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Synthesis and Characterization of Silver Nanoparticles

Prepared with Honey: The Role of Carbohydrates

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Abstract

Silver nanoparticles were synthesized using honey at pH 5.0 and 10.0 by employing a rapid, low cost, and simple technique. The influence of the honey carbohydrates (glucose and fructose) in the synthesis was characterized. Moreover, the kinetic variables in the synthesis at room

temperature and at pH 5.0 and 10.0 were analyzed by measuring surface plasmon resonance at 411 nm by absorption spectroscopy. Transmission electron microscopy, thermal gravimetric analysis, and differential thermal analysis were employed in order to characterize the metal nanoparticles and the capping agents. The synthesized nanoparticles were obtained for the first time at pH 5.0. This fact allows evaluating the kinetics and reaction mechanism. The obtained nanoparticles were spherical, monodispersed and smaller than 20 nm. The results show that glucose serves as a reducing and capping agent while fructose has a limited reducing effect.

Keywords: differential thermal analysis, silver nanoparticles, surface plasmon resonance, thermogravimetric analysis

INTRODUCTION

There is an increasing interest in silver nanoparticles due use in biotechnology, biomedical applications, bioengineering, catalysis, water treatment, energy storage, optics, electronics, and in food science due to low toxicity, biocompatibility, antimicrobial activity, and optical and electrochemical properties (Maiyalagan 2008; Pradeep 2009; Marambio-Jones and Hoek 2010; Farhadi et al. 2012; Ravi et al. 2013; Kumar and Anthony 2014; Abd-Elaal, Tawfik, and Shaban 2015). Several methods have been introduced for synthesizing and stabilizing silver nanoparticles, including chemical or physicochemical reduction and electrochemical techniques. Usually, these methods are expensive, energy consuming, and dangerous for the environment (Janardhanan et al. 2009; Wojtysiak and Kudelski 2012).

Recently, the developments of environmentally friendly methods that employ natural compounds to synthesize silver nanoparticles have attracted the interest of many researchers. Raveendran et al. performed the synthesis of silver nanoparticles using glucose as the reducing

agent and starch as stabilization agent. Under these conditions, the complete reaction required heating at 40°C for 20 h (Raveendran, Fu, and Wallen 2003). Other authors synthesized silver nanoparticles in an aqueous medium using bamboo hemicelluloses as the stabilizing agent and glucose as the reducing agent by microwave irradiation (Peng, Yang, and Xiong 2013). Dhand et al, carried out a study employing dried roasted coffee seed in the form of a hydroalcoholic extract to prepare silver nanoparticles. The mixture was stirred continuously for 10 min and the solution was incubated at room temperature for 2 h. The silver nanoparticles were dried under an infrared lamp for characterization (Dhand et al. 2016).

Begum et al. used black tea leaf extracts, requiring several steps of filtration to remove the insoluble matter. In addition, reaction time was over an hour (Begum et al. 2009). Baghizadeh et al. used the seed extract of *Calendula officinalis* in the liquid phase. In order to obtain the extract, several washes and filtrations were performed. The synthesis required heating and 24 h to complete the reaction (Baghizadeh et al. 2015). Monodisperse spherical silver nanoparticles were synthesized by Philip at pH 8.5, employing high honey concentrations. A disadvantages of this approach is that the honey constituents do not participate in the reaction, interfering with the subsequent purification of silver nanoparticles. By the other hand, the author does not describe which components are responsible for the reduction of silver ions and the role of sugars was not been evaluated (Philip 2010). Sreelakshmi et al. synthesized silver nanoparticles using high honey concentrations and the reaction takes place within three hours. Nevertheless, the influence of pH, the role of sugars, and the reaction kinetics were not evaluated (Sreelakshmi et al. 2011).

