The Antibiotic Capability of Silver Nanoparticles Depending on Different Sizes

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Abstract

Nano materials, for their minute sizes and variable shapes, possess special properties. Current Studies have discovered that silver nanoparticles can be used as broad- spectrum antibiotic to which bacteria are not able to evolve resistance, providing multiple possibilities of practical application of silver nanoparticles in medical field. Literary review shows that the antibiotic capability of silver nanoparticles is influenced by several factors including sizes, but the qualitative research on the relationship between antibiotic effect and the diameter of silver nanoparticles is scarce. Therefore, in this research, silver nanoparticles are produced in different sizes controlling by the preparation period, and are tested with Escherichia Coli to demonstrate the antibiotic capability of silver nanoparticles in each size. A qualitative conclusion is drawn in the research about the relationship between the antibiotic capability of silver nanoparticles and their diameter.

Key Words: Silver nanoparticles; Antibiotic capability; Diameter; Escherichia Coli; Liquid Phase

Content

1 Introduction

1.1 Literature Review

1.1.1 Sterilization Mechanism of Silver Nanoparticles

Existing studies agree on a clear sterilization mechanism of silver nanoparticles. The first damage caused by silver nanoparticles is interrupting the duplication of DNA molecules. Xiaobao Xie[1] and other authors in their thesis states that silver nanoparticles force DNA molecules of Escherichia Coli to concentrate in the cell nucleus, rather than dispersing randomly, a state in which DNA molecules are not able to conduct regular duplication process, therefore impeding the reproduction of bacteria.

Silver nanoparticles can also function as catalyst to ignite the process of producing other substances with germicidal activity. Inoue y, Hoshino m, Takahashi H, and other authors [2] in their study discovered that silver nanoparticles can activate the oxygen in water or air to produce hydroxyl free radical and reactive oxygen species that inhibit the growth of bacteria or eliminate them.

Silver nanoparticles also target the cytomembrane of bacteria. In the research of Kim K J, Sung W. S., Suh B. K. [3], researchers unveiled that under the examination of TEM, the cytomembrane was severely destructed by silver nanoparticles and became porous, with the change of membrane permeability. Large amount of glycoprotein, protein, and potassium ions leak from the inside of bacteria, depleting ATP and leading to the death of bacteria.

However, questions that in what state silver nanoparticles exist inside the bacteria and with what substance on the cytomembrane silver nanoparticles attach still remain unsolved and require further discussion, which have significant meaning to reach more comprehensive understanding of sterilization mechanism of silver nanoparticles.

1.1.2 Methods of Producing Silver Nanoparticles

As the development of technique in nanomaterial sphere, a variety of methods of producing silver nanoparticles was invented recently. Classified by the state in which the chemical reaction occurs, the methods can be divided into gas phase, solid phase, and liquid phase; categorized by the reaction condition, means of producing silver nanoparticles includes redox reaction, electrolysis, photocatalysis etc. [4] Preparing silver nanoparticles in gas phase requires large investment and energy consumption but low productivity; silver nanoparticles produced by solid phase often have relative large diameter and broad range of sizes; therefore, liquid phase method is always adopted to produce small amount of silver nanoparticles with minimum cost[5]. Furthermore, compared with redox reaction, electrolysis and photocatalysis require complicated equipment and are less controllable. Thus, silver nanoparticles in this research are prepared by redox reaction in liquid phase, with primary reagents of $AgNO_3$ and NaH_2PO_2, which have minor effects on environment and toxicity [5].

1.2 Purposes of Research

Since the discovery of antibiotics, it has become a common method to cure the diseases caused by bacteria. However, the bacteria may evolve resistance to traditional antibiotics, Penicillin for example. To be effective, new generation of antibiotics have to be invented, which may results in stimulating the emergence of superbugs- this has become an issue for modern medicine. As the development of nano-technology, nanoparticles-especially those of silver- are founded to possess antibiotic property without causing drug resistance of bacteria. The antibiotic effects of silver nanoparticles are influenced by several factors, according to the completed studies, including diameter, shape, concentration, and coating substances [6]. Each factor can affect the contact between nanoparticles and bacteria, leading to the different strength of germicidal activity. Therefore, accurate explanation of the effects of each factor is vital to practical application of nanoparticles in medical field. Among these influential factors, size of silver nanoparticles has a major effect on the antibiotic ability. Research has proven that the antibacterial effect of silver nanoparticles is inversely proportional to the diameter, for that specific surface area increases as the diameter decrease, which enhance the permeability of nanoparticles [7]-[8]. Thus, studying the relationship between diameter of nanoparticles and their antibiotic effects can make contribution to further the function of silver nanoparticles in eliminating bacteria.

