Growth inhibition of tropical fungi by silver nanoparticles incorporated polyurethane coating

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Abstract. Polyurethanes (PUs) have been widely used in the coating industry due to their high flexibility and good UV resistance. Extensive formulations of PU coatings have been reported previously in the literature. Scientists nowadays improvise products whereby nanomaterial additives such as silver nanoparticles are incorporated to obtain antibacterial and antifungal properties. In this study, spherical shaped silver nanoparticles with an average size of 46 nm were added into polyol (polypropylene glycol) which was cross-linked with toluene isocyanate to obtain PU nanocomposite coating films. Characterizations of the coating film were carried out using Fourier transform infrared (FTIR) spectroscopy to observe the urethane linkage that was formed during the cross-linking and Field Emission Scanning Electron Microscope with Energy Dispersive X-Ray (FESEM-EDX) Spectroscopy analysis to determine size, shape and distribution of the nanoparticles. The antifungal activity of the nanocomposite coating was studied against \textit{Cladosporium cladosporiodes}, \textit{Penicillium oxalicum} and \textit{Aspergillus aculeatus}. The results showed that the AgNPs-incorporated PU coating film has the ability to inhibit the tropical fungi growth.

Keywords: digital image processing, fuzzy inference system, histogram equalization, mammography
1. Introduction

Polyurethanes (PUs) were discovered by Otto Bayer in the 1930s along with the introduction of basic ingredients for PUs synthesis; isocyanates, polyesters, polyethers and acrylates [1]. They are known for their wide range of applications including as rigid and flexible foams, thermoplastics and waterborne PU [2][3][4] due to their outstanding performances, such as highly flexible, good abrasion resistance and great adhesion to a surface. However, the drawback of these materials is that they can be degraded by fungi, bacteria and/or other microbes that can cause microbiologically-influenced-corrosion (MIC), hence limit their extensive industrial applications[5]. In the coating industry, PUs are favorable due to their chemical resistant properties which are contributed by the crosslinked structure. This behavior depends on polyol’s molecular weight whereby a higher molecular weights leads to a more cross-linked structure of the coating thus exhibits excellent chemical stability[6][7]. For outdoor applications, the ability to resist chemical and microorganism attacks is necessary to prolong the coating effectiveness. Nowadays, a high-performance PU coating have been invented by incorporating of active ingredients into the PUs. Common additives that can inhibit microbial growth are metal cation (e.g. Ag⁺, Au⁺ or Cu²⁺) complexes, nanomaterials (e.g., metal nanoparticles or carbon nanotubes) and antibiotics[8][9][10]. The use of these additives has been demonstrated to boost the coating performances as well as improves the lifespan of the coating.

Silver (Ag) has been acknowledged a long time ago as one of the most effective germicides to prevent or reduce the growth of bacteria as well as fungi. To keep drinks fresh and unspoiled, the Greeks and Romans used containers made from silver. After years of researches, the nanomaterial experts discovered that silver works best in the form of nanoparticles (AgNPs) due to the high surface area to volume ratio. Furthermore, a small concentration of AgNPs, for example, less than 2 microgram/mL, does not show significant toxicity towards mammalian cells which in this case, monocytic cell [11].

AgNPs and PUs resin are commonly first synthesized individually, then mixed to form AgNPs-incorporated polyurethane composites (Atay et. al [12]). FTIR analysis which shows that the peak intensity of the organic bonds is independent on AgNPs concentration indicates that the presence of AgNPs does not affect the organic PUs structure. This suggests that the AgNPs are free to interact with the microorganisms. It has also been reported that Ag has the potential to reduce bio-aerosolization process of human pathogens in its surrounding by incorporating the nanoparticles into PU foams that were used as a part of air filtration device[13]. Lee et. al (2010) reported that at a high concentration of AgNPs, the probability of nanomaterial to be agglomerated is higher, hence the AgNPs were unable to penetrate effectively into the microorganism’s cell membrane [14]. Other than that, a high concentration of AgNPs in polymeric coatings leads to a porous and rough coating surface [12].

The study on fungi growth inhibition by AgNPs that exist in the tropical climate has been carried out recently[15][16][17]. These nanomaterials possess an inherent ability to terminate the fungi cell through a reaction that damages protein and nucleic acid [18]. Therefore, AgNPs have a potential as an additive for coating materials which can be used to inhibit or prevent the fungi growth. This kind of concept is still limited as most works of literature were more involved with bacterial inhibition function. This report describes results of our work on synthesis of the AgNPs incorporated PU coating and its ability to inhibit three tropical fungi (Cladosporium cladosporiodes, Penicillium oxalicum, Aspergillus aculaetus) growth of on potato dextrose agar.

