Characterization and Evaluation The Biological Activity of Prepared Nano-Gentamicin Nanoparticles

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Abstract

Nanotechnology is a capable approach to enhance the activity of common antimicrobial agent mainly used in human and veterinary drug. Gentamycin is a broad-spectrum antibacterial against Gram-positive and Gram-negative bacteria. The study aims to determine the characterization and the biological activity of gentamicin in the Nano-form prepared by Sol-gel application using an ultrasound device by aqueous solution at temperature (80)°C. Nano gentamycin examined by Scanning electron microscopy (SEM), transmission (TEM), atomic force microscopy (AFM) and antibacterial effect of both Nano and stander gentamycin were analyzed. The results showed that the average size of gentamycin nanoparticles was 68.51 nanometers with homogeneous distribution. The EDX showed large number of elements present in the Nano solution. The antibacterial effect of Nano gentamycin took a wide inhibition range of Gram-positive and Gram-negative bacteria compared to the standard substance, where the inhibition diameter for E.coli was 29 mm, and for st. epidermidis was 27 mm. The MIC of gentamicin nanostructures was the concentration 0.00125 mg/ml.

Keywords: Gentamicin, Bioactivity, SEM, TEM, MIC, Nanoparticles.

1.Introduction

1.1. Gentamycin Sulfate

Chemical formula : C21H43N5O7, molecular weight = 477.60, Gentamycin: It is an antibiotic belonging to the family of aminoglycosides, which is a mixture of sulfate microbial substances produced from the growth of bacteria Micromonaspora purpurea. They are many kinds of gentamycin: C1, C1a, C2, C2a, Cb, it is a broad-spectrum bacterial antibiotic (except for anaerobic bacteria and streptoxoxy) [1]. Gentamycin Sulfate is a white powder that is freely soluble in water and insoluble in alcohol, acetone, chloroform and ether. It is involved in the reversible binding of the S30 ribosome unit, as it works to inhibit the formation of the mRNA complex, thus leading to preventing protein synthesis, which results in the death of the bacterial cell [2].

Figure 1. Gentamicin sulfate molecule.
1.2. Sol - gel technology

The name sol-gel is derived from the fact that the micro-particles or molecules in a solution (sol) agglomerate and under controlled conditions, finally bond together to form a cohesive network (gel). There are two types of the sol-gel technique: the first type is called the colloid method, and the second type Polymeric method [3]. Both types include dissolving or suspending substances in a suitable liquid, usually the water in the colloidal type and alcohol in the polymeric type, so that the active substances interact together to form the gel and the gel grows over time and heat and also the volume of the container. At this point, the viscosity of the liquid will increase until it reaches to gel state [4].

Sol - gel technology has many advantages [5] that made it used instead of other technologies, including: It can be operated or sterilized at high temperatures, It does not swell or shrink when in contact with liquids, It is more resistant to corrosion, further low costs.

The application of the antibiotic nanoparticle mechanism has been shown to provide several advantages over conventional administration and methods, including: the ability to deliver the drug to a specific site in the cells of the body[6,7]. Nanoparticles can also be exploited to facilitate the continuous release of the antibiotic and to reduce the amount and the number of doses [8]. Moreover, nanoparticles can block the drug inside the body, which it reduces the systemic toxicity caused by conventional administration of the drug. The present study aims to evaluate the bioactivity of Gentamicin Nano-prepared.

2. Matrails and Methods

2.1. Gentamicin nanomaterial’s preparation and measurement

2.1.1. Preparation of gentamicin Nano Sol-gel method

Nanoparticles have prepared by using the Sol gel method, which is the simplest method by reducing the particle size using materials such as acetic acid, distilled water and ethanol and at a temperature of 80°C using the Probe Sonicator as a catalyst and a hydrolysis agent that transforms the material in the final stage with increasing temperature’s solution into the Nano form[9].

2.2. Atomic force microscopy (AFM)

To observe the surface roughness and topography of the sedimentary thin films, they are examined with a Scanning Probe Microscope (SPM), which was examined at Baghdad University, College of Science [10].

2.3. Scanning Electron Microscopy (SEM)

A scanning electron microscope was used to view the nanostructures and examine the surface morphology of the samples, as the samples were examined with a scanning electron microscope Inspect S50 with a magnification power of (X2000).

2.4. Transmission electron microscopy (TEM):

The transmission electron microscope was used to understand the properties and size of nanoparticles. Testing was performed with a Tecnai G2 20 at 200 kV.

