

# Facile formation of colloidal silver nanoparticles using electrolysis technique and their antimicrobial activity

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In this research work, colloidal silver nanoparticles were successfully produced via an electrolysis method and their antibacterial activity was evaluated. This study aims to determine the optimum concentration of colloidal silver nanoparticles to produce an effective inhibition zone for *Escherichia coli* bacteria growth. Based on the results obtained, the applied current and reaction times during the electrolysis played a crucial role in determining the colloidal silver nanoparticles' concentration (ppm). It was found that a minimum content of colloidal silver nanoparticles (0.02 ppm) was required to perform the 0.1 mm inhibition zone diameter on agar medium spread with *E. coli* bacteria. Interestingly, high concentrations of colloidal silver nanoparticles could exhibit high conductivity ( $\mu\text{S}/\text{cm}$ ) as well as the high intensity of the maximum plasmon peak from the UV/vis absorption spectra at about 390 nm. In conclusion, this research work revealed the destruction of the cytoplasmic membrane and the rupture of the internal organisation of *E. coli* bacteria, leading to the leakage of the cytoplasmic contents and cell death after having contact with sufficient concentration of colloidal silver nanoparticles.

**1. Introduction:** In recent years, the germs or bacteria are becoming more resistant towards the present antimicrobial agents [1]. Therefore, executing research for generating a bacteria-free environment area has been the passion for scientists, which can provide us a healthy and clean environment. In fact, the finding of the antibacterial source is crucial in leading a high quality of life, which is in harmony with nature. To date, the colloidal silver solution has been established as a potential antibacterial agent [2]. Indeed, colloidal silver nanoparticles had been used in the past to cure wounds and infections as it possessed antibacterial potency.

Indeed, colloidal silver is a suspension of nano-sized particles in distilled water [3]. The capability of the silver nanoparticles to inhibit bacterial growth is related to the fact that silver nanoparticles can bind to the thiol group in bacteria protein. A thiol group is basically a group of proteins responsible for the enzymatic activity. Thus, when silver nanoparticles combined with the thiol group it caused the deactivation of enzyme and protein in bacteria cells. Besides that silver nanoparticles can also denature the DNA molecules in bacteria by causing the DNA molecules to condense and lose their replication abilities [4]. On the other hand, Brown and Smith reported that silver nanoparticles were found to be deposited in the vacuole and cell wall as granules which caused the bacterial cells to increase in size and exhibited structural abnormalities and finally lead to cell death of the bacteria [5]. This literature showed that colloidal silver nanoparticles are a potential weapon in eliminating bacteria growth. Nevertheless, the production of colloidal silver nanoparticles by the conventional method is not that environmentally friendly and costly. For instance, silver nanoparticles that originate from silver nitrate are produced by the reaction where silver foil reacts with nitric acid and resulting in silver nitrate, water and oxides of nitrogen ( $3\text{Ag} + 4\text{HNO}_3$  (cold and diluted)  $\rightarrow 3\text{AgNO}_3 + 2\text{H}_2\text{O} + \text{NO}$ ) [6]. In fact, the production of toxic nitrogen oxide gas is not an eco-friendly move and expensive maintenance cost is required in order to treat the gas in order to convert it into something harmless [7].

In fact, electrolysis is a process of forcing electricity through the cell to induce a non-spontaneous redox reaction to occur. At the anode, the silver electrode is oxidised to silver ions ( $\text{Ag} \rightarrow \text{Ag}^+ + \text{e}^-$ ) and some of the silver ions were carried by the

electrolyte and further reduced at the cathode and resulted in colloidal silver nanoparticles ( $\text{Ag}^+ + \text{e}^- \rightarrow \text{Ag}$ ) [8]. In this research, colloidal silver nanoparticles were generated by electrolysis due to the simple procedure and yet environmental friendly. In this study, several important parameters such as applied voltage, time of electrolysis reaction and mass change in silver electrodes will be studied in order to produce the optimum concentration of colloidal silver nanoparticles for high antibacterial performance. It is expected that the higher concentration of colloidal silver nanoparticles produced, the higher the potential of antimicrobial activity.

