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RESEARCH ARTICLE

The Effect of Deposition Plasma Current For Gold Nano Thin Film on Bacteria Using Cold Plasma Sputter

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Abstract

New and special physical mechanism for Synthesizing Gold Nano particles purely and directly was used to deposit Gold Nano particles directly on a bacteria surface to study the effect of the Gold particles in Nano scales with changes in deposition current of plasma sputter (discharge current) on the bacterial behavior according to the effect of antibiotic, pH and temperature on the bacteria and compare it to the effect of the Gold without using chemical methods or assisting agents (catalysis) in Synthesis of them. For this purpose, two types of bacteria have been selected, gram negative *Escherichia coli* (*E.coli)*, and gram positive *Staphylococcus aureus*. (12) Types of antibiotics was used, it was found that the bacterial sensitivity to the antibiotics changes by Gold deposition rate, and every antibiotic have its own effect. Noted that Gold deposition in nano scale on bacteria decrease bacterial activity when grown in different pH rate and temperature. Concluding that Gold nano particle have marked effect on bacteria without causing changes in the size of the bacterial cell, and bacterial sensitivity variable to each antibiotic particularly.

Introduction

Modern researches are interested in materials that have effective Nano scale such as Gold and Silver because of their natural and chemical characteristic which lead the researchers to investigate and study these elements in a frame called Nano technology, which have favorable applications in many fields like medicine, as for prof. Mostafa A. El-Sayed (Receives National Medal of Science in Chemistry-U.S.A)for treating skin cancer %100 on animals by using very small and ultramicroscopic (Nanorods) bars of Gold and Silver in finding cancer cells and attaching to these cells then applying low energy laser rayon the bars, which become hot enough to destroy the diseased cells without causing any damage to the healthy cells of the body.[1]

The Nano technology researches include all metalloids and crude materials, but Gold and Silver have a medical property in which the body cannot resist them when these Nano particle penetrate the living cells easily.

Many researches were done using Nano particles on different types of bacteria and bounding it [3], so it's used in treating and diagnosing bacteria through specialized protein agent that bind to the cell wall(selective interaction)[5].Because Gold and Silver Nano particles have the ability to breakdown the bacterial cell wall revealing the poisoning effect of the Nano particles on the bacteria such as *E.coli* and *Staphylococcus aureus* through breaking down there cell wall[6].

Most of these studies that have been done in this field used the chemical methods as a solutions to prepare Nano Gold particles through using assisting agents which bind between the Gold Nano particles and the bacteria, or synthesis of Gold Nano particles by Using Leaf Extract of Tephrosia purpurea [7]…etc.

In this research we intended to use a special physical mechanism to Synthesis Gold Nano particles purely and directly and deposit it directly on the bacteria to study the effect of the Gold particles in Nano scales on the bacteria without using chemical methods or assisting agents (catalysis) in Synthesis them, and studying its sensitivity change against antibiotics and its growth in different pH and temperatures degrees.

Two types of bacteria have been selected *E.coli*, and *Staphylococcus aureus* one of them is gram negative and the other is gram positive, both of them are pathogenic to the human.

The first species of bacteria in this study was *E.coli* is, a well-known gram-negative rod-shaped bacterium. It is a member of the normal intestinal flora, but it becomes pathogenic only when they reach tissue outside of their normal location. The most frequently sites of clinically important infection are the urinary tract, biliary tract and other sites in the abdominal cavity, also it may reach any part in the body including blood stream, prostate gland, lung, bone and eyes. [10, 9]

The second species of bacteria in this study was *Staphylococcus aureus*, which is gram-positive and shaped as a cluster-forming coccus. *Staphylococcus aureus* is the most spreader bacteria in the nature, which is present on the skin, mucous membrane, upper respiratory tract, air and soil. And regarded as one of the bacteria that can cause dangerous harms when there is a defect or a disturbance in host body immune system. The species of this type get a special importance among the other *Staphylococcus aureus* bacteria because of being the most important reason for causing many clinical diseases to the human differ in severity between simple skin diseases to dangerous systemic disease that may lead to death. [4, 2]

Materials and Methods

The bacterial isolates have been obtained from laboratories of biology department, section of Microbiology, college of science, university of Mosul in pure culture and tested to make sure of purity of culture and diagnosis of bacteria. The deposition of Gold nano particles on bacteria is performed, by using cold plasma deposition device (Sample Preparation System, Quorum Technology, made in UK. Model: Q150R, seril No.:11009) for different deposition currents(20to80mA) with limited pressure which the devise works at it ($1x10^{-1}$ mBar) and constant deposition thickness (6 nm).

