Approaches for development of new Nano-silver printing ink.

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ABSTRACT

Decaying books and papers were collected from Erbil city; later decayed portions were excised and inoculated on nutrient agar plates, potato dextrose agar, dubo's cellulose agar for microorganism isolation. Both Bacillus subtilis and Acrodictyssp. were mainly isolated organisms and given positive test on Carboxyl Methyl Cellulose (CMC) media due to producing cellulase enzyme. In this study Nano-silver particles was prepared locally and used in preparation of mixture with writing ink in ratio of (1:1, 1:2 and 2:1) respectively. The best growth inhibition gate when used equal volume (volumes: volume) of both writing ink and Nano-silver particles. Result presented that the mixture inhabit microbial growth, as a results which Nano-silver particles affect the cellular compose and biological activity. This study concluded ability to prove new ink which can control microbial contamination texture papers.

Keywords: Nano-silver, printing ink, Carboxyl Methyl Cellulose.
INTRODUCTION

Nano-silver particles (NSPs) generally present at 1 to 100 nm in size in at least one dimension, and different synthetic NSP routes lead to variable sizes, shapes, morphology, and even stability [1]. As particle size decreases, the surface area-to-volume ratio of NSPs increases dramatically, this leads to significant changes in their physical, chemical, and biological properties [2].

Nano-silver particles (NSPs), are among the most attractive nano-materials, and have been widely used in a range of biomedical applications, including diagnosis, treatment, drug delivery, medical device coating, and for personal health care [3].

The microbial activity due to the presence of various secondary metabolites. Hence, plants used to discover bioactive natural products that may serve as development of new pharmaceuticals [4].

Recently, NSPs have become of intense interest in biomedical applications because of their broad antibacterial effect on a range of Gram-negative and Gram-positive bacteria, antifungal, antiviral, and anti-inflammatory activity [5]. The antimicrobial effects of silver (Ag) ion or salts are well known, but the effects of Ag nanoparticles on microorganisms and antimicrobial mechanism have not been revealed clearly, however the antimicrobial efficacy of NSPs depends on their size and concentration [6].

Generally, the paper ink is a complex medium, which is act as favorable medium for microorganism contamination which cause of paper degradation by their extracellular cellulase enzyme [7]. Depending on that fact most modern book papers have a relatively short life span, which can be further reduced by improper storage environments.

In this paper, we first try to synthesis of NSPs, then the unique physiochemical properties of NSPs, such as antibacterial, antifungal activity. Further, recent applications of NSPs in printing ink as a preventive.

MATERIAL AND METHOD

1- Preparation of Nano-silver[8,9]

The procedure is to place a plate of Silver of a very high purity (>99.99%) immersed in 10 ml of double distilled deionized water (DDDW) inside a glass vessel and then irradiated. Please note, the Silver plate as well as ablation cell were cleaned using ultra sonic path for that purpose.

The laser used for ablation is Nd:YAG system with 20 ns pulse width with repetition rate of 5 Hz. The fundamental (1064 nm) and second harmonic (532 nm) output of Nd :YAG laser were used to irradiate Silver plate. The required exposure time of laser was 10 minutes and this Silver Nanoparticles are produced. Successful preparation procedure can be immediately noticed via the color change of the solution. Normally, the color changes from yellowish to greenish solution which can be directly attributed to the Ag-NPs size.

The Ag-NPs were thus characterized using UV-VIS optical spectral spectrometer. Therefore, 5 cc of Ag-NPs solution was taken onto a quartz substrate as a sample holder stage.

2-Preparation of Nano-silver ink:

Stock solution of mixed dyes: printer dye + Nano-silver particles in a ratio (1:1), (1:2) and (2:1), (or at concentrations 50, 25, 75 %) for testing the quality of the printer dyes and filter paper without any addition as control were prepared.

3-Isolation and preparation of pure cultures of bacteria from textile samples:

Decaying books papers were excised and inoculated on nutrient agar plates. The plates were incubated at room temperature (~35°C). Pure cultures were subculture on agar plate once in a week[10].
a- Screening bacteria for cellulose activity:

After the pure culture formation, individual bacterium was inoculated on CMC agar medium containing carboxy methyl cellulose (5g/L), Peptone (5g/L), NaCl (5g/L), beef extract (3g/L), and agar (20g/L). The pH of the medium was adjusted to 7.0. The CMC agar plates were incubated at 37°C for 24 h.

A preliminary qualitative assay for cellulolytic activity was carried out using Congo red dye. At the end of the incubation, the agar medium was flooded with an aqueous solution of Congo red (0.1% w/v) for 15 min. The excess Congo red solution was poured off, and the plates were further treated by flooding with 1M NaCl for 15 min.[11].

b- Evaluation of antimicrobial activity of Nano-silver (disc diffusion method):

The agar disc diffusion method was employed for indicator bacteria. The filter paper disc prepared by using ordinary office two-hole puncture, paper discs with approximate diameter of 6mm. were punched out one by one from a sheet of filter paper, the disks placed in vials, sterilized by oven and allowed to cool.

