chemistry proceedings

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Proceeding Paper

Antibacterial Activity of $Ag_2O/SrO/CaO$ Nanocomposite †

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† Presented at the 26th International Electronic Conference on Synthetic Organic Chemistry; Available online: https://ecsoc-26.sciforum.net.

Abstract: The increase in bacterial resistance to one or several antibiotics has become a global health problem. Nanocomposite have become a tool against multidrug-resistant bacteria. A nanocomposite, $Ag_2O/SrO/CaO$ was prepared from $AgNO_3$, $SrCl_2.6H_2O$, $CaCl_2$, and a solution of Na_2CO_3 via calcination of the salts mixture. The nanocomposite was successfully prepared by the co-precipitation method and completely according to green chemistry, both in terms of synthesis method, solvent and precursors. The nanocomposite was characterized by XRD, XRF, FESEM analyses. Afterwards, the nanocomposite was applied for antibacterial activity against gram-positive and gram-negative bacteria including PS Aeruginosa, Keleb peneumonia, Staph coccus aureus, Staph saprophyticus, and Esherichia coli.

Keywords: nanocomposite; green chemistry; antibacterial; metal oxides

Citation: Aghaee, M.; Manteghi, F. Antibacterial Activity of $Ag_2O/SrO/CaO$ Nanocomposite. *Chem. Proc.* **2022**, *4*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Firstname Lastname

Published: date

1. Introduction

The mixture of metal oxide nanoparticles and the combination of two or more metal oxides has an improved set of properties. In this way, we can modify the physical, chemical, biological and morphological properties of the oxides. The combination and calcination of metallic compounds can create diverse properties and applications in the product, for example, it is shown that silver compounds are severe against bacteria.

Metal oxides, especially CuO, NiO, CoO, ZnO, and Cu_2O in their nano-forms, have been considered as potential biocide agents. Most of these metal oxides and their antibacterial activity has been often related to the production of reactive oxygen species (ROS) [1,2].

Metals and metal oxides shows antibacterial properties in the following ways: protein dysfunction, production of ROS and antioxidant depletion, impaired membrane function, interference with nutrient upake, genotoxicity [1].

2. Experimental

2.1. Peparation of $Ag_2O/SrO/CaO$ Nanocomposite

The $Ag_2O/SrO/CaO$ nanocomposite metal was prepared by the co-precipitation of corresponding carbonates from the aqueous solution of metal salts. Initially, 0.25 M, 30 mL solution of each of $AgNO_3$ (1.274 g), $SrCl_2.6H_2O$ (1.999 g), $CaCl_2$ (0.832 g), and a solution (1.00 M, 50 mL) of Na_2CO_3 (5.299 g) were prepared with distilled water. Next, $AgNO_3$, $SrCl_2.6H_2O$, and $CaCl_2$ solutions were mixed and the resulted mixture was stirred vigorously at room temperature for few minutes. In the next part, the solution of 1.00 M Na_2CO_3 was added slowly to the above mixture with agitation until the precipitation of the carbonates was complete. The final mixture was stirred for 4 h at 55–60 °C with constant stirring. Then, the white metallic precipitate was filtered and washed several times with distilled water. Then the produced compound was dried at room temperature. To obtain a multi-metal nanocomposite ($Ag_2O/SrO/CaO$ nanocomposite), the obtained dried precipitate was calcinated in a muffle furnace at 600 °C for five hours. As a result of the calcination, a metal oxide nanocomposite was formed from the carbonates [3].