By taking a drop of recently growing bacteria in Nutrient agar medium that have been sterilized by an autoclave devise (HIRAYAMA,HICLAVE-HVE-50)and putting it on a small sterilized petri dish(Dolphi MD, made in S.A.R- 3.5 x 1.2 cm) and smear it by a loop on the dish in sterilized circumstances inside the hood to prevent contamination by other types of bacteria. Then placing the dish that contain the bacteria in the depositing devise so that the Gold Nano particles deposit on it in different circumstances.

After the Gold Nano particles deposition process, a swap is taken by a loop and implanted in a growth medium (Nutrient Broth) that is sterilized by an autoclave devise then placed inside the incubator (Binder GmbH Borgstr.14D-78532 Tuttiingen) for 24h at 37c.

After that the sensitivity against antibiotic discs (Bioanalyse, for in Vitro Diagnostic Use) have been tested on the bacteria according to NCCLS [8], by using a swab from the cultured bacteria inside the Nutrient Broth medium (the bacterial suspension equivalent to the 0.5 McFarland standard) using a sterilized swab(Transport SWAB, CITOSWAB-made in China) and swabbing a Petri dish (Dolphi MD, made in S.A.R- 9 x 1.5 cm)contained Mueller Hinton Agriculture medium then placing the antibiotics on it,12 types of antibiotics were used shown in the table(1).

Table(1) antibiotics:

No	Name	Symbol	concentration
1	AMOXICILLIN	AX	25 mcg
2	Ampicillin/oxacillin	APX	(25/5) mcg
3	CARBENICILLIN	PY	25 mcg
4	CEFOTAXIME	CTX	10 mcg
5	CEFTRIAXONE	CRO	10 mcg
6	CEPHALOTHIN	KF	30 mcg
7	IMIPENEM	IPM	10 mcg
8	METHICILLIN	ME	10 mcg
9	OXACILLIN	OX	10 mcg
10	PENICILLIN G	P	10 U
11	PIPERACILLN	PRL	30 mcg
12	Vancomycin	VA	10 mcg

Then the dishes placed inside the incubator for 24h at 37c.To measure the sensitivity and resistance against the antibiotics a fine ruler is used to measure the diameter of the sensitive area around antibiotic discs passing through the center of the discs and comparing the result before and after deposition of Gold nano particles on the bacteria. In another side using tubes with volume 5ml filled with Nutrient Broth sterilized culture medium, in which the bacteria with and without Gold nano particles implanted, and by holding it with a loop and placing it in tubes in equal quantities, he tubes are divided in to 5 groups according to the preparing and incubating circumstances: as presented in table (2). [11, 12]

Table (2):

Group	Temp./ C^0	pH
1	37	3
2	37	9
3	37	7
4	45	7
5	45	7

And then the tubes were placed in the incubator for 24h. Measuring bacterial growth rate through turbidity degree of the medium, which is measured using visible spectroscopy (Libra S4-seril No.114908-manufacturedbyBiochromLtd, Cambridge CB4 OFJ England), from its absorption rate, the growth rate was measured and the results were compared. All used culture mediums are production of (Lab M Limited 1 Quest Park , Moss Hall Road, Heywood, Lancashire BL9 7JJ, United Kingdom)company .

