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Antibacterial activity with eggshell nano-particles activated by microwave plasma

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The eggshell cuticle is the proteinaceous outermost layer of the eggshell which regulates water exchange and protects against entry of micro-organisms. Outer eggshell and cuticle protein was extracted from domestic chicken. The aim of the research is to find out the effect of the treated and untreated nano particles of egg shells with micro wave cold plasma on the effectiveness of E. coli (negative bacteria) that infect the skin and measure the diameter of bacterial inhibition zone, the eggshell has been prepared by a chemical method (sol gel) and measure the level of acidity and the PH is neutral. The result of Atomic Force Microscope (AFM) shows that the particles diameters become smaller with nano-particles solution than for egg shell tests (powder). The nano-particles solution for egg shell exposed to cold plasma. The microwave plasma used in this search has voltage "175v" and the gas flow at " 5liter /min " and frequency (2.45 GHz) at the room temperature for five minutes' time. The E. coli bacteria were treated with nano-particles solution egg shells. The results indicated that after 24 hours of placing the bacteria in the incubator, the inhibition zone for the nano-particles eggshell that for the is greater than that for nano-particles eggshell exposed to the plasma, and in both cases there were a good response.

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1. Introduction

Nanoparticles (NPs), that is, particles measured at the nanometer scale, are materials that have nanoscale limits that are of very great importance and useful in various fields of life as engineering, biological and medical applications, and in chemistry depending on the principle of surface-to-volume ratio and there are several methods in order to obtain nanomaterial, including methods Physical mechanical and chemical methods such as the sol-gel method, because it is considered an easy and fast method [1]. The egg shells are a source of elements, so the eggshell is used in the manufacture of some industrial and food products, such as calcium additives and other elements in some countries such as Japan [2][3]. The sol-gel chemical technique used to produce nanomaterials on a large scale because it is considered an efficient process for producing egg shell nanoparticles, being a low-cost method, energy efficiency, high production rate and fast yield of fine homogeneous powder were examined the structural and chemical properties of the materials by atomic force microscopy technique [4][5]. The atomic force microscope (AFM) method is an advanced technique of fundamental importance in order to study the atomic properties of the subject matter on an atomic scale. This method has occupied great and advanced horizons in the number of disciplines and fields of science such as medicine, molecular biology, solid-state physics and materials [6][7]. The basis of the work of the atomic force microscope (AFM) depends on the principle of excluding Pauli by drawing a topographic map of the studied material and studying its properties on the nanometer scale by measuring the interaction or the force of attraction or repulsion between the studied sample and the protruding tip that is similar to a spring. These forces give details and information about the surface [8]. The method of examination using the scanning probe microscope (SPM) was used because it is one of the important and effective devices in many recent researches that adopt the nanoscale because it gives results and information with infinite accuracy of the local characteristics of the surfaces of solid bodies [9]. Escherichia

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coli (E. coli) is a facultative, rod-shaped, Gram-negative bacterium, of the family enterobacteriaceae, it naturally inhabits the digestive system and skin. It causes infections and skin infections [10] [11].

1.1. Microwave plasma

Microwave plasma sources can be designed for either high or low pressure operation and take on somewhat different physical configurations depending upon the pressure of operation. the major advantage of microwave discharges is their ability to create intense discharges in chemically active gases with low maintenance [12]. The microwave plasma used in this search has voltage "175v" and the gas flow at " 5liter /min" and frequency (2.45 GHz) at room temperature for five minute

1.2. Materials

The chemical synthesis of the Eggshell sample has been obtained via the technique of XRF examination. The metal oxides present in the sample are SiO_2(0.327%), Al_2O_3(0.0708%),CaO(56.71%),MgO(0.693%), Na_2O(1.301%), SO_3 (0.873), and P_2O_5(0.393%).

2. Experimental

1-The eggshells were collected from domestic chicken at first, then cleaning by insert in boiling water to separate white matter.

2-The eggshells are dried by using oven with 30 C^0.

3- The eggshells are converted to powder.

4- The Nano eggshells powder was made by using chemical method (Sol-gel).

5- The Nano eggshells powder was examined by atomic force microscope (AFM).

6- (5 ml) of Nano eggshell are exposure to microwave plasma with voltage (175 v) for 5 mints.

7-The (E.coil) bacteria are prepared in pitradish and made two holes.

8- First hole treated with Nano eggshells which are treated with microwave plasma. Another hole was treated with Nano eggshell without exposure to microwave plasma.

9- The diameter of inhibition zone for the E. coli bacteria are measured to detect the effect of nano-eggs shell solution

3. Results and Discussion

Figure (1) shows homogeneity structure of particles of eggshells powder table (1) shows Surface Roughness parameters, Hybrid Parameters, Functional Parameters and spatial Parameters for the eggshells powder. The average diameter is 98.62 nm as shown in table (2), Figure (2) shows The Granularity accumulation distribution of eggshells powder particles.

