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The study of antibacterial properties of NiO thin film using Sol-gel synthesis

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ABSTRACT: Nickel oxide thin film of various preferred orientations was deposited using sol-gel method using solvents including methanol. The resulting film was analyzed using X-ray diffraction (XRD), scanning electron microscopy (SEM), contact angle measurement, and ultraviolet spectroscopy (UV-vis). Antibacterial activities of nickel oxide-based film against gram-negative bacteria (Escherichia coli) has been evaluated using drop test method under UV light irradiation (photocatalyst) and dark conditions (catalytic). The antimicrobial activity of the film was determined through counting the number of colonies on each plate which was reported as colony-forming unit (CFU) per milliliter. Results showed that NiO has high photocatalyst and catalytic activation efficiency against *Escherichia coli.*

Keywords: antibacterial properties, scanning electron microscopy (SEM), Sol-gel synthesis, UV light irradiation, colony-forming unit (CFU)

INTRODUCTION

Bacteria can successfully proliferate everywhere. However, bacteria are exposed to many environmental variations such as temperature, osmolarity, radiation, toxins, and limitations in nutritional sources. Therefore, to be able to survive in different environments, they must develop different approaches. Increasing bacterial resistance to antibiotic treatment is a growing concern for physicians. One mechanism by which this bacterial resistance can occur is biofilm formation. Biofilms are bacterial communities coated with a self-produced hydrated polymer matrix. An important feature of microbial biofilm is the intrinsic resistance to elimination by the immune system and antibiotics. With the aim of overcoming the intrinsic resistance of biofilms, recent researches were conducted to design suitable coatings with antibacterial activities using nano-based techniques of changing the properties of surfaces. Examples in this regard include the direct antibacterial properties of colloidal nano particles with respect to a wide range of micro-organisms (Krasner *et al.*, 2006).

In recent years, the improvement of antibacterial agents with no negative effects or little impact on the natural environment is of particular importance in nanotechnology. Industrial effluents are exposed to contamination with microorganisms and organic compounds. Therefore, water purification requires a major technology in the biochemical and biological industries.

Traditional methods include the use of chlorine oxide, ozone, UV radiation, and advanced filtration processes which are effective in the removal of most bacteria. Nevertheless, especially in wide areas, these methods entail high costs. On the other hand, the formation of harmful disinfection by-products (DBPs), many of which are carcinogenic, during the purification process is the main problem of the traditional methods. More than 600 harmful by-products have been reported (Qi *et al.*, 2004). Nickel oxide, as an absorbent material with high chemical stability and appropriate chemical, electrolytic, and optical properties, has attracted much attention in electrochromic devices, smart windows, (Wu and Yang, 2007, Arshak *et al.*, 2007), gas sensors (Park *et al.*, 2008), as a catalyst (He *et al.*, 1999), in electrochemical capacitors, and fuel cells (Li *et al.*, 2008, Xi *et al.*, 2008, Xi *et al.*, 2008). While many applications and properties of nickel oxide-based materials have been studied and proven, the antimicrobial activity observed in other metal oxides has not been clearly studied in nickel oxide to this date (Vidotti *et al.*, 2006, Tam *et al.*, 2008). However, the antimicrobial activity of in organics has considerable importance due to the need to control infections and excessive antibiotic resistance.

Therefore, this study was conducted with the aim to investigate the antibacterial activity of nanostructured nickel oxide with emphasis on the importance of using new methods based on cost-effective, environmentally friendly, inorganic materials with simple synthesis.

MATERIALS AND METHODS

A. Preparation of sol - gel

In order to prepare the sol solution, 13 grams of nickelnitrate6AbeNi $(NO_3)_{2\cdot 6}H_2O$ was dissolved in 80mL of methanol. Then, the solution was placed at 67°C for 4.5 hours under refluxing oil bath (for indirect heating). After completing the desired time, the solution was cooled and filtered. The filtered solution was then washed with ethyl acetate. Subsequently, 4,2-pentanedione and nonionic surfactants was added to the solution. The solution was stored for 24 hours and then transformed into a gel at ambient temperature to be digested.

Thin film preparation

In the deposition stage, glass substrates were placed in the above gel solution for 5 minutes. They were removed using immersion machine with 130 min/1 mm speed. After each deposition, the coating was exposed to dry air for 10 to 20 minutes, and then, it was placed in the oven at 150°C for 20 minutes. Finally, after the last cycle of deposition, film calcination was performed at 550-600°C in the oven.

B. Identification of nickel oxide thin films

To determine the crystal phases, the average size grading, surface morphology, passing properties, and the band gap energy of the prepared samples and identify the samples, X-ray diffraction spectroscopy (XRD), scanning electron microscope (SEM), and ultraviolet spectroscopy (UV-vis) were performed.