Sputter Coating:

Deposition of Gold Nano particles on bacteria using a device made for preparing samples in form of metal slide with Nano size by cold plasma deposition system in the presence of Argon gas, in which Nano particles is generated and placed on the target as shown in figure(1),

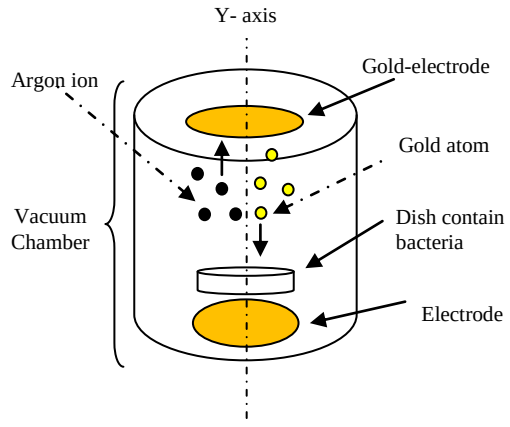

Figure (1): deposition chamber.

The system composed of a cylinder with vacuum room contain two vertical electrical electrodes the upper electrode covered by a layer of pure Gold sheet which represent the cathode electrode, and the lower electrode represent the anode electrode. When applying potential between these two electrodes the inert Argon gas (heavy atoms) is entered to the vacuum room and becomes ionized to produce cold plasma. The Argon ions accelerated toward the Gold sheet and by hitting it, Gold atoms ejected to deposit on to the surface of bacteria within coating unit. A major disadvantage of simple sputter coaters is the excessive amount of heat generated in the sample. To overcome this problem, permanent magnets are utilized to deflect the high energy electrons generated in the glow discharge away from the sample. The magnetic lines of force cause enclosed loops at the cathode surface, increasing the interaction path length of the high energy electrons in the discharge [13].Deflection and retardation of electrons result in increased ion yield and sputtering efficiency. The bacteria is placed in a dish between the two electrodes as a target for Gold atoms to deposit on it, at room temperature in low plasma current (0 to 80 mA) in order to form well regulated Gold grains in Nano scale. The thickness of deposited Gold atoms can be measured by specialized crystal

detector in Nano scale, called Film Thickness Monitor (FTM). This device is made to measure and control deposition thickness from 1 to 2000 nm (*Quorum Technology*).

Deposition Current& Deposition Rate:

The deposition current have distinct role in sputtering atoms, by increasing the deposition current the kinetic energy of the Argon ions increased i.e. accelerated, and ejected more Gold atoms to deposit on the surface of bacteria within coating unit. This means that increase the sputtering deposition rate.

Figure (2) shows the Correlation between sputter rate and deposition current. This is limited to sputtering system used in this work, and given by Quorum Technology Company [20].