The goal of the present work was to study the synthesis of silver nanoparticles using low honey concentrations in acidic and alkaline media. The synthesis at acid pH has a slow reaction

rate, which allows evaluating the kinetics and mechanism. The influence of operational variables on the reaction kinetic and the role of glucose and fructose in the synthesis were studied. The obtained nanoparticles were analyzed by absorption spectroscopy, transmission electron microscopy, and differential thermal analysis. The synthesis is ecofriendly and does not require washing, filtering, heating, sonication, or microwave treatment. This methodology is simple, low cost, rapid and prepares small silver nanoparticles.

EXPERIMENTAL

Reagents

All reagents were of analytical grade. Ultra pure water (18 M Ω) was used throughout. 5.88×10^{-3} mol L⁻¹ silver was prepared by dissolving 0.0998 g of AgNO₃ (Merck 99.9%) in 100.0 mL in water. Sodium hydroxide solution (0.1 mol L⁻¹) was prepared by diluting 0.40 g in 100 mL with water. Honey aqueous solutions were prepared from commercial honey purchased in local markets from Buenos Aires, Argentina. An enzymatic analysis (Wiener kit Glicemia enzimática AA, Argentina) was employed to determine glucose in honey. Aqueous solutions of glucose and fructose (Sigma-Aldrich) 78.20 and 95.50 g L⁻¹, respectively, were prepared.

Synthesis of Silver Nanoparticles

The honey stock solution was prepared by dissolving 25.0 g of honey in 100.0 mL of water. An appropriate volume of this solution was added to 265 μ L of AgNO₃ solution (5.88×10^{-3} mol L⁻¹) and the pH was adjusted with sodium hydroxide. The volume was reduced to 10.0 mL with water. This diluted honey solution shows a very pale yellow color, almost colorless and was stirred for one minute and the appearance of typical intense brown-yellow

color was observed which indicates the formation of silver nanoparticles. The synthesis was carried out at pH 5.0 and 10.0. The synthesized silver nanoparticles were separated by centrifugation at 12,000 rpm for one hour. The supernatant does not exhibit the characteristic surface plasmon resonance band which indicates the absence of silver nanoparticles.

Characterization of Silver Nanoparticles

The surface plasmon resonance bands of silver nanoparticles were recorded on an Agilent Carry 60 spectrometer from 300 to 700 nm. The size and distribution of silver nanoparticles were obtained with a transmission electron microscope (JEOL 100 CX II). The qualitative effect of the glucose on the synthesis of the silver nanoparticles was performed by thermogravimetric analysis and differential thermal analysis. These analysis were carried out under nitrogen on a Rigaku Thermoflex TG 8110 coupled to a Thermal Analysis Station TAS 100. Centrifugation was performed using a Servall centrifuge with a SM 400 rotor.

RESULTS AND DISCUSSION

Characterization of Honey

Honey is composed primarily of monosaccharides (simple sugars) such as glucose and fructose. This product also contains other numerous types of sugars, as well as acids, proteins, and minerals. The glucose concentration in honey depends on the floral origin of this product (Musa, Özcan, and Ölmez 2014). In silver nanoparticle synthesis, glucose reduces the silver cations from the silver nitrate. For this purpose, glucose content in honey was evaluated by an enzymatic analysis due to its high sensitivity and specificity against other sugars in honey. In this method, glucose oxidase oxidizes the glucose to gluconic acid with the release of hydrogen

peroxide. The quantitative release of hydrogen peroxide produces a secondary reaction that involves the reduction of the colorless 4-aminophenazone to a colored quinine (Trinder 1969). The obtained value was 34.5%.

Absorption Spectroscopy

Silver nanoparticles were synthesized in aqueous solution and their stability was confirmed by absorption spectroscopy. **Figure 1** shows the characteristic surface plasmon resonance band at 411 nm for the silver nanoparticles prepared with equal honey concentrations at two pH values. Silver nanoparticles synthesized at pH 10.0 have narrower surface plasmon resonance band than those obtained at pH 5.0, compatible with monodispersed spherical nanoparticles (Kelly et al. 2003; Philip 2010). These nanoparticles are stable for more than 1 year, as can be seen in the surface plasmon resonance band of silver nanoparticles synthesized at pH 5.0 after this time interval (**Figure 1**).

