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Preparation of ($ZnO/Cr_2O_3/NiO$) Nanocomposite by Photo Irradiation Method and Study of its Ability to Inhibit Certain Harmful Isolates and Activity of Serum ALP Enzyme

Shaimaa H. Jabber, Athraa S. Ahmed and Rasha S. Mahmood
Department of Chemistry, College of Science, University of AL-Mustansiriya, Baghdad, Iraq
rashashakir.m@uomustansiriyah.edu.iq

Abstract: In the present study, nanocomposite ($ZnO/Cr_2O_3/NiO$) was synthesized by photo irradiation. The synthesized nanoparticles characterized by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and X-Ray Diffraction (XRD). Results show that to nanocomposite ($ZnO/Cr_2O_3/NiO$) have average particles size of ($ZnO/Cr_2O_3/NiO$) nanocomposite of TEM is (29.6) and SEM is (34.2). The average diameter that obtained from AFM is about 55 nm while the diameter of 60 nm is the lowest diameter and the diameter of 42 nm is the highest percentage in the sample and diameter of the crystal nanoparticles (25 nm) was found ($ZnO/Cr_2O_3/NiO$) nanocomposite were applied to study the inhibition of bacterial using *Staphylococcus epidermidis* (Gram positive), *Escherichia coli* (Gram negative). The antibacterial activity of ($ZnO/Cr_2O_3/NiO$) nanocomposite show a higher inhibition of *Escherichia coli* bacteria when a compared with *Staphylococcus epidermidis* also study the effect of nanocomposition ($ZnO/Cr_2O_3/NiO$) on the enzyme activity of ALP. The activity of serum ALP in patients with CKD in presence of nanocomposition ($ZnO/Cr_2O_3/NiO$) was lower than its activity in this group without nanocomposition ($ZnO/Cr_2O_3/NiO$).

Key words: ($ZnO/Cr_2O_3/$ NiO) nanocomposite, antibacterial activity, CKD, ALP, *In vitro*, AFM, XRD, TEM and SEM

INTRODUCTION

Nanotechnology is a qualitative jump in the world of technology which holds a great promise for the design and development of many types of new products with its potential medical applications on early illness detection, treatment and protection. Nanoparticles produced and used in a wide range of commercial products throughout the world. The field of nanotechnology is an active area of research. Nanoparticles exhibit new or improved properties based on specific interest such as size (100 nm), distribution and morphology. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use for human benefits in the last years, noble metal nanoparticles have been the topic of center research due to their special optical, electronic, mechanical, magnetic and chemical properties that are significantly various from those of bulk substances (Sivaranjani and Meenakshisundaram, 2013; Mani *et al*., 2017).

The most important results of nanoscience are the nanotechnology. Newly, nanotechnology is consider to be one of the key technologies of the 21st century 1. The word ‘nano’ indicates one billionth or 10^{-9} units. It is vastly agreed that nanoparticles are group of atoms in the size range of 1-100 nm[2].

Frequently, nanometer-size metallic particles show singular and highly changed in the chemical, physical and biological properties compared to their macro scaled counterparts due to their small size and high surface-to-volume ratio. Thus, these nanoparticles have been the fundamental object of research in recent years (Paul *et al*., 2017).

Nanoparticles are known to be many-sided and have a large range of applications in recent biology, medicine and bio-revelation of pathogens. Nanoparticles derived from biological exporter have been shown to have antibacterial and antifungal properties. Due to high health-care costs and high in incidents of infections and antibiotic resistance, there is an important need to develop new antimicrobial molecules (Alaa El-Dien *et al*., 2017).

Serum Alkaline Phosphatase (ALP) is a metalloenzyme that catalyses the hydrolysis of organic pyrophosphate. Vascular calcification is a major

Corresponding Author: Rasha S. Mahmood, Department of Chemistry, College of Science, University of AL-Mustansiriya, Baghdad, Iraq, rashashakir.m@uomustansiriyah.edu.iq

component of atherosclerosis and pyrophosphate provides integrity for vessels by inhibiting medial vascular calcification. Elevated total serum ALP levels are associated with mortality in patients with chronic kidney failure (Edem, 2018).

