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Synthesis, Characterization, and Antibacterial Activity of Chitosan Crosslinked Ethylenediaminetetracetic Silver Nanocomposite (CCESN)

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ABSTRACT

The increasing resistance to antibiotics has necessitated the development of alternative antimicrobial agents. This study synthesized silver nanocomposites using chitosan synthesized from shells of *Archachatina marginata*. Chitosan was crosslinked with EDTA, serving as both a reducing and capping agent. The resultant product was characterized through Energy Dispersive X-ray (EDX), Transmission Electron Microscopy (TEM), X-ray Diffractometry (XRD), and Ultraviolet-Visible (UV-Vis) Spectrometry. The silver nanoparticles formation was initially shown by a colour change to brown which was further established by a peak absorbance at 435 nm, from a UV-Vis spectrophotometry scan. TEM and XRD analyses confirmed that the silver nanocomposite has a face-centred cubic structure, with an average size of 45.30 nm. EDX analysis identified silver as the primary element in the nanocomposite. The antimicrobial efficacy of the silver nanocomposites was tested against strains of *Escherichia coli* O157 and *Staphylococcus saprophyticus* DSM 18669. The antimicrobial tests revealed higher susceptibility of *Staphylococcus saprophyticus* DSM 18669 compared to *Escherichia coli* O157, demonstrating the nanocomposites' targeted efficacy. Future studies should investigate the molecular mechanisms of antibacterial activity while exploring scalable production and biocompatibility for applications in healthcare, food preservation, and wound care.

KEYWORDS: Nanocomposite, antimicrobial resistance, chitosan, nanoparticles, nanomaterials.

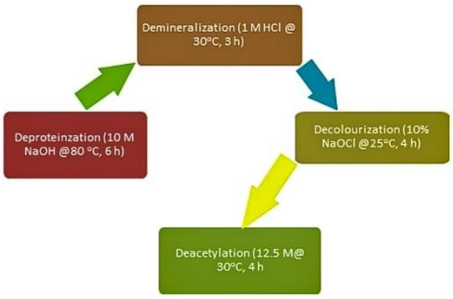
1. INTRODUCTION

Nanotechnology, which involves the manipulation of materials at the molecular scale, has found diverse uses in areas such as biomaterials, nanomedicine, and energy[1]. However, traditional methods for synthesizing nanoparticles often employ toxic chemicals[2], prompting an increased interest in eco-friendly biological approaches[3]. Among these, silver nanoparticles (AgNPs) are known to possess strong antimicrobial properties, though their precise mechanisms remain under investigation[4]. Chitosan, a biodegradable and biocompatible polymer derived from the shells of crustaceans and molluscs, is especially useful in nanoparticle synthesis[5]. In this work, chitosan from the shells of *Archachatina marginata*, combined with ethylenediaminetetracetic acid (EDTA), was used to produce silver nanoparticles. The primary objective of this study is to determine the characteristic properties of a synthesized chitosan-EDTA silver nanocomposite, and evaluate its antibacterial efficacy against *Staphylococcus saprophyticus* DSM 18669 and *Escherichia coli* O157, with the aim of developing sustainable nanomaterials and addressing antibiotic resistance.

2. MATERIALS AND METHODS

2.1. Chitosan Synthesis

Chitosan was obtained from *A. marginata*'s shell via deproteinization, demineralization, decolorization, and deacetylation processes[6], as outlined in Scheme 1.



Scheme 1: Synthesis of chitosan from the shells of *Archachatina marginata*

2.2. Preparation of Cross-Linked Chitosan-EDTA

Chitosan (1 g) was dissolved in acetic acid (100 mL), and EDTA (0.34 g) was added to form cross-linked chitosan-EDTA, which was then separated from the supernatant via centrifugation[7].

2.3. Synthesis of Chitosan-EDTA Silver Nanocomposites (CCESN)

The cross-linked chitosan-EDTA suspension (0.001%) was mixed with silver nitrate (5 mM), the pH was adjusted to 10 with NaOH, and the reaction proceeded for five hours to synthesize CCESN[7].

2.4. Characterization of CCESN

The synthesized CCESN was characterized using UV-Vis spectrophotometry, TEM, XRD, and EDX.

2.5. Antibacterial Study of CCESN

Antibacterial activity of the synthesized CCESN was assessed against *S. saprophyticus* DSM 18669 and *E. coli* O157 using disk diffusion and broth microdilution methods[8].