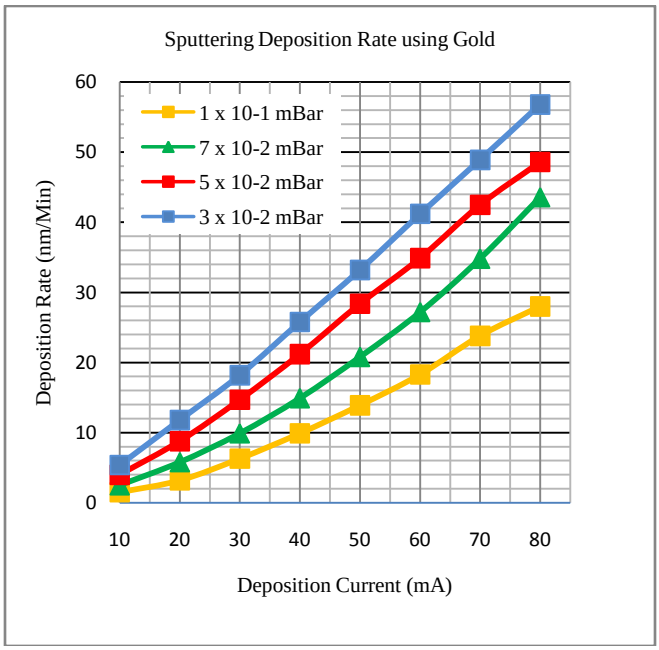

Figure (2): Sputtering Deposition Rate using Gold,
Gold tooling factor = 2.7

Result and Discussion

Gold particles have been deposited on two types of recently grown bacteria by cold plasma at deposition currents (20 to 80 mA), with constant Gold nano particles thickness (6 nm) under pressure ($1x10^{-1}$mBar), and to test the effect of the Gold particles on the bacteria, sensitivity change of bacteria against antibiotics is used, for this purpose 12 types of antibiotics is used as we mentioned previously, and observed the bacteria grows at three specific temperature degree(4, 37 ,45 C^0) and three specific pH rate(3 , 7 ,9).

Antibiotic test:

It have been noted that sensitivity of the 12 antibiotics to the bacteria *Staphylococcus aureus* after deposition of Gold Nano particles revealed different behavior, generally its divided to three group according to how its inhibition zone changed with increasing Gold deposition thickness on the bacteria, first group include (CRO, ME, VA, APX, PRL, OX, IPM) second group include (KF, AX, P) third group include (CTX, PY).

In figure (3) the inhibition zone area of the first antibiotic group increased slightly by increasing Gold deposition current. While in figure (4) the inhibition zone area of the second antibiotic group decreased slightly with increasing Gold deposition current.

The inhibition zone area of the third antibiotic group increase by increasing gold deposition current as in figure (5).

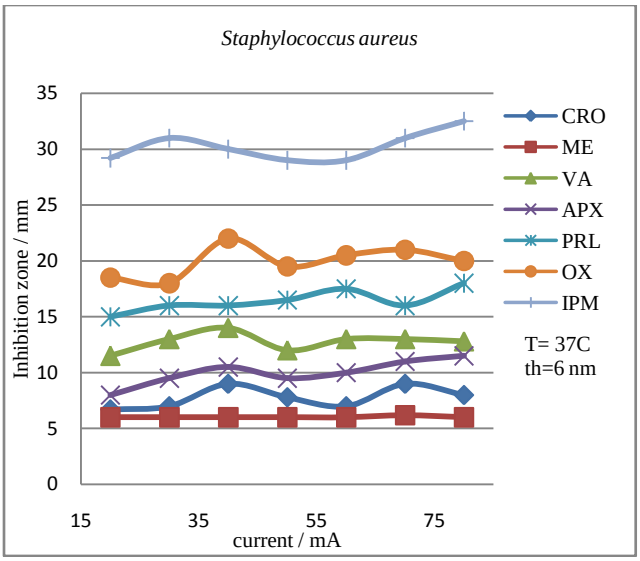

Figure (3): change in sensitivity of *Staphylococcus aureus* with first antibiotic group by increasing Gold deposition current.

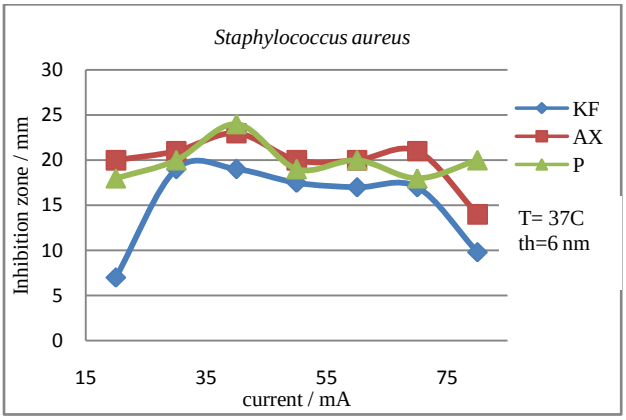

Figure (4): change insensitivity of *Staphylococcus aureus* with second antibiotic group by increasing Gold deposition current.