Among the most important nanoparticles commonly used in nanotechnology are zinc oxide with its limportant physical and chemical properties such as high electrochemical coupling coefficient, high chemical stability, high photostability and broad range of radiation absorption is a multifunctional material. In materials science, zinc oxide is classified as a semiconductor in group II-VI. ZnO have energy band (3.37 eV), large bond energy (60 meV) and big thermal and mechanical stability at room temperature make it likable for potential use in laser technology, electronics and optoelectronics. So, the properties of ZnO make it used as a converter, energy generator, sensor and photocatalyst in hydrogen production. Because of its solidity, hardness and piezoelectric constant it is a specific material in the ceramics industry while it is have a little toxicity, biocompatibility and make it a material of interest for biomedicine and in proecological systems (Kolodziejczak-Radzimska and Jesionowski, 2014).

NiO is a p-type oxide semiconductor with a large band gap (3.6-4.0 eV) and ~1.8 eV conduction band energy which has been looked as a good materials for electronic, optical and catalytic applications (Kunz, 1981; Sato *et al*., 1993).

NiO is a very important material and has attracted growing interest due to its wide important chemical and physicochemical methods have been employed to produce NiO nanomaterials including nanosheets, nanowires, nanorods and nanoparticles (Tao *et al*., 2012).

MATERIALS AND METHODS

Synthesis of ($ZnO/Cr_2O_3/NiO$) nanocomposite: Photo irradiation method was used to prepare nanoparticles composite of zinc oxide, nickel oxide and chromium oxide using irradiation cell for this purpose we used (20 mL from 0. 1 M for $CrCl_3.6H_2O$ salt with 20 mL of 0.1 M for $ZnCl_2$ salt with 20 mL of 0.1 M for$NiCl_2$ salt) as a source for the production of composites nanoparticles. Then we add to the previous salts 30 mL of urea at a concentration of 1 M very slowly (drop per second) and put the mixture inside the tube pierx and irradiated for a period of time half an hour using a snow bath.

After that we got a green precipitate, we wash the precipitate several times with distilled water then we filtered and dried the precipitate for a day after then we burned the precipitate to a temperature of 400°C. For a period of 2 h. Then we obtained a triple composite nanoparticles.

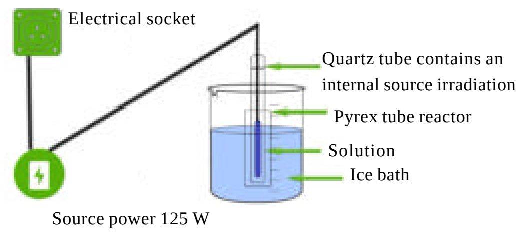

Fig. 1: Scheme of photo irradiation cell

Photo irradiation cell: This cell is used to irradiate the salts of zinc, nickel and chromium which were the source of zinc oxide, nickel oxide and chromium oxide nanocomposit. Immersed UV source is used (mercury medium pressure lamp with power of 125 W) has a maximum light intensity at wavelength at 365 nm. The cell contains a quartz tube as a jacket for immersion UV source in the salts solutions of zinc, nickel and chromium Pyrex tube is used as a reactor as show in Fig. 1. The reactor is cooled by ice bath to avoid the rising in temperature as a result of the UV irradiation.

Preparation Mueller Hinton agar: The central of Mueller Hinton agar was prepared by dissolving a certain weight of the central in a specific volume of distilled water, sterilized and put it in a (petridishes), so, it is ready as a central for bacteria. We made the bacterial suspension by transferring colonies from cultivated and diagnosed bacterial on alimental dish then dissolved it in the test tube containing a solution containing a sterile nutrient and then mixture using a vorte device to obtain a bacterial suspension. Then we compared it with the McFarland solution to calibrate the number of bacterial cells that give an approximate number of cells (1.5×108) sand cell.

Application of ($ZnO/Cr_2O_3/NiO$) nanocomposite on some isolates of pathogenic bacteria: Prepared small tablets of filtration papers and saturated them with a suspension of the nanocomposite each of them individually by placing these tablets in a dish contain a solution stuck from these nanocomposite. After this, put the dish in the incubator for half an hour until saturation with nanoparticles, then planted the bacteria on the Mueller Hinton agar dish by using the swab and put the saturated tablets with nanomaterials on the agar surface using the forceps sterilized and put the dishes in the incubator for 24 h, after which the results were recorded by measuring the diameter of the inhibition zones in millimeters.

