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Application of Thiourea Polyurethane@Copper Sulfide Composite for Antibacterial Potential

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Abstract
This work demonstrated the synthesis of a new composite by coupling copper sulfide nanoparticles and isothiouronium polyurethane foam to enhance antimicrobial activity. Fourier transform infrared (FTIR), ultraviolet-visible spectra (UV/Vis), point zero charge (pHpzc), acidic and basic sites, iodine number, methylene blue value, energy disperse (EDX), X-ray diffraction (XRD), and scan electron microscopy (SEM) were used to characterize isothiouronium polyurethane@copper sulfide foam composite (ITPU@CuS). The antibacterial activity of ITPU@CuS was tested against Gram-positive (*Bacillus cereus*) and Gram-negative (*Pseudomonas aeruginosa*). The biocidal action of ITPU@CuS against the microbial strains was significantly higher at the concentration of 150 µg/ml than at lower concentrations. Also, transmission electron microscopy (TEM) for the ITPU@CuS-treated bacteria was studied. TEM micrographs of the treated bacteria confirmed the bactericidal action of ITPU@CuS by showing malformation action, wrinkling, and bacterial cell wall damage. The obtained composite could be considered to be a promising material aimed at industry, especially in the field of food packaging, where it is effective against pathogenic microbes.

***Keywords*:** polyurethane foam composite; copper sulfide; antibacterial activity; *Bacillus cereus*; *Pseudomonas aeruginosa*

1. Introduction

Microbial infection persists as one of the most critical problems in different fields such as human health including healthcare products, dental equipment, hospitals, water purification systems, and food packaging [1]. In the last decades, many scientific papers have tended to prepare new safe, and effective antimicrobial agents especially antimicrobial polymers [2]. Polymers have been reported as chemically stable antimicrobial agents with low toxicity and high selectivity and efficiency [3]. Polyurethane composite is one of the most diverse families of such polymers [4].

Recently, nanoparticle compounds were used as antimicrobial agents due to their high efficacy against a broad spectrum of microorganisms [5,6]. This is because of their unique properties, including higher surface area, catalytic, magnetic, optical, and electrical properties, and their stability than their bulk state. Copper sulfide nanoparticles (CuSNPs) are a class of semiconductor nanomaterials that demonstrate interesting characteristics for biomedical applications [7,8]. Mutalik et al. demonstrated the antibacterial activity of CuSNPs against *Escherichia coli* [9]. Also, Liang et al. reported the potent antibacterial effect of CuSNPs as well as their capacity to promote wound healing through re-epithelialization and collagen deposition [10].

Isothiouronium compounds exhibited a strong antimicrobial action on top of their important roles in many chemical and biological processes [11].

Polyurethane composites such as polyurethane-salvadora persica, magnetic-isothiouronium polyurethane, and iminodiacetic polyurethane-carbon nanofibers were developed and used in dye wastewater treatment [4, 12, 13]. The polyurethane

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composites could be prepared by using a condensation process between the reactive functional groups of polyurethane foam and its derivatives with specific compounds [12]. These composites are very important due to their high flexibility, high surface-to-volume ratio, and thermal insulating properties. Also, the cationic groups of polyurethanes such as imidazolium, ammonium, or pyrrolidinium have shown a good antimicrobial action against a broad range of bacteria and fungi [14]. This study developed new photocatalytic material to achieve an efficient antibacterial effect. The isothiouronium polyurethane foam composite (ITPU@CuS) was synthesized by immobilization of CuSNPs on polyurethane foam via the seeding method. CuSNPs were anchored on the surface of polyurethane foam to sharply improve the photocatalytic antibacterial ability.

2. Materials and methods

2.1. Materials and Reagents

Commercial flexible polyurethane foam sheets (open cell polyether type, d = 12 kg/m^3), were brought by the Foamex factory, New Damietta, Egypt. Before use, 10 g of PUF have soaked in 200 mL of HCl (1 mol/L) at room temperature overnight. NaOH (pellets, ≥98%), $CuSO_4.5H_2O$ (salt, ≥98%), Na_2S (scales, ≥60%), thiourea (ReagentPlus®, ≥99.0%), and benzylpenicillin potassium salt (penicillin G, powder, BioReagent, suitable for cell culture) were purchased from Sigma–Aldrich Chemie, Steinheim, Germany. HCL (37%), H_2SO_4 (95-97%) and ethanol (96%) were obtained from PioChem, Egypt. Mueller-Hinton agar medium (MHA, NutriSelect® Plus), used in the antibacterial test, was purchased from Sigma–Aldrich Chemie, Steinheim, Germany.