Silver nanoparticles were synthesized with various honey concentrations at pH 5.0 and 10.0 and the surface plasmon resonance bands are shown in **Figure 2** and **3**, respectively. The plasmon spectral band shifted to shorter wavelength with increasing concentration of honey, indicating a decrease in particle size. Furthermore, nanoparticles were synthesized from various honey samples available in the province of Buenos Aires, Argentina. In all cases, the characteristic surface plasmon resonance bands at 411 nm were obtained.

In order to evaluate the effect of glucose and fructose on the formation of silver nanoparticles, the synthesis at pH 5.0 and 10.0, was carried out employing both sugars and a mixture at the same concentrations found in honey employing the same procedure. Surface plasmon resonance bands were characterized in all cases. **Figure 4** shows the obtained surface

plasmon resonance bands of the resulting silver nanoparticles. When fructose was used as the reducing agent, no characteristic surface plasmon resonance band was observed at 400 nm at pH 5.0. However, at pH 10.0, a broad band from 350 to 600 nm was observed (**Figure 5**). Silver nanoparticles synthesized by glucose at pH 5.0 showed a broad surface plasmon resonance band with a shoulder at 350 nm, probably due to the presence of nanoprisms (Kelly et al. 2003; Millstone et al. 2009; He and Yu 2015). Furthermore, at pH 10.0, a characteristic and narrow surface plasmon resonance band with a slight shoulder was obtained. A similar response at pH 5.0 and 10.0 was found when a mixture of glucose and fructose was used to obtain silver nanoparticles. The addition of fructose in the synthesis of silver nanoparticles at pH 10.0 did not produce a significant change in the surface plasmon resonance band. However, a slightly narrower surface plasmon resonance band was observed when the synthesis is performed at pH 5.0. Glucose acts as the reducing agent in the formation of the silver nanoparticles, while the fructose has a limited reducing effect since its antioxidant capacity is limited by the kinetics of tautomeric shifts (Makarov et al. 2014).

The comparison of silver nanoparticles synthesized with honey, glucose and fructose shows that those prepared with honey provide a symmetric and narrow surface plasmon resonance band at both pH values. These results correlate with small, spherical, monodisperse nanoparticles (Kelly et al. 2003), probably due the contribution of minor honey components, such as amino acids, proteins, aroma compounds, organic acids, phenolic compounds, minerals and trace elements (Lachman et al. 2007).

Transmission Electron Microscopy

Transmission electron microscopy was employed to determine the particle size and size distribution of the synthesized silver nanoparticles. For each sample, two suspension drops were placed in copper grids (200 mesh with a Formvar film), and dried at room temperature. **Figure 6** and **7** shows the images of silver nanoparticles. The size distribution was obtained at 0.10% honey at both pH values. The average sizes of silver nanoparticles were 18.4 ± 0.9 nm and 12.3 ± 0.6 nm for pH 5.0 and 10.0, respectively. These results demonstrate that no aggregates of silver nanoparticles were present.

Transmission electron microscopy images of silver nanoparticles obtained with glucose at pH 10.0 are shown in **Figure 8**. Various shapes, such as nanoprisms and polyhedral forms larger than the silver nanoparticles, were synthesized with honey at the same pH. However, the nanoparticles were primarily spherical.

Thermal Analysis

In order to identify the role of glucose as capping agent, thermogravimetric analysis and differential thermal analysis with the silver nanoparticles obtained with glucose and honey at pH 10.0 were performed under nitrogen. The silver nanoparticles suspensions were lyophilized and weighed on an analytical balance prior analysis. **Figure 9** shows that the thermogravimetric results from silver nanoparticles synthesized with honey and glucose were similar and show an initial weight loss from 50 to 170°C due to the loss of adsorbed water. The second weight loss, which accounted for 40% of the total silver nanoparticle weight, is probably due to pyrolysis of glucose on the surface of the silver nanoparticles (Pavlath, and Gregorsky 1985). Similar differential thermal analysis results between silver nanoparticles synthesized from glucose and honey were obtained. An endothermic peak around 300°C was present, as shown in **Figure 10**.

