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Physical properties of some nanometal oxides: Synthesis and laser-anti-bacterial application

Safaa A. Yaseen , We'am Sami

Department of Physics, College of Education, University of Al-Qadisiyah, Qadisiyah, Iraq.

*Corresponding Author E-mail: Safaaameer05@gmail.com

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ABSTRACT

In the present work, metal oxides CdO, NiO and $Cd_xNi_{1-x}O$ nanopowders (where x = 0, 0.5, and 1) were created by a simple chemical co-precipitation technique followed by calcination to be used against Staphylococcus aureus. The synthesized nanoparticles were characterized by XRD, FE-SEM, and PL spectroscopy analysis. Also, Diode laser with (650 nm wavelength and 50 mW power) was used as antibacterial agents. Calcination affects lead to improve crystallinity and decreasing of the impurities. Substitution of Cd^{2+} by Ni^{+2} with 1000 ppm increase the killing of bacterium compared with pure nano oxides. Increasing the irradiation time led to increase the killing of bacterium. The antibacterial activity of the substitute Cd^{+2} by Ni^{+2} with present laser enhanced antimicrobial efficiency in *S. aureus* bacterium compared to that of pure metal oxides.

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الخواص الفيزيائية لبعض أكاسيد المعادن النانوية: تصنيع وتطبيق الليزر المضاد للبكتيريا

صفاء ياسين، وئام سامي

قسم الفيزياء، كلية التربية، جامعة القادسية، القادسية، العراق.

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الـــخُـــلاصـــة

تم في هذا العمل تحضير مساحيق نانوية من أكاسيد المعادن CdO و NiO و $Cd_xNi_{1-x}O$ (حيث 1, 0 = x ،0.5) و باستخدام تقنية ترسيب كيميائي مشترك بسيطة، متبوعة بالتكليس، وذلك لاستخدامها ضد بكتيريا المكورات العنقودية الذهبية. تم توصيف الجسيمات النانوية المُصنّعة باستخدام تقنيات XRD و FE-SEM و PL. كما استُخدم ليزر الدايود (بطول موجي 650 نانومتر وقدرة 50 ميلي واط) كمضاد للبكتيريا. يؤثر التكليس على الرصاص لتحسين التبلور وتقليل الشوائب. يؤدي استبدال Cd^{2+} بـ Ni+2 بتركيز 1000 جزء في المليون إلى زيادة في قتل البكتيريا مقارنةً بأكاسيد النانو النقية. كما أن زيادة زمن التشعيع تؤدي إلى زيادة في قتل البكتيريا. تظهر

نتائج الخاصية المضادة للبكتيريا أن النشاط المضاد للبكتيريا لبديل Cd^{+2} بواسطة Ni^{+2} مع الليزر
الحالي يعزز الكفاءة المضادة للميكروبات في بكتيريا S. aureus مقارنة بأكاسيد المعادن النقية.

1. INTRODUCTION

By adjusting their size to change their effect, nanotechnology provides a way to re-examine the biological characteristics of known antibacterial chemicals. Bacteria may lead it to be resistant to a variety of organic antibiotics. The production of enzymes by certain bacterial species that harm antibiotics is one of the main causes. Since a new membrane keeps the antibiotics from entering, a change in cell composition or protein secretion may occur. As a result, scientists are considering using nanoparticles as anti-bacterial agents in several medical domains to find an alternative to antibiotics [1].

The nanoparticles (NPs) were considered for antibacterial therapeutics, if several key requirements have to be fulfilled such as dealing with the engineered materials nanoparticles of well characterized composition, size, morphology and crystallinity , as well as the most crucial requirement is their biocompatibility [2]. Gram staining, which primarily relies on the thickness of the bacterial cell membrane, which is made of peptidoglycan (PG), is the most widely used method for classifying bacteria. Bacteria will be classified as gram-positive if their cell wall thickness falls between 20 and 50 nm, and gram-negative if it less of this range. The bacteria's cell wall is attacked by metal nanoparticles to cause structural damage [3].

