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Effect of Temperature on the Synthesis of *Centella asiatica* Flavonoids Extract-Mediated Gold Nanoparticles: UV-Visible Spectra Analyses

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Abstract. Many plants have been reported for the nanosynthesis of metal nanoparticles by virtue of the reducing potential of their bioactive compounds. *Centella asiatica* (CA) is one of the widely used plants, as claimed by traditional system of medicine, to have its positive effects on a variety of ailments. However, no research evidence indicates the synthesis of CA flavonoids extract (CACrF)-mediated gold nanoparticles (GNPs). In the present study, the initial synthesis of gold nanoparticles (GNPs) mediated by *Centella asiatica* crude flavonoids extract (CACrF) has been discussed. The protocol involves a one-step, non-toxic and cost effective procedure based on green nanotechnology avoiding the use of any synthetic chemicals potentially harmful for environment and biomedical applications. The CACrF was reacted with gold chloride, trihydrate (HAuCl₄.3H₂O) for the synthesis of GNPs. The reaction was carried out at room temperatures. The formation of GNPs was visually observed by a change in the color of solution from pale yellow to ruby pink. UV-visible (UV-vis) spectrophotometric analysis was performed to verify the synthesis of CACrF-mediated GNPs. As a function of time, the surface plasmon resonance (SPR) behavior of GNPs was evaluated to study the reaction kinetics and the UV-vis spectra were recorded after every 10 minutes up to 70 minutes. A single peak at 542 nm with absorbance of 0.475±0.039 indicated the synthesis of GNPs.

INTRODUCTION

The temperature plays a crucial role in tuning the size and shape of gold nanoparticles (GNPs). The effect of temperature on the surface plasmon resonance (SPR) feature of metal nanoparticles is a critical factor in pure and applied science of the nanoparticles [1], [2]. The variation in temperature of the reaction medium for the process of nanosynthesis has considerable effect on tuning the size of the nanoparticle synthesis. In literature, an increase as well as a decrease in GNP size has been reported in relation to the increase in reaction temperature [3]–[7]. It has been reported that the high reaction temperature leads to a rapid nucleation process of metallic nanoparticles involving the enhanced consumption of most of the metal ions with least secondary reduction of the preformed nuclei [8], [9].

Centella asiatica (CA) is a perennial herb of family Apiaceae that is found in many countries including Malaysia, Thailand, China, India along with Africa. It is one of the important medicinal plants used by traditional system of medicine. It has been reported to have a variety of bioactive compounds. Main bioactive compounds of CA include phenolic compounds (flavonoids, tannins), alkaloids and terpenes. The medicinal value of CA has a proven record in various disorders by virtue of its diversified biological actions. The use of CA is reported since ancient times in Ayurvedic and Traditional Chinese Systems of Medicine for a number of medical problems including the cognitive disorders. The therapeutic effects of various CA extracts have been proved efficacious for wound healing, memory problems, respiratory and digestive disorders, kidney problems and skin ailments [10]. CA has tremendous potential of being a natural source of antioxidant (AO) as its AO activity has been found comparable to that of Rosemary and sage [11]. Protective role of CA extract against γ -radiation induced DNA damage in vitro has been revealed by plasmid relaxation assay and the radioprotective function of CA has been suggested to its AO property [12]. In a comparative study involving forty three edible plants of Thailand, CA showed a comparatively high level of natural AO compounds including vitamin C, vitamin E, carotenes, tannins and total phenolics [13].

In recent years, considerable efforts have been put into the use and application of nanotechnology for achieving the goal of effective drug delivery into the brain for neurological disorders [5–7]. Nanoparticles (NPs) have been described as effective drug delivery system (DDS) for drug delivery to brain for the treatment of AD [8–10] and they have emerged as valuable tools also in imaging, diagnosis and drug delivery approaches [11-12]. There is also substantial amount of research evidence to exhibit the potential of NPs bound with some anticancer drugs to cross the blood-brain barrier (BBB) and release in brain at therapeutically effective concentrations [22]. So, there is convincing evidence regarding the use of NPs as effective DDS to overcome the shortcoming as far as drug delivery to the affected tissue is concerned. NP technology offers two-fold benefits related to the therapeutic potential of drugs: 1) hiding the limiting properties of potential drug molecules thereby facilitating their passage across BBB, and 2) slowing the release of drug molecules resulting in low toxicity to surrounding tissue. GNPs-mediated therapeutic drug delivery treatment is a promising area of research at present [23].

At present, there is a convincing amount of research data that supports the effectiveness of different extracts of CA regarding its anti-oxidant activity [15–22]. However, no research data are available in current scientific literature regarding the use of CA flavonoids for the synthesis of GNPs by green nanotechnology approach. In this study, the synthesis of GNPs mediated by CACrF has been discussed and the effect of different temperatures has been studied on the synthesis of CACrF-GNPs. The process of GNP synthesis has been monitored and preliminarily verified by UV-vis spectral analysis.