2 Experiment

2.1 Equipment and Reagents

Table 1.1 Equipment

Name	Type	Manufacturer

Table 1.2 Chemical reagents

Name	Type	Manufacturer
PVP	K90	BOSF
Escherichia coli	ATCC25922	LUWEI CHEMISTRY
Sodium hexametaphosphate	AR	FUCHEN FINECHEMISTRY
Sodium hypophosphite	AR	FUCHEN FINECHEMISTRY
Acetone	AR	-----
Ethanol	AR & 75%	-----
Distilled water	-----	Watons
Silver nitrate	AR	-----
Nitric acid	AR	-----
Peptone	AR	-----
Beef extract	-----	-----
Agar	AR	-----
Sodium chloride	AR	-----

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Ethanol	AR & 75%	——
Distilled water	——	Watons
Silver nitrate	AR	——
Nitric acid	AR	——
Peptone	AR	——
Beef extract	——	——
Agar	AR	——
Sodium chloride	AR	——

2.2 Synthesis of Silver Nanoparticles

Silver nanoparticles in this research are prepared by redox reaction in liquid phase in following procedures:

Prepare oxidizing agent (1.60g $AgNO_3$ +10mL distilled water) and reducing agent (50mL distilled water + 0.44g NaH2PO2 + 0.40g PVP + 0.20g $(NaPO_3)_6$+1mL 1M HNO_3 solution); under 40 ℃, drip oxidizing agent into reducing agent at the rate of 12 drops per minute while stirring at 400 rpm for 90 minutes, 150 minutes, and 360 minutes; the product is purple black solution. Centrifuge the solution at 12000 rpm for 20 minutes to acquire the grey deposit; wash the deposits with 2ml acetone three times and centrifuge the sample 2 minutes for each run; dry the final product in the vacuum drying oven for 7 hours and disperse the powder silver nanoparticles in water and PBS by ultrasonic cleaner.

2.3 Preparation of Biofilm

To make biofilms for the test of silver nanoparticles' antibacterial property, Escherichia coli is chosen as experiment strain. The following are the specific experiment procedures.

1) All equipment that will be used is sterilized in an autoclave sterilizer for 20 minutes. Then, E. coli is cultivated in the fluid medium (the medium without agar) that is prepared after the sterilization for 24 hours.
2) The bacteria solution is diluted in three different concentrations: original solution, ten percent of original solution and one percent of original solution. Then mediums are divided into three groups, each of whom is inoculated with $300\mu L$ of bacteria solution.

2.4 Antibiotic Effect Test of Silver Nanoparticles

Pieces of circle paper saturated with solution of silver nanoparticles in different sizes are placed on medium. Then, solid mediums carried with bacteria and silver nanoparticles are stored in a constant temperature incubator for 24 hours to observe the extent of bacteria's reproduction. The biofilms are divided into three identical groups, each group containing three biofilms in bacteria

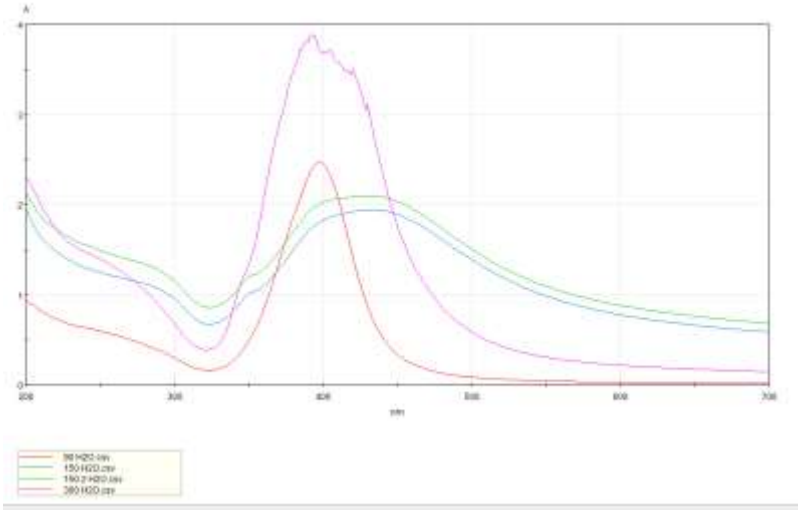
concentration of 100%, 10%, and 1% respectively.

3 Results

To exam the product of silver nanoparticles, this research adopts UV-VIS to verify that the final product belongs to silver nanoparticles, size analyzer to determine the diameter of nanoparticles, and TEM to validate the result of UV-VIS and size analyzer with additional information about the structure and shape of nanoparticles; the results of antibacterial test reveal the antibiotic effect of silver nanoparticles.

