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GMA/Ag COMPOSITE AS ANTIMICROBIAL AGENT

Sandra Bulatović* ID, Natalija Nedić ID, Tamara Tadić ID, Bojana Marković ID, Aleksandra Žerađanin ID, Aleksandra Nastasović ID
University of Belgrade, Institute of Chemistry, Technology and Metallurgy (ICTM), National Institute of the Republic of Serbia, Belgrade, Serbia

Silver, a precious metal used across various industries, can be released into the environment as a byproduct of industrial activities, potentially leading to environmental pollution. Consequently, removing silver from wastewater is crucial for enhancing environmental quality. Porous synthetic polymers (composites), with their high specific surface area and unique physico-chemical properties, have garnered interest as effective sorbents in environmental protection. Glycidyl methacrylate (GMA)-based composites are widely used in various applications such as sorbents (of metals, organic compounds, etc.), enzyme supports, and in biomedicine. The main objectives of this study were the synthesis, characterization, and investigation of the antimicrobial activity of a novel GMA/Ag composite. For the synthesis of the composite, GMA as the monomer, and the crosslinker trimethylolpropane trimethacrylate (TMPTMA) were used, followed by functionalization with diethylenetriamine (DETA). Silver was incorporated into the composite by sorption from 0.1 M $AgNO_3$ solution at pH 5, and 25°C, for 24h. The synthesized composite was characterized using Fourier-transform infrared spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). The antimicrobial activity of the GMA/Ag composite was assessed using the agar-well diffusion method against different microorganisms, including representatives of Gram-negative (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*), yeast (*Candida albicans*), and fungi (*Aspergillus niger*). The results of antimicrobial tests indicated that the GMA/Ag composite displayed good antimicrobial activity against the analyzed microbes, and it can potentially be used for biomedical applications, in the food and pharmaceutical industries , in the treatment of wastewater, etc.

Keywords: GMA/Ag composite, FTIR, SEM, antimicrobial activity.

* Author address: Sandra Bulatović, University of Belgrade, Institute of Chemistry, Technology and Metallurgy (ICTM), National Institute of the Republic of Serbia, Njegoševa 12, Belgrade, Serbia
e-mail address: sandra.bulatovic@ihtm.bg.ac.rs

INTRODUCTION

Silver is being increasingly utilized across diverse industries, such as jewelry, photography, catalysis, electronics, biomedicine, and chemical manufacturing, owing to its exceptional physicochemical properties [1]. However, increasing demand and improper handling have led to the generation of silver-containing wastewater [2]. According to the World Health Organization (WHO), silver(I) ions in aquatic environments are classified as hazardous substances, making their removal from wastewater a critical step prior to discharge [3]. Silver(I) ions interact with biomolecules, causing toxic responses comparable to those induced by other heavy metals and persistent organic pollutants. Their bioaccumulation presents significant environmental and health risks, as silver can be transferred through the aquatic food chain [4]. Accordingly, the U.S. Environmental Protection Agency has set the maximum allowable silver concentration in drinking water at 0.1 mg/L [2]. Among various water treatment methods, sorption is recognized for its simplicity, cost-effectiveness, and environmental sustainability [3]. Owing to its low energy requirements and minimal secondary pollution, it has attracted considerable attention for the removal of metals from wastewater [2].

The glycidyl methacrylate (GMA)-based composites serve as efficient chelating agents that efficiently bind metal ions, facilitating their removal from aqueous solutions, and thereby contributing to the reduction of toxic metal concentrations in the environment [5]. These porous synthetic polymers, characterized by high specific surface area and unique physicochemical properties, are widely used not only as sorbents but also as reactive composites. Because the GMA molecule contains an epoxide group susceptible to nucleophilic and electrophilic ring-opening reactions, various functional groups can be introduced to tailor polymer properties for diverse applications [6]. In this study, an amino-functionalized GMA composite was used for silver(I) ion sorption. Incorporating metal ions into polymers enables the creation of biocidal materials with expanded applications. Silver is well known for its antimicrobial properties, acting through various mechanisms, including protein denaturation, and disrupting the bacterial respiratory chain by interfering with membrane-bound respiratory proteins. Additionally, silver alters membrane permeability and generates reactive oxygen species (ROS), leading to oxidative stress and eventual microbial cell death [7].

The aim of this study was to investigate the application of an amino-functionalized composite based on glycidyl methacrylate (GMA) as a potential sorbent for silver(I) ions, which was synthesized and characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) analyses. Additionally, the antimicrobial efficiency after sorption of silver(I) ions was evaluated. This approach aims to achieve a dual purpose: removal of silver(I) ions from wastewater and imparting antimicrobial activity to the analyzed GMA/Ag composite.

