International Journal of Nanoscience and Nanoengineering
2018; 4(3): 29-34
http://www.openscienceonline.com/journal/nano

Biosynthesis of Silver Nanoparticles Using Starch Isolated from *Nypa fruticans (Thunb.) Wurmb* (Nipa) Seeds

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To cite this article
Vanessa Jane Rotamula Calderon, Karina Milagros Rebadulla Cui-Lim. Biosynthesis of Silver Nanoparticles Using Starch Isolated from *Nypa fruticans (Thunb.) Wurmb* (Nipa) Seeds. *International Journal of Nanoscience and Nanoengineering.* Vol. 4, No. 3, 2018, pp. 29-34.

Received: May 31, 2018; **Accepted**: June 29, 2018; **Published**: August 6, 2018

Abstract

Nanotechnology is still on its early stage of development and yet, it already constitutes vast range of applications but only few methods are known to synthesize these nanomaterials. In this study, another biosynthesis of silver nanoparticles (AgNPs) was exemplified and is more efficient and economical alternative for large-scale resolves. Starch from Nypa fruticans (Nipa) seeds had ministered the synthesis of AgNPs as both reducing and capping agent. Nipa starch first undergone series of physical property tests and compared it to commercial corn starch. Thereafter, the synthesis of AgNPs through reduction method was done in a thirty-minute reaction period and the resulting AgNPs were further characterize through UV-Vis Spectroscopy in which resulting peak confirms the presence of AgNPs and Scanning Electron Microscopy (SEM) in which the surface morphology of the sample was determined. The AgNPs was identified to have a rod-like shape and has a diameter of 30um at x2000 and x25000 magnification. The present study shows a new way of synthesizing AgNPs which can further be applied to various applications and more importantly a potential breakthrough for the field of nanotechnology.

Keywords

Biosynthesis, Silver Nanoparticles, Nipa Starch

1. Introduction

Nanotechnology involves the study of minute particles with sizes ranging from one to one hundred nanometers (1-100 nm). As compared to its size, nanotechnology has developed widespread to different fields of science such as physics, biology, and chemistry.

Due to its extremely small size, these nanoparticles can present more different properties than that of a typical large particle. It exhibit effective catalytic activity and enable them to produce potential applications.

Syntheses of nanoparticles are widely developed and various researches came up with different ways of preparing nanoparticles. As stated in various literatures, there are two general methods used in the synthesis of nanoparticles namely: "Top Down" and the "Bottom Up" processes. The "Top Down" process is the broken down of starting material to reduce into smaller particles, while in "Bottom Up" process, nanoparticles are built by joining together smaller particles such as atoms and molecules.

This study used plant as raw material in synthesizing silver nanoparticles. Lots of articles and researches have used plants to synthesize AgNPs due to its convenience and it could prevent high risk of hazards compared to the earlier physical and chemical methods used in synthesizing AgNPs.

This study used starch isolated from Nypa fruticans seeds to synthesize AgNPs. Nypa fruticans is a native in the Philippines particularly in Northern Samar. It is the only species of palm considered adapted to the mangrove biome. Only the leaves and fruits appeared above the water and its stems and the lower part are all submerged in the water and the stem and the lower part are all submerged in the water.

In this study, starch was first isolated from the mature seeds of Nypa fruticans. The starch obtained undergone tests to determine its properties and then used it to synthesize

AgNPs; the synthesized AgNPs undergone characterization through Ultra-Violet Visible (UV-Vis) Spectroscopy and Scanning Electron Microscopy (SEM).

This study aimed to broaden the people's knowledge about the seeds of Nypa fruticans and the potential applications of AgNPs and unveil its hidden abilities that are soon be needed in different industries.

2. Methodology

2.1. Collection and Preparation of Plant Material

Collected fruits of Nypa fruticans (Nipa) were washed to remove the dirt and dehulled manually to get its seeds. The seeds were washed and air-dried.

2.2. Starch Isolation

1) About 1.5 kg of ground nipa seeds was weighed and soaked in 1% (w/v) of sodium metabisulfite solution for an hour.
2) After soaking, seeds were placed in the osterizer with distilled water and grinded to paste and then squeezed using cheesecloth.
3) Filtrate was soaked with 1% sodium metabisulfite and allowed to settle.
4) Supernatant was then discarded and the starch mucilage was washed four times with 1% sodium metabisulfite and allowed to settle for 90 min after each wash.
5) Washed starch mucilage was then dried at 60°C in a drying oven for 12 hours
6) Dried starch was then pulverized and stored for further analysis.

