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Proceeding Paper

Synthesis, Characterization, and Biological Activity of a Novel *SA-g-p(AAc-co-AM)/ZnO NP* Hydrogel Composite †

Aseel M. Aljeboree [1,*], Hadeel K. Albdairi [2], Mohammed Abed Jawad [3], Sarah A. Hamood [4], Firas H. Abdulrazzak [5] and Ayad F. Alkaim [1]

1 Department of Chemistry, College of Sciences for Girls, University of Babylon, Hilla 5001, Iraq; alkaimayad@gmail.com
2 Babylon Police Command, Ministry of Interior, Hilla 5001, Iraq; hadeel.kalaf.gsci61@student.uobabylon.edu.iq
3 Anesthesia Techniques Department, Al-Nisour University College, Baghdad 10011, Iraq; mohammed.a.medical.lab@nuc.edu.iq
4 Department of Medical Laboratories Technology, Al-Esraa University College, Baghdad 10011, Iraq; sara.ashour@esraa.edu.iq
5 Forensic Evidence Department, College of Science, Al-Karkh University of Science, Baghdad 10081, Iraq; firas_habeb2000@yahoo.com
* Correspondence: annenayad@gmail.com
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Abstract: This research investigated the preparation and efficacy of a *SA-g-p(AAc-co-AM)/ZnO* hydrogel composite with enhanced biological activity. The hydrogel was synthesized using the sodium alginate biopolymer through the co-polymerization method. Our findings indicate that introducing zinc oxide nanoparticles to the hydrogel amplified its biological activity. The disc diffusion technique was applied to evaluate the antimicrobial properties against two Gram-positive bacteria isolates (*Staphylococcus aureus, Streptococcus epigenetics*) and two Gram-negative bacteria (*E.coli, Klebsiella* spp.). The antimicrobial activities of three surfaces—ZnO, *SA-g-p(AAc-co-AM)* hydrogel, and *SA-g-p(AAc-co-AM)/ZnO* hydrogel composite—were assessed. Characterization of these prepared surfaces was executed using FE-SEM and EDX. The results highlighted that the *ZnO NPs* exhibited minimal antibacterial activity against both types of bacteria. Conversely, the *SA-g-p(AAc-co-AM)/ZnO* hydrogel composite demonstrated heightened antibacterial effects against *Staphylococcus aureus* (30 mm) and *Streptococcus epigenetics* (25 mm). The Gram-negative bacteria, *E.coli* and *Klebsiella* spp., recorded inhibition zones of 13 mm and 12 mm, respectively. The *SA-g-p(AAc-co-AM)* hydrogel showed diminished antibacterial activity relative to the composite, attributed to the absence of zinc oxide. Overall, the isolated effect of zinc oxide nanoparticles indicated a minimal antibacterial influence on all Gram-positive and Gram-negative bacteria strains.

Keywords: hydrogel; sodium alginate; zinc oxide; antibacterial; Gram-positive; Gram-negative

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1. Introduction

Hydrogels are composed of poly-saccharide-based biopolymers, among which sodium alginate (SA) stands out as the most abundant globally. Its significance stems from its role as an effective adsorbent, attributed to its hydroxyl and amino functional groups. Moreover, the non-toxicity, low cost, biocompatibility, and biodegradability of sodium alginate enhance its utility. Numerous biocompatible functional monomers, when employed, result in SA-based hydrogels possessing desirable physical and chemical properties [1]. Specifically, acrylic acid, a monomer frequently utilized in hydrogel preparation, possesses a carboxylic acid group that can be leveraged to modulate the swelling and mechanical strength of sodium alginate (SA)-based hydrogels [2,3]. Microorganisms encompass a vast array of living entities, including bacteria, fungi, and algae. This study focuses on two primary bacterial classifications: Gram-positive and Gram-negative bacteria [4,5]. *Escherichia coli*, a Gram-negative and facultatively anaerobic bacterium, is commonly found in the intestines of animals and humans and extends its presence to certain foods and plants [6,7].

In contrast, *Streptococcus pyogenes*, typically categorized under "Group A Streptococcus" (GAS), is a Gram-positive bacterial pathogen. This bacterium has a propensity to cause a spectrum of ailments ranging from non-invasive to invasive infections. Some notable infections include cellulitis, pharyngitis, impetigo, scarlet fever, necrotizing fasciitis type II, acute rheumatic fever, post-streptococcal glomerulonephritis, and streptococcal toxic shock syndrome [8–10]. Within this context, GAS is a subset of *Streptococcus pyogenes* and stands as a pivotal class within hemolytic streptococci. This bacterium has been implicated in numerous human diseases across different stages. Notably, it has been linked to over 600 million cases of strep throat ("strep throat") [11,12].

