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To cite this article: Anuoluwa Abimbola Akinsiku *et al* 2018 *IOP Conf. Ser.: Earth Environ. Sci.* **173** 012019

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Room temperature Phytosynthesis of Ag/Co bimetallic nanoparticles using aqueous leaf extract of *Canna indica*

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Abstract. Continuous quest for safe environment has led to ‘green’ approach of synthesizing nanoparticles. The method is easy, eco-friendly and cost-effective. Locally sourced medicinal plant *Canna indica* was used as capping/ stabilizing agent instead of toxic chemicals. Secondary metabolites in the plant extract acted as reducing agents. Optical measurements were carried out using Uv-vis spectrophotometer and photoluminescence (PL). Formation of core-shell was detected in the TEM micrograph, which was supported by two peaks observed in surface plasmon resonance shown in the Uv-vis spectra. FT-IR spectrophotometric analysis indicated the presence of some specific functional groups in the phytochemicals which were adsorbed on the surface of nanoparticles. This analysis depicted the presence of hydroxyl group (O-H stretching) with a broad strong peak at 3360 cm^{-1} , C-H stretching at 2938 cm^{-1} , C=C stretching at 1659 cm^{-1} , C=N stretching at 1557 cm^{-1} and C-O deformation at 1065 cm^{-1} . Crystalline phase of the nanoparticles was determined using X-ray diffraction (XRD). The plant-mediated green synthesized silver/cobalt nanoparticles are potential optical materials as a result of their broad absorption band and emission.

1. Introduction

Recently, nanoparticles and nanomaterials are products of a rapidly growing technology. Paradigm shift has led to the development of ‘green’ nanoparticles as they are biocompatible; form stable nanoparticle products and they possess less adverse effects on health and environment. Different fields such as nanobiotechnology, catalysis, chemical, bio-sensing, photo-catalysis among others explore metallic nanoparticles due to their unique optical, electronic, magnetic and chemical properties [1-5]. Biosynthesized metallic nanoparticles have been explored in biomedical for antimicrobial coatings, imaging and drug delivery applications. Catalytic application includes removal of environmental contaminants, they are also used as sensor in electrochemical application. Silver nanoparticles are applied as colorimetric sensor in chemical sensing as a result of their sharp localized surface plasmon resonance [6-7]. The high surface energy and high ratio in the surface to volume characteristics possess by nanoparticles are also of great advantage [8].

Bimetallic nanoparticles (BNPs) involves combination of two different metals to develop novel properties of the two metals present. They are more important as they possess enhanced specific



properties than their corresponding monometallic nanoparticles (MNPs). Features of BNPs include additional degree of freedom; higher surface area, directly proportional to their adsorption power which qualifies them as effective catalysts [9]. Moreover, synergistic effects of bimetallic nanoparticles have also made them to be highly active in catalysis; but, transition-metal nanoparticles are reported to be unstable (as a result of their high surface energy), self-aggregate, expensive and difficult to separate from reaction mixture [10-11].

From research, synergism has been reported to occur when BNPs are used for catalysis, as interaction between the two metals is key. Shapes or arrangements of BNPs depend on the synthetic method and thermodynamic equilibrium of the two metals (precursors). Studies have shown that BNPs with few tens and hundreds of atoms possess active surfaces, and they can serve as green catalysts, as this could solve the challenges encountered in transition-metal nanoparticles. Ismail *et al.* [12] used an adsorption method to synthesize Cu/Ag and Cu/Ni bimetallic nanoparticles loaded on ginger powder. Antibacterial and antibiofilm activities of Ag, Au and Ag/Au BNPs were carried out by Gopinath *et al.* [13] using leaf extract of *Gloriosa superba*. Plant-mediated green syntheses of silver nanoparticles and bimetallic Ag/Ni nanoparticles were carried out in our previous works [14-16]. However, few studies have been carried out on green synthesis of Ag/Co bimetallic nanoparticles. Pt/Ag, Pt/Rh, Ag/Ni, Au/Pt, Pd/Au, Ni/Au, Ag/Co BNPs synthesized using chemical and physical methods have been reported [17-21].

Unfortunately, use of toxic organic solvents and strong reducing agents during nanoparticles synthesis has posed great threat to the environment. Hence, development of eco-friendly and cheap nanomaterials is a necessity [22]. In view of the synergistic properties of BNPs, we report for the first time, phytosynthesis of Ag/Co BNPs at room temperature, using leaf extract of *Canna indica* as a reducing agent instead of toxic chemical. Secondary metabolites in the plant extract acted as capping/stabilizing agents for the newly formed nanoparticles. Optical measurements were carried out using Uv-vis spectrophotometer and photoluminescence (PL). Further characterizations were done using x-ray diffraction (XRD), transmission electron microscope (TEM) and Fourier transformed infra-red (FT-IR).

2. Experimental details

2.1. Materials and reagents

Cobalt chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$), silver nitrate (AgNO_3) were purchased from Sigma-Aldrich Company, UK, and used as received. Distilled-deionized water, Whatman number 1 filter paper and fresh leaf extract of *Canna indica*.

