⁻¹³</td>
</tr>
<tr>
<td>PP/OBEN-Cu15</td>
<td>40</td>
<td>7.26</td>
<td>5.64 x 10⁻¹³</td>
</tr>
<tr>
<td>PP/OBEN-Cu20</td>
<td>46</td>
<td>6.54</td>
<td>5.84 x 10⁻¹³</td>
</tr>
</tbody>
</table>

Although PP is hydrophobic in nature that has good barrier property against water, the water vapor would penetrate and diffuse into polymer matrix through free volume in amorphous region. There are three main factors that determine the permeability of a polymeric material: the degree of crystallization, the structure compactness, and the polarity. Adding ionic aggregates from Surlyn into non-polar PP matrix decreased water vapor permeability slightly. This could be attributed from increasing in crystallinity of PP matrix having Surlyn to be the nucleating sites. Although the SEM result (Fig.2) shows immiscible blend between PP matrix and ionic aggregates providing flow channel for water vapor to pass through their interfaces, the polar water molecules could not adsorb well into the relatively nonpolar PP matrix to diffuse through the existing channels. Thus, it becomes like breathable film.
Similarly, from Table 2 and Figure 3, oxygen permeability increases with CuP content (4.55-5.21 x 10^{-6} mol/m\cdot s\cdot Pa). This attributes to the presence of voids at the interfaces which become the opening for gas transportation.

**Table 2** Oxygen transmission rate and oxygen permeability of neat PP and PP nanocomposite blown films

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Film thickness (µm)</th>
<th>Oxygen Transmission rate (cc/m²•day)</th>
<th>Oxygen Permeability (mol/m²•s•Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat PP</td>
<td>52</td>
<td>1717</td>
<td>4.55 x 10^{-6}</td>
</tr>
<tr>
<td>PP/Surlyn</td>
<td>49</td>
<td>1910</td>
<td>4.77 x 10^{-6}</td>
</tr>
<tr>
<td>PP/OBEN</td>
<td>34</td>
<td>2754</td>
<td>4.77 x 10^{-6}</td>
</tr>
<tr>
<td>PP/OBEN-Cu5</td>
<td>59</td>
<td>1643</td>
<td>4.94 x 10^{-6}</td>
</tr>
<tr>
<td>PP/OBEN-Cu10</td>
<td>46</td>
<td>2162</td>
<td>5.07 x 10^{-6}</td>
</tr>
<tr>
<td>PP/OBEN-Cu15</td>
<td>56</td>
<td>1796</td>
<td>5.13 x 10^{-6}</td>
</tr>
<tr>
<td>PP/OBEN-Cu20</td>
<td>56</td>
<td>1824</td>
<td>5.21 x 10^{-6}</td>
</tr>
</tbody>
</table>

In PP/OBEN blown film, the water vapor permeability increased slightly compared to PP/Surlyn film but it was still lower than those of neat PP. This could be result from relatively increasing in crystallinity of PP matrix when OBEN nanoclay was dispersed with the help of Surlyn. It is reported [1] that organoclay caused flow path of water vapor to be more tactoids and thus reduced water vapor flow rate compared to homogeneous polymer matrix. In PP/OBEN-CuNP blown film, the increasing trend of water vapor permeability with respect to CuNP content was observed. Although the crystallinity of PP matrix was increased with the nucleating effect of CuNP, the hydrophilicity of PVP coated on CuNP would provide better adsorption of water vapor and diffuse through the existing flow channels between the interface of PP matrix, ionic aggregates, and nanoparticles.

Table 2 presents oxygen permeability of neat PP and PP nanocomposite blown films and graphically presented in Fig.3. In contrast to water vapor permeability (WVP), oxygen permeability of PP/Surlyn blown film was higher than neat PP. This result is correlated to the incompatibility between PP matrix and Surlyn ionomer dispersed phases which Dumont et al. [3] argued that the lack of interactions between PP chains and inorganic tactoids might lead to the formation of voids in the structure that boost gas diffusivity. The main reason for this behavior in the PP nanocomposite film is the presence of Surlyn. As it is known the better dispersion of nano-particles can be achieved by adding more compatibilizer but this sort of compatibilizer has also a reverse effect on permeability. It increases the diffusion coefficient of the nanocomposite due to its low molecular weight [3].

The contradiction between water vapor and oxygen permeability in the PP nanocomposite films could be explained based on the difference of molecular sizes and polarity between water molecules and oxygen molecules. Molecular size of non-polar oxygen molecule is about 0.292 Å, while polar water molecule is about ten times bigger (2.75 Å). Since oxygen is much smaller, their flow path through PP/Surlyn and OBEN-CuNP blown films would occur in either the exiting flow channels along the interface between PP matrix and Surlyn dispersed phases or the amorphous region of PP matrix. Although crystallinity of PP was increased with respect to CuNP content, the relatively lesser amount of clay aggregates and individual platelets dispersed in the PP matrix provided less tortuosity for oxygen molecules to pass through the PP matrix. Therefore, these combined actions lead to an overall
decrease in the oxygen barrier properties of the PP nanocomposite blown films.

3.2 Antimicrobial activity of neat PP and PP nanocomposite films
Figures 4-6 reveal the agar diffusion tests for antibacterial property of the nanocomposite films. The antibacterial activity of OBEN and OBEN-CuNPx pastes for E. coli was measured by the agar diffusion method. Colonies of E.coli could not be viewed in the clear zone (inhibition zone) around the holes having OBEN-CuNP pastes, whereas such colonies were formed all over the control holes (water) and just OBEN. The microbial inhibition indicates that there was antibiotic released from the pastes and diffused into the agar layer, retarding the development of microbial cells in the agar. This clearly demonstrates that the antimicrobial activity is only due to copper nanoparticles impregnated into OBEN organoclay. Ruparelia et al. [6] reported that the bactericidal effects observed in their study were impacted by the release of Ag⁺/Cu²⁺ ions in solution. They stated that the presence of nanoparticles in suspension would ensure continuous release of ions into the nutrient media. Silver or copper ions released by the nanoparticles may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death. However, the exact mechanism behind bactericidal effect of copper nanoparticles has not been reported yet and needs to be studied further.

Figure 4  Agar diffusion test showing clear zone around OBEN-Cu5 pastes compared to OBEN powder and water (reference)

Figure 5  Agar diffusion test showing clear zone around (a) OBEN-Cu10, OBEN-Cu15, and (b) OBEN-Cu20 pastes compared to OBEN and water (reference)

Figure 6  Agar diffusion tests of neat PP and PP nanocomposite blown films

Around the control reference (antibiotic agent). This could result from very low diffusivity of copper ion into agar to inhibit the grown of E.coli. It was noticed that there was none bacterial colony on the nanofilms suggesting the bacterial could not grow on the films.
4 Conclusion
Having ionic aggregates (Surlyn), the PP film was less water vapor permeate due to increasing in crystallinity. Organoclay caused flow path of water vapor to be more tactoids and thus reduced water vapor flow rate compared to homogeneous PP matrix. Adding OBEN-CuNP into PP films increased water vapor permeability due to hydrophilicity of PVP coated on CuNP. In contrast, all PP films adding Surlyn or nanoparticles increased oxygen permeability because there were flow channels at the interfaces between Surlyn and PP matrix. From the agar diffusion tests, it is found that there were inhibition zones surrounding OBEN-CuNP pastes. However, the clear zone was not observed when using film samples which would be result from very low diffusivity of copper ion from the film samples into agar.

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References: