










Research Article

Vancomycin as an Antibacterial Agent Capped with Silver Nanoparticles: An Experimental Potential Analysis

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For the treatment of various infections, a variety of antimicrobial drugs are formulated. Nevertheless, many bacterial infections now exhibit antibiotic resistance due to the widespread utilization antibiotics. Methicillin-resistant among the most dangerous multidrug-resistant bacteria is *Staphylococcus aureus* (MRSA). Vancomycin became a viable therapy option due to MRSA resistance to methicillin medicines. One of the well-informed antibacterial compounds with wideband antibacterial activity is silver nanoparticles (AgNPs). AgNPs are thus suitable candidates for usage in conjunction alongside vancomycin to increase its antibacterial effect. The goal of the present research work is to boost the antibacterial potency of the glycopeptide antibiotic vancomycin towards Gram-positive (*Staphylococcus aureus*) but also Gram-negative (*Escherichia coli*) bacteria. The chemical reduction approach is used to create a colloidal solution of silver nanoparticles utilizing silver nitrate as a precursor in the environment of the ionic surfactant trisodium citrate that serves as covering including reducing reagent. Vancomycin was used to functionalize the synthesized nanoparticles and create the nanodrug complex (Van@AgNPs). The synergistic antibacterial potential of silver nanoparticles coated with vancomycin on both test pathogens was investigated using the agar well diffusion technique. The antibacterial potency for both classes of bacteria has significantly increased, according to the well diffusion test. It has been noted that this improvement is synergistic instead of additive.

1. Introduction

As more bacterial infections develop multidrug resistance (MDR), the availability of antibiotics has been a critical issue

for the healthcare professionals [1]. The most difficult issues for leading experts in the field of biomedicine are the rise in multiple-drug resistant confined of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*,

TABLE 1: Compounds employed and their uses.

Compounds	Uses
Silver nitrate (AgNO_3)	A precursor
Trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$)	In the production of silver nanoparticle, it is employed as reducing agent as well as capping agent
Vancomycin ($\text{C}_{66}\text{H}_{75}\text{Cl}_2\text{N}_9\text{O}_{24}$)	A medication utilized for storing on the surface area of formulated silver nanoparticles
Double deionized water	Each of the solutions was prepared using water that had been twice deionized

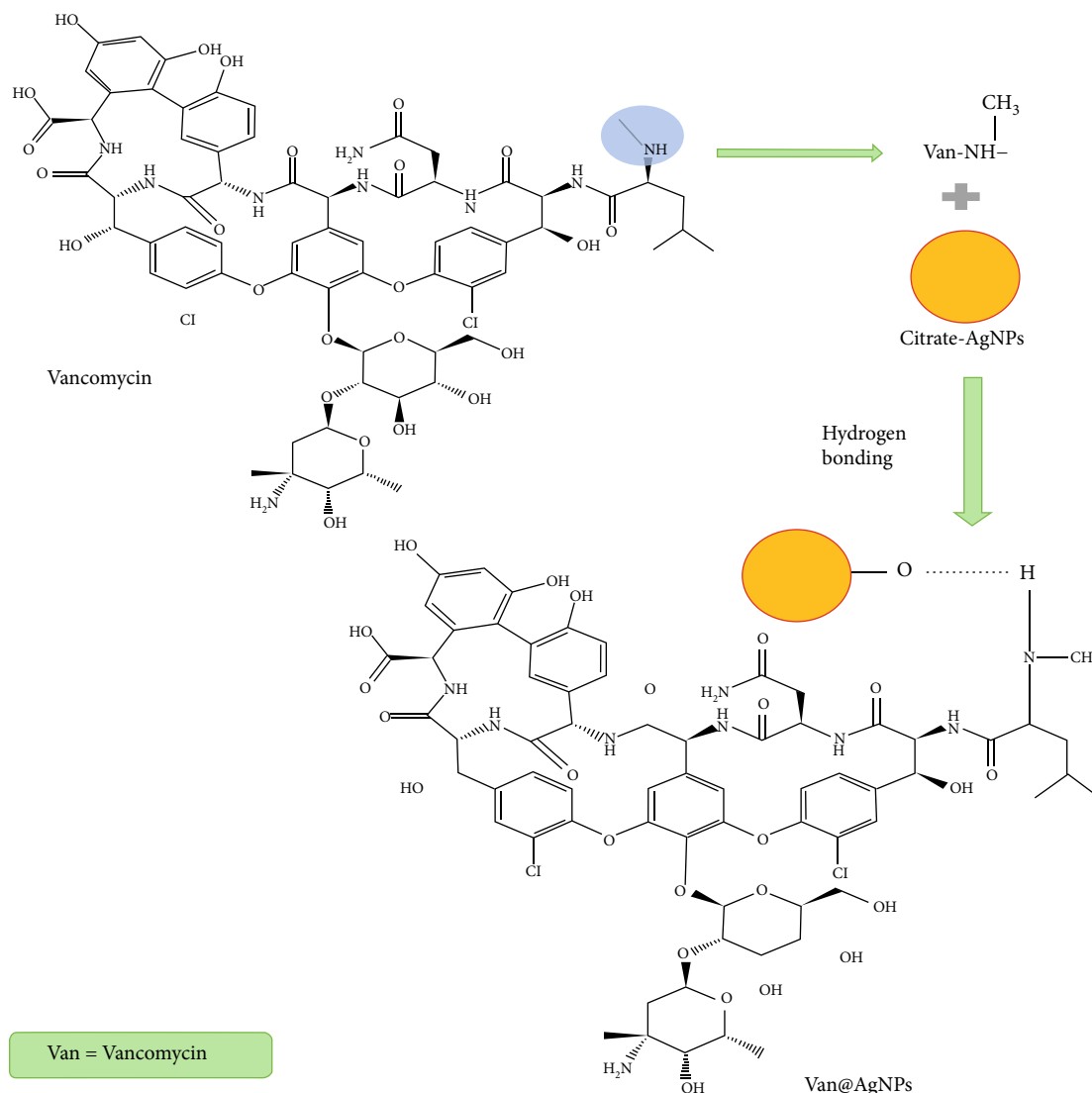


FIGURE 1: Reaction expressing the addition of vancomycin with AgNPs.

Enterococcus faecium, and *Enterobacter* species to the numerous traditional antibiotics. Antibiotic usage in any form can put more strain on bacteria due to selection. This causes vulnerable bacteria to perish while allowing resistant bacteria to thrive [2, 3]. Alternative therapies are becoming more and more necessary as antibiotic resistance spreads. Nanoparticles offer enormous opportunity for medicinal delivery systems for tiny molecules including medicines, DNA, and proteins. The antibacterial effectiveness of noble metal nanoparticles, that is, because of their huge surface area permitting large synergistic effect which is derived by several interactions, has recently

emerged as one of the most potential tools in the antibacterial inventory [4]. Some antimicrobial agents can be made more efficient by diminishing the cell membrane or enhancing cellular penetration. So, by reducing the negative effects related to medication molecules, metal nanoparticles combined with antibacterial pharmaceuticals may demonstrate better efficacy in particular therapies [5]. Since the earliest times, silver was already thoroughly studied for its ability to prevent infections, and silver nanoparticles (AgNPs) were established antibacterial properties towards a variety of bacterial species [6].

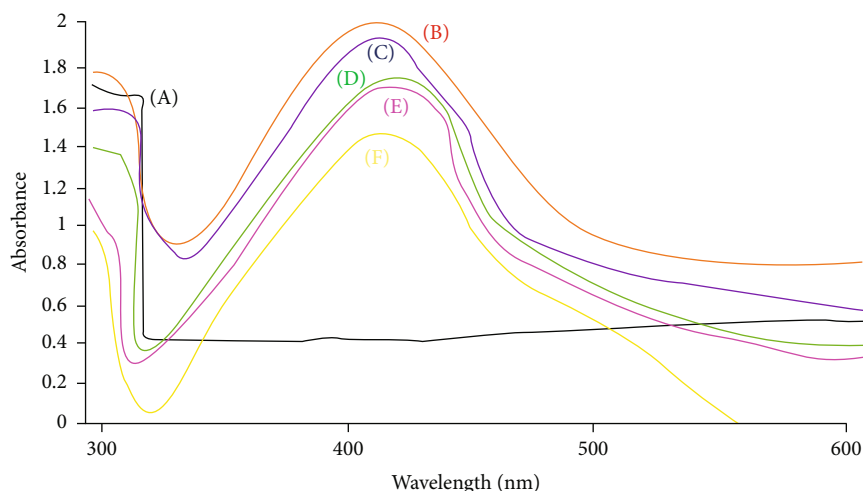


FIGURE 2: UV absorption spectra of (a) vancomycin, (b) citrate-AgNPs, and (c-f) Van@citrate-AgNPs at concentrations 0.05 mM, 0.07 mM, 0.1 mM, and 0.3 mM, respectively.

Vancomycin has been the dominant antibacterial medication employed to cure infections brought on by methicillin-resistant *Staphylococcus aureus* (MRSA) in the early 1950s. The widespread burden of vancomycin resistance among Gram-positive cocci is getting worse nowadays. Vancomycin advancement research showed that staphylococci vancomycin resistance proved challenging to establish *in vitro* [7]. In 1988, the first types of vancomycin-resistant enterococci (VRE) were discovered. Ever after, an increasing number of glycopeptide-resistant strains are also identified, indicating that such bacteria will possess the ability to spread the genes that give them vancomycin resistance to other bacterial species. Nanomedicines have aided in the advancement of new therapies being developed to combat VRE. Due to their acceptable biological deeds, silver nanoparticles (AgNPs) have several uses as antibacterial therapeutics [8]. The utilization of the silver nanoparticle along with vancomycin to increase the potency of the vancomycin specifically in MRSA is the main goal of this research paper.

2. Resources and Procedures for Experiments

2.1. Resources. Every one of the compounds included in this experiment would be of the analytical grade as well as were not subsequently purified before usage (Table 1).

2.2. Generation of Silver Nanoparticles with a Citrate Capping (Figure 1).

- (i) Silver nitrate (AgNO_3) was used like a precursor in a chemical reduction process to create colloidal citrate-capped AgNPs (citrate-AgNPs), with sodium citrate acting like a reducing and shielding factor
- (ii) In this procedure, a 50 mL solution of AgNO_3 (1 mM) in deionized water was brought near boiling while being continuously stirred on a magnetic hot plate

- (iii) During at that time as quickly as possible, the AgNO_3 solution started to boil; 350 L sodium citrate solution (20 mM) has been poured dropwise
- (iv) The solution gradually becomes brownish yellow that also shows a sign that the Ag^+ ions have been reduced
- (v) The solution was heated for a little while longer while being stirred, then thereafter cooled to room temperature for additional experiments

2.3. Vancomycin-Coated Silver Nanoparticle Formulation. A separate aqueous solution of vancomycin (5 mg/5 mL) has been produced beneficial to the manufacturing related to vancomycin loaded citrate-AgNPs (Van@ citrate-AgNPs). Vancomycin was utilized at different quantities like 0.05, 0.07, 0.1, 0.3, and 0.5 mM. Concentration of citrate-AgNPs remained unchanged, i.e., 1 mM. Using a magnetic stirrer which is set at room temperature, the solution then permitted to agitate for 20 to 25 minutes. The solution was left overnight, after which it was refrigerated for future testing but also characterisation. UV-Vis spectra of vancomycin, citrate-AgNPs, and Van@citrate-AgNPs at different concentration is presented in Figure 2.

2.4. Microbiological Evaluation Processes. Employing of agar well diffusion technique, the antibacterial action of vancomycin-capped silver nanoparticles is evaluated with reference to medication. The media and glassware utilized throughout this test were sterile for twenty minutes at 120°C in an autoclave. This procedure is utilized for calculation of the zone of inhibition with both Gram-positive bacteria (such as *S. aureus*) and Gram-negative bacteria (such as *E. coli*) in order to study antibacterial action. Both bacteria had been cultivated for 24 hrs at 37°C in Luria broth (LB) medium. The whole area of the agar plate was covered with a certain amount of microbial inoculation. Thereafter, tiny holes having a diameter of about 5 mm had been made using a sterile

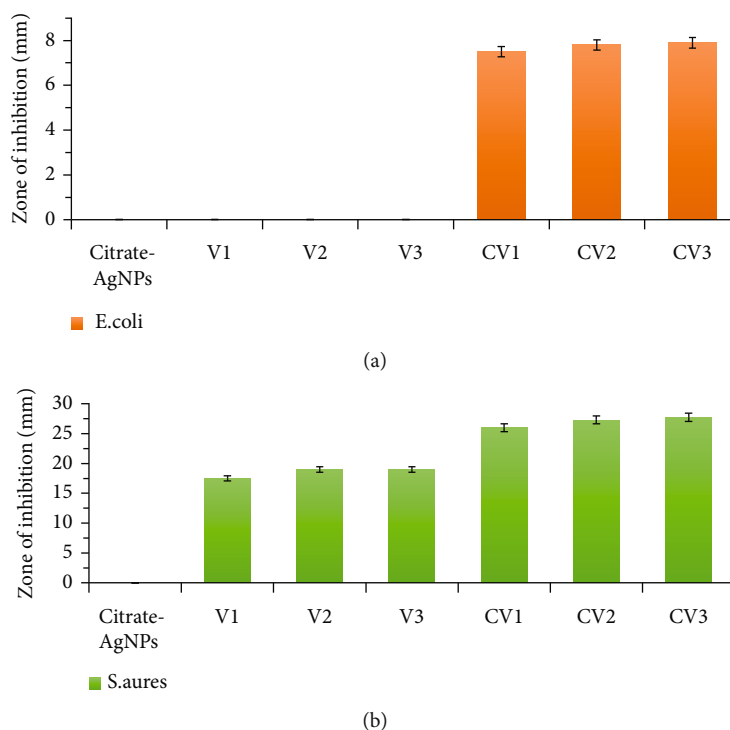


FIGURE 3: Antibacterial action of bacterial strain in the form of zone of inhibition \pm S.D.

TABLE 2: Zone of inhibition (mm) \pm S.D. of AgNPs, Van, and Van@AgNPs against *S. aureus* and *E. coli*.

	Gram-positive bacteria (<i>Staphylococcus aureus</i>)	Gram-negative bacteria <i>Escherichia coli</i>
AgNPs	0 \pm 0	0 \pm 0
Van1: 0.05 mM	17.5 \pm 1.0	0 \pm 0
Van2: 0.1 mM	19 \pm 0.6	0 \pm 0
Van3: 0.3 mM	19 \pm 0.6	0 \pm 0
Van@AgNPs1: 0.05 mM	26 \pm 0.6	7.5 \pm 0.5
Van@AgNPs2: 0.1 mM	27.3 \pm 0.5	7.8 \pm 0.9
Van@AgNPs3: 0.3 mM	27.7 \pm 0.4	7.8 \pm 0.9

cork borer. 50 L of each of the following solutions was added into the holes separately: vancomycin, silver nitrate (AgNPs), vancomycin-AgNPs (Van@citrate-AgNP complex), and also regular saline solution.

2.5. Study on In Vitro Antibacterial Action. By employing standard agar well diffusion approach, the synergistic antibacterial impact produced from the citrate-capped AgNPs containing vancomycin is examined under this work. Independent effects of the unbounded vancomycin and uncovered silver nanoparticles, targeting both kinds of Gram-positive (*S. aureus*) as well as Gram-negative (*E. coli*) strains, are examined. Citrate functions as linkage between the vancomycin and the AgNPs. Because citrate is anionic capping agent boosts AgNP stability throughout an electrostatic stabilizing mechanism, its usage is rationalized. At the evaluation concentration 60 g/mL, the bare AgNPs which do not contain drug had no antibacterial impact on either strain.

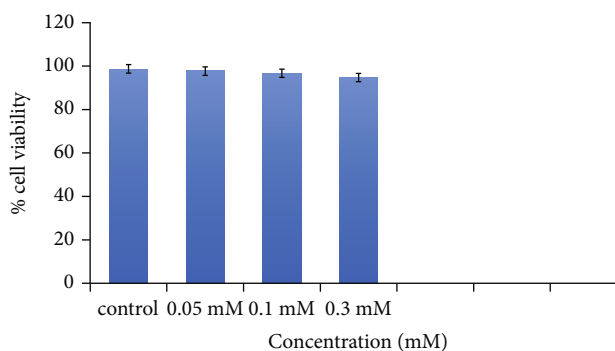


FIGURE 4: Percentage of cell availability in different concentration.