Test for Properties of Starch

Foam Capacity

1) About 0.5g of the starch obtained from nipa seeds was blended with 25 mL distilled water in a domestic blender for 5 minutes.
2) Homogenate was poured into a 100 mL graduated cylinder and the initial volume was recorded after 30 seconds final volume was recorded.
3) Foam capacity was expressed using the formula:

$$\frac{final\ volume - initial\ volume}{initial\ volume} x100$$

Emulsion Capacity

1) About 0.5g of nipa starch was dispersed in 3 mL distilled water using a mixer for 30 seconds.
2) After complete dispersion, 5 mL of vegetable oil was gradually added and continued mixing for another 30 seconds.
3) Suspension was centrifuged at 1600 rpm for 5 minutes. The amount of oil from the sample was directly read from the tube.
4) Emulsion capacity was determined with the formula:

$$\frac{g\ starch}{oil\ emulsified} x100$$

Gelatinization Temperature

1) About 0.5g of starch sample was placed in a 20 mL beaker and 10 mL distilled water was added.
2) Dispersion was heated on a hotplate with a thermometer suspended until the solution thickened and became sticky and thus temperature was recorded as its gelatinization temperature.

Water Holding Capacity

1) Five percent w/v of starch solution was dispersed in pre-weighed centrifuge tube.
2) Tube with starch solution was then agitated for 10 minutes.
3) After agitation, the solution was centrifuged for 20 minutes.
4) Supernatant was then discarded and the weight of paste was taken. As the weight of water bound by dry starch, the water holding capacity was expressed by:

$$\frac{weight\ of\ water\ bound\ by\ starch}{weight\ of\ original\ sample}$$

Swelling Power

1) About 0.1g of starch was placed in the pre-weighed test tube and 10 ml of distilled water was added.
2) The starch solution was then subjected to water bath with starting temperature of 50°C with continuously stirring for 30 minutes.
3) Test tubes with starch solution was then cooled and centrifuged for 20 minutes. Weight of the paste was recorded.
4) The procedures were then repeated for different temperatures until 90°C and swelling power was expressed by:

$$\frac{g\ starch\ paste}{g\ dry\ starch\ sample}$$

Determination of pH

Dispersed 20% w/v starch solution was shaken in water and the pH was determined using a pH meter.

Browning and Charring Temperature

Small amount of starch was put into a capillary tube. Browning and charring temperatures was determined using a melting point apparatus.

All parameters were taken in three trials and the average was obtained. Corn starch was used and undergone series of tests with these similar parameters to compare and affirm the results of the nipa starch's properties. The significance of results of both starches was analyzed using T-test.

Synthesis of Silver Nanoparticles (AgNPs)

1) About 1g of starch was added to 100mL of distilled water and heated with stirring on a hotplate until starch was dissolved.
2) Ten milliliters of the starch solution was added to 50 mL of 1 milimole (mmol) silver nitrate solution and was kept on hotplate with stirring for 30 minutes.

3) The overall reaction process was carried out in an improvised dark room to avoid unnecessary photochemical reactions.
4) Synthesis of AgNPs was determined by the change of color of the solution; from colorless to yellowish brown.
5) Synthesized AgNPs was purified through repeated centrifugation for 20 minutes and then washed with distilled water.
6) The purified AgNPs was weighed in analytical balance and then diluted into 100 mL solution in a volumetric flask and served as a stock solution for characterization.

Characterization through UV-Vis Spectroscopy

1) UV-Vis spectrophotometer was warmed up for 15 minutes. Wavelength was calibrated and started blanking the instrument.
2) Different concentrations (0.08 M, 0.04 M, 0.02 M) was made from the previous stock solution.
3) Wavelength of 350nm-550nm was used as ranged for each concentration.
4) After each concentration, the wavelength was calibrated before working for the next concentration.
5) Absorbance was read after 1 min of placing the sample cuvette in the sample compartment.