Thermal analysis was also performed with pure glucose. Thermogravimetric analysis shows that no weight loss was observed before the melting point (**Figure 9**) followed by significant changes due to the decomposition of glucose (Orsi 1973). **Figure 10** shows the endothermic melting of glucose at 170°C. The thermal behavior of glucose upon silver nanoparticles shows that the sugars are responsible for the reduction of silver ions and participate in the stabilization of nanoparticles (capping action).

Kinetics

A study of the changes in the surface plasmon resonance band as a function of reaction time of silver nanoparticles synthesized at both pH values was performed. The synthesis of nanoparticles at pH 10.0 occurs instantaneously and is associated with a high formation rate. On the other hand, at pH 5.0, absorbance was found to increase as a function of reaction time.

Figure 3 shows that surface plasmon resonance band at 411 nm rapidly increased at the beginning of the reaction, then reaching a plateau which indicated that the synthesis was completed. Three stages may be distinguished (Fedlheim and Foss 2002). The first one (nucleation), is slow, but continuous and homogeneous. It can be observed at the beginning of the curve before the near-linear region. This step implicates metal reduction and the formation of critical nuclei. The second step, named autocatalytic surface reduction, implies a rapid reaction where the product is also reactive. This stage is associated with a jump in the curve. Lastly, in the growth stage, the nanoparticles are agglomerated. If a stabilizing agent is not present, microparticles may be formed. This is the final stage of the curve.

In the synthesis of silver nanoparticles, the first stage may be accelerated in alkaline media (pH 10.0). The presence of hydroxyl reacts with Ag^+ to form Ag_2O and then the $\text{Ag}(\text{OH})_x$

complex. Lastly, Ag^0 is obtained, increasing the rate of nucleation (Nishimura et al. 2011). The hydroxyl in solution also opens the glucose ring. **Figure 11** shows that the reaction rate increases with the honey concentration (reducing agent), showing that higher reactant concentrations increase the reaction rate (Nishimura et al. 2011). However, at high concentration of honey (0.125 and 0.200 g% w/v), the hydroxyl groups may decrease due to the buffering capacity of honey, preventing the formation of the intermediate complexes mentioned above and decreasing silver reduction and nucleation (Buba, Gidado, and Shugaba 2013). In conclusion, the linear form of glucose reduces Ag^+ to Ag^0 through the mechanism detailed above. Subsequently, glucose molecules are adsorbed on the reduced silver (Ag^0) acting as the capping agent.

CONCLUSIONS

The synthesis of silver nanoparticles using honey was studied in acid and alkaline aqueous media at room temperature. The glucose in honey acts serves as the reducing agent and thermal analysis suggest that it also was a stabilizing agent. Furthermore, fructose has no significant effect on silver nanoparticle formation. Silver nanoparticles synthesized with glucose are larger and more diverse than those synthesized with honey, showing that the minor components of honey have a significant effect on the nanoparticle formation. A study of the kinetics was performed using surface plasmon resonance. The rate of the reaction was dependent upon the pH and honey concentration. Transmission electron microscopy showed that the silver nanoparticles prepared with honey had average sizes of 18.4 ± 0.9 nm and 12.3 ± 0.6 nm for pH 5.0 and 10.0, respectively. The images also showed the nanoparticles were primarily spherical. The synthesis was simple, low cost, and avoids the use of hazardous and toxic substances, contributing to the goals of green chemistry.

