Development and physico-chemical characterization of Polyvinylidene fluoride (PVDF) flat sheet membranes with antibacterial properties against *E. coli* and *S. aureus*

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Abstract. The use of silver-modified montmorillonite (Ag-MMT) nanoclay from local montmorillonite ore as an additive for the development of PVDF flat sheet membranes with antibacterial properties for use in water disinfection was the focus of this study. It covers the development of PVDF flat sheet membranes with Ag-MMT nanoclay. The physico-chemical characterization was done through XRD, AFM, contact angle measurement, and FE-SEM while the antibacterial properties against gram-positive *S. aureus* and gram-negative *E. coli* were through inhibition zone and contact inhibition assessment. XRD results showed exfoliation of the Ag-MMT nanoclay in the PVDF flat sheet membrane, with minimal intercalations and similar functional group interactions. AFM results showed an increased surface roughness for every increase in Ag-MMT nanoclay which correlates to the contact angle measurement of membranes, demonstrating high contact angle measurement and high hydrophobicity for rougher surfaces, showing high hydrophilicity for the 0.250% Ag-MMT nanoclay membrane with a contact angle of 79.5 degrees. FE-SEM results reveal the morphology of the membrane. All experimental membranes are negative in contact inhibition against *E. coli* and *S. aureus*. However, the Ag-MMT nanoclay has been found to have antibacterial properties with the formation of inhibition zones, showing a higher sensitivity against *E. coli*.

1. Introduction.
The use of PVDF flat sheet nanocomposite membranes with antibacterial properties is a potential solution to solve the water pollution problem in the Philippines caused by *E. coli* and *S. aureus*. Synthesized nanocomposite membranes from Philippine montmorillonite nanoclay had shown to exhibit additive properties such as increased tensile strength. Further research on the development of these said membranes, specifically in modifying it with silver for antibacterial properties against *E. coli* and *S. aureus* is needed. Montmorillonite composition is also known to vary according to location, which implies that studies done by other countries regarding the physico-chemical properties of their
montmorillonite nanoclay may be different from the properties of montmorillonite ore that originated from the Philippines. The aim of the study is to be able to develop and characterize PVDF flat sheet membranes modified with silver-modified montmorillonite (Ag-MMT) nanoclay in order to improve its antibacterial properties. Specifically, the objectives are (1) to develop PVDF flat sheet membranes modified with Ag-MMT nanoclay; (2) to characterize the physico-chemical properties of the membrane, and (3) to determine the antibacterial effects of the membrane and the Ag-MMT additive on both *S. aureus* and *E. coli*, which are the most common bacteria found in water, and are representatives of gram-positive and gram-negative bacteria respectively. The agar plate method was used to measure inhibition zones with contact inhibition analysis.

2. Related Work
In the Philippines, material use, waste, and emissions were found to grow at 2.4% yearly, with 89% emission towards the air (Martinico-Perez, Schandl, & Tanikawa, 2018) [1]. These include common bacterial pollutants such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), which according to Gaho and Abouni (2015) [2], are two of the leading causes of global mortality. Thus, improved water disinfection systems are needed. By a recent study of the University of the Philippines – Diliman, the antibiotic resistance and extended-spectrum beta-lactamase (ESBL) production of *E. coli* that is isolated from irrigation waters are explored (Vital et al., 2018) [3], which show that highly polluted surface waters were constantly seen to show that they serve as proliferation sites for the emergence of antibiotic-resistant bacteria in the environment. *S. aureus* in drinking water may also serve as a source for colony-forming bacteria exposed to contaminated water, with selective antibiotic pressure for multi-drug resistant pathogens (Schaumburg, Alabi, Peters, & Becker, 2014) [4].

A membrane is one of the most popular materials for membranes in water application, and most are made of polymers and are found in commodity plastics to highly specialized materials for use in aircraft, computers, and healthcare (Shete, Thakar, Abraham, & Paranjape, 2010) [5]. The phase inversion method is a very versatile technique in preparing polymeric membranes, with a variety of morphologies that suit different applications for porous structures (Mulder, 1985) [6]. Most commercial membranes are cast using phase inversion, wherein a polymer is transformed from a liquid, soluble state to that of a solid state. PVDF is a polymer that exhibits high mechanical strength, good chemical resistance, and thermal stability with aging resistance, which is very important for the actual application of membranes (Kang & Cao, 2014) [7]. In a study by Said (2018) [8], silver nitrate was used as a reducing agent for the formation of the AgNP. Results show that the addition of silver nitrate into membrane has an effect on membrane structure which reduces pore size, forming a dense and sponge membrane. All experimental membranes were found to show an increase in water flux. In a study by Zhang (2013) [9], the possibility of AgNPs as disinfectant agents was explored and results show that AgNP shows strong antibacterial action in all water conditions. The results of the study provided information about the foreseen environmental impact of AgNP on endemic viruses. In the study of Yunis (2011) [10], silver nanoparticles (AgNPs) were synthesized which demonstrated that AgNPs can be used as growth inhibitors of bacteria because of their size as they can easily lyse through the nuclear content of bacteria wherein they present a huge surface area, that initiates wide contact with bacteria.