Figure (3) shows homogeneity structure of particles of Nano eggshells particles. Table (3) shows Surface Roughness parameters, Hybrid Parameters, Functional Parameters and spatial Parameters for the eggshells powder. The average diameter is 57.83 nm nm as shown in table (4), Figure (4) shows The Granularity accumulation distribution of Nano eggshells particles.

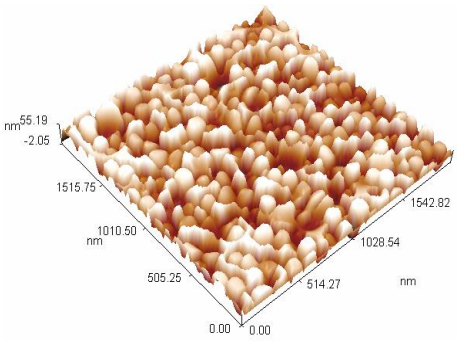


Fig. 1. The distribution and the homogeneity structure of particles eggshells powder.

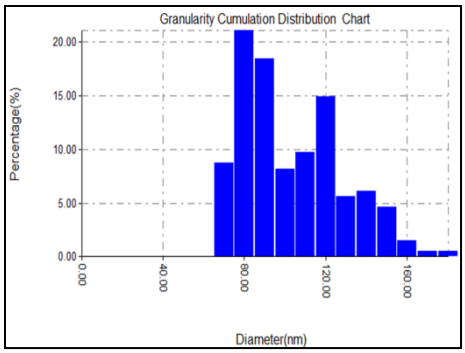


Fig. 2. The Granularity accumulation distribution of the particles.

Table 1. Surface Roughness parameters for the eggshells powder.

1 Sa (Roughness Average)	12. 7(nm)
2 Sq (Root Mean Square)	14.8(nm)
3 Ssk (Surface Skewness	-0.178
4 Sku(Surface Kurtosis)	1.95
5 Sy (Peak-Peak)	57.2(nm)
6 Sz(Ten Poi Height)	32.6(nm)
Hybrid Parameters	
1 Ssc (Mean Summit Curvature)	-0.1(1/nm)
2 Sdq(Root Mean Square Slope)	1.22(1/nm)
3 Sdr(Surface Area Ratio)	49.7
Functional Parameters	
1 Sbi(Surface Bearing Index)	6.18
2 Sci (Core Fluid Retention index)	1.49
3 Svi(Valley Retention index)	0.0962
4 spk(Reduced Summit Height)	0 (nm)
5 Sk(Core Roughness Depth)	46.7
6 Svk(Reduced Valley Depth)	9.33(nm)
7 Sdc (0-5 % height intervals of Bearing Curve)	2.4(nm)
8 Sdc (5-10 % height intervals of Bearing Curve)	2.29(nm)
9 Sdc 10-50 (10-50 % height intervals of Bearing Curve)	19 (nm)
10 Sdc 50-95(50-95 % height intervals of Bearing Curve)	26 (nm)
spatial Parameters	
1 Sds (Density of Summits)	0($1/um^2$)
2 Fractal dimension	2.46

Table 2. The average diameter of the eggshells particle

Avg. Diameter:98.62 nm	<=10% Diameter:70.00 nm
<=50% Diameter:90.00 nm	<=90% Diameter:130.00 nm

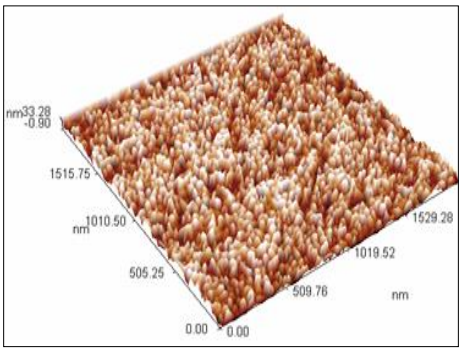


Fig. 3. The distribution and the homogeneity structure of Nano eggshells powder particles.

Table 3. Surface Roughness parameters for the Nano eggshells powder.