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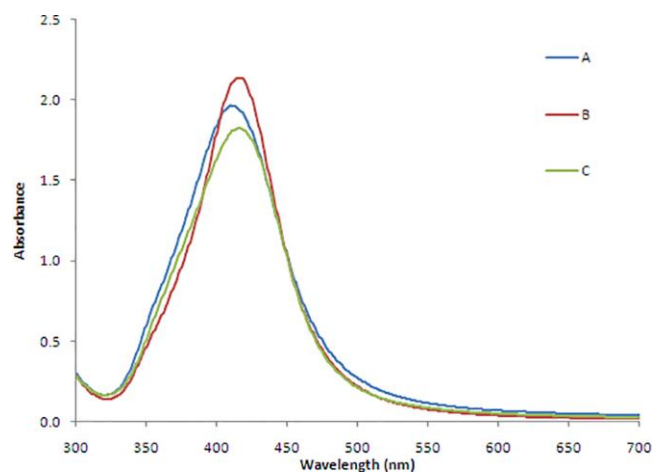
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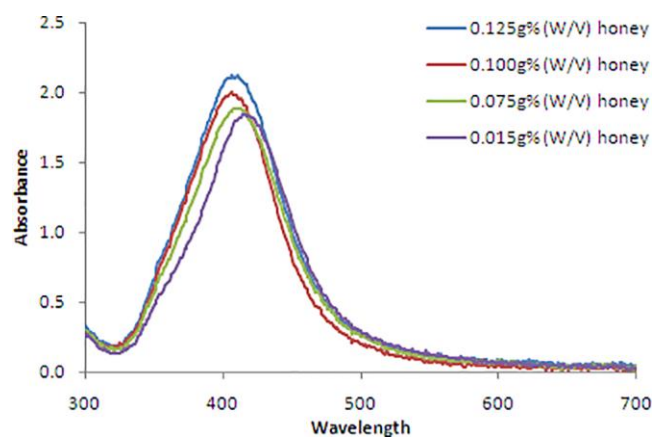
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Figure 1. (A) Silver nanoparticles synthesized with honey at pH 5.0. (B) Silver nanoparticles synthesized with honey at pH 10.0. (C) Silver nanoparticles synthesized with honey pH at 5.0 after 1 year.



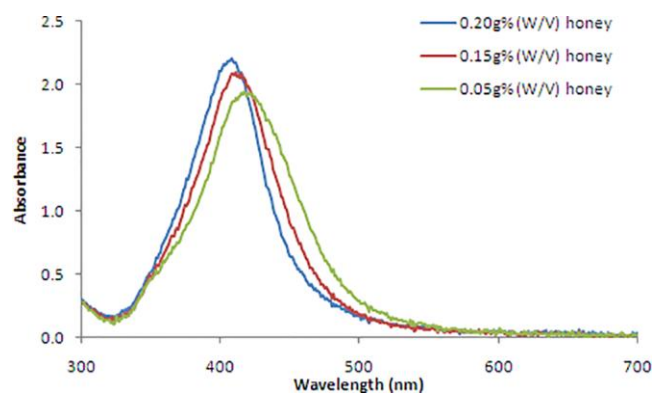
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Figure 2. Silver nanoparticles synthesized with various concentrations of honey at pH 5.0.



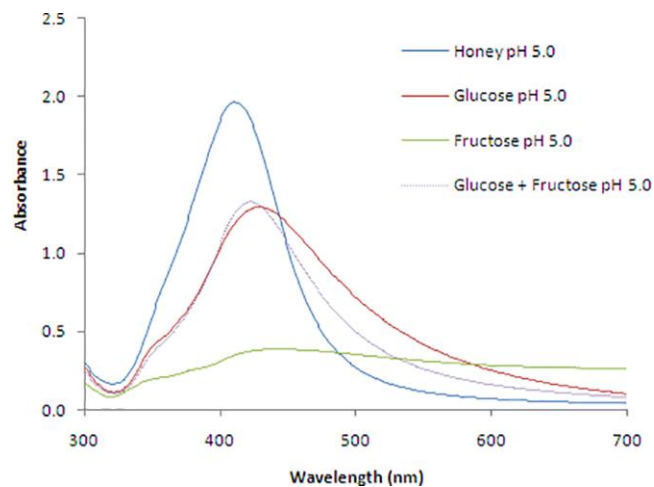
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Figure 3. Silver nanoparticles synthesized with various concentrations of honey at pH 10.0.