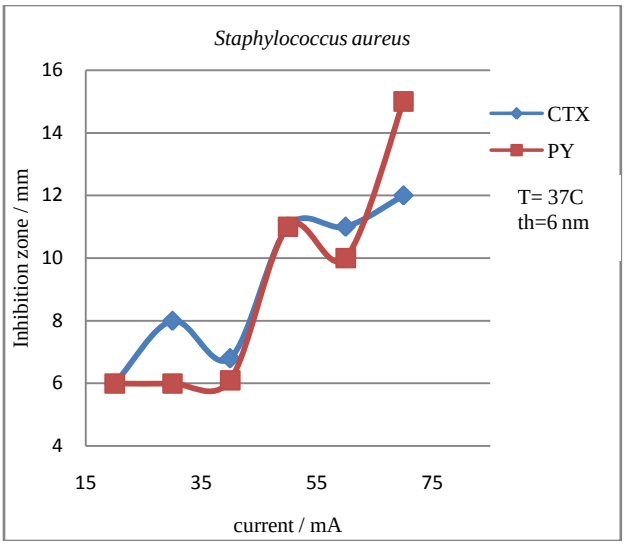

Figure (5): change in sensitivity of *Staphylococcus aureus* with third antibiotic group by increasing Gold deposition current.

E-coli bacteria is sensitive to three types of antibiotics only (IPM, CTX, CRO) and resistance to the other (9) antibiotics, the activity of the bacteria did not changed by the other 9 antibiotic after deposition of Gold. As shown in figure (6) in which inhibition zone area of (CRO, CTX ,IPM) antibiotics increased by increasing Gold deposition current.

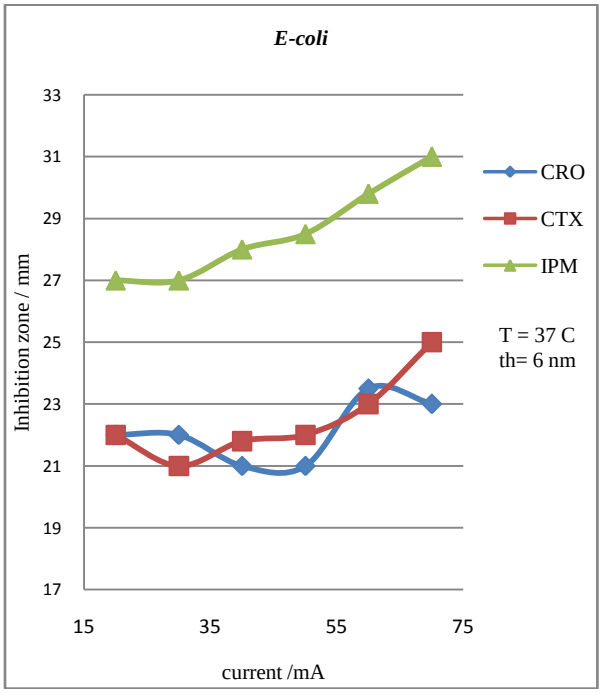

Figure (6): change in sensitivity of *E-coli* with antibiotics by increasing Gold deposition current.

The reason of this bacterial behavior may be due to the existence of Nano Gold particles across the negatively charged cell wall to the cell or the attachment of the particles to the cell wall that change the reactions of the antibiotics to the bacteria due to changes in the cell wall composition[10], this factor appear according to the type of

antibiotic used so the effect of any antibiotic must be studied on many types of bacteria after deposition of the particles as a result of a chemical reaction between the antibiotic and the bacterial cell wall on the other hand the chemical composition of the antibiotic and presence of subgroups such as presence of Sulfate and Nitrogen that combined with Gold[14].

Bacterial resistance to heavy metals (Au) as in figure (4) is due to some genes on plasmed DNA where there is a relationship between resistance to antibiotics and heavy metals as in reference[15].Figure (7) shows changes in inhibition zone of the antibiotics at different current deposition compared with control sample of *Staphylococcus aureus*, the effect of deposition currents on bacteria is obvious when compared with the control, but we do not mention the effect of Gold deposition and pressure because this two factors in presence of Argon gas is stable (constant) during deposition process .Generally, Gold Nano particles deposition at different deposition currents lead to slightly decreasing bacterial activity as a result of attachment or entrance of Gold particles to negatively charged cell wall that lead to decreasing potency of the cell wall for the transmission of substances between the bacterial cell and its surrounding.