Among the most promising of novel antibiotic agents are metal NPs, which have shown strong antibacterial activity in an overwhelming number of studies, high attention to the nano-metals, because of their potential bactericidal effects. Inorganic NPs, including metals and metal oxides, possess great antibacterial properties and might be alternatives for treating of infection by antibiotics [4].

Cadmium oxide is classified as an inorganic compound with the chemical formula Cd-O. Cadmium oxide nanoparticles (CdO NPs) have noteworthy antibacterial efficacy for a wide range of pathogenic microorganisms that have developed resistance to conventional antibiotics. CdO shows a narrow direct band gap between the Cd 5s-based conduction band and O 2p-based valence band which makes it to be photoactive [5].

Nickel oxide is a p-type semiconducting material has a broad band gap between 3.4 and 4 eV. Additionally, it is an antiferromagnetic substance. Its exceptional magnetic characteristics make it a unique material [3]. NiO nanoparticles have cytotoxic effects because of their unique properties like surface area, metal ion releasing and adsorbing ability [6,7].

Low level lasers intensity (LLLI) range between (1 to 1000 mW) has been used for the treatment of many medical conditions including wound healing and soft tissue injuries [8]. The biological effects of low-intensity lasers are related to the exposure factors used. High mono- chromaticity, directionality, densities of energy, and laser emission mode properties are characteristics that enable semiconductor laser devices to treat various diseases [9]. Diode Laser is highly compact and reliable. The mechanism that responsible for causing death of bacteria has been revealed at long irradiation time [10].

Depend on these properties , in this study CdO, NiO and substitution involving $Cd_xNi_{1-x}O$ where (x=0, 0.5, 1) has been synthesized by Co-precipitation method. Samples were analyzed by techniques such as XRD, FE-SEM, and PL., antibacterial activities with Diode laser were performed against Staphylococcus aureus.

2. Experimental work:

2.1. Synthesis of Metal Oxide NPs

0.94 M of aqueous Cadimum chloride $CdCl_2.H_2O$ [Thomas backer India (98.0 %)] was created by dissolving 11g in 100ml of deionized water (DW) according to molarity equation (1) [11].

$$M = \frac{m/Mw}{V/1000} \qquad (1)$$

Then, placing the solution on a magnetic stirrer hot plate at a constant temperature of 60°C for 30 min. until the substance was completely dissolved.

37 ml of Sodium hydroxide NaOH [Lobachemie India (99.9%)] with a concentration of 3.57 M was added drop by drop through burette to bring the pH to 10 with constant stirring with the help of magnetic stirrer at a constant temperature of 60°C that was confirmed by a change of solution color to white, then the mixed materials were washed with deionized water.

The precipitate was placed in watch glass then dried by oven at 100 °C for 6 hours. Next, it was grind to change the precipitate into powder. After that, the precipitate was placed in furn and burned at 450 °C for 3 hours to obtain the desired nanoparticles.

The same steps followed in preparing CdO were adopted in preparing NiO except that (0.94 M) of aqueous Nickel chloride NiCl2.6H2O [Lobachemie India (99.9%)] was created by dissolving 22.2g of it in 100 ml of DW, and 20 ml of sodium hydroxide NaOH with a concentration of 3.57 M was added drop by drop to bring the pH to 9 which was confirmed by a change of solution color to green [12].

Cadimum and Nickel Chloride were used as a raw metal sources. To prepare $Cd_xNi_{1-x}O$ where [x= 0, 0.5 and 1] nanoparticles, the following steps were taken: i). Each substance was weighted separately in stoichiometric ratio and dissolved in a suitable quantity of DW. At 60°C, the solutions were mixed and stirred for 30 min.

ii) Stoichiometrically amount of sodium hydroxide NaOH was added into the mixture drop wise to bring the pH to 9 under stirring. iii) The precipitate was filtered and washed using deionized water. iv) The precipitate was transferred to oven and dried for 6 hours at 100°C. v) To achieve fine powders, Mortar was used to grind the obtained powder for 2 min. Finely the precipitate was placed in furn and burned at 450 °C for 3 hours to obtain the desired nanoparticles.

All synthesized samples then processed by calcination at 700˚C for 3hr.