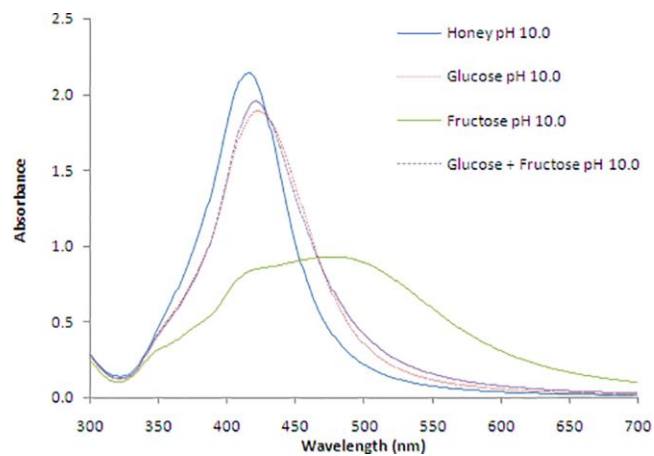
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Figure 4. Surface plasmon resonance of silver nanoparticles synthesized with various sugars and honey at pH 5.0.



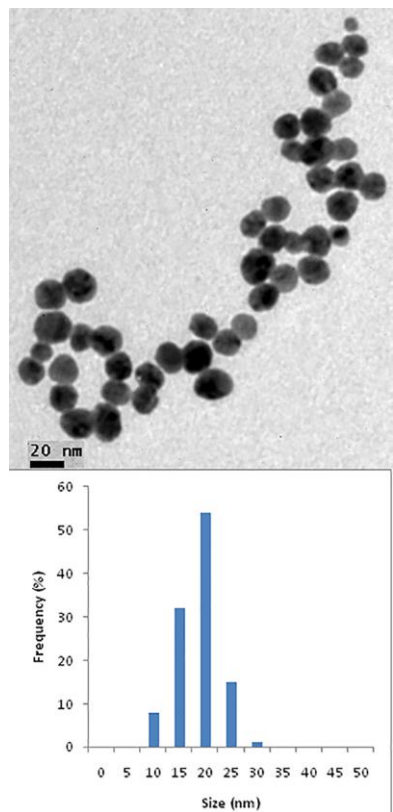
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Figure 5. Surface plasmon resonance of silver nanoparticles synthesized with various sugars and honey at pH 10.0



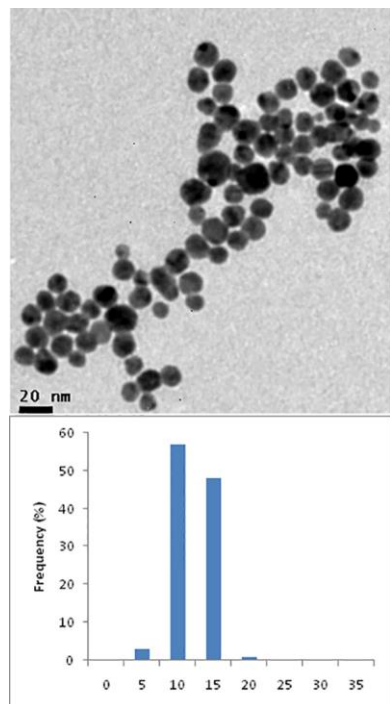
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Figure 6. Transmission electron micrograph and size distribution of silver nanoparticles synthesized with honey at pH 5.0.



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Figure 7. Transmission electron micrograph and size distribution of silver nanoparticles synthesized with honey at pH 10.0.



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Figure 8. Transmission electron micrograph of silver nanoparticles synthesized with glucose.