2.2. Characterization

The XRD patterns of the title composite, shown in Figure 1, was applied for the investigation of crystalline structure of nanomaterials. In Figure 1, the 2θ peaks appear at 25.67°, 27.705°, 33.246°, 36.55°, 41.8°, 42°, 45°, 51.849°, 54.724°, 57.8°, 62° and 76.5°, respectively, due to the formation of monoclinic (P2$_1$/C) Ag_2O in this nanocomposite. The diffraction peaks observed at 32°, 35.8°, 37.5° 38.2°, 48°, 54.5°, 57.9° are for the crystallographic planes of SrO, the cubic structure of SrO nanoparticles was detected (JCPDS file#6-520). The peaks at 32.5°, 36.6°, 38.2°, 44.5°, 45.4°, 64.2°, 67.8°, 77.4° and 81.8° (JCPDS card no.00-004-0777) are resulted from the cubic structure of CaO. The phase purity of SrO, CaO and Ag_2O in the Ag_2O/SrO/CaO seen in the XRD pattern is an evidence for the presence of all three oxides in the nanocomposite.

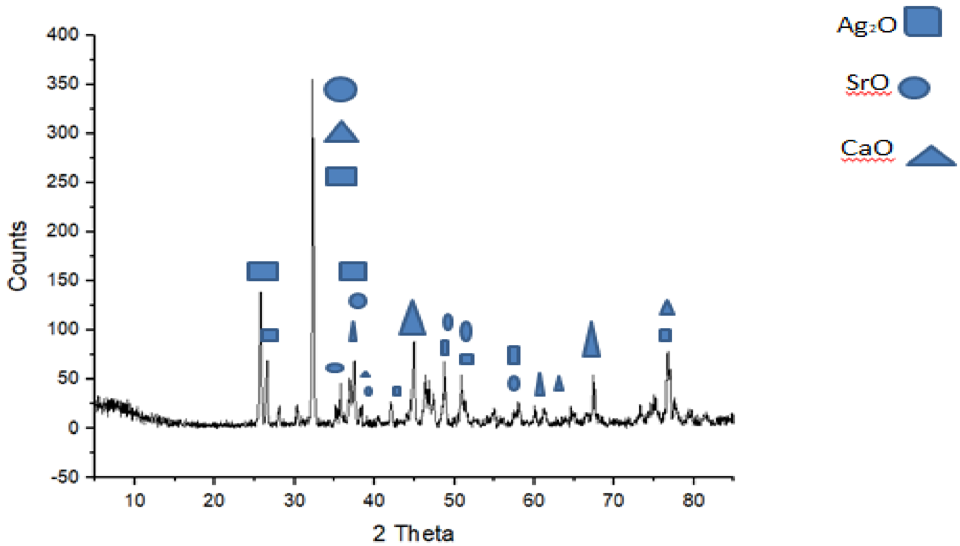


Figure 1. XRD pattern of Ag_2O/SrO/CaO.

In XRF analysis of Ag_2O/SrO/CaO, shown in Table 1, the percentages of pure CaO, SrO and Ag_2O are calculated equal to 10.77, almost 45.16 and, 42.7, respectively.

Table 2. The XRF results as percentage of elements.

Elements	Ag	Na	Al_2O_3	SiO_2	P_2O_5	SO_3	K_2O	CaO	TiO_2
wt %	56.062	>>	-	-	-	-	-	9.627	-
Elements	Fe_2O_3	V_2O_5	MnO	Cr_2O_3	Ba	Sr	Zn	Se	Nb
wt %		-	-	-	-	31.985	-	-	>>
Elements	F	Cr	Cl	Ce	Co	Mo	Ca	Cu	Ho
wt %	-	-	2.325	-	-	-	-	>>	-

The SEM micrographs of the sample (scales 200 nm and 5µm) show the spherical morphology of Ag_2O/SrO/CaO observed in Figure 2b.