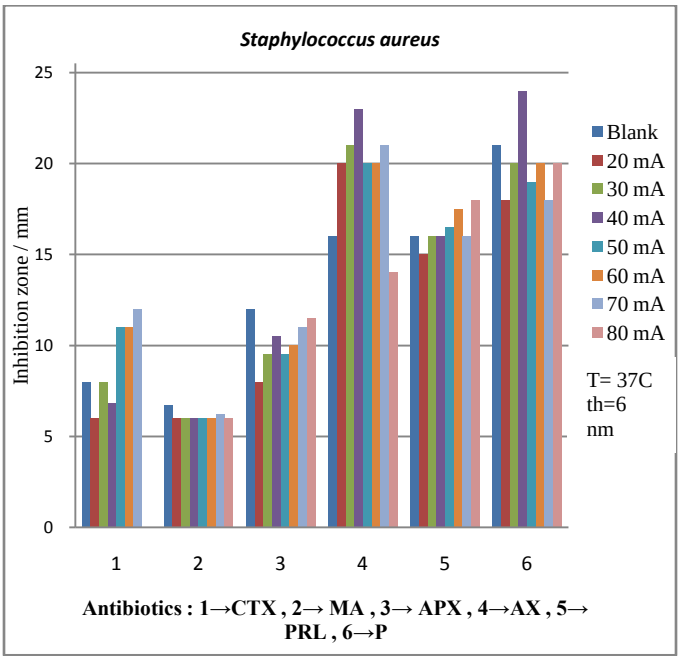

Figure (7): changes in inhibition zone of the antibiotics with eight situation of *Staphylococcus aureus* circumstances.

Figure (8) shows change in inhibition zone of the antibiotics on *E-coli* for circumstances control and deposition currents. We note that Gold Nano particles deposition lead to decreasing *E-coli* activity as a result of close encounter of Gold particles to negatively charged cell wall that lead to decreasing potency of the cell wall for the transmission of substances between the bacterial cell and its surrounding [16]. This observation agreement with Chatterjee, which showed that the Gold Nano particle nontoxic nature on *E.coli*. Chatterjee noted the length of *E-coli* increase when add gold and iron oxide nano particles [17]. But in this research the size of *E-coli* not changes because of adding the Gold atoms is done directly without using any chemical agents as shown in figure(9).

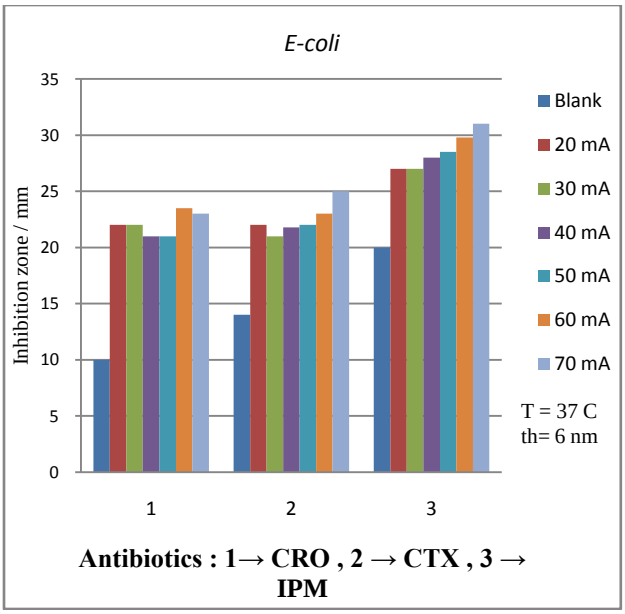

Figure (8): changes in inhibition zone of the antibiotics for *Staphylococcus aureus* by deposition of Gold Nano layer with different deposition current.