2.2. Preparation of Bacterial Samples

The taken bacterium sample was Staphylococcus aureus (S. aureus) that collected, isolated and identified by the Al-Ameen Researches Lab. in Al Najaf city, Iraq. Then, they were subcultured in nutrient broth agar tubes using a sterile loop in a hood with a benzene burner to create a sterile environment, then incubated at 37°C for 24 hrs for growth.

2.3. Anti-bacterial Application

The prepared nano-oxides was converted to colloidal with concentrations 500 and 1000 ppm depending on dilution equation [13] and used as antibacterial in the following steps:

I) Sub culture was made in the small tubes of nutrient broth that prepared previously. II) 0.2 ml from the colloidal NPs was placed in each tube according to the prepared concentration above. III) The tubes was placed in the incubator at 37°C for 24 hrs.

The following process made use of a diode laser with 650 nm wavelength and 50 mW power: I)With a distance of 10 cm between the laser source and the tube, the laser device was positioned vertically to regulate the direction of the beam that would be administered directly to the samples. II) Other bacterial-containing tubes were exposed at varying laser exposure times (5, 10, 15, 20) minutes. III) The tubes were placed in an incubator was set to 37ċ for 24 hours.

For applying Diode laser on bacteria with nano-oxides, the following steps were adopted:

The laser device was placed in the same position that was mentioned previously. II) Other tubes that contain S. aureus bacteria were taken with nano-oxides (with concentrations 500 and 1000 ppm), and irradiate them separately with exposure times (5 and 10) min. III) The tubes were then placed in an incubator that was set to 37ċ for 24 hours.

To obtain homogeneous mixture, each tube in this study was placed in Vortex device for shaking the mixture. Then, 0.2 ml from each tube by micro pipette was taken and place them in TCP to measure the amount of killing by ELISA test.

The absorption of wave length (630 nm) of the device light was used depending on the sample.

The amount of absorption determine the density of the sample that indicate the amount of turbidity of the liquid containing the bacteria.

3. Results and Discussion

3.1. Phase Formation and Structural Analysis

The patterns of XRD for nano CdO, NiO, and $Cd_xNi_{1-x}O$ samples revealed sharp diffraction peaks, confirming the formation of phase cubic structures as shown in Fig.1 before calcination.

Different impurities can result during incomplete combustion, there was impurities that can be appeared during preparation and can be shown in some peaks appeared in XRD (show small peaks at low angles) out of standard peaks for each nano-oxides mainly due to hydroxide group (-OH), and secondly may related for carbonate group (CO_3) and NaCl product during chemical reaction [14,15].

Calcination at 700 °C for 3 hrs (Figure-2) was significantly reduced any residual hydroxide phases and improved crystallinity in the synthesized samples' structure, as evidenced by the enhanced peak intensities, calcination led to decrease of the impurities and give good purity in the nano oxides, that approved with other researchers [16].

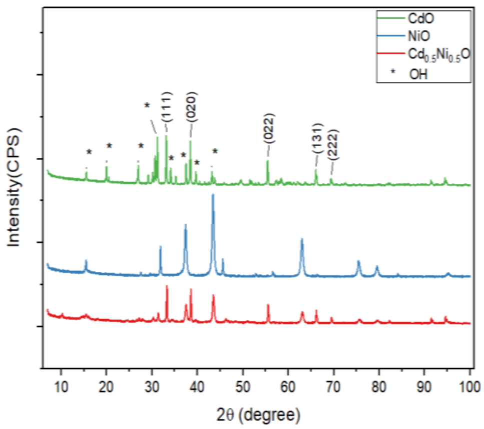


Fig. 1: The X-ray diffraction for CdO, NiO, and $Cd_xNi_{1-x}O$ nanoparticles before calcination.