3. Proposed Approach
Silver-modified montmorillonite nanoclay (Ag-MMT) was obtained through cation exchange between sodium montmorillonite nanoclay and silver nitrate crystals. The components used were Polyvinylidene fluoride 6010 polymer (PVDF Solef 6010) as polymer, N-methyl pyrrolidone solvent (NMP), Polyvinylpyrrolidone K30 (Alderchem PVP K30) as pore former, and technical grade Polyethylene glycol 400 (Fluka PEG 400) as additives for flexibility. A post-treatment of 20% glycerin solution was also formulated as an immersing medium for freeze-fracture of the membrane. Liquid nitrogen was used for fracturing the membrane. Figures 1, 2, and 3 show the schematic diagram of the research methodology.
4. Results and Discussion

4.1. X-ray Diffraction (XRD) results

Figure 4 shows the XRD spectrum results. The results indicate that the majority of the Ag-MMT nanoclay was dispersed into the PVDF membrane, with the least exfoliation seen at the highest nanoclay concentration of 0.500%. The most exfoliated sample was the 0.250% nanoclay membrane sample with no other formation of peaks. The XRD pattern also confirms the crystalline structure of the Ag-MMT nanoclay and the PVDF membrane’s amorphous structure. All membranes are confirmed to retain their amorphous structure due to exfoliation while the presence of some peaks indicates semi-crystalline properties due to the presence of intercalation, especially with the highest nanoclay concentration of 0.500% having an amorphous structure approaching a crystalline structure.

4.2. Atomic Force Microscopy data for surface roughness and contact angle measurement

The contact angle value is used to assess the interaction between water and the membrane formed or the wettability of the membrane. An acute contact angle (<90 degrees) indicates hydrophilicity (high wettability and high free energy) while obtuse and reflex angles (>90 degrees) indicate hydrophobicity (low wettability and low free energy). From the contact angle results, it can be inferred that increasing nanoclay concentration up to 0.250% induces hydrophilicity, decreasing contact angle value until
reaching the lowest peak which shows induced hydrophilicity in the hydrophobic PVDF polymer. The maximum hydrophobicity can be measured in the contact angle for 0.375% nanoclay, with a decrease seen in 0.500%.

Table 1. AFM data and contact angle measurement.

| Sample  | Percentage of Nanoclay (%) | Surface Roughness, µm | Percent increase of surface roughness from Sample A (%) | Contact angle, (deg) | Percent increase of contact angle from control(%) |
|---------|----------------------------|-----------------------|--------------------------------------------------------|---------------------|-----------------------------------------------|
| Sample A | 0.000                      | 0.107                 | ---                                                    | 117.5               | ---                                           |
| Sample B | 0.125                      | 0.112                 | 4.672897                                               | 92.0                | -21.702128                                    |
| Sample C | 0.250                      | 0.112                 | 4.672897                                               | 79.5                | -32.340426                                    |
| Sample D | 0.375                      | 0.158                 | 47.663551                                              | 120.5               | 2.553191                                      |
| Sample E | 0.500                      | 0.191                 | 78.504673                                              | 112.5               | -4.255319                                     |

An increase in nanoclay concentration shows an increase in surface roughness as shown in Figure 5. The contact angle value measured for the membrane samples has shown a decreasing trend which displays increasing hydrophilic properties with contact angle values of 92 degrees and 79.5 degrees as shown in Figure 6. These values also showed hydrophobic properties at 120.5 degrees and 112.5 degrees. The results also showed that increasing surface roughness contributes to the increasing hydrophobicity of the membrane with a higher contact angle, while insignificant changes in surface roughness seen up to 0.250% nanoclay concentration was found to induce hydrophilic properties, with smoother surfaces demonstrating greater hydrophilic capacity. A more hydrophilic property encourages more interaction with the membrane which may yield more antibacterial action of silver against the bacteria E. coli and S. aureus.

4.3. Field Emission-Scanning Electron Microscopy (FE-SEM) Images from ADMATEL

The FE-SEM images of the membranes show the morphological properties of the membrane after being subjected to the freeze-fracture method. The control sample shows an abundance of horizontal macrovoids in the membrane and finger-like structures underneath and a relatively flat surface with low surface roughness. The sample with 0.125% nanoclay seems to display more vertical macrovoids and horizontal finger-like structures. The 0.250% nanoclay membrane sample showed a more compact thickness with horizontal macrovoids yet intact finger-like structures, demonstrating low surface roughness. The 0.375% nanoclay membrane demonstrates similar morphology to the control membrane yet shows two layers of finger-like structures, with one overlapping the other. Lastly, the 0.500% nanoclay sample shows a much varied, larger void size and inconsistent finger-like structures. All membranes have demonstrated asymmetrical geometries in terms of its structure.