Application of ($ZnO/Cr_2O_3/NiO$) nanocomposite on Serum Alkaline Phosphatase (ALP):

Samples collection: Thirty samples were collected from Chronic Kidney Disease (CKD) patients, their age ranged between (16-70 years). About 5 mL of venous blood samples were taken using plastic disposable syringes. Blood samples were left for 30 min at room temperature. After coagulation, the sera were separated by centrifugation at 704 xg for 10 min. Hemolysed samples were discarded and the sera were stored and frozen at -20°C until analysis.

Serum Alkaline Phosphatase (ALP) assay: ALP was measured by Biuret colorimetric method using a kit supplied by Spinreact.

Statistical analysis: Statistical analysis was done using Microsoft Office (SPSS Version 14) which include the following: mean±standard deviation, student t-test and $p<0.05$ was considered significant and <0.001 was highly significant.

RESULTS AND DISCUSSION

In the practical part, the use of photolysis is also provided for the preparation of ($ZnO/Cr_2O_3/NiO$) nanocomposite. The source of differentiation in this method is the quality of the product. The quality here is the trend towards increasing the amount of small nanoparticles in diameter relative to the total amount of nanoparticles prepared. This statistical study was carried out in a special laboratory using the atomic force microscope (Fig. 2).

The statistical analysis of the examination by the atomic force microscope of nanocomposite found that the average diameter is about 55 nm while the diameter of 60 nm is the lowest diameter obtained in this method and the diameter of 42 nm is the highest percentage in the sample. Through, the practical results, it has been shown that what distinguishes the method of photolysis is obtain the diameters of a small range and this consider a preference form the other methods for the preparation of nanoparticles.

X-ray diffraction technique was used to determine the crystalline shape as well as to identify the nanoparticles prepared. The sample was checking by using X-ray diffraction which has the characteristics of a continuous size model (step size of 2 = 0.05°) and at a speed of 5°/min). The processed voltages for the X-ray tube were 40 kV and the tube current (30 mA). X-ray was generated using a copper objective and the wavelength of the X-ray generated (1.54). The results confirmed that we

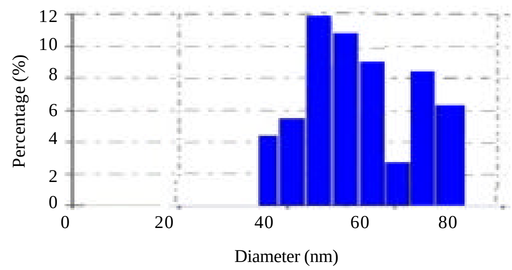

Fig. 2: Distribution of diameters of composites nanoparticles of zinc oxide, nickel oxide and chromosome nanocomposite ($ZnO/Cr_2O_3/NiO$) (Granularity cumulation distribution chart)

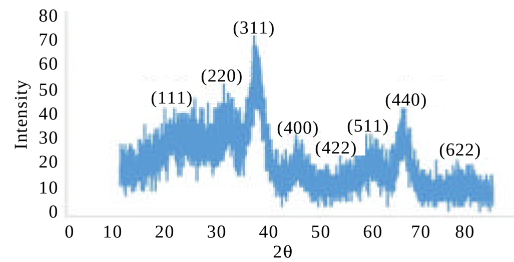

Fig. 3: X-ray diffraction of three composites nanoparticles of zinc oxide, nickel oxide and chromo oxide nanocomposite ($ZnO/Cr_2O_3/NiO$)

had three composites nanoparticles zinc oxide, nickel oxide and chromo oxide are also shown in Fig. 3. The X-ray diffraction of the triple composites peaks showed a deviation at angle, 2• = 18.4, 30.3, 35.7, 43, 53.9, 57.4, 63.1, 75.6, corresponding to Miller, 220, 311, 400, 422, 511, 440, 622, 111. Where through the results of the measurement by X-ray diffraction which was confirmed that these particles are nanoparticles through the application of the Eq. 1 Scharr:

$$D = \frac{0.9\lambda}{\beta\cos\theta} \quad (1)$$

Where:

- • = The wavelength of X-ray
- • = The highest peak from the middle or
- • = The angle of diffraction of the peak

By applying this equation, the diameter of the crystal nanoparticles (25 nm) was found. The average particles size and distribution were determined randomly on the Transmission Electron Microscopy (TEM) image (Fig. 4). The average particles size of ($ZnO/Cr_2O_3/NiO$ nanocomposite) of TEM is (29.6) and SEM is (34.2).