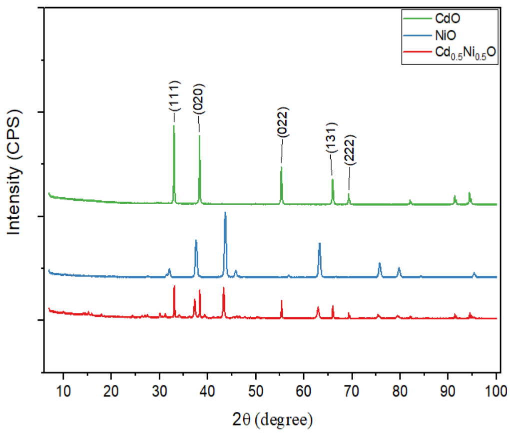


Fig. 2: The X-ray diffraction of CdO, NiO, and $Cd_xNi_{1-x}O$ nanoparticles after calcination

All the distinctive peaks of CdO were observed at 33.05°, 38.35°, 55.36°, 66.01° and 69.35° which were indexed to (111), (020), (022), (131) and (222). The distinctive peaks of NiO were observed at 37.28°, 43.34°, 63.00°, 75.51°, 79.48° which were indexed to can be easily indexed to (101), (10-2), (104) and (113), (202). The distinctive peaks for substitution of Cd^{2+} by Ni^{+2} were observed at 33.11°, 38.42°, 55.46°, 66.14°, 69.49° and 82.30° which were indexed to can be easily indexed to (111), (020), (022), (131), (222) and (040) crystal planes. Crystal planes for three synthesized samples

CdO, NiO and $Cd_xNi_{1-x}O$ nanoparticles before and after calcination; were comparable with the standard values and suggested by literatures [17, 18 and 19] respectively.

Structure parameters such as crystallite size was determined by using Scherrer"s formula and lattice constant [21,22] of the as-synthesized powdered metal oxide nanoparticles were computed from the highest peak intensity for each XRD plane, and summarized in Table (1).

Table 1: Structure parameters of CdO, NiO, and $Cd_xNi_{1-x}O$ nanoparticles before and after calcination.

Sample		2θ (deg)	FWHM (deg)	Lattice Constant a (Å)	Crystal Size DXRD (nm)
CdO	as-synthesized	32.9101	0.2716	4.7101	31.88
	Calcination	32.976	0.1481	4.7009	58.47
NiO	as-synthesized	43.3777	0.2501	4.6607	35.73
	Calcination	43.5959	0.3773	4.1488	23.7
$Cd_{0.5}Ni_{0.5}O$	as-synthesized	38.5237	0.179	4.6700	49.14
	Calcination	33.0209	0.1137	4.6947	76.17

The crystallite sizes (D) were ranged from 23.7 nm (for NiO after calcination) to 76.17 nm (for $Cd_{0.5}Ni_{0.5}O$ after calcination) depending on the specific metal oxide.

It's clear from the results in Table (1) that there was an increase in crystallize size of CdO and $Cd_{0.5}Ni_{0.5}O$ after calcination.

This result agreed with the result of other studies, that revealed the crystallite size increases gradually for CdO sample calcined at 700 °C [16].

While the crystallize size and lattice constant of NiO show decrease after calcination, that may related to the type of metal oxide and calcination time. A study conducted by (Shanaj BR and John XR) observed that increasing the calcination time from 4 to 5 hrs for NiO has to increase in crystallite, and they were clearly suggested that the increase of calcinations time results in decrease of lattice parameter [22].

The lattice constant for $Cd_{0.5}Ni_{0.5}O$ was increased and this may be related to the composition of the two metals that affect the crystal dimensions.

There was inverse relationship between crystallize size and lattice constant for CdO, and that may related to the ionic radius of the material. In contrast, the lattice constant of NiO and $Cd_{0.5}Ni_{0.5}O$ is directly proportional to the crystalline size.

3.2. Morphological analysis

The morphology of the surface for all synthesized powdered metal oxide samples are demonstrated in Fig. 3 for synthesized metal oxide samples that followed by calcination at 700°C for 3hrs.

The figure show an increase in average particle size for all synthesized nano oxides after calcination and that agreed with (Essam F. et al.) revealed that the particle size of NiO-CdO nanocomposite (with calcination 800 °C for 3 hr.) increases with the increase of CdO content in the synthesized samples. Also, the study shows systematic changes in CdO particle size with calcination processing [23]. A study of (Sonam Dwivedi et al.) revealed that systematic particle size increase from 350°C to 650°C calcination temperatures [24].