The prepared mixtures of Nano silver ink were evaluated for its antimicrobial activity against bacterial and yeasts isolates. The tested bacteria were *Escherichia coli* and *Staphylococcus aureus*. The media of Muller Hinton agar for bacteria were poured into plates containing one ml cell suspension of bacteria (1.5 x 10^4 cells /ml).

4-Isolation and preparation of pure cultures of fungus from textile samples.

Various bio-deteriorated textile samples were collected from storage rooms in some library in Erbil city. All textile samples collected composed of linen fibers only. Samples mycoflora were isolated by using Agar Plate Method (APM), cotton swab technique and biodeteriorated textile part technique.

a. Agar Plate Method: Paper from books cuts into small cubes then transferred directly with sterile forceps into Petri dish contain sterilized Potato Dextrose Agar (PDA) and Dubois Cellulose Agar (DCA). Three replicates were made and the plates were incubated at 25°C for 5-7 days. Fungi colonies were identified according to morphological and microscopic characteristics [12].

b. Biodeteriorated textile part technique: In biodeteriorated textile part technique very small paper separated from the original ancient textile objects, paper cuts into small cubes transferred using sterilized tweezers into SDW then 1ml of water added to the media (Potato Dextrose Agar (PDA) and Dubos Cellulose Agar (DCA)) and the plates were incubated at 25°C for 5-7 days. Fungi colonies were identified according to morphological and microscopic characteristics [13].

c. Cotton swab technique: In the cotton swab technique, the fungal species were isolated using sterile moist cotton buds swabbed over the surface of ancient textile objects where fungal growth or fungal structures were observed. Cotton swabs were then used to distribute fungi on the media PDA and DCA, in Petri dishes. The dishes were incubated at 25°C for 5-7 days [13].

d. Preparation of spore suspension: The spore suspension of the selected fungus was prepared by adding 10 ml of SDW on the fungal plate, the spore was scraped by using a sterilized glass rod, and the spore mixture was then placed in a small sterilized vial put stir for 10 minutes. The spores were quantified using Hemocytometer and a light microscope. The spore suspension was then adjusted to ideal concentration of 1x10^6 spores / ml [14].

e. Evaluation of antifungal activity of Nano-silver: While, the tested yeasts were: *Candida albicans* and *Rhodotorula glutinis*, Nano-silver particles were tested by using filter paper disc diffusion method, Yeast suspension prepared from 24h colony by using phosphate buffer saline (PBS) in compare with standard control with concentration 41.5x10^6 cell/ml of yeast suspension. 0.1 ml of yeast suspension were inoculated on SDA then spread using sterilized L shaped glass rod, then incubated at 37°C for 24-48h. The diameter of the zone of
inhibition around each of the discs (disc diameter included) was taken as measured of the antimicrobial activity. Measurements were made from both sides of the slope and their average accepted [15].

RESULT AND DISCUSSION

The UV-VIS optical spectral distribution show that the Ag-NPs sizes range up to 20 nm, however, the smallest particles was about 8 nm in size. Different laser spot were applied, nevertheless, 80-85 mJ / pulse was the best energy used that ensured long term Ag-NPs stability and no coagulate over long time period. Thus with this method, it is shown that Ag-NPs were obtained without the necessity for any chemical additives which in turn makes it very favorable in terms of avoiding whatever Chemicals that are harmful[8-16].

Isolation of bacteria on deteriorated historical textile

As a result of isolation of microorganism from decaying books and papers in Erbil city were collected from; Both Bacillus subtilis (98%) and E.coli (2%) were isolated. Bacillus subtilis (98%) were given positive test on Carboxyl Methyl Cellulose (CMC) media due to producing cellulase enzyme, while E.coli (2%) was gave a negative test.

Figure (1) shows the growth of Bacillus subtilis after 24 hrs. of incubation on CMC agar and demonstrated positive results. The applied bacteria has shown positive results for dyes degradation / decolourization, as that indicated by the change and disappearence of color of the dyes from the dye-containing media of the Petri plates which indicates the production of extracellular enzymes by the applied bacteria, during the biodegradation of tested dyes. This result is agreement with that found by [17].

These results are agreement with that of Jafari et al., (2015) who found that microorganisms had sensitivity against nanoparticles; however, E. coli showed higher sensitivity than S. aureus.

The antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms. The small size and the high surface to volume ratio i.e., large surface area of the nanoparticles enhances their interaction with the microbes to carry out a broad range of probable antimicrobial activities (Gutierrez, et al., 2010).

Figure (1) shows the growth of Bacillus subtilis on CMC agar (A) without and (B) with Congo red stain.

Congo red binds with carboxymethyl cellulose and turns into bright red. Cellulase produced by individual bacterium hydrolyzed carboxymethyl cellulose around the bacterial colony and the dye Congo red unable to stain it. Therefore, the hydrolyzed zone appears transparent while the un hydrolyzed regions appear bright red.