C. Preparing the bacterial culture

Escherichia coli (*E. coli*) (PTCC 1399) was purchased from the Institute of Biotechnology and was revived using the liquid medium nutrient broth (NB). First, a culture of the bacteria was prepared on nutrient agar medium (NA), and then, with asterile loop, 4-5 colon bacteria was removed and was added to 5 ml distilled water to prepare the microbial substance. It must be noted that the amount of cultured bacteria added to distilled water was to the extent that its turbidity would be equivalent to 5.0Mc Farl and (1.5 × 108) standard.

D. Antibacterial test

Using samplers, 100 µl of the prepared microbial substance was placed as drops onto two films of nano structured nickel oxide catalyst. Then, one of them was placed in the incubator at 37°C (absence of radiation, catalytic route) and the other one was placed under UV radiation (photo catalytic route). After different times the catalysts were removed from the incubator or under UV exposure, and they were drip washed with distilled water. Next, 200 µl of the washing water (water that the catalysts were washed with) was inoculated in sterile plates containing pre-prepared nutrient agar (NA) and it was spread using a crooked bar (loop) which was sterilized with alcohol and heat. Subsequently, the samples were placed in the incubator for 0, 2, 4, 6, and 8 hours at 37°C temperature. Finally, the remaining number of colonies was counted by the researcher.

RESULTS AND DISCUSSION

Identification of the nickel oxide sample with XRD pattern is presented in figure 1. The XRD peak in 2θ = 37.3° is in agreement with NiO cubic phase (rhombohedral, structure model NaCI, JCPDS no. 44-1159) (Burnstein, 1954, Moss, 1954). The relative severity of the peak (111) represents the high crystallinity of nickel oxide nanostructure. Granulation size is approximately 90 nm.

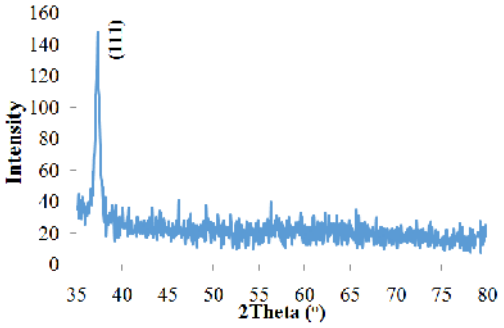

Fig. 1. X-ray diffraction (XRD) spectrum of nickel oxide sample.

SEM micrograph (Fig. 2) indicated that the granulation sample size was even at the nano scale. Nickel oxide film transmission spectrum is presented in figure 3. Sample band gap energy was estimated as 4.17 eV from Eg of the wavelength absorption band edge in the visible ultraviolet region.

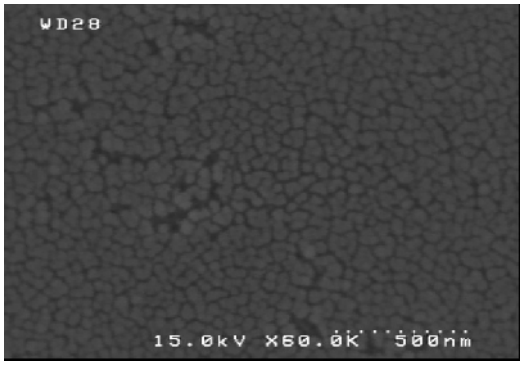

Fig. 2. Scanning electron microscope (SEM) image of nickel oxide.

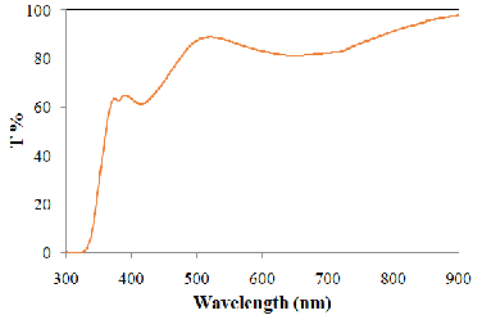

Fig. 3. Nickel oxide film passing spectrum.

There was a suitable match between the energy band gap of prepared thin films and the results reported in the NiO nanoparticles (4.3 eV) (Tasker, 1979).

A. Evaluation of the antibacterial activity of nickel oxide catalysts

Antibacterial evaluation of nickel oxide film samples was conducted using "drip method", under ultraviolet light and darkness and at various times. For a more accurate comparison between the inactivation of catalytic efficiency and photocatalytic nickel oxide film sample, the two parameters of survival ratio and discriminative factor were calculated and are reported in Table 1.

Table 1. Comparison of the efficiency of nickel oxide film samples' catalytic and photocatalytic in activation of *E. coli*.