3.1 Data of Silver Nanoparticle

3.1.1 UV-VIS



According to the graph above, the maximum absorbance wavelength occurs at 400nm, 420 nm, and 400 nm for 90 minutes, 150 minutes, and 360 minutes samples respectively. The wavelength range from 400 to 420 nanometers directly meets the optimum maximum absorbance wavelength of silver nanoparticles [9], showing that the colloid contains mainly silver nanoparticles of size 1-100 nm.

3.1.2 Size-analyzer

Table 1.3 The size result of silver nanoparticles

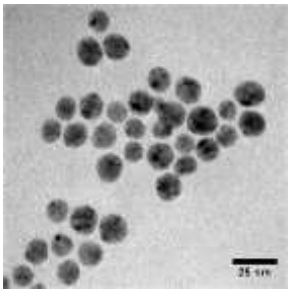
Reaction time(min)	Dispersing agent	Average diameter(nm)	Cumulative frequency at 20nm	Cumulative frequency at 50nm	Maximum absorbance wavelength (nm)	Shape
90	Water	64.946	0.1223	0.6658	400	Round
150	Water	53.866	0.3345	0.8300	420	Round
360	Water	72.588	0.0707	0.5676	400	Round

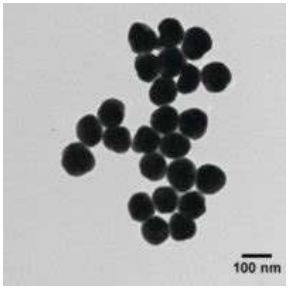
From the chart, the average diameters of 90, 150, and 360 minutes reaction time are 64.95 nm,

53.87 nm, and 72.59 nm. As reaction time increases, the size of nanoparticle is expected to increase [9]. However the 150 minute sample is inconsistent with this finding, because to avoid silver mirror reaction, the pH value in the 150 minute sample is higher than the other two counterparts, which caused a smaller diameter size. Excluding human error in the experiments, the result still shows positive correlation between reaction time and diameter of silver nanoparticles.

3.1.3 TEM

The TEM result validates diameter of silver nanoparticles obtained from size analyzer. It also shows that most silver nanoparticles are spherical, which is in favor of effective functioning as antibiotic to silver nanoparticles.





3.2 Antibacterial Capability

The antibiotic effect of silver nanoparticles is directly reflected by the width of inhibition zone. According to data in table 1.4, the antibacterial capability of silver nanoparticle is directly proportional to its diameter. The 150 minutes samples, however, do not follow the trend: with smallest particle diameter, they do not produce the broadest inhibition zone. Referring to process of the experiments and literature review, it is conjectured that different pH value in 150 minutes samples may influence the result of antibiotic test.

Table 1.4 Width of Inhibition Zone

Bacteria Concentration / Stirring Time	1%	10%	100%
90 min.	0.30	0.13	
	0.40	0.19	<0.1
	0.20	0.15	
	Ave. 0.30	Ave. 0.16	DNE
150 min.	0.20	0.22	
	0.30	0.10	<0.1
	0.25	0.14	
	Ave. 0.25	Ave. 0.15	DNE
360 min.	0.15	0.12	
	0.2	0.12	<0.1
	0.18	0.20	
	Ave. 0.18	Ave. 0.14	DNE
Control Group	0	0	0
	0	0	0
	0	0	0

*The width of inhibition zone, in centimeter, is determined by the largest width of each loop; the experiments for 100% bacteria result in tiny loops whose widths are less than 0.1 centimeters, which is below the minimum range of measuring tool.

4 Discussion

Silver mirror reaction occurs when the silver nanoparticles are prepared. pH value is adjusted to

avoid the precipitation of silver nanoparticles, but the unexpected phenomenon cannot be totally eliminated from the experiments. It is speculated that temperature may be influential factor in producing process that determines whether the silver mirror will appear. The silver nanoparticles are relatively large in the final product, and form cluster randomly. According to literature review, under high reaction temperature, Brownian movement combined with long reaction time lead to frequent collision of particles and the formation of cluster eventually [8]. Therefore, the quality of silver nanoparticles can be improved by reduce the reaction time.

Some silver nanoparticles are plate-shape or irregular shape in the product. The shape of silver nanoparticles is affected by the type and usage of dispersing agent [10]. Thus, the shape of silver nanoparticles can be amended by change the dispersing agent in the experiments.

Because of unsatisfactory diameter and shape of silver nanoparticles, the trend in results is not very distinct as expectation. This is the aspect that can be improved by in further research and experiments.

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