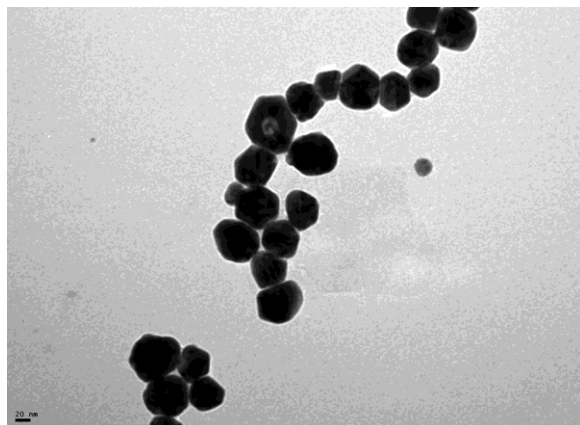
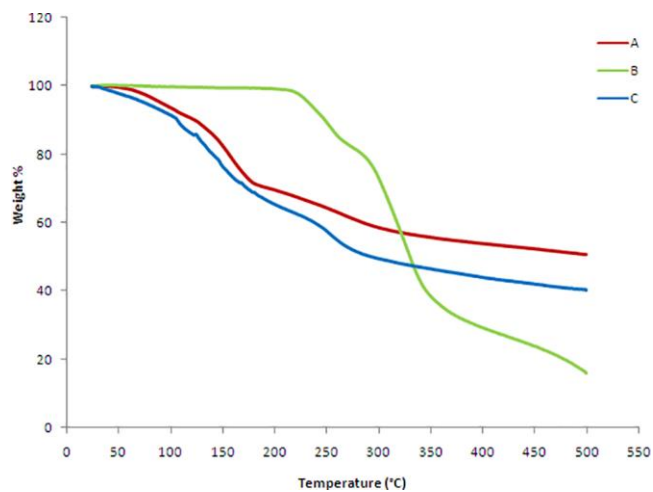


Figure 9. Thermal gravimetric analysis: (A) silver nanoparticles synthesized with glucose at pH 10.0, (B) glucose, and (C) silver nanoparticles synthesized with honey at pH 10.0.



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Figure 10. Differential thermal analysis: (A) silver nanoparticles synthesized with honey at pH 10.0, (B) silver nanoparticles synthesized with glucose at pH 10.0, and (C) glucose.

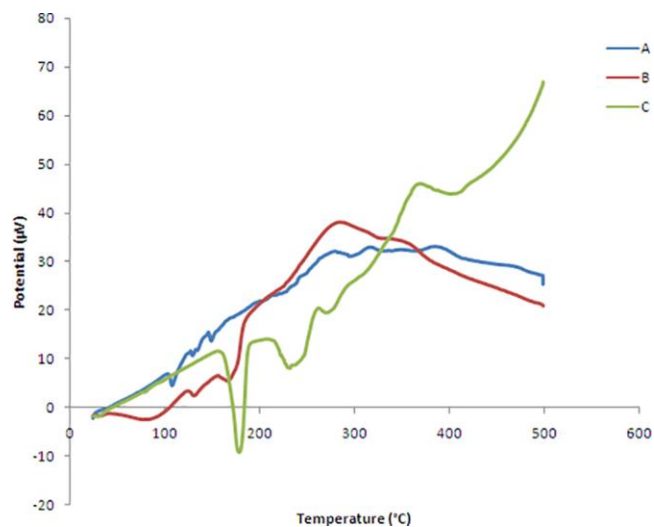
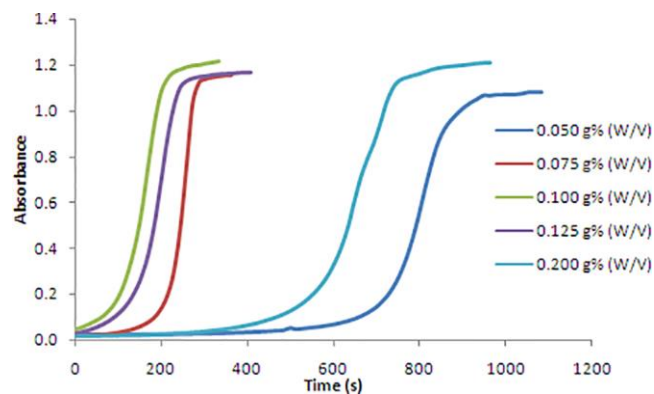


Figure 11. Kinetics of silver nanoparticles synthesized with honey at pH 5.0 as a function of honey concentration.



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