2. Method

2.1. Preparation of ZnO Nanoparticles

Nanoparticle zinc oxide was synthesized using a hydrothermal process. Zinc acetate (15 gm) and oxalic acid (7 gm) were combined and diluted to 100 mL with distilled water. This mixture was stirred for 1 hr to achieve a homogenous solution. Subsequently, the resultant mixtures were autoclaved at 160 °C for 24 h. The formation of a white precipitate indicated the creation of zinc oxide. This precipitate was repeatedly washed with distilled water, sonicated for 10 min, and dried in an oven at 105 °C for 12 h.

2.2. Preparation of (SA-g-p(AAc-co-AM)/ZnO) Hydrogel Composite

Sodium alginate (SA, 1 gm) was dissolved in 20 mL of distilled water. Separately, ZnO nanoparticles (0.1 g) were mixed with 20 mL of distilled water. This ZnO solution was then incrementally introduced to the SA solution, with constant stirring at 25 °C for 3 h. Following the attainment of a homogenous mixture, acrylic amide (AM, 0.5 g) and acrylic acid (AAc, 3 mL) were added, and the blending continued at 25 °C. Subsequently, potassium persulfate (KPS, 0.03 g) dissolved in 1 mL of distilled water was introduced with continuous mixing for 5 min. N,N-dimethyl acrylamide (0.05 g) was added under a nitrogen gas purge. The reaction mixture was then placed in a water bath at 75 °C to finalize the co-polymerization, yielding the (SA-g-p(AAc-co-AM)/ZnO) hydrogel composite. The (SA-g-p(AAc-co-AM)) hydrogel was synthesized similarly but without the ZnO nanoparticles.

2.3. Bacterial Isolates

The study employed four pathogenic bacterial isolates: Gram-positive bacteria, namely, Streptococcus epigenes and Staphylococcus aureus, and Gram-negative bacteria, such as *E. coli* and *Klebsiella* spp. These isolates were collected from clinical samples and identified based on their macroscopic and microscopic characteristics, and antibiotic sensitivities.

2.4. Bacterial Biological Activity Test

Gram-positive (*Streptococcus epigenetics* and *Staphylococcus aureus*) and Gram-negative (*E. coli* and *Klebsiella* spp.) bacteria were procured from the Department of Life Sciences, College of Science, University of Babylon. Cultures utilized Mueller–Hinton agar and mannitol salt agar for growth, isolation, and differentiation.

2.5. Preparation of Standard Solutions for Bacteria

The Mueller–Hinton agar medium was prepared by dissolving 37 gm of the medium in 1 L of distilled water. This mixture was heated until the agar dissolved completely. It was then autoclaved at 120 °C for 15 min. The medium was poured into sterilized Petri dishes at a volume of 15–20 mL per plate and allowed to solidify. To ascertain sterility, the plates were incubated at 37 °C for 24 h.

3. Results and Discussion

3.1. Physicochemical Characterization of Adsorbent Surfaces

Figure 1 illustrates the surface characteristics of the studied hydrogels. Figure 1a depicts the surface of the (SA-g-p(AAc-co-AM)) hydrogel, which is smooth with a cloud-like appearance and numerous folds and clumps. In contrast, Figure 1b displays the surface of the (SA-g-p(AAc-co-AM)/ZnO) hydrogel composite after the addition of zinc oxide nanoparticles, which appears rougher and consists of spherical assemblies, indicative of the incorporation of zinc oxide nanoparticles [13]. Figure 1c presents the EDX spectrum of the hydrogel, confirming the absence of zinc oxide. Conversely, Figure 1d reveals the EDX spectrum of the hydrogel with zinc oxide nanoparticles, with peaks corresponding to zinc and oxygen, further confirming the incorporation of zinc oxide onto the surface [14–17].

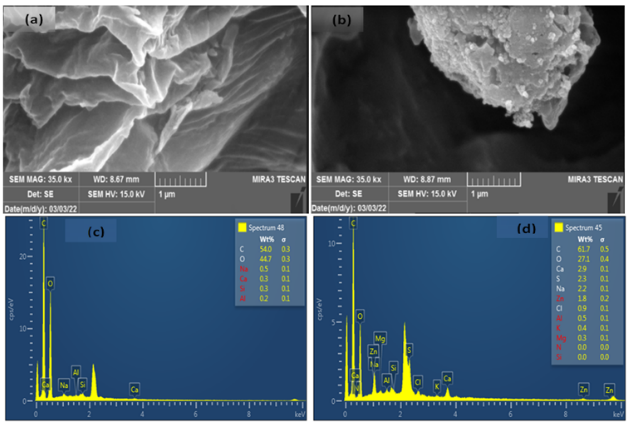

Figure 1. FESEM images of (**a**) (SA-g-p(AAc-co-AM)) hydrogel, (**b**) (SA-g-p(AAc-co-AM)/ZnO) hydrogel composite, (**c**) EDS of (SA-g-p(AAc-co-AM)) hydrogel, and (**d**) EDS of (SA-g-p(AAc-co-AM)/ZnO) hydrogel composite.