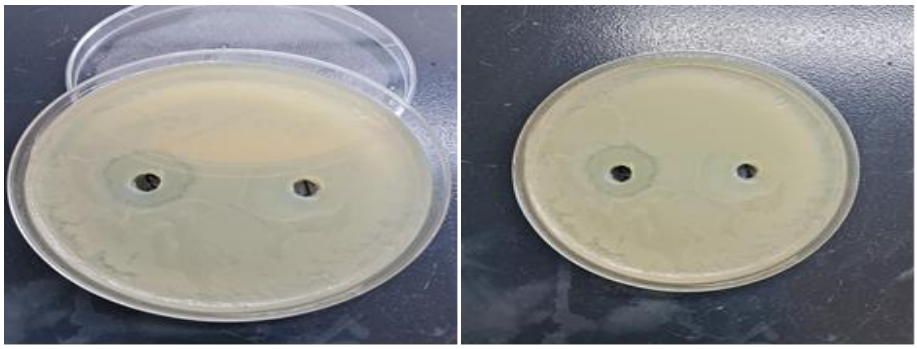
1 Sa (Roughness Average)	8.54 (nm)
2 Sq (Root Mean Square)	9.87 (nm)
3 Ssk (Surface Skewness)	3.18
4 Sku(Surface Kurtosis)	1.8
5 Sy (Peak-Peak)	34.2 (nm)
6 Sz(Ten Poi Height)	34.2(nm)
Hybrid Parameters	
1 Ssc (Mean Summit Curvature)	-0.25 (1/nm)
2 Sdq(Root Mean Square Slope)	1.85 (1/nm)
3 Sdr(Surface Area Ratio)	121
Functional Parameters	
1 Sbi(Surface Bearing Index)	5.58
2 Sci (Core Fluid Retention index)	1.49
3 Svi(Valley Retention index)	0.0692
4 spk(Reduced Summit Height)	3.64 (nm)
5 Sk(Core Roughness Depth)	29.7(nm)
6 Svk(Reduced Valley Depth)	0.87 (nm)
7 Sdc (0-5 % height intervals of Bearing Curve)	1.77 (nm)
8 Sdc (5-10 % height intervals of Bearing Curve)	1.7 (nm)
9 Sdc 10-50 (10-50 % height intervals of Bearing Curve)	13.7 (nm)
10 Sdc 50-95(50-95 % height intervals of Bearing Curve)	15.4 (nm)
spatial Parameters	
1 Sds (Density of Summits)	707($1/um^2$)
2 Fractal dimension	2.7

Table 4. The average diameter of the eggshells particle.

Avg. Diameter:57.83 nm	<=10% Diameter:0 nm
<=50% Diameter:55.00 nm	<=90% Diameter:60.00 nm

3.1. Antibacterial Activity

The antibacterial of the eggshell solution exposed to plasma for 5 mints the measure based on the inhibition zone. As it is observed that the diameter decreases when the solution exposed to plasma compare with Inhibition zone without microwave plasma. Figure 4(a, b), shows the activity of nano- eggshell (a) without plasma exposed and (b) with plasma.



(a) *(b)*

Fig. 4. The inhibition effect of Eggshells solutions on E. coli bacteria: E. coli without plasma, (b) E. coli with plasma.

Table 3. Diameter of inhibition of solutions for E.Coli bacteria.

Concentration %	Types of bacteria	Inhibition zone(mm) Without plasma microwave	Inhibition zone (mm) With plasma microwave at 5 minute
100	E.Coli	17	25

4. Discussion

The surface roughness of all amplitude parameters is lower for eggshells nano particles, so are the hybrid parameters of nano particles are lower than that of eggshell nano particles, the spatial parameters (density of peaks) are lower for the eggshell particle than that of nano particles, while for fractal parameter itis higher than for eggshells nano particles, the surface of nano particles is smoother than that of eggshells. The nano particles eggshells has been exposed to plasma for five minute, it shows The reason for the high effectiveness of the of nano particles solution is due to the size and surface area of these particles, which enable them to reach the DNA of the microbial cell and communicate with it, the result shows there is Inhibition zone about 17 mm for the region without plasma microwave and 25mm for eggshells solution effected with plasma, The antibacterial of eggshell is due to alkalinity and activated oxygen species in its minerals presented in the eggshell.

Conclusions

Alkalinity and activated oxygen species in eggshell nanoparticles are associated to their antibacterial properties. The surface area of the minerals in the of eggshells increased by converting the solution to nano particles. SiO, Al_2O_3, CaO, MgO, Na2O, SO_3, and P_2O_5 nanoparticles have the ability to damage cell membranes, enabling internal contents to get out and causing bacterial cell death. Mineral solutions such as CaO, MgO, and Na_2O have a good antibacterial effect in water beside the other mineral. Using an Atomic Force Microscope (AFM), the average diameter of the NPS was measured, revealing that the solution with plasma effect has a

lower diameter than the solution without plasma, and that the diameter decreases with time when exposed to plasma. Because the efficiency of solutions that have not been exposed to plasma is smaller than that of solutions that have been exposed to plasma, the effect of plasma is to improve the effectiveness of metal oxides present in various quantities in the eggshells.

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