2.2. Instruments

All absorbance measurements were performed with a Jasco UV/VIS Spectrometer v-630 (Jasco, Japan). The pH measurements were carried out using a Jenway 3510, UK, pH meter. The X-ray diffraction (XRD) patterns of the ITPU@CuS composite were recorded by X-ray X' Pert powder diffractometer (Philips, D8-Brucker Model), equipped with Ni filter and Cu kα-radiation (λ= 1.5418 Å) at 40 kV and 30 mA. Infrared (IR) spectra were carried out using a KBr disc (KBr pellet) on a JASCO FTIR-410 spectrometer in the 4000–400 cm^{-1} region. TEM and SEM analyses were carried out at the Electron Microscope Unit, Mansoura University. SEM (JSM-6510LV, USA) supported with EDX unit, operating at 20 kV accelerating voltage, was used to detect morphology and the main constituent elements of ITPU@CuS.

2.3. The Bacterial Strains Used for Antimicrobial Activity

The bacterial strains including *Bacillus cereus* ATCC 6633 and *Pseudomonas aeruginosa* ATCC 27853 were purchased from American Type Culture Collection (ATCC), USA.

2.4. Experimental Procedure

2.4.1. Preparation of Isothiouronium Polyurethane@Copper Sulfide Foam Composite

CuNPs were prepared according to the Rawat et al. method [13]. ITPU@CuS composite was prepared by soaking 10 g of cubic polyurethane foam (0.125 cm^3) in 250 mL of $CuSO_4.5H_2O$ (0.5 mol/L) and H_2SO_4 (0.5 mol/L) followed by adding Na_2S (0.25 mol/L) dropwise and the mixture stand overnight at room temperature. The product was refluxed with 15 g thiourea in 200 mL ethanol at 50°C and then washed with ethanol and air-dried.

2.4.2. Antibacterial Activity Using Agar Well Diffusion Method

The antibacterial potential of the prepared composite was tested against Gram-positive bacteria (*B. cereus*) and Gram-negative bacteria (*P. aeruginosa*) using the agar well diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute [16]. MHA medium was prepared and autoclaved. Then, the cool moulted agar media were inoculated by 100 µL culture of each strain (0.5 McFarland standard (1-2×10^8 CFU/mL) and then poured into sterile Petri dishes in triplicates. After solidification, 200 µL of 50, 100, and 150 µg/mL of ITPU@CuS and penicillin G (as standard antibacterial) were prepared and added separately into the small wells (5 mm). Plates were incubated at 37°C for 48 h. After the incubation, zones of inhibition (ZOI) were measured in millimeters (mm).

2.4.3. Ultrastructure Study of the Treated Bacteria

TEM (JEOL JEM-2100, Japan) was used to study the ultrastructure of ITPU@CuS-treated bacteria. The exponential phase cultures of bacteria were subjected to 150 µg/mL of the prepared composite for 2 h at 37°C. The untreated bacteria

were included as controls. The cell cultures were centrifuged at 8000 rpm for 15 min and then washed 3 times with distilled water. The samples were prepared, cross-sectioned using an ultra-microtome (50 μm), and then loaded on carbon-coated copper grids (Type G 200, 3.05 μM diameter, TAAP, U.S.A.). TEM micrographs of the samples were taken using the JEOLJEM-2100 device operated at an accelerating voltage of 200 kV [17].

Statistical analysis

SPSS software version 18 was used for all the statistical analyses. All values in the experiments were expressed as the mean±standard deviation (SD) and were analyzed with aone-way Analysis of Variance (ANOVA) with a significant level set at $p<0.05$.