Table (1) and figure (2) show the inhibitory effect of bacteria growth in filter paper disc soaked in (ink: Ag-NPs) in a ratio (1:1), (1:2) and (2:1) (v/v) , as shown in the figure(2) the filter paper used in the present study soaked in different concentration of mixed dyes for testing their inhibitory effects and the result was 2.3
mm., 1.1 mm, and 0.4 mm, respectively, filter paper stoked in ink used as control, the result shows that the pen ink allowed adequate colony development of the bacteria.

That mean the dyes used in the printing and writing on paper have no any inhibitory effect. While in the case of plates contains filter paper soaked in the mixed dyes at different concentration which shows different inhibitory effect of bacterial growth that mean these dyes have been reported as anti-bacterial effect. The results show that the highest inhibition with diameter or action was at ratio one volume ink: one volume Ag-NPs.

Figure (2) show the inhibitory effect of Bacillus subtilis growth in filter paper disc soaked in (ink(I): Ag-NPs (N) (v/v)).

| Table (1): Showing the inhibitory effect of Ag-NPs ink on the Bacillus subtilis growth |
|-------------------------------------|-------------------------------------|-------------------------------------|
| Bacillus subtilis                   | 2.3 mm                             | 1.1 mm                             | 0.4 mm                             |
| Ratio of paper ink (V): Nano-Silver (V) | Ink 1: Nano1                   | Ink 1: Nano 2                      | Ink 2: Nano1                       |

Effect of Nano-ink on Fungus in historical textiles

Result shows the tested fungus Acrodictys fimicola isolated and demonstrated positive results in the Congo red test. The applied fungus has shown positive results for dyes degradation/decolourization, which indicates the production of extracellular enzymes by the applied fungus, during the biodegradation of tested dyes. This result is matches with that found by [18] who found Aspergillus flavus has which the ability for biodegradation of two commercially used textile dyes, bromophenol blue and Congo red.

The result was also similar to biodegradation of Congo red and Bromophenol blue by the fungus Trichoderma harzianam in semi-solid medium [19]. In the present study, dyes might be degraded by the production of extracellular enzymes as well as adsorption of dyes by the mycelium of Acrodictys fimicola during its growth in the dye-containing medium.

Figure (3) demonstrated the effect of different concentrations of a mixture ink and silver nanoparticles on the growth of fungus Rhodotorula glutinis compared to model control negative without the use of silver nanoparticles, where the presence of heavy growth while the growth rates have less when using equal volumes of ink and stuck silver nanoparticles which inferred the presence of inhibitory effect on the growth of fungi. Thus, we conclude from the previous tests of the current study that the use of silver nanoparticles with ink is the preferred means which are approved in preserving the documents to resolve the problem of documents without damage affected when stored in libraries.

Jafari et al., (2015) found that copper chloride and silver nitrate had a lower inhibitory effect in their nanoparticles, especially against the tested fungi. Also concluded in current study the possibility of developing dyes printing by preparing a mixture of printing ink and silver nanoparticles in the inhibition of isolated growth.
(Acrodictys fimicola) from the damaged bond, and the prevention of pollution in documents. Our results agreed with results of previous studies [7, 10].

RESULTS

Results obtained in this study could make the development of new antifungal stain possibly happen. AgNPs can be mixed with paper printing and writing ink for preventing paper and book deterioration in the libraries and archives.

Metal nanoparticles with antimicrobial activity when embedded and coated on to surfaces can find immense applications in water treatment, synthetic textiles, biomedical and surgical devices, food processing and packaging (Gutierrez, et al., 2010).

For many decades, silver has been known for its antimicrobial prospective and its speculated that the AgNPs exert their effects by inhibiting enzymatic respiratory system of microbes, alteration of DNA replication and interaction with S-H bonds of proteins leading to inactivation[20,21]. However, the mechanisms are still obscure; Chamakura et al.[ 22]have demonstrated how E. coli cells absorb AgNPs through cytoplasm membrane.

These AgNPs are also involved in formation of reactive oxygen species thereby inhibiting respiratory enzymes and proteins leading to physiological malfunctioning responsible for mortality of E. coli [23].

The toxicity of silver ions, though not very clearly understood, could be either due to adhesion to the cell membrane and further penetration inside or by interaction with phosphorus containing compounds like DNA disturbing the replication process or preferably by their attack on the respiratory chain, Some studies also reported that the attachment of the nanoparticles on to the surface of the cell membrane disturbs the permeability and respiration functions of the cell [10].

CONCLUSION

The sources of deterioration and degradation of historical textiles and stored book paper in Erbil city were the bacterial (Bacillus subtilis) and fungal (Acrodictys fimicola). It should be emphasized that mixture of paper ink with AgNPs is the best method to prevent fungal and bacterial growth on historical textiles in order to protect textile surfaces from any contamination.

REFERENCES