**2. Experimental procedures:** The experimental set up consists of immersing two polished 99.99% in purity of silver rods (7 mm in diameter) being 10 mm apart from each other in 50 ml distilled water as an electrolyte and connected to a direct current (DC) power supply and ammeter. The electrolyte was stirred at a constant rate of 100 rpm to homogenise the electrolyte and maintain a uniform current during the electrolysis process. The two silver rods act as a source of colloidal silver nanoparticles and a DC power supply was used as a power source to force the dissolution of silver electrodes to occur since it is a non-spontaneous reaction. Next, the ammeter was employed to measure the current which indicates the rate of reaction or the rate of dissolution of silver electrodes. On the other hand, the electrolyte used was distilled water in order to produce higher purity of colloidal silver nanoparticles and the small amount of impurities present in distilled water actually increases the conductivity of the electrolyte that facilitates the rate of dissolution of silver electrodes.

The effect of different applied voltage used in electrolysis was studied in the first trial where the electrolysis process was carried out at room temperature using voltage in the range of 2 to 10 V for a period of 15 min. On the other hand, in the second trial, the effect of different time of electrolysis reaction on the conductivity of the solution and the production of colloidal silver nanoparticles (ppm) was investigated by fixing the maximum voltage at 60 V at room temperature and varying the reaction time from 30 min to 5 h. In both of the trials, the mass change in the silver electrodes was measured accurately using a weighting balance. On the other hand, the conductivity of the solution was measured using a Mettler Toledo conductivity meter and 0.1 demal potassium

chloride (KCl) as the calibration standard. The silver nanoparticles colloidal solutions produced were stored under ambient conditions in glass containers.

The concentration of silver nanoparticles in solutions were determined using flame atomic absorption spectroscopy (iCE™ 3300 AAS Atomic Absorption Spectrometer) using air-acetylene flame. The wavelength selected was 328.1 nm, lamp current of 4 mA, spectral band pass of 0.5 nm and detection limits of 0.007 (µg/ml) when  $n=4$ . The certified silver standard was purchased and used for calibrating the instrument. The aggregation state of silver nanoparticles was observed with a UV-vis spectrometer (Perkin Elmer UV-vis spectrometer) within the wavelength of 200–800 nm. Next, different concentrations of silver nanoparticles solutions were then subjected to antibacterial activity. The antibacterial test was performed using disk diffusion assay [9]. This test evaluates the ability of the colloidal silver nanoparticles to inhibit the bacterial growth by measuring the diameter of the zone of inhibition. Briefly, different concentrations of colloidal silver nanoparticles were applied to the agar medium spread with *Escherichia coli* bacteria using paper discs. The diameters of the zone of inhibition induced by the solution were compared with the agar plate spread with bacteria in the absence of silver nanoparticles as a control.

**3. Results and discussion:** In this part of the experiment, the influence of different applied voltage during electrolysis on the formation of colloidal silver nanoparticles is discussed. The average current from the electrolysis at a different applied voltage ranging from 2 V up to 10 V for 15 min is summarised in Table 1. The obtained results showed that applied voltage affected the current of the electrolysis process significantly. It was found that the current generation rate increased linearly with the applied voltage. This reveals that the high reaction dissolution rate of the silver electrode occurred when applying a higher voltage. Nevertheless, a maximum current generation is <1 mA was achieved with a high applied voltage of 60 V.

Besides, electrolysis reaction time is known to play an important role in the formation of colloidal silver nanoparticles [10]. Therefore, in this part of the experiment, the electrolysis experiment was fixed at 60 V and different electrolysis reaction time was investigated. Based on the aforementioned discussion, the high current generation at 60 V was preferred due to the high concentration colloidal silver nanoparticles formation from the silver electrode itself. The aim of this section is to observe the colloidal silver nanoparticles formation by optimising the electrolysis reaction time. It is noteworthy to mention that the mass change of silver electrode before and after the electrolysis reaction was insignificant. The main reason might be attributed to the charged silver formed when an atom, or a group of atoms, lose or gain electrons during the electrolysis reaction. In the present investigation, the colloidal silver nanoparticles for different electrolysis reaction times were further evaluated with a conductivity meter to indicate the presence of silver nanoparticles within the colloidal. In general, high concentration colloidal silver nanoparticles solution showed higher conductivity value depend upon the purity of the water, silver, and cleanliness of all equipment used during colloidal silver