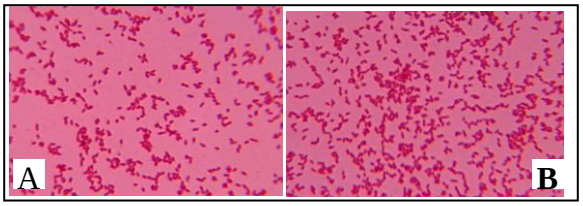

Figure (9): *E-coli* bacteria
A-before gold Nano layer deposition
B-after gold Nano layer deposition(20 mA, 6 nm). (magnification100X).

pH test:

From figures (10 and 11) of both types of bacteria we note that they grow best at pH degree 7,also we note that when the deposition current increased absorption decreased. The bacterial growth is decreased means the Gold decrease the bacterial activity as a result of attachment or entrance of Gold particles to negatively charged cell wall [18], and decreasing potency of the cell wall for the transmission of substances between the bacterial cell and its surrounding.[19]

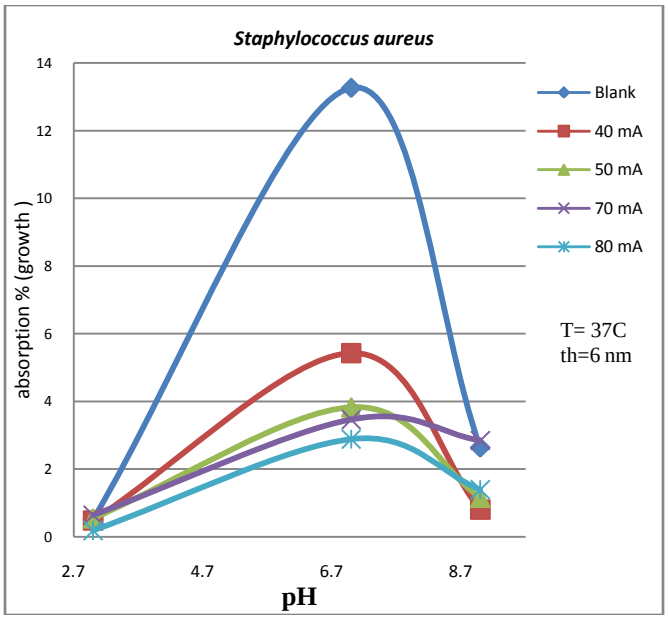

Figure (10): growth of *Staphylococcus aureus* in different pH rate and Gold deposition current.

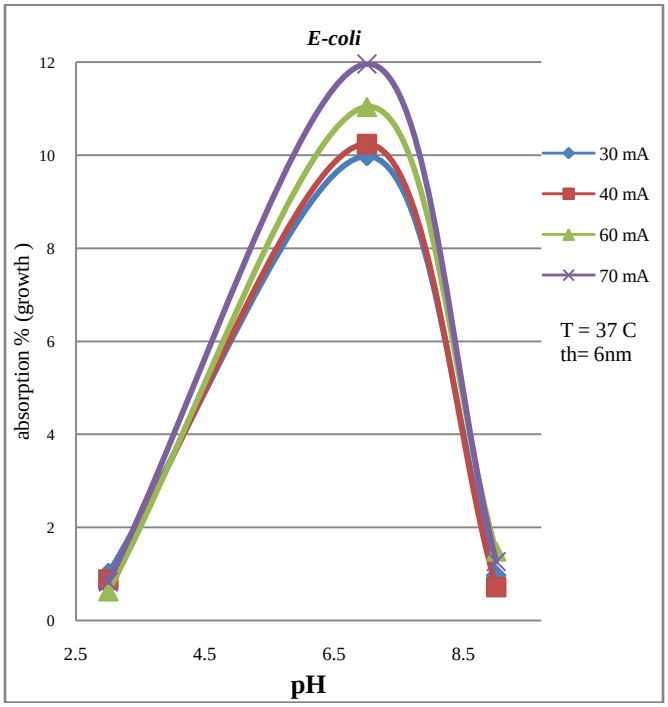

Figure (11): growth of *E-coli* in different pH rate and Gold deposition current.

Temperature test:

Figures(12 and 13) of both types of bacteria revealed that the best grow was at $37C^0$, also that increasing the deposition current may result in decreasing absorption, i.e. the decrease of the bacterial activity.