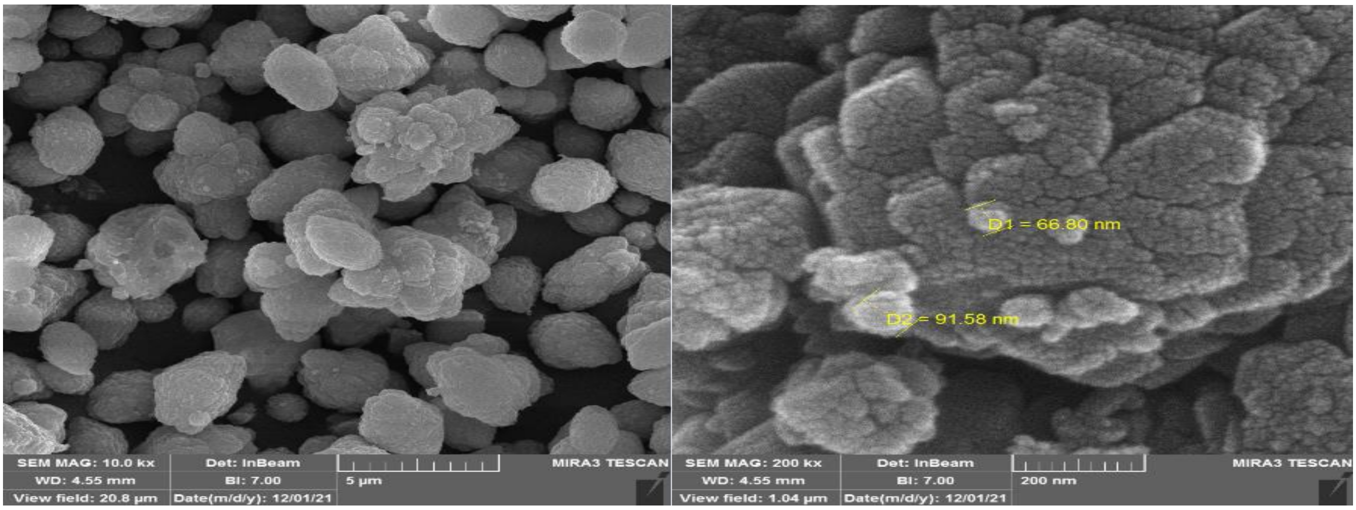
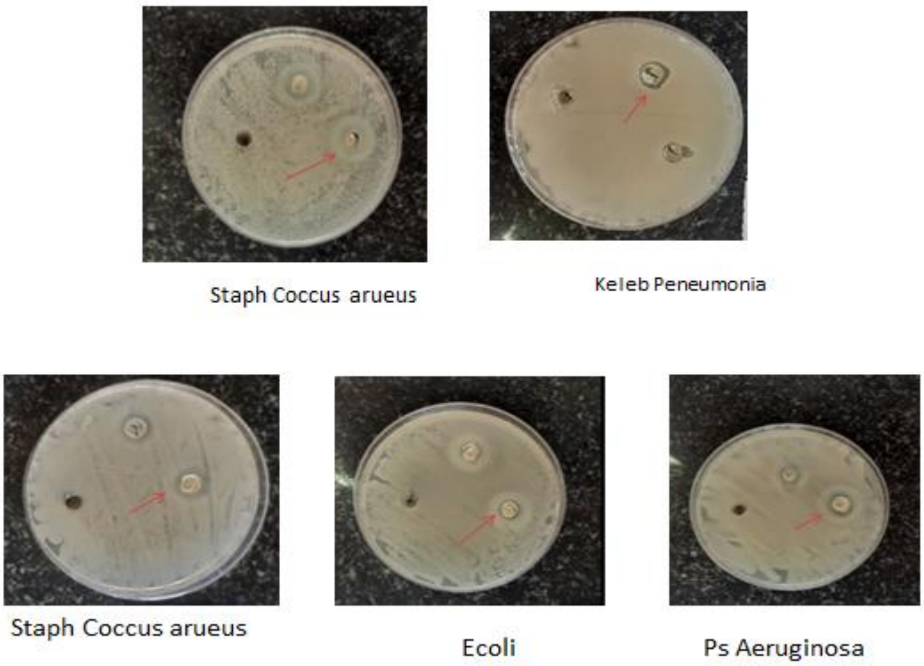


Figure 2. The FESEM images $Ag_2O/SrO/CaO$.