production [11]. The average conductivity reading (µS/cm) for different electrolysis reaction times based on the conductivity meter are summarised in Table 2. The primary conductivity of distilled water used in our study was about 0.5 µS/cm before conducting the electrolysis process. The current through the distilled water as an electrolyte, two silver metallic rods were in it which, in turn, is linked with the ends of a power source. In this case, the movements of the free ions ( $\text{Ag}^+$ ) generated during the electron flow in and out of the electrolysis cell were observed. These furnished cations ( $\text{Ag}^+$ ) move towards the cathode (negative electrode) and anions towards the anode (positive electrode) [12]. This free motion of both types of ions gives rise to electrolytic conduction by producing the silver nanoparticles within the colloidal solution.

AAS analysis was conducted to determine the average parts per million (ppm) of the silver nanoparticles present in the colloidal with different electrolysis reaction times. The results obtained from AAS were tabulated in Table 3. Interestingly, it could be found that the increase in average parts per million (ppm) of the silver nanoparticles up to 0.5575 ppm was found after 5 h of electrolysis reaction times is likely attributed to the more silver nanoparticles were successfully formed at this longer hour of electrolysis reaction time [13]. In fact, a high concentration of silver nanoparticles within the colloidal solution will provide a higher conductivity value [13, 14]. Indeed, the electrons from the power source were flowing out through the negative electrode (silver) of the electrolysis cell. These flowing electrons were used up in the reduction reaction

**Table 2** Average conductivity reading (µS/cm) for different electrolysis reaction times obtained by a conductivity meter

Voltage, V	Time of reaction, h	Conductivity readings, µS/cm	Average conductivity, µS/cm	Current, mA
60	0.5	3.08 3.09 3.13	3.10	6.23
60	1	3.82 3.81 3.83	3.82	6.23
60	2	4.34 4.33 4.33	4.33	6.23
60	3	4.58 4.55 4.56	4.56	6.23
60	4	4.86 4.90 4.89	4.88	6.23
60	5	5.04 5.06 5.06	5.05	6.23

**Table 3** Average parts per million (ppm) of the silver nanoparticles present in colloidal at different electrolysis reaction times obtained by AAS analysis

Different time of electrolysis reaction, h	Concentration of silver ions produced, ppm
0.5	0.0165
1	0.0193
2	0.0429
3	0.2225
4	0.2344
5	0.5575

**Table 1** Different applied voltages and different currents produced during the electrolysis

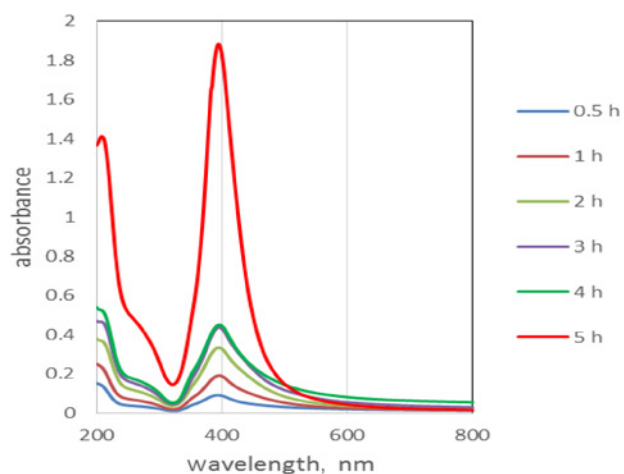
Voltage, V	Time, min	Current, mA
2	15	0.01
4	15	0.26
6	15	0.50
8	15	0.74
10	15	0.97

which took place at the cathode (silver rod). The number of electrons accepted at the negative terminal of the electrode was reverted back to the positive terminal of the power source via positive electrode of the electrolysis cell, where electrons are coming out due to oxidation [15]. In this case, more electrons were flowing in and out from the electrolysis cell and resulted in more silver nanoparticles formation within electrolyte (distilled water).