3. Results and Discussion

3.1. Characterization of ITPU@CUS

The surface morphology of ITPU@CuS was investigated using SEM at magnifications from 100x to 20,000x (Fig. 1A, 1S). Fig. 1 represents the SEM image of the ITPU@CuS at a magnification of 100x, in which the particles of CuSNPs appeared regularly distributed inside the spaces of the polyurethane foam (PUF) matrix. As indicated in Fig. 1B, the surface of PUF contains many crystals (CuSNPs) which are irregular in size (20-150 nm) and shape.

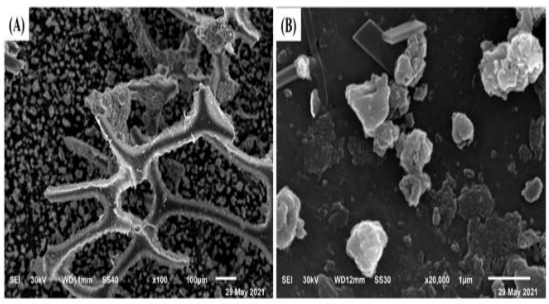

Fig. 1. SEM for ITPU@CuS composite.

Fig. 2 shows the EDX spectrum of the ITPU@CuS composite. It shows that the element contents of ITPU@CuS were C (58.1%), O (31%), S (7.2%), and Cu (3.7%). EDX result proposes that the chelating percentage between PUF and CuSNPs was 89.1: 10.9. The molar ratio of these elements (C, O, S, and Cu) was 4.84: 1.94: 0.23: 0.06 with the formula $C_{250}O_{100}S_{20}Cu_3$.

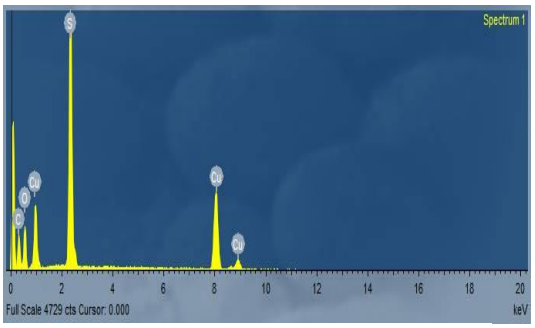

Fig. 2. EDX For ITPU@CuS composite.

The FTIR spectra of ITPU@CuS were shown in Fig. 3, the characteristic absorption peaks of the ITPU@CuS were observed at stretching vibrations 3778 and 3698 cm^{-1} (O-H), 3280 cm^{-1} (N-H), 2965 cm^{-1} (C-H_{Ar}) and 2868 cm^{-1} (C-H_{Aph}), 2348 and 2089 cm^{-1} (–SC($NHNH_2$)) and stretching vibrations 1601 and 1596 cm^{-1} (C=C). Also, the characteristic peaks at 1089, 650, and 610 cm^{-1} are due to Cu-S and bonding between CuSNPs with ITPU. The electronic spectrum of the ITPU@CuS composite was recorded using the Nujol mulls method (Fig. 2S). A higher energy band at 437-529 and also a small peak at 673 nm were observed.

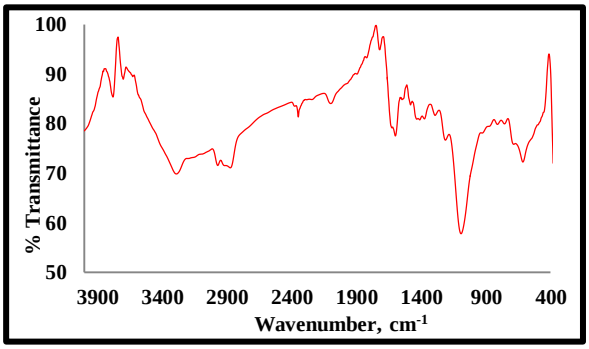

Fig. 3. IR spectrum of ITPU@CuS

The acidic and basic sites of ITPU@CuS were determined using 0.1 mol/L of NaOH and HCL, respectively. The results obtained indicate that the acidic sites of ITPU@CuS (2 mmol/g) are more than the basic sites (1 mmol/g). Also, the pH_{pzc} was found to be 3 for the ITPU@CuS composite (Fig. 3S). At pH < pH_{pzc}, the surface of the composite has a net positive charge, while at pH > pH_{pzc} the surface has a net negative charge. The internal surface area of ITPU@CuS was determined with iodine number which is related to the microporosity of the composite. The iodine number of ITPU@CuS was calculated to be 3 mmol/g; this value indicates that the ITPU@CuS is a high microporous surface.