Surface Characterization of the Nipa Starch with AgNPs Using Scanning Electron Microscopy (SEM)

1) SEM was used to determine the surface morphologies and the size of AgNPs of the prepared sample.
2) Gold sputtering of the sample was done using a gold coater to enhance conductivity of the sample.
3) Afterwards, it was placed pn copper stubswith carbon tape (RS).
4) Microscope chamber maintained a pressure below 10^{-6} Torr.
5) SEM filament was set at a voltage of 10 kV and a current of 5 nA using 750x and 3500x magnification.

3. Results and Discussion

Starch was successfully isolated from nipa seeds and the results of its properties and its comparison to the properties obtained from corn starch which served as a reference, clearly proved its potential use in industries.

Table 1. *Summary of Results for Starch Properties.*

PARAMETERS	NIPA STARCH	CORN STARCH (Reference)
Foam Capacity	7.25%	0.40%
Emulsion Capacity	2.67g/mL	1.33g/mL
Gelatinization Temperature	84.33°C	77.33°C
Water Holding Capacity	8.75 mL	13.50 mL
pH	5.07	5.31
Browning Temperature	193.33°C	234°C
Charring Temperature	266.67°C	252°C

Table 1 shows the summary of results obtained from the different tests done for the physical properties of the starch obtained from the nipa seeds as compared from the corn starch. This study recorded that the properties of nipa starch has greater value than the corn starch in terms of its Foam Capacity, Emulsion Capacity, Gelatinization Temperature, and Charring temperature. On the other hand, the Water Holding Capacity, Swelling Power, pH and Browning Temperature of Nipa starch has lesser value compared to the Corn Starch.

Foam Capacity of starch is a direct indication of its fat content. This directs that the nipa starch has higher fat content than that of the corn starch and could further suggest nutritional value.

The result in emulsion capacity implies that nipa starch has higher emulsion capacity than the corn starch making it more preferable as an emulsifying agent.

Gelatinization is due to the irreversible change that starches undergo when heated. It absorbs water making granules swell more and become paste (Kolawole, 2013). Gelatinization temperature is also an indication of the minimum cooking temperature. The results indicate that the Nipa starch needs to be cooked much longer at higher temperature than the corn starch.

Water Holding Capacity is the amount of water a starch can hold at room temperature; the result implies that Corn starch (standard) can hold much water than the Nipa starch and can further be observed in its swelling power in Figure 1.

Browning and charring temperatures are the temperatures to which starch can be heated without any color change (Kolawole, 2013). This means that the Nipa starch can withstand high temperatures than the Corn starch and are preferable in industries because of its capability to withstand high temperatures.

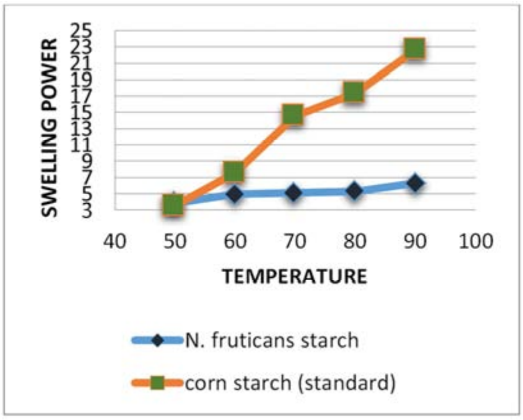

Figure 1. *Swelling Power of Corn and Nipa Starches.*

The result for the swelling power of N. fruticans was plotted as swelling power against temperature. This shows that N. fruticans starch is capable of swelling as temperature increases. Although, there are two swelling pattern that can be observed in the graph; from 50°C -60°C swelling power tends to increase rapidly as well as that in 80°C -90°C. This trend points to the possible two sets of internal bonding forces that ease at different temperatures. On the series, the results of the swelling power for corn starch shows a rapid increase of swelling power from 50°C until 70°C, then from 70°C to 80°C the swelling power slightly slows down its pace and again a rapid increase until 90°C. The result clearly shows that the nipa starch has a less swelling power than that with the standard nevertheless; both starches were seen to have an increasing swelling power as temperature increases to 90°C.

This swelling power of N. fruticans was ascertained of its relevance being used in in pharmaceutical industry as a disintegrant in the formulation of tablets and this high swelling power also means high in digestibility therefore can be used in dietary applications.