The FE-SEM images show the formation of all synthesized samples possessed near-spherical or slightly agglomerated morphologies. The agglomeration ensues in nanoparticles owing to its magnetic nature and the binding of primary particles held together via fragile surface interaction such as Vander Waals force [25].

3.3. Optical Properties

Photo Luminonus (PL) spectra for three synthesized samples showed the relation between PL intensity related to the wave length before and after calcination (Figure.4 and Figure.5).

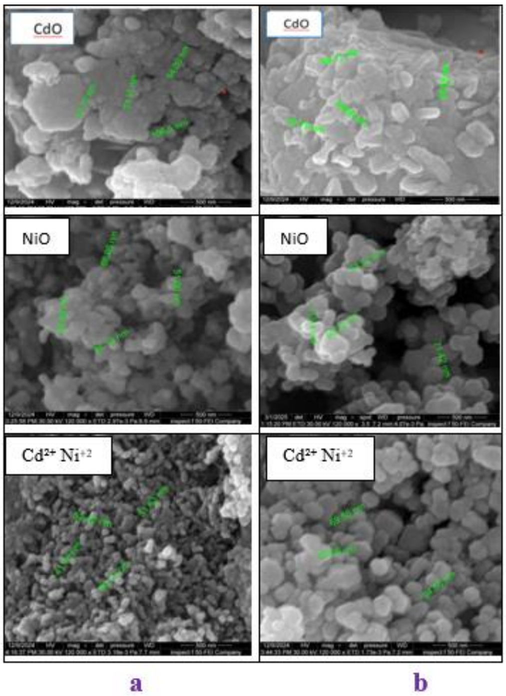


Fig. 3: FE-SEM images of all synthesized metal oxide samples (a) as-synthesized (b) followed by calcination at 700°C for 3hr.

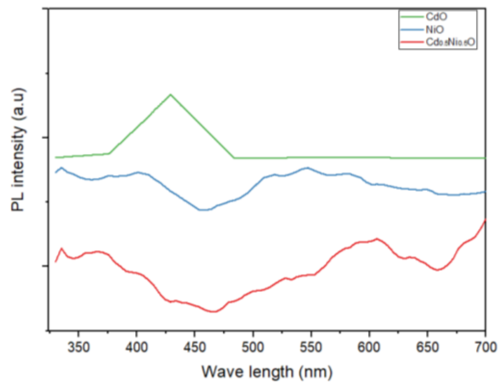


Fig.4. PL of CdO, NiO, and $Cd_xNi_{1-x}O$ nanoparticles before calcination.

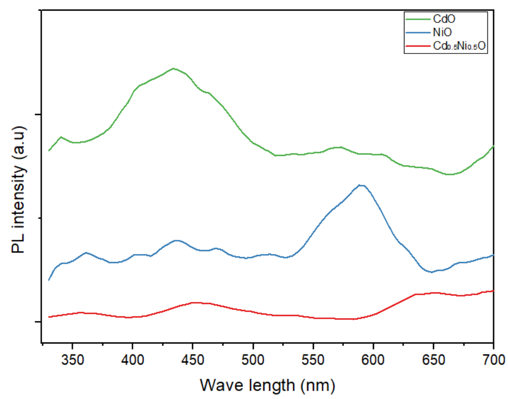


Fig.5. PL of CdO, NiO, and $Cd_xNi_{1-x}O$ nanoparticles after calcination.

From the two figures, the energy gap (E_g) was concluded depend on equation (2)[28]. The results were summarized in Table 2.

$$Eg = \frac{1240}{\lambda\ (nm)} \qquad (2)$$

Table 2: Calculation of energy gap from PL spectrum before and after calcination.