Table 1: Serum ALP in CKD patients with nanocomposition compared to patients CKD without nanocomposition

Groups	Sample number	ALP Mean±SD (U/L)	p-values
Pat.without nanocomposition	30	250.26±8.81	p<0.001
Pat.with nanocomposition	30	120.36±7.91	

p<0.001 highly significant

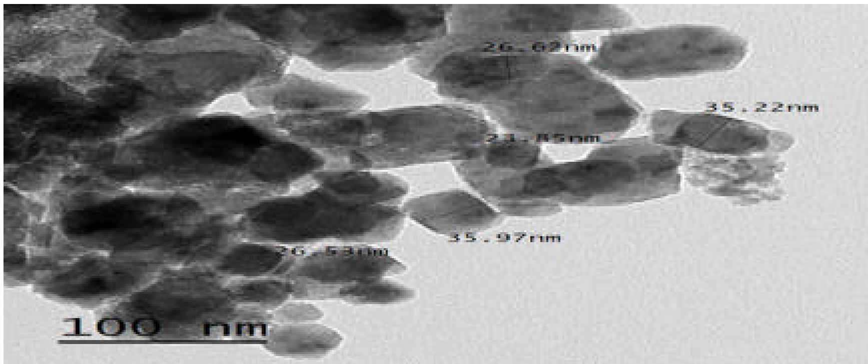

Fig. 4: TEM of $ZnO/Cr_2O_3/NiO$ nanocomposite

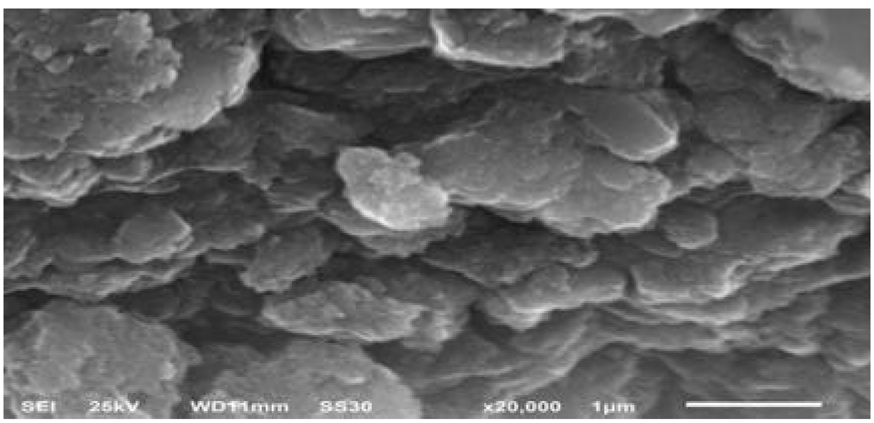

Fig. 5: SEM of $ZnO/Cr_2O_3/NiO$ nanocomposite

Effect of nanocomposite ($ZnO/Cr_2O_3/NiO$) on some isolates of pathogenic bacteria: The effect of zinc oxide, nickel and chromium particles in some isolates of harmful bacteria was studied by studying the effectiveness of these nanoparticles in inhibiting the growth of these isolates. *Staphylococcus epidermidis* (Gram positive), *Escherichia coli* (Gram negative).

We are found from the practical part results the nanoparticles have been a hightly resistant to bacterial isolates by killing the bacterial cell and inhibiting its growth through the dispersion of the external electric voltage of the membrane and this will end cell life.

These nanoparticles have a high efficiency and high susceptibility to kill bacteria. A high inhibition of these nanoparticles was also observed on *Escherichia coli* (Gram negative) where the diameter of the inhibition was 16 mm compared to Gram positive (*Staphylococcus epidermidis*) which had a diameter of inhibition of mm14 because of the thickening of the cell wall of Gram positive.