MATERIALS AND METHODS

Materials and instrumentation

For the synthesis of the GMA composite, the following reagents were used: glycidyl methacrylate (GMA, p.a., Merck, Germany), trimethylolpropane trimethacrylate

(TMPTMA, p.a., Sigma-Aldrich, Germany), 2,2′-azobisisobutyronitrile (AIBN, 98%, Sigma-Aldrich, Germany), cyclohexanol (Cy6, p.a., Merck, Germany), and tetradecanol (C_{14}, p.a., Merck, Germany). The GMA composite sample was functionalized with diethylenetriamine (DETA, p.a., Merck, Germany) in toluene (p.a., Sigma-Aldrich, Germany). A solution of $AgNO_3$ in deionized water was used for Ag (I) sorption. Mueller-Hinton agar for bacteria (casein hydrolysate 17.5 g/L, meat extract 2 g/L, starch 1.5 g/L, agar 17 g/L) and Sabouraud Dextrose agar for yeast and fungi (agar 15 g/L, glucose 4%, D (+)-glucose 40 g/L, peptone 10 g/L) were used for the determination of antimicrobial activity.

GMA/Ag composite was characterized with a Nicolet SUMMIT FTIR Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), in ATR mode, in the range 4000–500 cm^{-1} and Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM-EDS) (JEOL JSM-6390 LV, JEOL Ltd., Tokyo, Japan). Before SEM analysis, the composite had been coated with a thin layer of gold.

Synthesis of composite

The GMA-based composite was synthesized via suspension copolymerization in a 500 mL flask equipped with a condenser and mechanical stirrer, under nitrogen and heated in an oil bath. The aqueous phase (225 mL) was heated to 75 °C, followed by the addition of the monomer phase (GMA, 29.2 g), the crosslinker (TMPTMA, 19.5 g), the initiator (AIBN, 0.5 g), and inert components (Cy6, 51.0 g; C_{14}, 12.8 g). The reaction was carried out for 2 h at 75 °C, and 2 h at 80 °C. The product was isolated by decantation, washed, dried, and purified via Soxhlet extraction with ethanol (24 h). Amino-functionalization was performed by treating 7.2 g of the composite with 31.4 g DETA, in toluene (350 mL), stirred at room temperature for 24 h, then heated at 80 °C for 6 h. The resulting amino-functionalized GMA-based composite was filtered, washed, and dried [6].

Sorption of Ag(I) ions from aqueous solution

An aqueous solution of silver(I) ions (0.1 M) was prepared by dissolving $AgNO_3$ in deionized water. Sorption of Ag(I) ions using the amino-functionalized GMA-based composite was performed at room temperature (25 °C), pH 5, for 24 hours, resulting in the amino-functionalized GMA/Ag composite, which was further characterized and used to determine antimicrobial activity.

Agar diffusion method

The microorganisms used in this study were: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 2433), and *Aspergillus niger.*

The nutritious microbiological media were heated to boiling in a water bath to ensure complete dissolution, then sterilized for 25 minutes at 120 °C. After solidification of the media, the microorganisms were directly streaked onto the surface of the agar plates with a sterile inoculating loop, using the zig-zag method. Also, the wells for the GMA/Ag were formed in the agar plates. The GMA/Ag (30 mg) was mixed with 100 μL sterile distilled water, and added to the wells. After 24 hours of incubation at 28°C, the Petri dishes were examined, and the zone of microbial growth inhibition was measured with a millimeter ruler [8, 9].

RESULTS AND DISCUSSION

In Figure 1, the FTIR spectra of the amino-functionalized GMA-based composite before and after silver(I) ions binding are shown. In the spectrum of the analyzed composite before silver sorption, a broad peak is observed in the region around 3500–3300 cm^{-1}, with a maximum at 3365.7 cm^{-1}, which is attributed to –OH groups and N–H stretching vibrations. After silver sorption, the intensity of this peak changes, suggesting that hydroxyl and amino groups likely participated in the binding of silver(I) ions. The region around 2944.4 cm^{-1} is characteristic of asymmetric and symmetric stretching of $–CH_2$ and –CH bonds from aliphatic CH_2 and CH_3 groups, while the band at 1465.9 cm^{-1} corresponds to $–CH_2$ and –CH bending vibrations. The most prominent peak in the spectrum appears at 1726.7 cm^{-1} and corresponds to the C=O stretching vibration, characteristic of ester groups. Additionally, peaks corresponding to the vibrations of the epoxy ring are found at 1258.5, 1151.2, and 984.2 cm^{-1}; after sorption, changes in their intensity and slight shifts are observed, which may indicate a disturbance in local symmetry due to coordination with silver ions [10]. After sorption of silver(I) ions, a peak appears at 826.1 cm^{-1}, indicating the formation of an Ag–N complex [11].