2.3. Antibacterial Activity

Antibacterial activity of the title nanocomposite against gram-positive and gram-negative bacteria were tested. The bacteria involve PS. Aeruginosa, Keleb peneumonia, Staph coccus aureus, Staph sapropphyticus, Esherichia. The results are shown in Figure 3a–e and summarized in Table 2. It can be seen that the inhibition zone diameter from $Ag_2O/SrO/CaO$ is varied from 7.876 to 18.991 mm.

Test Bacteria	Inhibition Zone Diameter(mm)
	$Ag_2O/SrO/CaO$ Nano Composite
Ps.Aeruginosa	18.991
Keleb Peneumonia	7.876
Staph Coccus aureus	13.785
Staph Sapropphyticus	12.723
Ecoli	16.456



Proposed mechanism

According to the literature, three following mechanisms can be proposed for the antibacterial activity of the title compound.

Free radical's generations: Metal and metal oxides, when reacting with bacteria, follow a mechanism including action of nanoparticles and generation of reactive oxygen species (ROS). The amount of ROS depends on the metal or metal oxides that are synthesized as nanoparticles and the rate at which their ions are released. Some nanoparticles, such as ZnO, CuO and Ag Np release a wide range of ions and ROS against bacteria, ROS destroys the internal compounds of bacteria such as proteins, enzymes, DNA and bacterial respiration [4].

Cell membrane damage: Silver nanoparticles have physicochemical and biological properties that are different from the properties of silver in the from of bulk. In order to have toxic effects of silver nanoparticles, they must be able to interact with bacteria on the surface and even in the cytoplasm. Silver nanoparticles release silver ions that must be able to cross the bacteria cell membrane and this is not very easy. Bacteria are divided into two groups gram-positive (layers of peptidoglycans), gram-negative (layers of lipopolysaccharides), in both the groups, silver ions must be able to cross the membrane. Silver nanoparticles have their first encounter with the outermost part of the membrane, which is composed of protein with electron donors that include oxygen, phosphorus, nitrogen and sulfur atoms. Thiol-containing agents can block silver nanoparticles and inhibit their antibacterial activity. In some papers, it is stated that silver nanoparticles are adsorbed on cell membranes and the accumulation of nanoparticles in bacterial membranes causes abnormal structure, gaps are created in the surface of the membrane and the cell membrane is destroyed with the expansion of these cavities. Finally, the silver nanoparticles reach the cytoplasm and react with proteins, enzyme and also DNA [4].

DNA interaction: The effect of silver Np on DNA was not understood in detail. Oxidative pressure was expressed important mechanism proposed to damage DNA. Silver Np inhibit respiratory enzymes that conduct ROS formation. The reaction of the oxidized constituents of DNA with DNA was destroyed the DNA. Silver ions react with bases in DNA with higher affinity than phosphate groups, although AgNp also have an antibacterial effect without releasing silver ions. AgNP can penetrate bacterial cells, AgNp invade the surface of cell membranes and disport permeability by modifying cell potential and inhibiting cellular respiration. AgNp cations join to thiol groups in bacterial proteins, disrupting their activity and causing cell death. The most important part of the mechanism of AgNp against bacterial DNA is oxidative stress [4].

3. Conclusions

In this work, was synthesized a nanocomposite (Ag_2O/SrO/CaO) by an almost green method and green solvent i.e., water and used environmentally friendly salts of calcium, strontium and silver. The product was characterized by XRD, XRF, FESEM analyses, then the nanocomposite was examined as antibacterial activities against gram-positive and gram-negative bacteria. Including PS. Aeruginosa, Keleb peneumonia, Staph coccus aureus, Staph sapropphyticus, and Ecoli.

Author Contributions:

Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest:

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