2.5. Biological activity test

The diffusion hole method was used to detect the inhibitory effect of gentamycin against the Gram negative and Gram-positive bacteria. Muller-Hinton Agar culture medium has prepared, 1.0 ml of the bacterial suspension was spread and the plates were left at room temperature for 15 minutes after the holes were drilled in the culture medium inoculated with bacteria in a sterile piercing with diameter 7 mm. By a fine mechanical pipette, transferred 50 μl of the gentamicin solution prepared and its placed inside the pit, then the dishes were incubated at 37 °C for 18-24 hours, then the results were taken by measuring the diameter of the inhibition area, which represents Non-growth area of bacteria surrounding the hole by ruler [11].

2.5.1. Determine the minimum inhibitory concentrations (MIC)

The tube dilution method was used to determine the minimum inhibitory concentrations MIC of gentamicin nanoparticles against the negative and positive bacteria of the Gram stain which are E. coli , St.aureus and Ps.aurogenosa. Serial half dilutions were prepared from a standard concentration of 100 ml and using Muller Hinton Broth. It contained 8.9 ml of
Muller-Hinton broth, then inoculated with 1 ml of bacterial suspension at a concentration of $5.1 \times 10^5$ cells / ml and incubated at 37°C for 18-24 hours [12].

3. Results and Discussion

3.1. Nano properties

3.1.1. Atomic Force Microscopy (AFM)

The surface characteristic of gentamicin nanoparticles were determined by atomic force microscopy (AFM). The average surface roughness was 4.02 nm and the highest grain size was 17 nm were obtained by using AFM test. It was examined the gentamicin gel nanomaterial with SPM probe microscope, showing the homogeneous cumulative distribution of gentamicin nanoparticles and the average grain size equivalent to 68.51 nm, as shown in Fig. 2 and 3.

![Figure 2](image1.png)

**Figure 2.** The diagram shows the distribution of nanoparticles in the Gentamicin Nano gel.

![Figure 3](image2.png)

**Figure 3.** The AFM images shows cumulative homogeneous distribution of SPM-tested Nanoparticles of Gentamicin.

3.1.2. SEM and TEM electron microscope

The SEM assay confirmed that the gentamicin particles reached the nanoparticle shape and size. The test also showed four dimensions of the gentamicin nanoparticle ranging from 19-31 nm as shown in Figure (4). The gentamicin nanoparticles are relatively spherical and uniformly shaped particles and this result is agree with AFM test. There was a minimal amount of particle clumping as well. Agglomeration is a phenomenon that arises from the sticking of particles together one after the other, the reason for this is that gentamicin has a hydrophilic nature; Inevitably traces of water remain inside the particle or near the surface [13]. The samples were examined with an EDX device attached to a scanning electron microscope, through which the quality and quantity of elements present in each sample as shown in Figure (5), and the peaks of Cadmium and Calcium were observed in the figure, in addition oxygen that served as a cover for the nanoparticles.
The SEM images show gentamicin nanoparticles.

Figure 5. Shows the EDX pattern of gentamicin nanoparticles.

Table 1. Quantities of elements according to their presence in gentamicin Nano gel.

| Elt | Line | Int  | Error  | K      | Kr   | W%  | A%  | ZAF  | Ox%  | Pk/Bg |
|-----|------|------|--------|--------|------|-----|-----|------|------|-------|
| C   | Ka   | 482.9| 126.4111| 0.1743| 0.0671| 31.81| 43.91| 0.2108| 0.0671| 1545.62|
| O   | Ka   | 647.6| 126.4111| 0.2375| 0.0914| 38.69| 40.09| 0.2363| 0.0914| 220.55|
| Na  | Ka   | 40.1 | 145.9400| 0.0152| 0.0058| 1.21 | 0.88 | 0.4816| 0.0058| 37.43 |
| Mg  | Ka   | 145.1| 145.9400| 0.0558| 0.0215| 3.41 | 2.33 | 0.6293| 0.0215| 77.57 |
| Al  | Ka   | 353.1| 145.9400| 0.1376| 0.0530| 7.66 | 4.71 | 0.6914| 0.0530| 149.48|
| S   | Ka   | 470.9| 66.5366| 0.1928| 0.0742| 8.77 | 4.53 | 0.8458| 0.0742| 160.29|
| Cl  | Ka   | 57.8 | 66.5366| 0.0241| 0.0093| 1.16 | 0.54 | 0.7974| 0.0093| 18.90 |
| K   | Ka   | 40.6 | 43.0012| 0.0176| 0.0068| 0.80 | 0.34 | 0.8455| 0.0068| 11.82 |
| Ca  | Ka   | 327.8| 43.0012| 0.1450| 0.0558| 6.47 | 2.68 | 0.8625| 0.0558| 76.55 |

The transmission electron microscope is a good tool for confirming the structure and shape of materials. The images taken with TEM showed that the nanoparticles of gentamycin were spherical, uniformly distributed and have a rough surface as shown in Fig. (6) a, b. The result of the TEM assay confirmed the surface roughness of the gentamycin nanoparticles and the
homogeneous cumulative distribution. It is important, since surface roughness plays a major role in controlling the initial release of antibiotics, as rough surfaces establish a larger area for antibiotic release [14,15].