Fig. 1 shows the UV-vis spectra of silver nanoparticles colloidal prepared with different silver ion concentrations. With increasing the silver nanoparticle concentration, the colour of the solution still remained colourless. The absorption peak at around 390 nm as presented in Fig. 1. The presence of the absorption peak is attributed to the surface plasmon excitation of silver nanoparticles within the colloidal solution [16]. Interestingly, it could be noticed that a weak absorption maximum of surface plasmon peaks was observed at about 400 nm, showing that silver nanoparticles were produced at a relatively low concentration at the 30 min of electrolysis reaction time (0.0429 ppm). Whereas, the intensity of the maximum plasmon peak increased, indicating that higher concentrations of silver nanoparticles were existing within the colloidal solution with increasing the electrolysis reaction time [16].

In many clinical microbiology laboratories, an agar disk diffusion method is used routinely for testing common, rapidly growing, and certain fastidious bacterial pathogens [17]. Disk diffusion tests based solely on the presence or absence of a zone of inhibition without regard to the size of the zone are not acceptable for determining antimicrobial susceptibility. The principle of standardised methodology and zone diameter measurements correlated with the minimal inhibitory concentration of antibacterial agent (colloidal silver nanoparticles) [17, 18]. In the present investigation, the discs were dipped in different concentration of colloidal silver nanoparticles solution for the antibacterial test (*E. coli* bacteria) using disk diffusion assay. The obtained results were recorded and shown in Fig. 2. It can be seen that the increment of the inhibition zone diameter with an increase of the concentration's colloidal silver nanoparticles. Interestingly, an  $\sim 0.02$  ppm of colloidal silver nanoparticles is required in order to perform the 0.1 mm inhibition zone diameter whereas an  $\sim 0.5$  ppm of colloidal silver nanoparticles is required in order to exhibit the 0.2 mm inhibition zone diameter. The low concentration of colloidal silver nanoparticles (0.0165 ppm) was insufficient to produce obvious zone inhibition on agar medium spread with *E. coli* bacteria, which infers that ineffectiveness in killing *E. coli* bacteria.

It is a well-known fact that *E. coli* bacteria are Gram-negative bacteria with an outer membrane, which contains lipopolysaccharides to aid in creating a permeability barrier and protects the cell



**Fig. 1** UV/vis absorption spectra of the colloidal silver nanoparticles prepared via electrolysis of silver rod with different reaction times

[17, 19, 20]. This reduction of inhibition zone diameter phenomenon can be discussed by understanding the difference between the bacterial cell wall and cell membrane properties. The membrane of the tested *E. coli* bacteria disappeared and their surface became crumpled and distorted after having contact with colloidal silver nanoparticles ( $>0.02$  ppm). This study revealed the destruction of the cytoplasmic membrane and the rupture of the internal organisation, leading to the leakage of the cytoplasmic contents and cell death. This statement was in agreement with Ahmad Barudin and co-researchers in which the detachment of the cytoplasm membrane from the cell wall might be attributed to the influence of the colloidal silver nanoparticles that are attached to the bacterial cell membrane and cause structural changes [17]. The antibacterial efficacy of colloidal silver nanoparticles against *E. coli* is related to the presence of these outer membrane porin proteins [19, 20].

Concentration of colloidal silver nanoparticles (ppm)	Minimum inhibition zones produced (mm)	Representative images on zone inhibition produced on agar medium spread with <i>E. coli</i> bacteria
Control	—	
0.5 h (0.0165)	—	
1 h (0.0193)	0.1	
2 h (0.0429)	0.1	
3 h (0.2225)	0.1	
4 h (0.2344)	0.1	
5 h (0.5575)	0.2	

**Fig. 2** Inhibition zones produced on agar medium spread with *E. coli* bacteria by loading different concentration of colloidal silver nanoparticles (ppm) using paper discs

**4. Conclusion:** In summary, different concentrations of colloidal silver nanoparticles were successfully produced by controlling the movements of the free ions generated during the electron flow in and out of the electrolysis cell and eventually formed silver nanoparticles within the distilled water. It can be concluded that the increment of the inhibition zone diameter could be achieved when the concentration of colloidal silver nanoparticles increased. The detachment of the cytoplasm membrane from the cell wall may be attributed to the effect of the silver nanoparticles that are attached to the bacterial cell membrane and cause structural changes.

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## 6 References

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