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Antimicrobial and water-triggered release characteristics of a copper sulfate-polyvinyl acetate adhesive composite

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Abstract. Water-triggered release of antimicrobial solutions is advantageous in inhibiting the growth of bacteria and fungi in moist and wet environments. In this study, we fabricated a composite, by mixing polyvinyl acetate adhesive with copper sulfate solution, which exhibits antimicrobial activities against bacteria. Polyvinyl acetate adhesive serves as the binder and water soluble substance while copper sulfate serves as the antimicrobial agent. The composite was coated in an acetate film and air-dried. To monitor the rate of release of copper ions, the composite was submerged in water and the conductivity was measured. The conductivity saturation time was determined. The composite showed antimicrobial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive).

1. Introduction

Wet environments are conducive for the growth of microorganisms. The dependence of microorganisms in water makes it easier for them to thrive in these types of environment. For instance, their number rapidly increases in water-exposed surfaces (e.g., walls, woods, trees) after rainfall. Inhibiting the growth of microorganisms is essential in order to prevent possible causes of diseases that may harm humans, animals, and even plants.

Antimicrobial agents have been incorporated in different materials such as papers, textiles, fibers, films, and others [1-8]. However, if the intention is to release these agents on events wherein the environment becomes wet (a condition where microorganisms greatly multiply), the fabrication of materials that release antimicrobial agents in the presence of water is advantageous. This type of materials could have two integral components: (1) the water-sensitive component and (2) the antimicrobial component. The intention is that when the water-sensitive component interacts with water, the component releases the antimicrobial agents. Polymers have been made to interact with water for studies concerning ion release [9] and shape memory alloys [10]. Polyvinyl acetate (PVA) is a candidate material for the water-sensitive component. It is water-soluble; thus, it can release the encapsulated antimicrobial agents when dissolved in water. Furthermore, it is low-cost, widely available and its viscosity makes it easily be drawn into coatings and films.

Copper-based materials [11-14] have antimicrobial properties. Compared to silver metal which is a well-known antimicrobial agent, copper-based materials are cheaper. Copper sulfate [15-18] is a copper-containing salt which easily dissolves into ions in aqueous media. It can be used as molluscicide for the destruction of snails and slugs. It has antifungal applications and it can be used to inhibit algal growth in plant pots and swimming pools.

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In this study, we incorporate copper sulfate (CuSO4) in water-soluble matrix (polyvinyl acetate). We characterize its color and antimicrobial activity and determine its rate of release in aqueous solution.

2. Methodology

2.1 Preparation of Samples
Copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) salts were dissolved in water to prepare a 0.5 M copper sulfate solution. Varying ratios of polyvinyl acetate adhesive and copper sulfate solution were fabricated – 100:0, 95:5, 90:10, 80:20, 70:30, and 60:40. Each mixture was coated in an acetate film and was air dried. The copper sulfate pentahydrate serves as the antimicrobial agent while the polyvinyl acetate serves as a binder and water soluble substance in the sample.

2.2 Characterization
Transmission spectroscopy was done using a USB spectrometer to describe the color of each sample. The antimicrobial activity was determined through Disc Diffusion Method using *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) as test microorganisms. The samples were prepared in discs of 10.00mm in diameter. The test reference for this method was "US Pharmacopeia 30-NF 25, 2007".

For the conductivity measurement, acetate film was submerged in a beaker filled with water. Conductivity of water through time was monitored using OAKTON PC 510 pH/conductivity bench meter to determine the rate of release of copper ions.

3. Results and Discussion
We were able to fabricate a blue, translucent, paste-like composite containing polyvinyl acetate adhesive and copper sulfate. Transmission spectroscopy within the blue wavelength was done to characterize the color of these composites deposited on acetate films.

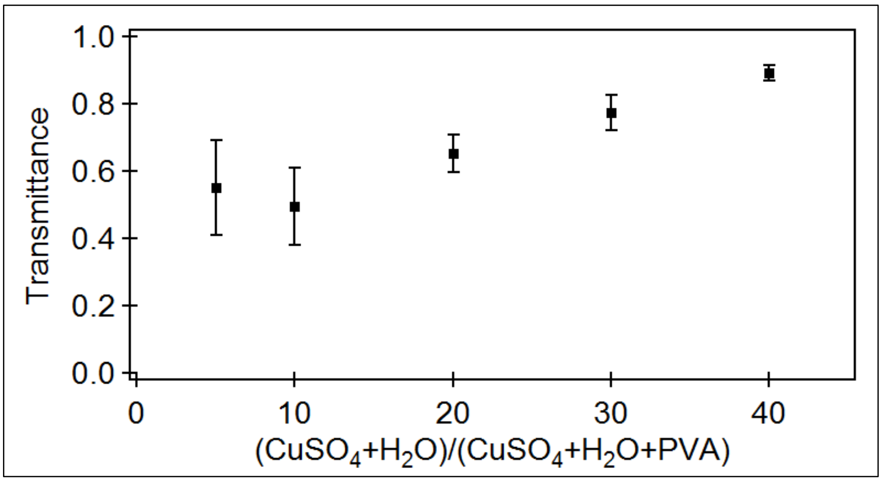

Figure 1. Transmittance of $CuSO_4$-PVA composite on film containing 5%, 10%, 20%, 30%, and 40% $CuSO_4$+H_2O.