The XRD pattern of the ITPU@CuS composite was obtained by scanning 2θ in the range 0.0–80° (Fig. 4). The amorphous nature of the polyurethane matrix was observed as broadband between 19° and 42°. Also, many sharp peaks were shown in ITPU@CuS

due to the overlapping of multifunctional groups of ITPU and CuSNPs.

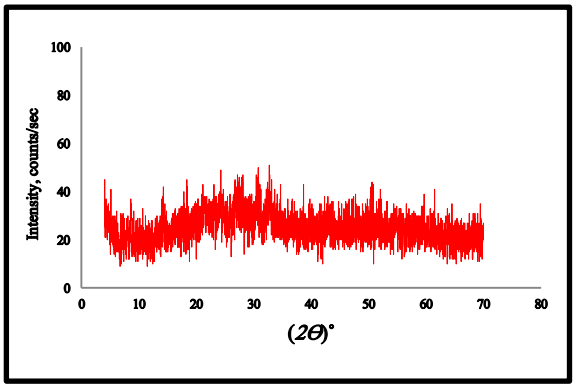

Fig. 4. XRD pattern of ITPU@CuS composite.

3.2. Antimicrobial Activity

Current approaches for fabricating antimicrobial polyurethane foams are commonly established on the microbicide additives [18]. Many researchers investigated the solo synthesis, and biological activities such as antibacterial, antifungal, and anticancer activities of isothiouronium and polyurethane [19-21]. Although copper-based materials have exhibited antibacterial potential [22-24], it is the first time to demonstrate the antibacterial activity of CuSNPs that are anchored on the surface of polyurethane foam.

The presented study quest for biologically active ITPU@CuS composite including synthesis and characterization of novel compounds that can be used in industry and medicine. The tested bacteria were found susceptible to the prepared ITPU@CuS showing a good antibacterial potential (Table 1 and Figure 5). There are significant differences in antibacterial effects between the samples with and without ITPU@CuS treatment. A highly significant difference ($P < 0.05$) was observed between the bacterial strains; *B. cereus*, and *P. aeruginosa*, and the diameter of the inhibition zone. The biocidal action of ITPU@CuS composite against the bacterial strains was significantly higher at 150 µg/mL concentrations than at lower concentrations that showed a dose-dependent manner of ITPU@CuS antibacterial potential.

Table 1 Antimicrobial activities of ITPU@CuS composite.

Compound	Concentration, µg/mL	Zones of inhibition (mm ± SD)	
		B. cereus	*P. aeruginosa*
ITPU@CuS	50	13 ± 0	-ve
	100	14 ± 0	9 ± 0.14
	150	15 ± 0	12 ± 0
Penicillin G	50	13 ± 0.14	11 ± 0.14
	100	15 ± 0.14	13 ± 0.06
	150	16 ± 0.06	14 ± 0.06

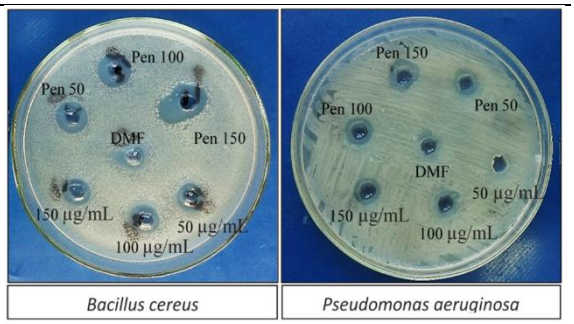

Fig. 5. Antimicrobial activities of ITPU@CuS composite; 50, 100 and 150 µg/mL, where DMF; dimethylformamide and Pen; penicillin.