No.	Sample	Synthesis T/t (Ċ/hr)	Calcination T/t (Ċ/hr)	Eg (ev) before	Eg (ev) after
1	CdO	450/3	700/3	2.88	2.81
2	NiO	450/3	700/3	2.25	2.10
3	$Cd_{0.5}Ni_{0.5}O$	450/3	700/3	2.03	1.90

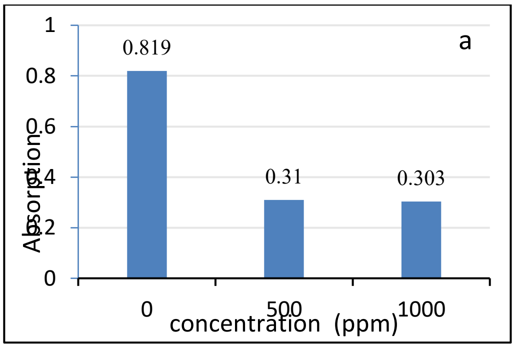
It was clear from the table that there was decreasing in the calculated energy gap after calcination, that agreed with (V. Pradeep Kumar et al.) was found that the optical band gap of the nickel oxide nanoparticles decreased due to size variation [27].

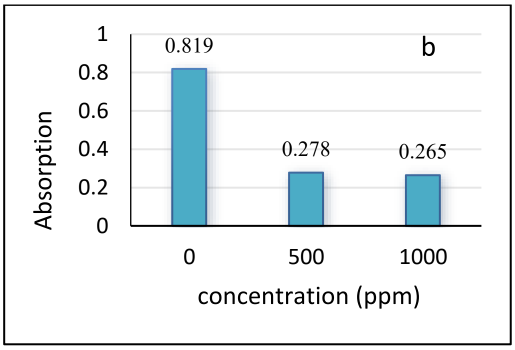
Essam F. et al. reported that For NiO-CdO substitution, there was a reduction in the energy band gap at the optimum content of CdO [23].

The behavior of CdO and NiO related to wave length shift in PL and reduction in band gap was appeared in the mix of them ($Cd_{0.5}Ni_{0.5}O$), where calcination improves crystallinity and perhaps reduces surface or internal defects, the PL emission tends to shift toward long wavelength, as shown in the Figure. 5.

3.4. Effect of Nano-oxides on *S. aureus* bacterium

Although the impact of the synthesized pure metal oxide nanoparticles (CdO and NiO) on the S. aureus bacterium exhibited varying effects, they ultimately produced a similar outcome. There was a notable inhibition of the bacterium at concentrations of 500 and 1000 ppm, yet no significant difference was observed between these two concentrations (Figure 6a and Figure 6b). In contrast, when substituting Cd^{2+} with Ni^{2+} (Figure 6c), the results indicated a significant inhibitory effect on the bacterium at both concentrations, with a pronounced difference between them; the higher concentration demonstrated a predominant effect.





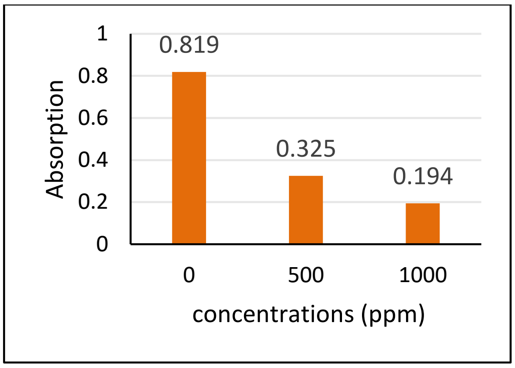


Fig. 6. Effect of synthesized metal oxides CdO (a) ,NiO (b) and $Cd_{0.5}Ni_{0.5}O$ (c) after calcination on S. aureus bacterium.

3.5. Diode laser light's impact on the S. aureus bacterium

Figure (7) illustrates the impact of a 50 mW diode laser at varying irradiation periods on S. aureus, that showed increasing the inhibition of bacterium with increasing irradiations times, and

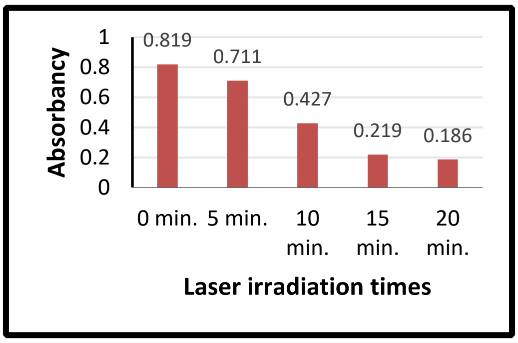


Fig. 7. Impact of Diode laser with varying irradiation times on S. aureus bacterium.