Figure 1 shows the plot of the transmittance per amount of copper sulfate. Wavelengths 450-495 nm were selected as the range of the blue color that will be accounted for the embedded copper sulfate in each sample. Based from the graph, the intensity of the blue color generally increases as the amount of copper sulfate increases. However, there is a slight difference between the sample containing 5% and 10% copper sulfate which can be attributed to the topography of the sample. The distribution of mixture in the surface of the air-dried sample was not uniform i.e., a portion of the sample appears to be bluer than the other portion.

The antimicrobial activity of the samples was also determined as shown in Table 1. Cefuroxime was used as positive control for *E. coli* while that of *S. aureus* is Clindamycin.

Table 1. Antimicrobial activity of samples against *Escherichia coli* and *Staphylococcus aureus.*

Sample/Control	*Escherichia coli*		*Staphylococcus aureus*	
	Zone of inhibition (mm)	Inhibitory activity	Zone of inhibition (mm)	Inhibitory activity
$CuSO_4$-PVA composite on film	19.41	complete	14.76	complete
Positive control: Cefuroxime 30 µg	17.28	complete	N/A	N/A
Positive control: Clindamycin 2 µg	N/A	N/A	19.68	complete

The composite containing polyvinyl acetate adhesive containing copper sulfate exhibited antimicrobial activity for both test organisms. The sample tested against *E. coli* (Gram-negative) produced a complete inhibitory activity within the zone of inhibition (ZOI) of 19.41mm which is higher than the positive control. The sample tested against *S. aureus* (Gram-positive) also produced a complete inhibitory activity within the zone of inhibition of 14.76 mm which is lower than the positive control. Gram-negative bacteria possess a thin peptidoglycan, a part cell wall which is responsible for bacterial survival. On the other hand, Gram-positive bacteria possess a thick peptidoglycan. Copper ions may adhere on the cell wall causing the rupture and killing of bacteria which possibly explains why the ZOI of *E. coli* is larger than that of *S. aureus.*

Conductivity of water containing the sample through time was also measured to determine the rate of release of copper ions. Figure 2 shows the plot of conductivity over saturation conductivity (σ/σ_{sat}) versus time over saturation time (t/t_{sat}) of samples containing 5% and 40% copper sulfate solution.

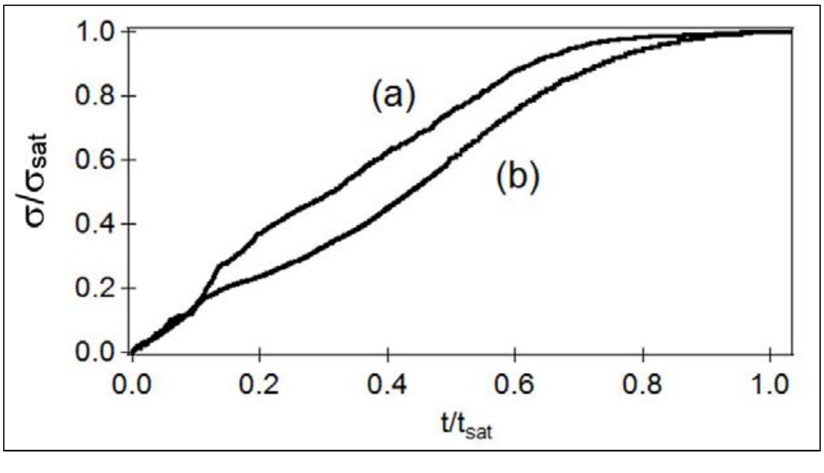

Figure 2. Conductivity of water through time with submerged film containing PVA to $CuSO_4 + H_2O$ ratio of (a) 95:5 and (b) 60:40 where σ/σ_{sat} is conductivity per saturation conductivity and t/t_{sat} is time per saturation time.

PVA molecules were dissolved which led to the dissociation of copper sulfate salts in water. As soon as the sample comes in contact with water, the PVA adhesive which acts as a binder starts to dissolve. Since the dissolution of PVA molecules is not abrupt, the copper ions are released in water gradually as seen in Figure 2. The conductivity of water continues to increase as the copper ions are released. After reaching a certain time, saturation occurs when all the copper ions are released in water resulting to an almost constant conductivity reading.

Table 2 shows the summary of the saturation time and saturation conductivity of the two samples. It can be seen that the sample with 40% copper sulfate solution has a significantly higher saturation conductivity and took longer time to saturate than the sample with 5% copper sulfate solution. This is due to larger amounts of copper ions released in water. The amount of copper ions is essential for its role to adhere and destroy organisms of interest.

Table 2. Comparison of saturation time and saturation conductivity of $CuSO_4$-PVA composite containing5% and 40% $CuSO_4$ + H_2O.

$CuSO_4$ + H_2O amount	Saturation time (mins.)	Saturation conductivity (μS/cm)
5%	869	9.84
40%	2117	33.04

4. Summary and Conclusion

Samples with different ratios of PVA to copper sulfate were prepared and deposited in film. The copper sulfate served as the antimicrobial agent while the PVA served as a binder for the fabrication of the sample. Between the two samples, the sample with 40% copper sulfate exhibited higher transmittance in the range of the blue wavelength. Antimicrobial activity against *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) were also evaluated resulting to more antimicrobial resistance by the latter organism. The conductivity of water increased through time as the $CuSO_4$-PVA composite on film is submerged in water. Also, the sample with 40% copper sulfate solution showed a higher saturation conductivity than the sample with only 5% copper sulfate solution. The fabricated mixture of PVA and copper sulfate can be smeared on wet surfaces for possible prevention of bacterial growth.

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