Effect of nanocomposite ($ZnO/Cr_2O_3/NiO$) on enzyme activity: The results have shown that there were a highly significant decrease in the serum level of ALP in CKD patients with nanocomposition ($ZnO/Cr_2O_3/NiO$) compared to patients CKD without nanocomposition ($ZnO/Cr_2O_3/NiO$) ($p<0.001$). These results are shown in Table 1. Results that obtained from Layasadat Khorsandi *et al.* are increasing of serum activity of ALT, AST and ALP in presence of zinc oxide nanoparticles in rats (Fig. 6 and 7). The obtained in this study are decrease of serum activity of ALP in presence of nanocomposition ($ZnO/ Cr_2O_3/NiO$). The results obtained are almost similar

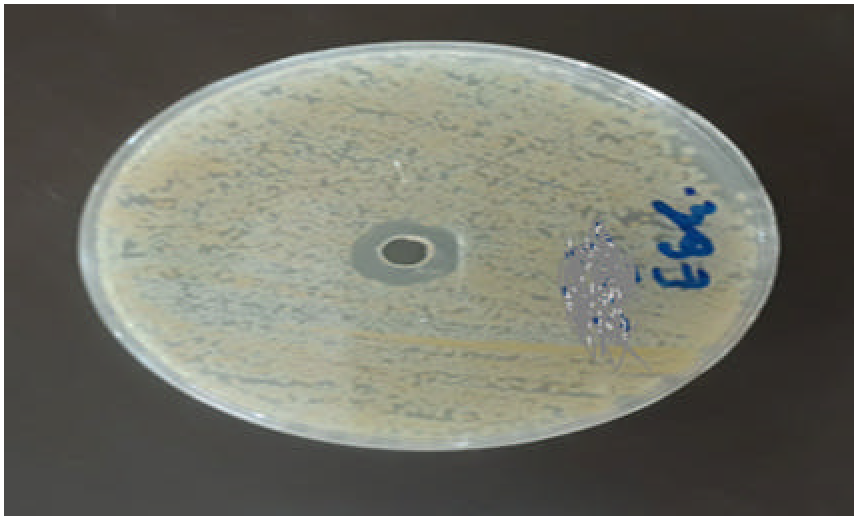

Fig. 6: Effect of $ZnO/Cr_2O_3/NiO$ nanocomposite in *E. coli* isolates

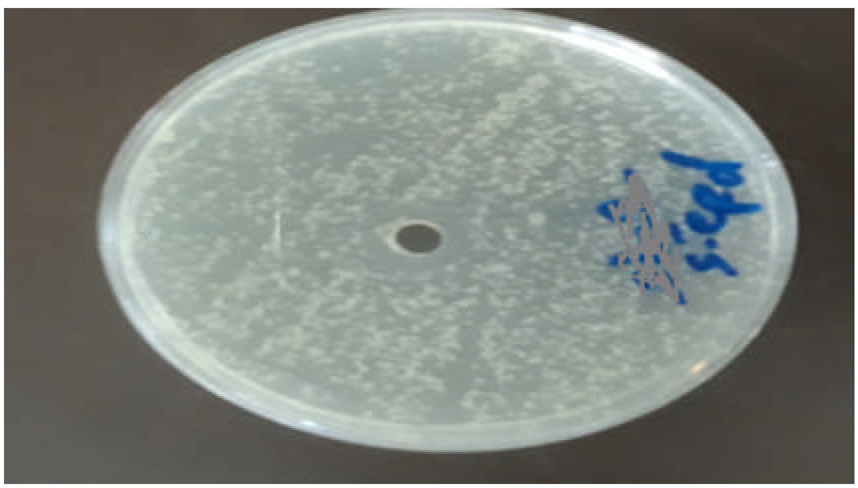

Fig. 7: Effect of $ZnO/Cr_2O_3/NiO$ nanocomposite in the isolates of *Staphylococcus epiderm*

to previous studies which found that The presence of ZnO NPs was lead to decrease the activity of ALP in abnormal case. In another study, TiO_2 NPs inhibited salivary ALP. Heavy metals strongly interact with thiol groups of vital enzymes and inactivate them. In addition, it is believed that the metal NPs like Ag bind to functional groups of proteins, resulting in protein deactivation and denaturation.

The results in this present study shown decreasing of serum activity of ALP in presence of nanocomposition ($ZnO/Cr_2O_3/NiO$). This effect may be attributed to the biological activity of this type of nanocomposition in addition to the conformational changes that can be occurred on the protein structure after interaction with nanocomposition.

CONCLUSION

Chromium (III) oxide with a large specific surface area has attracted big attention in lately. The nanoparticles of Cr_2O_3 can be very widely used in area such as catalyst, coating and corrosion wear resistance, advanced colorant, H_2 absorption material, humidity sensing (Meenambika *et al*., 2014).

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