The current study found that diode laser light inhibited the growth of S. aureus, which is consistent with finding by another study, that found diode laser irradiation cause reduction *S. aureus* growth [29].

The current study found that bacterial inhibition rises with longer laser radiation exposure times, the significant difference affected the bacterium

A

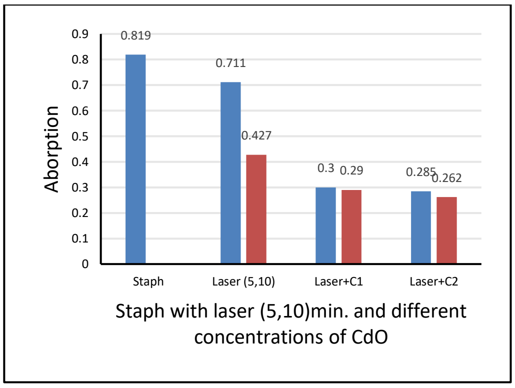
appear at 10 min., there was no significant difference between 10 min and other irradiations times (15 & 20 min).

which is consistent with findings of another research [10].

3.6. Impact of The Synthesized Metal Oxides With diode Laser

The inhibition of bacteria was demonstrated by the application of nano-oxide concentrations (500 and 1000 ppm) in conjunction with diode laser irradiation durations (5 and 10 minutes); however, no statistically significant difference was observed between the two concentrations utilized for CdO and NiO, as illustrated in Figure 8 (a and b). Conversely, a notable distinction was evident between the two concentrations employed for the substitution of Cd^{2+} by Ni^{2+}, as depicted in Figure 8c.

Bacterial species, the characteristics of lasers, the duration of laser exposure, and the type and concentration of nanoparticles collectively influence the synergistic antibiotic efficacy of lasers and nanoparticles [29,30].



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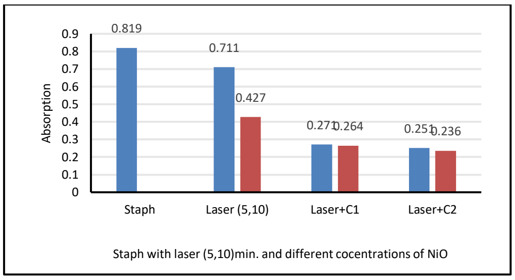

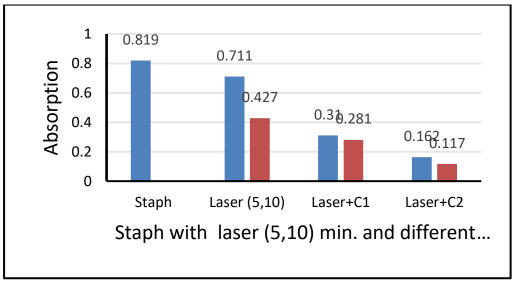

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Fig. 8. Effect of the synthesized metal oxides CdO (a) ,NiO (b) and $Cd_{0.5}Ni_{0.5}O$ (c) with diode laser on S. aureus bacterium. Where C1=500 ppm and C2=1000 ppm.

4. Conclusions

Calcination affects lead to improve crystallinity and decreasing of the impurities and give good purity in the nano oxides. This leads to enhanced transmission and finally increase agglomeration.

Substitution of Cd^{2+} by Ni^{+2} give more inhibition on *S. aureus* bacterium than synthesized of pure metal oxides nanoparticles (CdO and NiO) when used with 1000 ppm concentration.

Irradiation of *S. aureus* with Diode laser increase the killing with increasing the irradiation time. The dual effect of laser with nano-oxides can enhance the inhibition of *S. aureus* especially when using substitution of Cd^{2+} by Ni^{+2} with 1000 ppm concentration.

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