However, mentioned citrate-capped AgNPs containing antibiotic had a noteworthy synergistic impact. AgNP-vancomycin combination was shown to be more efficient towards *S. aureus* than *E. coli*.

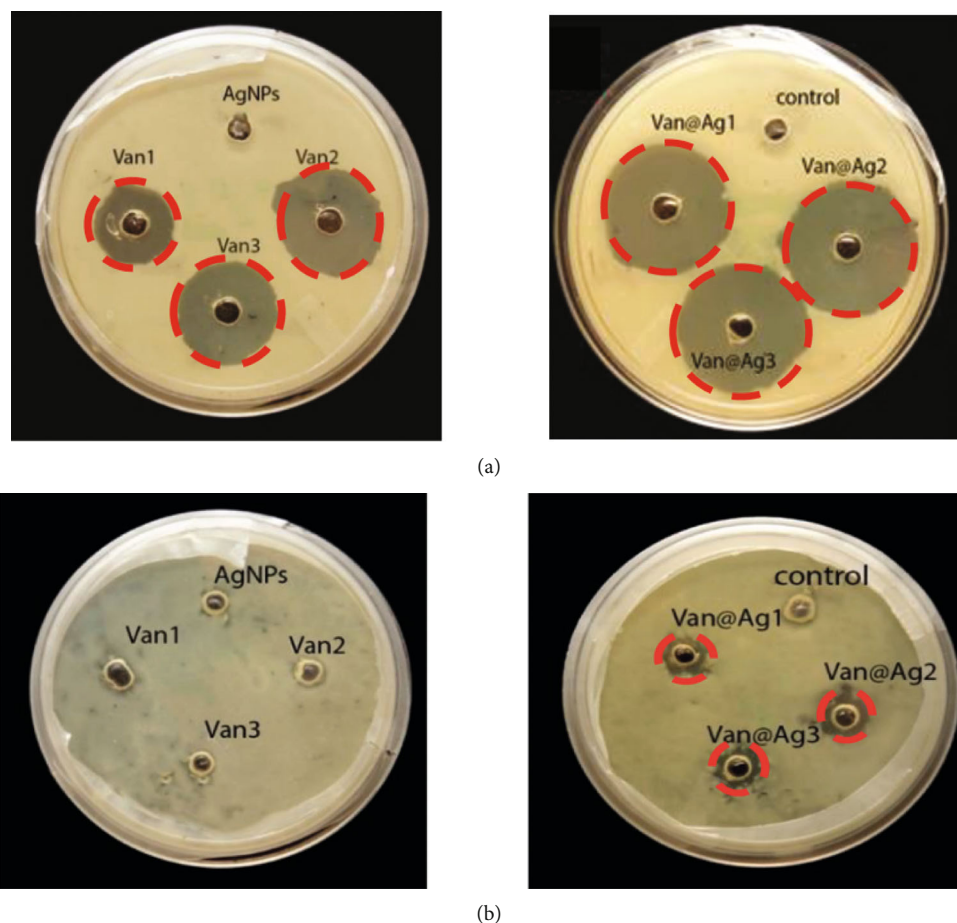


FIGURE 5: Illustration of agar plates demonstrating antifungal effect of vancomycin, AgNPs, and Van@citrate-AgNPs against (a) *S. aureus* and (b) *E. coli* at various concentrations.

Simultaneous testing of AgNPs, free vancomycin, and also vancomycin-coated silver nanoparticles (Van@AgNPs) was conducted versus both trial strains throughout the antibacterial investigation. When used over both Gram-positive and Gram-negative classes of bacteria, vancomycin-coated silver nanoparticles had a broader inhibition zone but also consequently had greater antibacterial activity (Figure 3). Vancomycin-coated silver nanoparticles inhibited *S. aureus* and *E. coli*'s growth more effectively than vancomycin did against these bacteria.

According to the prior studies, it is shown that vancomycin is exclusively efficient against strains of Gram-positive bacteria; it is ineffective towards strains of Gram-negative bacteria [9]. However, it was shown during this investigation that vancomycin showed zero effect supporting to the development of *E. coli*, whereas the AgNP-vancomycin combination somewhat suppresses it. As a result, AgNP-vancomycin combination became effective against vancomycin-resistant *E. coli*. Additionally, the impact was more potent towards Gram-positive than Gram-negative bacteria.