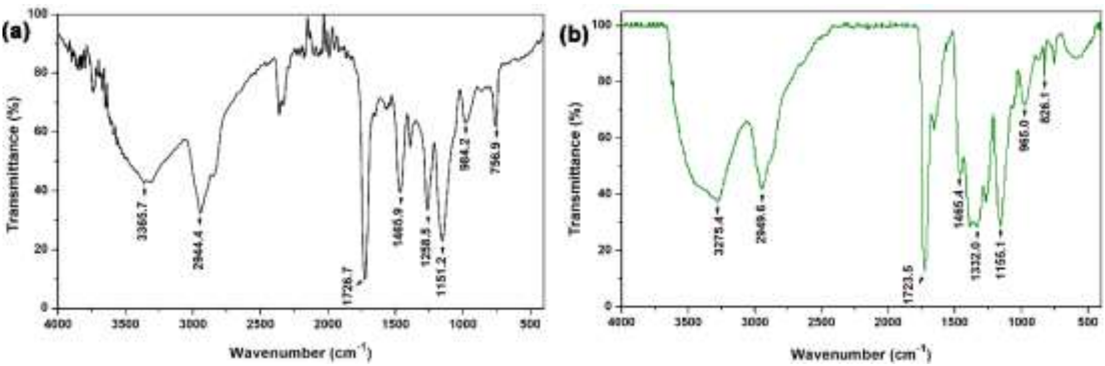

Figure 1. FTIR spectrum of the: (a) amino-functionalized GMA/Ag composite, and (b) after silver(I) ions binding.

In order to obtain morphological information, the surface of the GMA/Ag composite was examined using scanning electron microscopy (SEM) at magnifications of 1 μm and 100 μm (Figure 2). SEM analysis revealed that the samples consisted of spherical, opaque particles with a rough surface. The sample exhibited a three-dimensional porous structure with agglomerates of globules, typical for GMA porous composites [6].

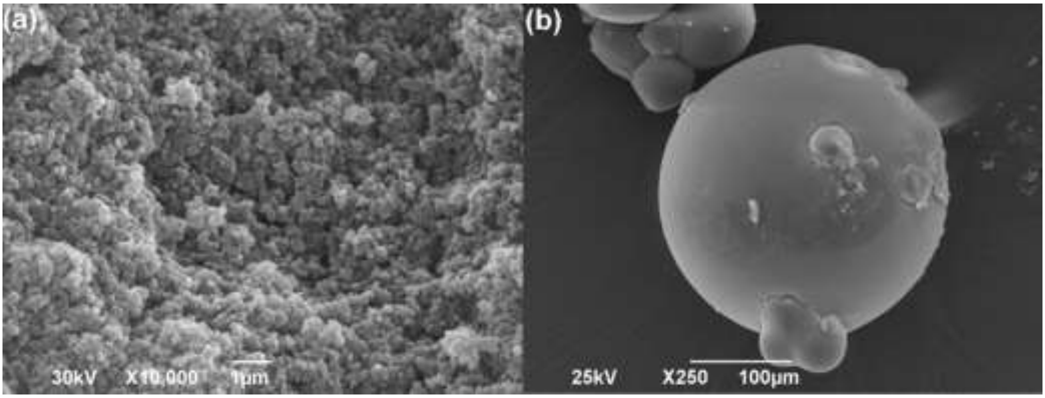

Figure 2. SEM images of GMA/Ag composite at: (a) 1 µm, and (b) 100 µm magnification.

Petri dishes with the test microorganisms used to evaluate the antimicrobial activity of the GMA/Ag composite after Ag(I) ion sorption, as well as control Petri dishes to which only water was added instead of the polymer, are shown in Figure 3. After 24 hours of incubation, the inhibition zones were measured by subtracting the radius of the well (5 mm) from the total radius of the clear inhibition zone (9 mm). The results of the antimicrobial tests demonstrated that the GMA/Ag composite effectively acted against Gram-negative bacteria (such as *E. coli*) and Gram-positive bacteria (*S. aureus*), with the same inhibition zone of 4 mm (Figure 3). The antimicrobial tests against yeast (*C. albicans*) and fungi (*A. niger*) were negative, probably due to the possibility that the silver concentration, or the form used in this study, wasn't adequate to exert a measurable inhibition effect. Therefore, it can be assumed that the analyzed composite is antibacterial because it showed a significant inhibition of bacterial growth.