2.6. Statistical Analysis of Antibacterial Studies

The results of antibacterial studies were analyzed for statistical significance using ANOVA in GraphPad Software Inc., with triplicate measurements expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

The study confirmed the successful formation of AgNPs, as evidenced by a visible color change (Figure 1) and a UV-Vis absorption peak at 435 nm (Figure 2). While the color shifts were less pronounced compared to earlier reports, they still indicated nanoparticle formation[9]. The distinct 435 nm peak, attributed to Surface Plasmon Resonance (SPR), further confirmed AgNP synthesis[10]. Additionally, peaks between 200–300 nm, corresponding to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, suggested chelation between chitosan and EDTA[11]

Figure 1: CCESN solution after completion of the synthesis

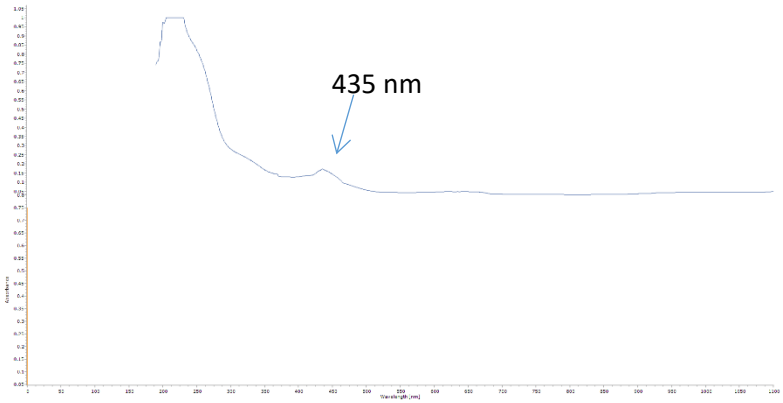


Figure 2: UV-Vis spectrum of synthesized CCESN highlighting the surface resonance plasmon effect

TEM images revealed spherical, polydisperse nanoparticles with an average size of 45.30 nm (Figure 3). The minimal particle aggregation suggests that the chitosan-EDTA cross-linking provided a stabilizing layer[12].

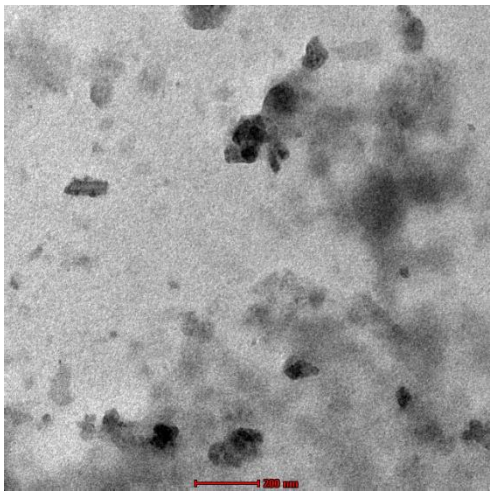


Figure 3: TEM micrograph of synthesized CCESN (200 nm)

XRD patterns (Figure 4) confirmed the presence of crystalline silver particles, with peaks at 38.62°, 42.95°, and 45.99° corresponding to the (111), (200), and (220) planes of the face-centered cubic lattice structure[13]. The crystallite size was calculated to be 22.20 nm[13]. Weaker peaks, likely due to the semi-crystalline nature and lower concentration of chitosan and EDTA, were also observed[14]

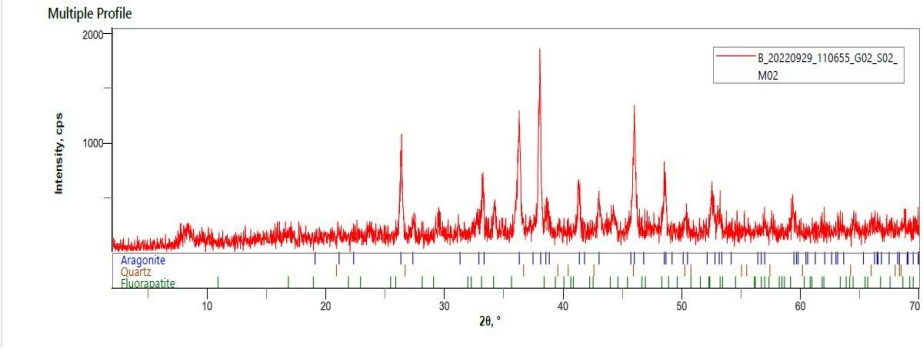


Figure 4: XRD pattern of synthesized CCESN

EDX profile (Figure 5) confirmed the presence of elemental silver (2.0–3.6 keV), aligning with UV-Vis and XRD results. Additional elements such as nitrogen, sulphur, copper, and iron were also detected, likely originating from chitosan, EDTA, or minor impurities[15,16].