2.5.1. Note. Van1:0.05 mM (V1),
 Van2:0.1 mM (V2),
 Van3:0.3 mM (V3),
 Van@AgNPs1:0.05 mM (CV1),

Van@AgNPs2:0.1 mM (CV2),
 Van@AgNPs3:0.3 mM (CV3).

The ZOI rises from 0 to 7.8 mm for *E. coli* while ZOI rises in between 0 and 27.7 mm for *S. aureus* (Table 2). Both the vancomycin-sensitive *S. aureus* and the vancomycin-sensitive *E. coli* were more delicate to the drug conjugate AgNPs after being cherished with Van@citrate-AgNPs. Vancomycin along with AgNPs has been found to have a synergistic impact against both classes of Gram-positive bacteria. Following treatment with Van@citrate-AgNPs, the morphological alterations in the bacterial cell walls of both *S. aureus* and *E. coli* have been observed (Figures 4 and 5). Similar findings have also been observed in other earlier publications [10, 11].

Based on diversity of the cell membranes of Gram-positive as well as Gram-negative bacteria, it is possible to explain why Van@citrate-AgNPs are more efficient against *S. aureus*. However, compared to Gram-negative bacteria which contain a thin cell membrane, Gram-positive microorganisms possess thicker cell walls consisting of peptidoglycan layers. However, Gram-negative bacteria have a lipopolysaccharide- (LPS-) based thick outer layer that covers their thin peptidoglycan wall that is missing in Gram-positive bacteria [12]. This thick outer layer has a diameter of about 10 nm. Due to the lack of an outer layer,