MATERIALS & METHODS

Chemicals & equipment

Centella asiatica whole plant powder was purchased from Ethno Resources Sdn. Bhd. (Selangor, Malaysia). $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ was procured from Sigma-Aldrich (USA). The UV-vis spectra were recorded on UV-vis spectrophotometer GENESYS™ 10S, Thermo Scientific, USA. Double-distilled Millipore water was used to make dilutions of CACrF and HAuCl_4 . All glassware were thoroughly rinsed with double-distilled Millipore water and dried before use.

Crude flavonoids extraction

The preparation of CACrF was done according to Chew et al. [32]. Briefly, 5 g of CA whole plant powder was extracted with 50 ml of 50% aqueous ethanol at 40°C for 2 hours followed by filtration with Whatman filter paper No. 1. The filtrate was concentrated near to dryness at 40°C in hot air oven to get a semisolid mass. The semisolid extract was weighed and stored at 4°C till further use.

Qualitative tests for flavonoids

CACrF was qualitatively tested for flavonoids. In brief, 10 ml of ethyl acetate in boiling water was heated with a small quantity of the extract for 3 to 5 minutes followed by filtration. The filtrate was used for following qualitative tests for flavonoids:

i. Ammonium test

Few drops of dilute ammonia solution (1%) were added to 1 ml of CACrF. The mixture was shaken and allowed to stand for separation of layer [33].

ii. Sodium hydroxide test

Few drops of dilute sodium hydroxide solution were added to 1 mL CACrF and mixture was gently shaken. The test tube was allowed to stand and the mixture was observed for change in colour to intense yellow. Then dilute HCl was added and disappearance of intense yellow colour was observed which indicated the presence of flavonoids [34].

Green synthesis and UV-vis characterization of CACrF-GNPs

Biosynthesis of GNPs was carried out based on protocol described by Das, Borthakur & Bora [35] with modifications. For GNP synthesis, different temperatures were selected based on the findings regarding the antioxidant activity of crude flavonoids extract prepared from CA [32]. 8% CACrF (pH 9.0) was mixed with 0.5 mM gold chloride (HAuCl_4) aqueous solution in equal volume and the solution was kept at 25°C, 40°C, 55°C and 70°C with constant magnetic stirring. The process of GNPs synthesis, that is, the reduction of Au^{3+} to Au^0 , was monitored by visual observation (change in solution colour from light yellow to ruby pink). The UV-vis absorption spectra in the range of 300 nm to 800 nm were recorded with an interval of every 10 minutes for 60 minutes.

RESULTS & DISCUSSION

As for the qualitative testing of CA crude extract for flavonoids, the ammonium test was positive as yellow coloration was observed at ammonia layer. Additionally, change in colour to intense yellow was observed in sodium hydroxide test which disappeared on adding dilute HCl indicating the presence of flavonoids in the crude extract of CA.

UV-vis spectra analysis is a widely used technique to monitor and verify the synthesis and stability of GNPs in solution due to their characteristic absorption in the range of 500 nm to 600 nm UV-vis range by virtue of the phenomenon of surface plasmon resonance (SPR). SPR indicates the size and morphology of GNPs [36]. The change in colour of the reaction medium is usually considered as the first indication of the process of GNPs synthesis. The CACrF was almost colourless while the colour of HAuCl_4 was pale yellow. Upon adding the HAuCl_4 to CACrF solution, the colour change in the reaction medium was visible within half an hour as shown in Figure 1.



FIGURE 1. Photo of the colour changes on addition of HAuCl_4 to CACrF

Temperature plays a crucial role in tuning the size and shape of GNPs. The effect of temperature on the SPR feature of metal nanoparticles is a critical factor in pure and applied science of the nanoparticles [28-29]. In the present work, the effect of temperature on GNP synthesis was studied at three different temperatures, that is, 40°C,

55°C and 70°C and the UV-vis spectra were recorded every 10 min up to 70 min considering the relatively high reaction rate. From the results obtained, it is clear that the rate of GNP synthesis and SPR band intensity increases with increasing temperature as shown in Figure 2. Earlier, similar findings related to the increase in reaction rate of GNP synthesis with increase in reaction temperature have also been reported by Das, Gogoi, & Bora [37] and Ghosh et al [38].