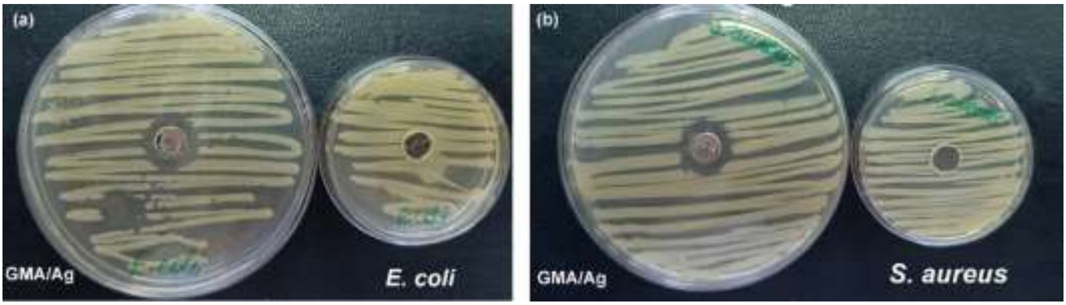

Figure 3. The antimicrobial activity of the GMA/Ag composite against: (a) *E. coli*, and (b) *S. aureus*.

Several studies have examined the antimicrobial activity of glycidyl methacrylate (GMA)-based composites functionalized with silver. Vukoje et al. (2015) demonstrated significant antimicrobial effects of poly(GMA-co-EGDMA) copolymers with immobilized silver nanoparticles against bacteria (*Escherichia coli, Staphylococcus aureus*) and fungi

(*Candida albicans*). Fan et al. (2011) demonstrated that silver-containing GMA-based dental resins exhibited strong antibacterial activity against *Streptococcus* mutants, while research by Gligorijević et al. (2022) showed that silver-modified polymethyl methacrylate (PMMA) resins effectively inhibited growth of *Staphylococcus aureus* and *Candida albicans*. These findings collectively underscore the antimicrobial potential of silver-functionalized methacrylate composites.

CONCLUSION

In this study, the antimicrobial activity of a novel silver-containing GMA-based composite (GMA/Ag) was evaluated using the agar diffusion method. SEM analysis of GMA/Ag revealed a three-dimensional porous structure, while FTIR spectra confirmed the amino-functionalization and incorporation of Ag(I) ions in the structure of the crosslinked composite through the formation of Ag–N bonds. Based on the obtained results, it can be concluded that the amino-functionalized GMA-based composite is effective as a sorbent of Ag(I) ions from aqueous solutions, as well as an antibacterial agent. However, the commercial application of this composite would require further studies, analyses, and evaluations to ensure its safe and effective use.

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Izvod

GMA/Ag KOMPOZIT KAO ANTIMIKROBNO SREDSTVO

Sandra Bulatović ID, Natalija Nedić ID, Tamara Tadić ID, Bojana Marković ID, Aleksandra Žerađanin ID, Aleksandra Nastasović ID
Univerzitet u Beogradu, Institut za hemiju, tehnologiju i metalurgiju (IHTM), Institut od nacionalnog značaja za Republiku Srbiju, Beograd, Srbija

Srebro je plemeniti metal koji se koristi u raznim industrijama, tako da može dospeti u životnu sredinu kao nusproizvod industrijskih aktivnosti, što potencijalno može dovesti do zagađenja. Shodno tome, uklanjanje srebra iz otpadnih voda je ključno za poboljšanje kvaliteta životne sredine. Porozni sintetički polimeri (kompoziti), sa svojom visokom specifičnom površinom i jedinstvenim fizičko-hemijskim svojstvima, izazvali su interesovanje kao efikasni sorbenti u očuvanju životne sredine. Kompoziti na bazi glicidil metakrilata (GMA) se široko koriste u različitim primenama kao sorbenti (metala, organskih jedinjenja itd.), nosači enzima i u biomedicini. Glavni ciljevi ove studije bili su sinteza, karakterizacija i istraživanje antimikrobne aktivnosti novog GMA/Ag kompozita. Za sintezu kompozita korišćeni su GMA kao monomer, i umreživač trimetilolpropan trimetakrilat (TMPTMA), nakon čega je usledila funkcionalizacija dietilentriaminom (DETA). Srebro je ugrađeno u kompozit sorpcijom iz 0,1 M rastvora $AgNO_3$, na pH 5, i 25°C, tokom 24 sata. Sintetisani kompozit je okarakterisan korišćenjem Furijeove-transformacione infracrvene spektroskopije (FTIR) i Skenirajuće Elektronske Mikroskopije (SEM). Antimikrobna aktivnost GMA/Ag kompozita je procenjena korišćenjem metode difuzije u agar-bunarićima za različite mikroorganizame, uključujući predstavnike gram-negativnih (*Escherichia coli*) i gram-pozitivnih bakterija (*Staphylococcus aureus*), kvasaca (*Candida albicans*) i gljiva (*Aspergillus niger*). Rezultati antimikrobnih testova su pokazali da GMA/Ag kompozit ispoljava dobru antimikrobnu aktivnost protiv analiziranih mikroba i da se potencijalno može koristiti za biomedicinske primene, u prehrambenoj i farmaceutskoj industriji, u tretmanu otpadnih voda itd.

Ključne reči: GMA/Ag kompozit, FTIR, SEM, antimikrobna aktivnost.