3.2. Biological Activity

Figure 2 presents the results of the antibacterial activity of the compounds, as determined by the disc diffusion method. Four bacterial isolates were used, including Gram-negative (*Klebsiella* spp. and *E. coli*) and Gram-positive (Staphylococcus aureus and Streptococcus epigenetics) bacteria. The results highlight that the (SA-g-p(AAc-co-AM)/ZnO) hydrogel composite exhibited superior antibacterial activity against all isolates when compared to both (SA-g-p(AAc-co-AM)) hydrogel and ZnO nanoparticles [18,19].

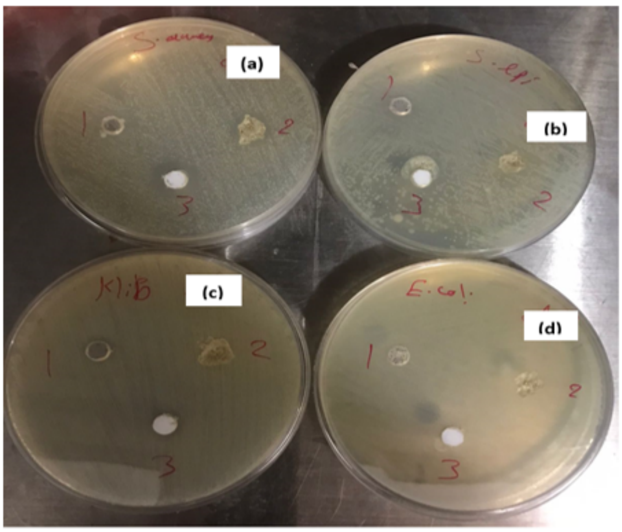

Figure 2. Antibacterial activity of the three compounds using the disc diffusion method. (**a**): *Staphylococcus aureus*; (**b**): *Streptococcus epigenetics*; (**c**): *Klebsiella* spp.; (**d**): *E. coli*.

Figure 3 displays the inhibition zones of the three compounds against pathogenic bacterial isolates. The (SA-g-p(AAc-co-AM)/ZnO) hydrogel composite demonstrated significant antibacterial activity, particularly against Staphylococcus aureus (30 mm) and Streptococcus epigenetics (25 mm), but exhibited reduced activity against *E. coli* (13 mm) and *Klebsiella* spp. (12 mm) [20,21]. The hydrogel composite displayed moderate antibacterial activity against Staphylococcus aureus (15 mm) and Streptococcus epigenetics (20 mm) but showed minimal activity against *E. coli* and *Klebsiella* spp. [22]. The ZnO nanoparticles exhibited minimal antibacterial activity against both Gram-positive and Gram-negative bacteria [23,24].

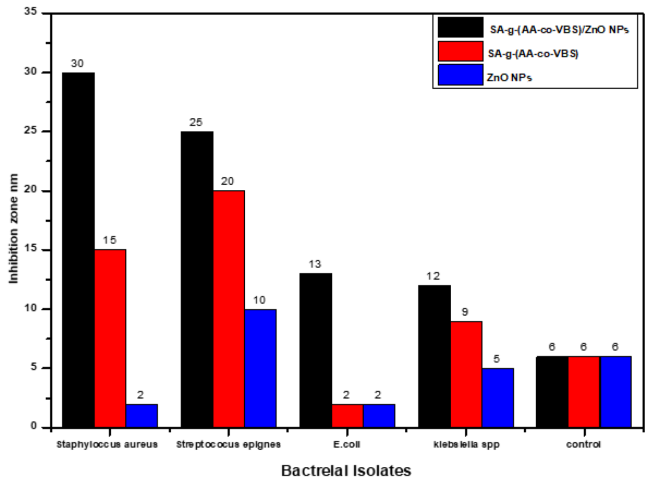

Figure 3. Effect of inhibition zones against pathogenic bacteria isolates.

4. Conclusions

The methodology employed in this study focused on the preparation of a hydrogel with significant efficiency against biological activity. Based on the data acquired, it can be concluded that the incorporation of zinc oxide nanoparticles into the hydrogel augmented its biological activity. This enhanced efficacy was particularly observed against the four bacterial isolates. Notably, the hydrogel composite demonstrated heightened effectiveness against Gram-positive bacteria as compared to Gram-negative bacteria. In contrast, the zinc oxide nanoparticles on their own exhibited minimal antibacterial activity against both Gram-positive and Gram-negative bacteria.

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