	Conditions	
The remaining number of colonies(CFU/ml)	Radiation	0.40×10^2
	Darkness	0.68×10^2
The parameters for evaluation of antibacterial activity		
S%	Radiation	0.026
	Darkness	0.065
DF		2.45

The survival ratio was calculated using $S = N/N_0 \times 100\%$, in which N_0 and N indicated the number of colony-forming units (CFUs) at the start (1.5×105 CFU/ml) and after 4 hours of UV radiation in photocatalytic conditions, respectively. Discriminative factor was calculated using DF = Nc/N formula, in which N and Nc indicated the number of CFU sremaining after 4 hours of exposure to the catalyst under ultraviolet radiation in photocatalytic conditions, and then, exposure to catalyst under darkness in catalytic conditions for the same duration. After 4hours of absence of light and radiation, the two parameters of survival ratio and discriminative factor were reported for *E. coli*. The survival ratio provides information on the overall antibacterial efficiency of the photocatalytic /catalytic system under review. Therefore, the smaller the S% is, the higher the antibacterial efficiency of the photocatalytic /catalytic system. The efficiency of nickel oxide catalyst film in *E. coli* bacteria in activation, using ultraviolet radiation and darkness for different durations is reported in figure 4. Fig. 5 presents the photographs of the remaining colonies of *E. coli* after exposure to nickel oxide catalyst for 1, 3, and 4 hours. Heterogeneous semiconductor photo catalysts produce reactive oxygen species (ROS) using light that can inactivate bacteria and reduce a wide range of chemical pollutants of water (Zhang *et al.*, 2007). These species include OH and O_2 radicals that have a high reactivity for bacterial inactivation.

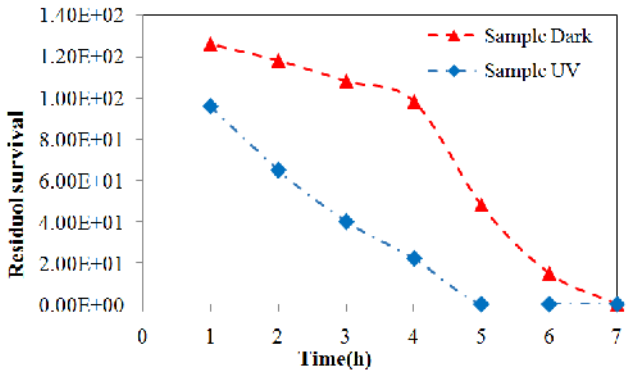

Fig. 4. The number of *E. coli* bacteria remaining in catalytic and photocatalytic reaction.

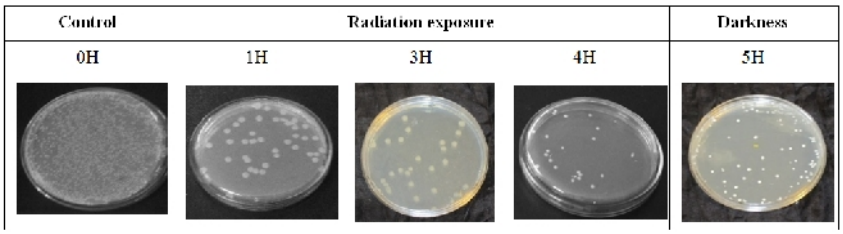

Fig. 5. Photographs of the remaining colonies of *E. coli* under different conditions

On the other hand, recombination of electron-hole pairs produced in semiconductor may occur as a result of excitation by ultraviolet light. This results in decreased photocatalytic activity. Thus, the antibacterial properties of the catalyst are determined through the competition among recombination of load carriers and transference of the species resulting from their reaction to the bacteria (Holt and Bard 2005).

Inactivation efficiency of ultraviolet light irradiation was higher than that of darkness. In activation through radiation is a combination of the antibacterial effects of metal ions and photo-induced antibacterial effects of ROS. However, under dark conditions, only the metal ions are effective in bacterial in activation. Ions released from the metaloxide nanoparticles penetrated into the bacterial cell membrane and combine within tracellular protease, and thus, inactivate bacteria.

The following possible mechanisms are suggested for the antibacterial activity of nickeloxide:

- Nickel nanoparticles penetrate into the bacteria and interact with compounds containing sulfur and phosphorus such as DNA and cause damage to the bacterial cell (Harish *et al.*, 2010).

-After nickel nanoparticles penetrated into the bacteria, they react with enzymes, produce hydrogen peroxide, and induce bacterial death. Ni^{2+} interacts with respiratory chain enzymes of the bacteria and impairs breathing (Harish *et al.*, 2011).

It seems that the mentioned in activation mechanisms based on the penetration of nanoparticles are effective on bacterial growth prevention in the absence of UV radiation. The higher efficiency of the photocatalytic method, in comparison to the catalytic method, is the result of the combination of photo-induced antibacterial effects of ROS and the antibacterial effect of ions released from metaloxide nanoparticles in this method. The nickel oxide catalyst has high activity in *E. coli* bacteria inactivation, and hence, can be presented as an efficient and cost-effective antibacterial coating in medical designs.

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