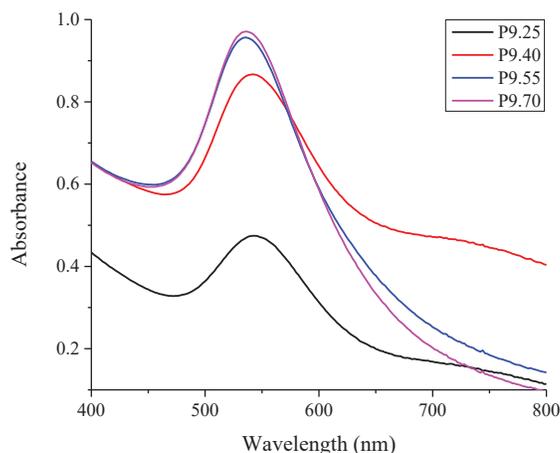


FIGURE 2. Effect of temperature on GNP synthesis: UV-vis spectra recorded at 70 minutes.

As per the results obtained regarding the UV-vis spectra of GNPs synthesized at different temperatures, it is clear that there is a blue shift to 542 nm at 40°C and 536 nm at 55°C and 70°C in comparison to the GNPs synthesized at 25°C. As for the UV-vis spectra recorded at 70 min reaction time, there seems to be a direct relationship between reaction rate and reaction temperature due to an increase in gold ions consumption that enhances the formation of nuclei [39]. The results suggest that the higher temperature leads to an increase in the activation energy of the molecules and faster rate of reaction. As a result, there is a decrease in the size of synthesized GNPs and, hence, monodispersed small nanoparticles are formed without undergoing the phase of particle size growth [9]. The results of this study are contrary to the findings reported by Du et al [40] regarding the effect of temperature on GNP synthesis reaction using flavonoid extract of *Lilium casa blanca*.

In literature, an increase as well as a decrease in GNP size has been reported in relation to the increase in reaction temperature [39–43]. However, the present study shows a decrease in the size of GNPs on increasing the reaction temperature as it is evident from the sharp and narrow SPR peaks with increased sphericity. These results are consistent with the previous findings discussing the same in context to the increased reaction rate of GNP synthesis upon increasing the reaction temperature. The high reaction temperature leads to a rapid nucleation process of metallic nanoparticles involving the enhanced consumption of most of the metal ions with least secondary reduction of the preformed nuclei [8], [9].

Rai et al [2] has described that on increasing the reaction temperature during the plant extract-mediated bioreduction of HAuCl_4 , the reduction rate of gold ions is fastened leading to an increased rate of nucleation with a consequent large concentration of spherical nanoparticles. Moreover, the majority of gold ions is consumed in nucleation process in response to a high reduction rate, thereby stalling the secondary reduction on the surface of preformed nuclei. As a result, a large population of spherical nanoparticles is formed under high temperature condition. In context to this, it is understandable that the GNPs synthesized during the 70 min reaction time at low temperature are polydispersed, anisotropic and large as shown by a broadened SPR peak in comparison to the GNPs synthesized at high temperatures. The GNPs synthesized at high temperature show a blue shifted, sharp and narrow SPR peak signifying the sphericity of nanoparticles based on the finding that secondary nucleation on preformed nuclei is generally favoured at low temperature [38], [45–46]. Some studies have shown that the activation energy and reducing power of the biological molecules increase in response to the increase in temperature that lead to a faster rate of reaction at higher temperature [44]. It has been shown that a low reaction temperature could be helpful

in controlling the rate of GNP synthesis and the uniform nanoparticles can be synthesized under optimum pH at different reaction temperatures [45].

CONCLUSION

In conclusion, the reaction rate of CACrF-mediated GNPs synthesis has a direct relation with increase in temperature. It is evident from the UV-vis spectral analyses of CACrF-mediated GNPs that every 15°C rise in temperature resulted in a faster rate of reaction, that is, the formation of GNPs was completed in a proportionately lesser period of time as indicated by UV-vis spectra. Also, a blue shift has been observed in the UV-vis spectra of GNPs synthesized with higher temperature. It indicates that by increasing the temperature of reaction medium, the size of GNPs is reduced. Another feature observed in the UV-vis spectra of GNPs synthesized at different temperatures is the broadening of peak which is considered as an indicator of polydispersity of nanoparticles. From UV-vis spectra, it can be concluded that the GNPs synthesized at high temperatures have more polydispersity in comparison to the GNPs synthesized at 25°C as indicated by its sharp and narrow peak of absorption maxima. As the value of absorption maxima of GNPs is directly related to their concentration, it can be concluded that increase in the temperature of reaction medium causes an increase in the concentration of GNPs as shown in the spectral graphs. In this study, we have presented a cost effective, fast, non-toxic and environment friendly green nanotechnology method for the synthesis of GNPs mediated by CACrF. To the best of our knowledge, it is the first report on GNPs synthesis mediated by CACrF that also includes the effect of temperature on GNPs synthesis. Further research related to the characterization of CACrF-GNPs is in progress.

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