Antibacterial action of ITPU@CuS composite against *B. cereus* and *P. aeruginosa* was examined by TEM analysis as shown in Figure 6. TEM micrographs of the untreated bacterial cells showed regular rods, normal cell walls, compact cytoplasm, and cell membranes. Whereas the treated bacteria micrographs revealed morphological changes in response to ITPU@CuS treatment in the form of malformation action, irregular morphologies, wrinkling, and wall damage, which led to the rupture of the bacterial cell membrane of the treated *B. cereus*. Also, treated *P. aeruginosa* micrograph showed malformation action, irregular rods, and lysed cell walls. It has been shown that the synthesized ITPU@CuS composite had bactericidal action by killing the bacteria. Although the exact mechanism of the antimicrobial action of ITPU@CuS is still unknown, some reported postulates hypothesized that the integration of the functional compounds into the polymer structure/composition throughout a chemical and/or physical bonding might increase the antimicrobial action [5, 26]. Ikeda et al. reported that polycations revealed antimicrobial action which might be due to the interaction between their high positive charge and the negatively charged microbial cell surface [27].

Copper and copper compounds could bind with sulfur-containing proteins in cell membranes and

attack the respiratory processes and cell division that leading to cell death [28]. The formation of reactive oxygen species (ROS) might also cause cell damage [2, 7]**.**

The doped CuSNPs in a polymer such as polyurethane increases their bactericidal action due to the large volume and lower electronegativity, which increase their molecular area [29]. Consequently, the effective area of the foam on the cell membrane increases resulting in a higher microbicidal activity.

It is worth noting that there was no antibacterial effect of the concentration of ITPU@CuS composite (50 µg/mL) on *P. aeruginosa* growth, suggesting that ITPU@CuS might more selectively induce cell death of Gram-positive *B. cereus* than Gram-negative *P. aeruginosa*. The morphologies of some *P. aeruginosa* cells persisted unaffected with smooth surface and rod shape in their TEM micrograph. This might be owing to the chemical compositions and different structures of their bacterial cell wall/membrane integrity disruption [30]. Future work is needed to study the antibacterial mechanisms of the ITPU@CuS composite.

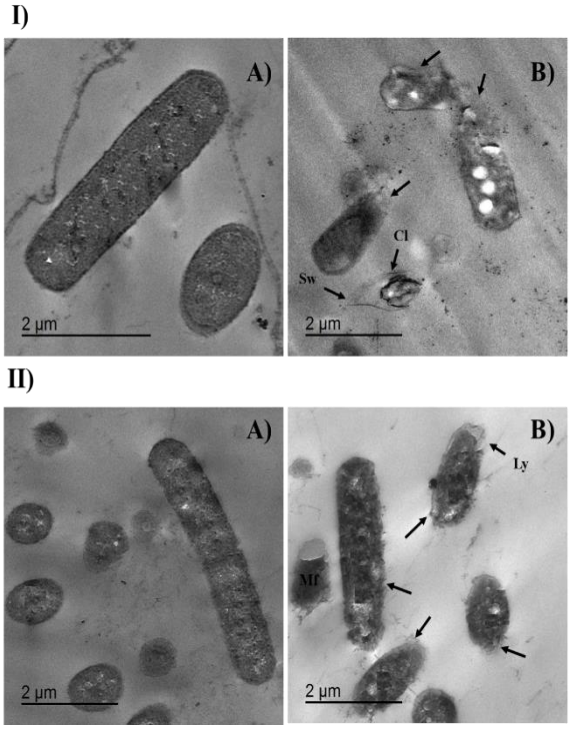

Fig. 6. The antimicrobial effect of ITPU@CuS composite; (B) on the ultrastructure of B. cereus; (I) and P. aeruginosa; (II). (A) is a negative control (without ITPU@CuS treating). There are irregular rods (arrows) with lysed cell walls (Ly), separated cell wall (Sw), malformed cells (Mf) and complete cell lysis (Cl).

4. Conclusions

In conclusion, ITPU@CuS synthesis, characterization and antibacterial activity were studied. The hexagonal phase of synthesized CuSNPs was observed from the SEM. The obtained results suggest that ITPU@CuS has a good internal surface area which is suitable for capturing pollutants and as antibacterial. ITPU@CuS can successfully inhibit the growth of the common such as *B. cereus* (Gram-positive) and *P. aeruginosa* (Gram-negative).

5. Conflicts of interest

"There are no conflicts to declare"

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