2. Materials and methods

2.1. Materials

Polypropylene glycol with an average molecular weight of 2000 g/mol was obtained from Sigma Aldrich; US. Toluene diisocyanate (TDI) (Toluene-2,4-diisocyanate, 80%, remainder 2,6-diisocyanate) was purchased from Alfa Aesar; US. Toluene, 99% purity was acquired from R&M; UK. Silicone oil was bought form ChemPur; Malaysia. Silver nanoparticles suspension was acquired form (Novolux; Spain). Potato dextrose agar (PDA) was purchased from Oxoid; UK. Fungi (Cladosporium
cladosporiodes, Penicillium oxalicum, Aspergillus aculaetus) samples were isolated from various corroded parts of an airplane used in tropical environment. All chemicals were used as received.

2.2. Methodology

2.2.1. Preparation of AgNPs incorporated polyurethane coating. Polypropylene glycol (4.0183 g) in toluene (2.0221 g) were first mixed in a beaker and heated in a silicone oil bath at 80.0°C with constant stirring. PU prepolymer was then prepared by adding toluene diisocyanate dropwise into the mixture. After 2 hours of stirring, AgNPs suspension was added and mixed for 1 hour. Then, the prepolymer was spread onto a glass surface, and cured in a vacuum oven at 60°C for 2 hours to obtain AgNPs incorporated PU coating film. The percentages of each material used are given in Table 1.

| Materials               | Mass percentage (%) |
|-------------------------|---------------------|
| Polypropylene glycol    | 54%                 |
| Toluene                 | 28%                 |
| Toluene diisocyanate    | 15%                 |
| AgNPs                   | 3%                  |

2.2.2. Antifungal activity. Three fungi (Cladosporium cladosporiodes, Penicillium oxalicum, Aspergillus aculaetus) were chosen for the assessment of antifungal activity of the AgNPs incorporated PU coating film. The antifungal test procedure was carried out by using PDA as the growth medium. The PDA was prepared by heating 39 g of PDA in 1000 mL of distilled water, poured into a 90 mm diameter petri dish just until the surface is covered, cooled it to a room temperature and stored at 4°C. In addition to AgNPs incorporated PU coating film, the PU film without the nanoparticles was also in the testing for a comparison. The agar plates were first divided into 4 areas that were used for control, Fungi 1 (Cladosporium cladosporiodes), Fungi 2 (Penicillium oxalicum) and Fungi 3 (Aspergillus aculaetus) (Figure 1). The PU film (1x1 cm²) were placed in the middle of the plates. Then by using a sterilized cotton swab, the fungi were streaked onto the agar medium and films. Each plate was sealed with parafilm and kept in an incubator with an average temperature of 26°C for 8 days. All of the procedures were carried out in a sterilized laminar flow to prevent contamination.

Figure 1. Preparation of antifungal activity.
2.3. Characterization of polyurethane coatings

2.3.1. Fourier transform infrared (FTIR) analysis. The interactions between polypropylene glycol and toluene diisocyanate were studied by using the FTIR spectrophotometer. The FTIR spectra were recorded by using Perkin Elmer Spectrum IR 10.6.1 and scanned from 4000 to 650 cm$^{-1}$. The cured sample that was peeled off from the glass surface was used for this FTIR study.

2.3.2. Field emission scanning electron microscope with energy dispersive X-ray spectroscopy (FESEM-EDX). The particle distribution of AgNPs in the PU coating films was examined using a field emission scanning electron microscope, FESEM-EDX (CARL ZEISS, Model: GeminiSEM 500) with gold-coated samples.

3. Results and discussion

3.1. Polyurethane synthesis

Figure 2 shows the chemical reaction equation for the PU synthesis from toluene diisocyanate (TDI) and polypropylene glycol (PPG) in the presence of toluene as solvent. The crosslinking process is through breaking of C=N bond in toluene diisocyanate and formation of urethane linkage (-RNHCOOR'-) in the final product.