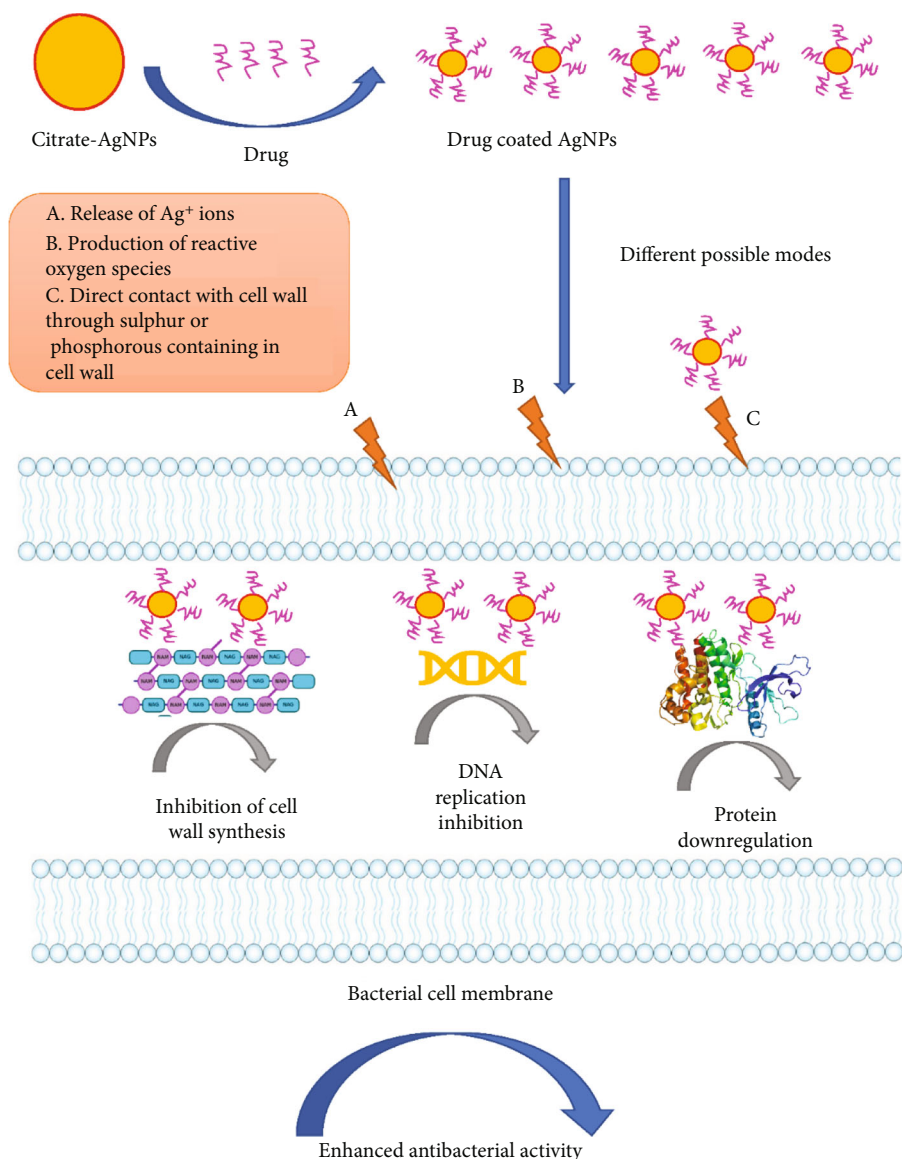


FIGURE 6: Proposed interaction between vancomycin-coated silver nanoparticle and bacterial cell walls.

Gram-negative bacteria are highly susceptible to AgNP-drug complexes although having a thicker peptidoglycan layer compared to Gram-positive bacteria [13].

2.6. Proposed Interaction between Vancomycin-Coated Silver Nanoparticle (Van@AgNPs) and Bacterial Cell Walls (Figure 6). Vancomycin's bactericidal action is dependent on the suppression of peptidoglycan-based bacterial cell wall production [14, 15]. However, this technique is no longer effective as some vancomycin-resistant bacteria (such as VRSA) have emerged. When combined with AgNPs, vancomycin has a synergistic antibacterial activity towards both experimental strains. As was before established, vancomycin forms the Van@AgNP complex via hydrogen bonding to AgNPs through citrate.

The stages in the process that result in a synergistic antimicrobial property might be used to describe the reaction mechanisms. These are the steps: vancomycin and AgNP

surface interaction. Ag^+ ions or AgNPs are released by a complex that is bounded with the bacteria [16]. AgNPs become poisonous when they bind to the phosphorous in DNA and the sulfur in proteins. It impairs a number of bacterial processes, including DNA replication along with protein synthesis; they ultimately result in bacterial or cell death. The main interactional mechanism is this [17, 18]. Additionally, oxidative stress that affords another pathway for cellular disruption can be commenced from the production of reacting oxygen substance on the surface of AgNPs [19, 20]. Nevertheless, the specific process through which silver nanoparticles fight microorganisms is still unknown. The electrostatic interaction among nanoparticles and the bacterial cell wall was also shown in earlier investigations [21].

A number of variables, such as particle size, shape, aggregation, and capping agent, affect the rate at which silver ions are released. In comparison to bigger particles, the tiny silver nanospheres discharge ions more quickly. The

surface's complexation has an impact on the ion release rate as well. Contrary to more readily displaced stabilizing compounds like citrate, firmly bound thiol-containing capping agents often reduce release rates [22]. As a result, the ion release rate is significantly influenced by the citrate coating on the silver surface. The histogram in Figure 3 depicts the improvement of antibacterial potency towards *S. aureus* and *E. coli*. Such findings taken together show that Van@citrate-AgNPs gather inside the bacterial membrane as well as cytoplasm, proving Van@citrate-AgNPs are successfully supplied to the bacterium where silver ions, vancomycin, or a combination of the two, that would be the main process, can communicate among the cell membrane to restrict bacterial development. In conclusion, Van@citrate-AgNP bioconjugate compounds function as an efficient antagonist of both Gram-positive and Gram-negative bacteria.

3. Conclusion

The chemical reduction approach was used to generate vancomycin-coated AgNPs in the existence of the ionic capping material trisodium citrate that intern serves like a connector and stability enhancer for the drug molecules. Van@citrate-AgNPs had improved significant ability towards both *S. aureus* (Gram-positive) and *E. coli* (Gram-negative), according to an *in vitro* antibacterial assay. The findings suggest that examined microorganisms are effectively inhibited by citrate-AgNPs. Depending upon the outcomes, we are able to say that citrate-AgNPs are effective transported systems for the medication vancomycin and that, with minimal adjustments, they may be employed for controlled administration to bacterial cells. In the end, it is concluded that the Van@citrate-AgNP is more potent than the single entity vancomycin towards both *S. aureus* and *E. coli*.

Data Availability

All data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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