At 5000x magnification as shown in Figure 7, all membranes demonstrated porous, sponge-like structures. Generally, more porous membranes are expected to show higher permeability and permeate flux, however, surface roughness and contact angle may affect membrane wettability and passage of liquids for certain water system processes and may inhibit antibacterial activity, thus high hydrophilicity is ideal for porous membranes.
Figure 7. FE-SEM images of the right cross-section of all membranes in top view at 5000x magnification

Figure 8. FE-SEM images of the right cross-section of all membranes in bottom view at 1000x magnification.

Figure 8 shows the bottom structures of the membranes do vary in size and shape that variate from the finger-like structures of membranes. The control membrane, the 0.125% nanoclay membrane, and the 0.250% nanoclay membrane has varying finger-like structures, while the 0.375% nanoclay membrane shows two layers of finger-like structures. The 0.500% seems to have the least amount of the finger-like structures. These finger-like structures form due to accelerated demixing processes which resulted in an increase in porosity and pore diameter, along with the decline of PEG rejection which encourages membrane elasticity that leads to the formation of said structures. These finger-like structures also enclose macrovoids which lead to an increase in membrane permeability. Thus, the membranes formed may allow quick permeation of materials in liquid mediums.

4.4. Antibacterial property and assessment of inhibition zone through agar-plate disc diffusion method and contact inhibition analysis

Each membrane sample was placed in a triplicate of Petri dishes to test for inhibition zone and contact inhibition analysis in bacterial growth of gram-negative E. coli and gram-positive S. aureus as shown in Figure 9 and Figure 10 respectively. After allowing bacteria to be cultured in a period of one week under high humidity conditions, bacterial growth has been observed to be formed on the experimental membranes with no observable reaction or antibacterial action. There has been no visible inhibition zone radius, with the membranes not able to inhibit bacterial growth. All experimental membranes were negative for contact inhibition.

While the antibacterial activity of membranes is observed, the effectiveness of antibacterial activity may need to be adjusted in terms of amount and formulation. A test was done with the placement of the Ag-MMT nanoclay in the agar plate with the formation of inhibition zones. A visible clearing due to the formation of inhibition zones was observed against both E. coli and S. aureus as shown in Figure 11 and Figure 12 respectively, with a larger inhibition radius formed in E. coli than S. aureus. Thus, E. coli is shown to be more sensitive to the Ag-MMT nanoclay than it is against S. aureus. This test validates the antibacterial property of the Ag-MMT nanoclay and also confirms the successful cation exchange with the display of antibacterial action against the challenge organisms.

The non-formation of inhibition zones with negative contact inhibition calls for a more effective formulation of PVDF membranes with Ag-MMT nanoclay that is proven to possess antibacterial mechanisms against both bacteria. However, this must be done in such a way that it will not sacrifice the structural integrity and physico-chemical properties of the membrane. Generally, an acceptable additive percentage of clay in membranes are only at a maximum of 1% to avoid affecting amorphous polymer structure.

Figure 9. Membrane test against E. coli.

Figure 10. Membrane test against S. aureus.
Figure 11. Ag-MMT nanoclay in agar plate method to test against E. coli.

Figure 12. Ag-MMT nanoclay in agar plate method to test against S. aureus.

5. Conclusion
The PVDF flat sheet membranes with Ag-MMT nanoclay were successfully developed and has displayed potential for antibacterial properties with physico-chemical properties that may be optimized for ideal properties according to size and function. Results of the XRD analysis confirmed that the Ag-MMT is exfoliated effectively throughout the membrane. The 0.250% nanoclay membrane sample had the most optimal exfoliation with almost no presence of aggregated nanoclay and was observed to retain its amorphous structure. The AFM and contact angle measurement showed that the surface roughness increases with increasing nanoclay concentration and hydrophobicity increases with surface roughness. The 0.250% nanoclay membrane also had the best antibacterial properties, with the lowest contact angle value of 79.5 degrees showing hydrophilic properties and low surface roughness. The FE-SEM images showed that all membranes have displayed macrovoids, finger-like, and sponge-like structures essential to membrane morphology with variations in arrangement and form that show high permeability. The antibacterial testing using the agar-plate method showed negative results for contact inhibition against both gram-positive S. aureus and gram-negative E. coli with non-formation of inhibition zones, yet the Ag-MMT nanoclay sample shows inhibition zone formation against both, showing potential for the PVDF membranes to achieve antibacterial properties with the additive’s proven antibacterial effects. A higher sensitivity of antibacterial activity was observed for gram-negative E. coli than for gram-positive S. aureus.

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