Figure 6. a,b Shows (TEM) images of gentamycin nanoparticles, as it shows the regular spherical shape and the homogeneous cumulative distribution.

3.2. Biological activity test

The bacteriological assay of gentamycin nanoparticles gel was performed by using two kinds of bacteria *Staphylococcus epidermidis* and *Escherichia coli* were obtained from the culture collection of Veterinary Drug Center, Ministry of Industry and Minerals, (Baghdad, Iraq). Figure (7) showed the clear zone represent the inhibition zone (no bacterial growth). Also, the nanoparticle gel showed super inhibitory ability, and the diameters of inhibition for *St. epidermidis* and *E. coli* were 27 mm and 29 mm, respectively and that is due to their different cell walls. In the other side the inhibitory ability for standard gentamicin for *E. coli* and *St. epidermidis* were 20 mm,22 mm , respectively as shown in Fig. (8), the zone S means standard gentamicin and the zone T means gentamycin nanoparticles. The reasons for the inhibitory ability of gentamycin nanoparticles higher than the inhibitory ability of standard gentamicin due to the Nano encapsulation gentamicin enhances its cellular accumulation and improves its effectiveness against intracellular bacteria[16] and also due to nanoparticles structures, and the antibacterial activity increased with increasing the anti-biotic content [17].

Figure 7. Show Inhibitory activity of Gentamycin gentamycin and Nanoparticles in E. coli and St. epidermidis. 

Figure 8. Show Inhibitory activity of the standard Gentamicin Nanoparticles in E. coli and St. epidermidis.

3.2.1. Determination of the MIC of gentamicin nanostructures

The minimum inhibitory concentrations (MIC) test is useful in order to define: if gentamicin maintains its activity, after transform it to the Nano form, by using it with three kinds of bacteria *Escherichia coli* ATCC10536 , *Pseudomonas aeruginosa* ATCC15442 they are Gram negative bacteria and *Staphylococcus aureus* ATCC6 538 it is Gram-positive bacteria, also it used fungus *Candida albicans* ATCC10231 were obtained from the culture collection of Ibn Sena Center for research, Ministry of Industry and Minerals, (Baghdad, Iraq). After 24/48 h of incubation at 37 °C, the test tubes were examined for possible bacterial turbidity and the MIC of each test compound was determined as the lowest concentration that could inhibit visible bacterial growth [18]. The results was found that gentamicin nanomaterial has inhibitory efficacy as
shown in table (2) the concentrations 0.01mg/ml and 0.005 mg/ml have no bacterial growth in all bacteria kinds and the fungus. But the concentrations 0.0025 mg/ml and 0.00125 mg/ml have bacterial growth in E.coli, St.aureus, Ps.aurogenosa and growth in Candida albicans. The MIC of gentamicin nanostructures was the concentration 0.00125 mg/ml.

**Table 2.** Shows the MIC of gentamycin nanostructures.

| Concentrations mg/ml | E.coli ATCC 10536 | St.aureus ATCC 6538 | Ps.aurogenosa ATCC 15442 | Candida albicans ATCC 10231 |
|----------------------|------------------|---------------------|--------------------------|---------------------------|
| 0.01                 | -                | -                   | -                        | -                         |
| 0.005                | -                | -                   | -                        | -                         |
| 0.0025               | +                | +                   | +                        | +                         |
| 0.00125              | +                | +                   | +                        | +                         |

(+) presence of bacterial growth; (−) absence of bacterial growth

**Conclusions**

1. The antibiotic Gentamicin transform successfully to the Nano form with the average grain size 68.51 nm with using Sol-gel method.
2. The MIC of the Nano-Gentamicin was 0.00125 mg/ml.
3. AFM, SEM and TEM tests confirm that is the Nano-Gentamicin particles have the surface roughness and the homogeneous distribution.
4. The biological activity of Nano-Gentamicin was superior than the biological activity of standard Gentamicin.

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