![Chemical reaction equation for the PU synthesis](image)

**Figure 2.** Synthesis of PU coating (n is ~2000).

In this polymer, polypropylene glycol and toluene diisocyanate represent as a soft segment and hard segment, respectively. In this study, polypropylene glycol with a molecular weight average of 2000 g/mol was used. According to the literature, polyols of an average of 1500-3000 g/mol molecular weights to get a smooth and flexible final product [19]. This is because low molecular weight of the polyol decreases the cross-linking density, hence increases the elasticity of the end product.

3.2. Fourier transform infrared spectroscopy (FTIR) analysis

The spectra of toluene diisocyanate, polypropylene glycol and cured PU coating, and their characteristics peaks are shown in Figure 3 and Table 2, respectively. The spectrum of toluene diisocyanate displays an intense and sharp peak at 2242 cm$^{-1}$ which represents the isocyanate functional group of R-N=C=O. The polypropylene glycol spectrum shows stretching vibration of carbonyl group from secondary alcohol and alkene groups which are at 1095 cm$^{-1}$ and 2858 cm$^{-1}$, respectively. Furthermore, the spectrum of the cured PU coating film displays the existence of a small and broad peak at 3300 cm$^{-1}$ suggesting the presence of amine groups which were produced from the cross-linking of polypropylene glycol by toluene diisocyanate. The absence of the isocyanate peak at 2242 cm$^{-1}$ also in line with the formation of urethane linkage. It also indicated that the diisocyanate was completely reacted in the PU formation. Carbonyl stretching of the urethane linkage was observed at 1725 cm$^{-1}$. All of the findings from the FTIR analysis were consistent and similar to that of the literature [7].
Table 2. FTIR spectral assignments of toluene diisocyanate, polypropylene glycol and PU coating.

| Functional groups          | TDI   | PPG   | PU coating |
|----------------------------|-------|-------|------------|
| -N=C=O                    | 2242  | -     | -          |
| C=C (aromatic)            | 1576  | -     | 1536       |
| C-H str. Sym.             | -     | 2868  | 2869       |
| C-H str. Asym.            | 2925  | 2970  | 2971       |
| C-H bending               | 1072  | 1453, 1373 | 1450, 1373 |
| C-O-C str. (polyester)    | -     | 1095  | 1093       |
| -NH (urethane)            | -     | -     | 3300       |
| C=O (urethane)            | -     | -     | 1725       |

3.3. Field Emission Scanning Electron Microscope with Energy Dispersive X-Ray (FESEM-EDX) Spectroscopy
FESEM-EDX analysis on the AgNPs incorporated PU coating film was used to determine the Ag nanoparticle distribution and, their size and shape. The blue dots which represent the AgNPs show that the particles are well distributed with no obvious agglomeration (Figure 4). The well distributed particles
are crucial as it will maximize the fungi growth inhibition function. The nanoparticles are in a spherical shape with the size range of between 58 – 74 nm (Figure 5).

![Figure 4. The blue dots represent silver (Ag).](image)

![Figure 5. AgNPs size characterized by FESEM-EDX.](image)

3.4. Antifungal activity
The antifungal activity was assessed using 3 fungi (Cladosporium cladosporiodes, Penicillium oxalicum, Aspergillus aculaetus) isolated from several corroded parts of an in service aircraft. As seen in Figure 6, the infected area for coating film without silver nanoparticles (Figure 6(a)) is wider than that of the silver nanoparticle incorporated film (Figure 6(b)). This indicates that the silver nanoparticle coating film inhibits the fungi growth. Silver nanoparticles has been known as one of the most effective germicides to inhibit the growth of bacteria and fungi. The presence of AgNPs damages to fungi’s mycelia and leads to mycelial growth delay[20], destruction of hyphae and conidia distortion [21][18]. The silver nanoparticles disrupt the fungi’s cell structure by damaging the cell membrane and stop the cell division process. This leads to cell death, hence inhibits the growth of fungi cells. Silver particles of nanosize is favorable because of their large surface area per volume ratio resulting in high number of cells’ termination, thus, enhancing the antimicrobial activity[11][22].

![Figure 6. Fungi growth with (a) PU coating film without AgNPs and (b) AgNPs incorporated PU coating film.](image)
4. Conclusion
This study has shown that AgNPs-incorporated PU coating film has the ability to inhibit the growth of fungi that are commonly found in tropical environment. The coating material can be prepared by entrapment of silver nanoparticles in a cross-linking reaction of polypropylene glycol by toluene diisocyanate. Initial observation indicates that the composite has the fungi growth inhibition behavior but further investigation is needed to determine the right formulation that is suitable to be used as an effective coating material.

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