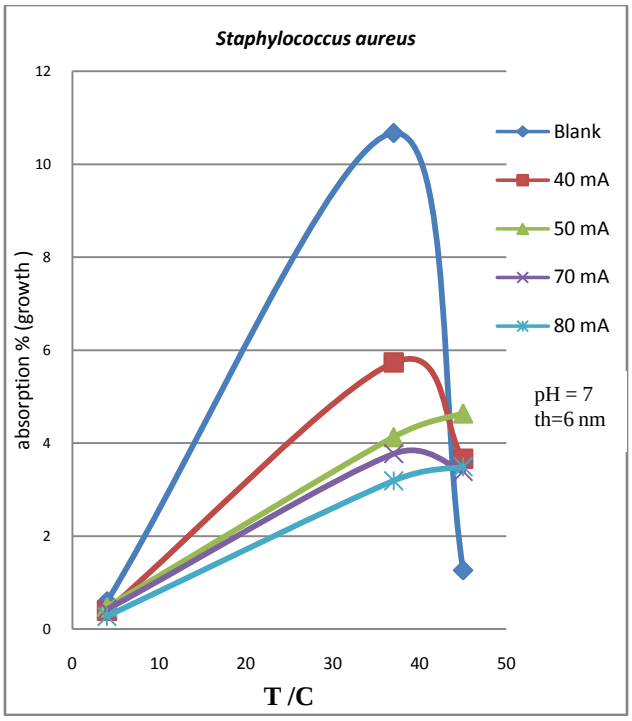

Figure (12): growth of *Staphylococcus aureus* in different temperature and Gold deposition current.

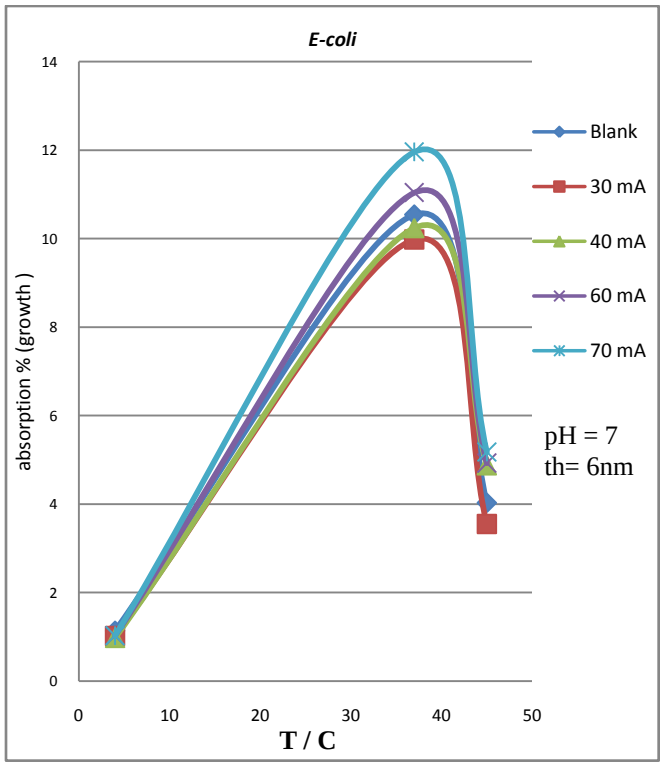

Figure (13): growth of *E-coli* in different temperature and Gold deposition current.

Conclusions

Plasma sputter coating could be used as a special physical mechanism to Synthesis Gold Nano particles purely and directly and deposit it directly on some bacteria's surface to study the effect of changes in deposition current of plasma sputter of Gold particles in nano scales on the bacteria without using chemical methods or assisting agents (catalysis) in Synthesizing them. This changes in deposition currents yields changes in bacteria's growing activity according to some applied physical parameters. Gold Nano particle at different deposition currents have marked effect on bacteria without changing the size of the bacterial cell, and bacteria sensitivity change to each antibiotic particularly.

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