As observed from the above results, the properties of two starches; the Nipa and the Corn starches had huge differences in terms of their values; a parametric test was done to show its significance.

Synthesis of Silver Nanoparticles

Synthesis of silver nanoparticles was done using 1g of starch isolated from *N. fruticans* dissolved in 100 mL distilled water added to 1 milimole (0.0085g $AgNO_3$ in 50 mL distilled water) of silver nitrate solution. The solution was then heated in the hotplate and after 30 min of reaction time the color change of the solution was observed. From the initial colorless solution, it turned out to become yellowish brown indication of the synthesized AgNPs as shown in Figure 2. This color change is due to the excitation of surface plasmon resonance (SPR) in AgNPs; the phenomena wherein free electrons present in the reduced AgNPs are collectively oscillating.

Before synthesis

After synthesis

Figure 2. *Before and After Synthesis of AgNPs.*

In this method, starch had a great influence in synthesizing AgNPs as a reducing agent at the same time as a stabilizer. Starch as known to have its two major components; the amylose and amylopectin; in aqueous solution gives off amylase. In hot water, amylase is gradually changing into smaller molecules and catalyses the reduction of silver ion (Ag^+) to elemental silver (Ag). Starch also acts as capping agent; it binds with the formed AgNPs and stabilizes it.

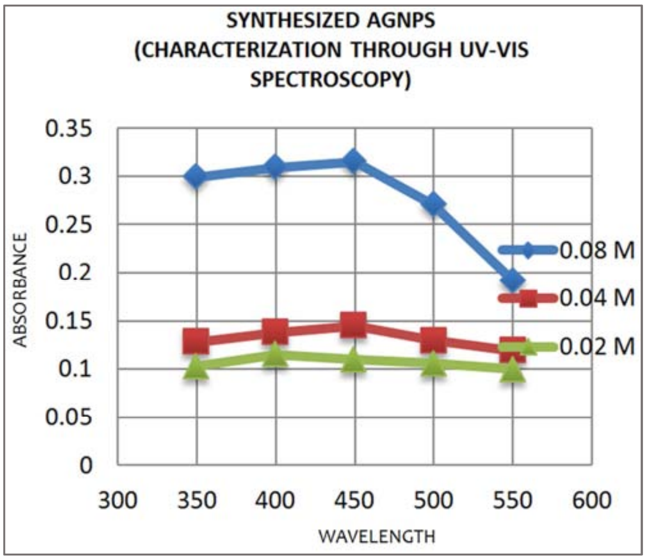

Figure 3. *Absorbance of AgNPs as Characterized trough UV-Visible Spectroscopy.*

Ultra-violet Visible Spectroscopy is the initial stage of characterizing Silver Nanoparticles (AgNPs). A typical AgNPs has a λ max value that is visible in 400-500 nm range. In this research, the absorption peak was identified between around 400-450nm. This result clearly proves the successful reduction of Ag^+ to Ag and of the synthesis of AgNPs. The formation of AgNPs was observed to have increased as concentration increases.

Due to the absence of standard AgNPs in this research despite of all the best efforts given by the researcher, $AgNO_3$ as a positive control and Nipa starch as negative control was used and characterized instead. $AgNO_3$ shows a slightly similar curve with the synthesized AgNPs. Figure 3 show that the absorbance of $AgNO_3$ had a maximum peak at 350nm to 450nm. It is also evident that the absorbance of $AgNO_3$ is much lesser than the absorbance of AgNPs, an indication that the concentration of Ag present in AgNPs is much greater than the concentration in $AgNO_3$.

Starch with AgNPs Morphology

Figure 4 shows the SEM micrographs of the nipa starch with silver nanoparticles (AgNPs) at (a) x2000, (b) x2500, (c) x5000, (d) x7000 magnification. The sample shows a rod-like shape and reveal that nipa starch with AgNPs has a diameter of (a) 30 um, (b) 30 um, (c) 20 um, (d) 10 um. The granules appeared in an agglomerated state.