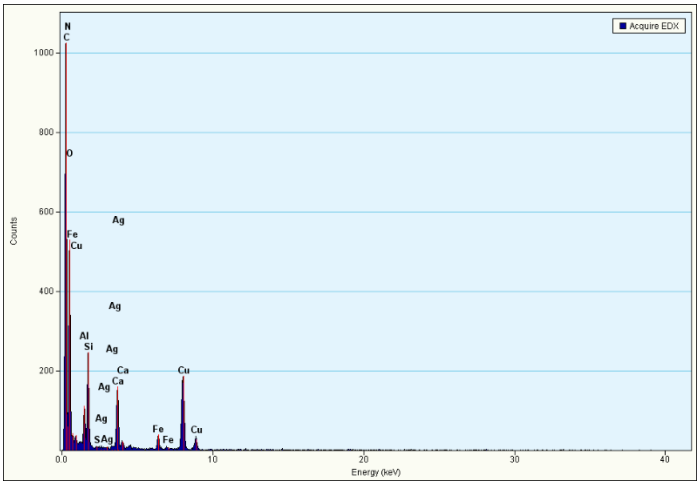


Figure 5: EDX profile of synthesized CCESN

The synthesized CCESN exhibited significant antibacterial efficacy, particularly against *S. saprophyticus* (46 mm), outperforming Levofloxacin at all tested concentrations[17]. (Figures 6a-b, Figures 7a-b;Tables 1-2). While CCESN was slightly less effective than Levofloxacin at the highest concentration, it demonstrated comparable efficacy at lower concentrations.

a b

Figure 6: Zone of inhibition of the synthesized CCESN against (a) *S. saprophyticus* DSM 18669 (b) *E. coli* strain 0157

c d

Figure 7: Zone of inhibition of Levofloxacin (c) *S. saprophyticus* DSM 18669 (d) *E. coli* strain 0157

Table 1*:* Inhibition zone for *S. saprophyticus* DSM 18669

Sample	Concentration (mg/mL)	Zone of inhibition (mm)
Silver nanocomposite	40	46.0 [a] ±0.82
Levofloxacin	0.05	31.33[c]±0.4
	0.025	27.33[c]±0.24
	0.0125	24.50[c]±0.41
	0.0063	18.33[b]±0.23

Columns with the different alphabets are statistically significant the same while those with same alphabet are statistically insignificant (p<0.05)

Table 2: Inhibition zone for *E. coli strain 0157*

Sample	Concentration (mg/mL)	Zone of inhibition (mm)
Silver nanocomposite	40	25.70[a]±0.25
Levofloxacin	0.05	30.33[b]±0.24
	0.025	25.50[b]±0.41
	0.0125	20.17[b]±0.24
	0.0063	0.00

Columns with the different alphabets are statistically significant the same while those with same alphabet are statistically insignificant (p<0.05)

The minimum inhibitory concentration (MIC) for both bacterial strains was 0.0781 mg/mL (Table 3), suggesting a bactericidal mode of action[17]. It is hypothesized that silver ions in CCESN interact with the negatively charged bacterial cell membrane, disrupting its integrity and inducing apoptosis[18,19].

Table 3: Minimum inhibitory concentration of the synthesized CCESN against the bacterial strains

Strains	MIC (mg/ mL)
Staphylococcus saprophyticus DSM 18669	0.0781
Escherichia coli strain 0157	0.0781

4. CONCLUSION

The chitosan-EDTA silver nanocomposite (CCESN) demonstrates potential as an effective and sustainable antibacterial agent, particularly against *S. saprophyticus*. While its efficacy against *E. coli* was slightly lower than Levofloxacin at the highest concentration, it performed comparably at lower doses. Future research should focus on optimization strategies and evaluate potential environmental and health risks associated with its application.

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