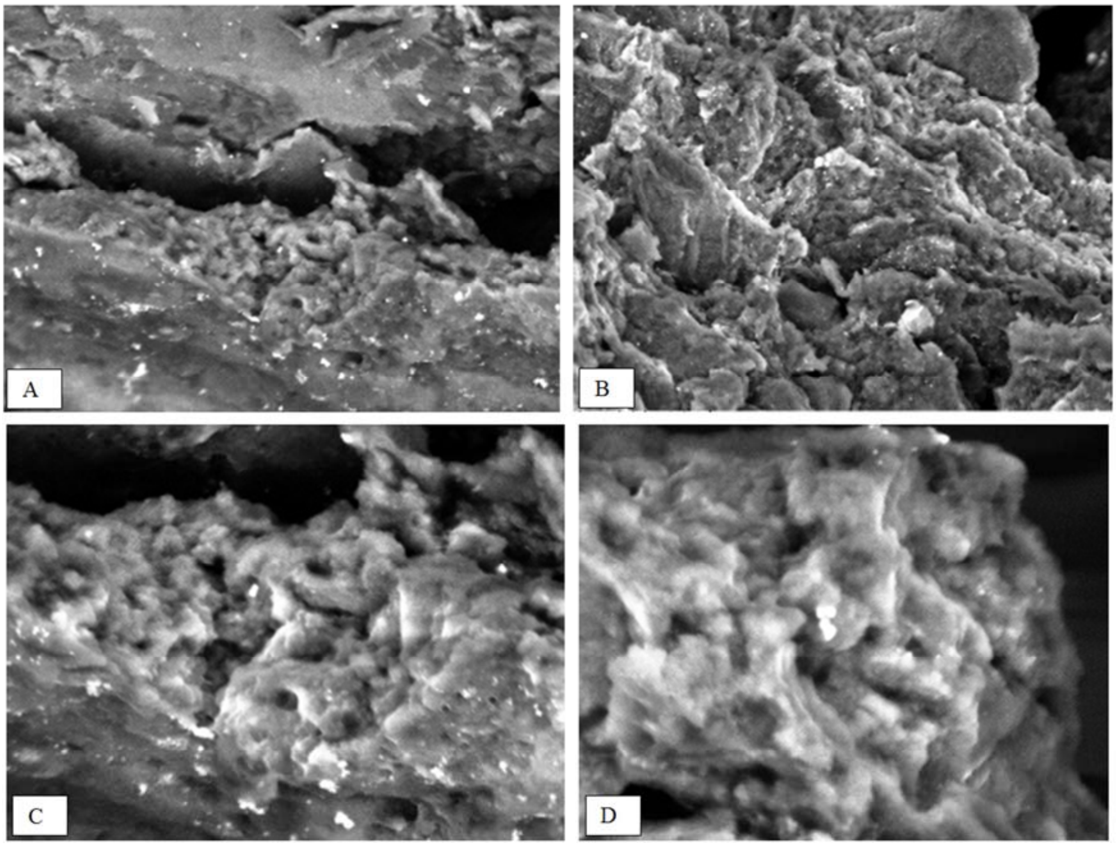

Figure 4. *SEM Micrographs of Nipa Starch with AgNPs.*

4. Conclusions

After all the experiments and analyses of the gathered data, the researcher further concludes the following:

1) Nypa fruticans (Nipa) starch has a greater value than with the Corn starch in terms of their properties namely; Foam Capacity, Emulsion Capacity, and Charring Temperature; therefore, it implies that Nipa is better than the Corn starch and the null hypothesis is rejected in terms of these properties. While the Water Holding Capacity, pH, and Browning Temperature of Nipa starch is lesser than the Corn starch and they have significant difference which means that Nipa is incomparable with the Corn starch in terms of these properties and the null hypothesis was also rejected in these properties. On the other hand, only the Gelatinization Temperature showed a not significant result, therefore the null hypothesis is accepted in this property.
2) Silver Nanoparticles was successfully synthesized using starch isolated from Nypa fruticans seeds.
3) Synthesized silver nanoparticles were initially characterized through UV-Vis Spectroscopy and show a similar maximum peak of 450nm to those typical silver nanoparticles. External morphology and structure illustrated through Scanning Electron Microscopy, reveals that the starch with AgNPs has a rod-like shape and has a diameter of 30 um at x2000 and x2500 magnification, 20 um at x5000 magnification, and 10 um at x7000 magnification. The sample appeared in an agglomerated state.

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