2.2. Preparation of aqueous leaf extract

Fresh *C. indica* leaves were collected from Ogun State, Nigeria (Fig. 1). They were washed thoroughly with distilled de-ionized water (d-d water), after which 20 g of the homogenized leaves were mixed with 200 mL d-d water at room temperature. Filtrate from the mixture was collected using Whatman number 1 filter paper and preserved at 4°C for nanoparticle synthesis and phytochemical screening [23].

2.3. Synthesis Ag/Co BNPs at room temperature

Ag/Co bimetallic nanoparticle formation was prepared via phyto-reduction method with modification to previous study [12,16]. Plant extract (50 mL) was added to precursor mixture (250 mL) in a beaker i.e. equal molar concentration containing 125 mL of AgNO_3 and 125 mL $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$. This reaction was carried out at varied concentrations (0.5-3.0 mM) of the precursor. Resulting mixture was stirred vigorously and left at 25°C, noting the initial colour. Metal ion reduction of Ag(I) ions to $\text{Ag}_s(0)$ and Co(II) to $\text{Co}_s(0)$ was monitored closely for any colour change. Appearance of surface plasmon resonance (SPR) was checked using double beam Uv-vis spectrophotometer by taking aliquot samples

at different time intervals, placed in quartz cuvette, operated at a resolution of 1 nm so as to measure the absorbance.

2.4. Isolation of metallic Ag/Co nanoparticles

After appearance of SPR band, the reaction mixture was transferred into a Thermo Fisher Scientific Centrifuge (Thermo Electron LED), which was operated at 5000 rates per minute (rpm) for 30 minutes, so as to collect the nanoparticles. Ag/Co BNPs were purified by re-dispersing in d-d water in order to remove the organics present. It was centrifuged again for 10 minutes. The suspension was dried in an oven at 70°C, collected and kept for structural and morphological characterizations.

2.5.Characterization

2.5.1. Optical characterization

Optical properties of Ag/Co BNPs were carried out using Uv-vis spectrophotometer (Thermo Scientific GENESYS 10S model) at wavelength range between 250 and 800 nm to measure absorbance maximum. Distilled-deionized water was the blank used. Also, photoluminescence (PL) emission of Ag/Co BNPs was measured by Perkin-Elmer 55 spectrophotometer at room temperature. The sample was placed in 1 x 1 cm quartz cell.

2.5.2. Structural characterization

Morphology, particle size determination and detailed structure of the biosynthesized Ag/Co BNPs were determined using transmission electron microscope (Technai G2), coupled with energy-dispersive x-ray spectrometer (TEM-EDX) to determine elemental composition of the nanocluster. The equipment was operated at 200 KeV (accelerating voltage) and 20 μ A current. Films on the TEM grids were left for 2 minutes to dry before taking any measurements, Ag/Co BNPs were prepared by drop-coating them onto carbon-coated copper TEM grids.

3. Results and discussion

Image of the utilized biodiversity and map of Nigeria indicating site of collection are shown in Figs. 1a and 1b respectively.



Fig. 1 (a): Image of *Canna indica* (FHI No. 109928)

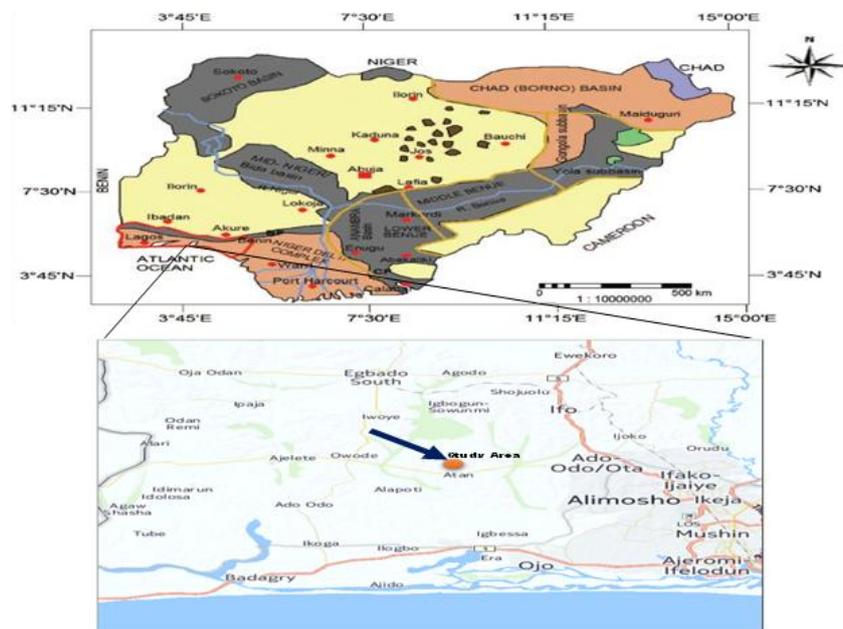


Fig. 1:(b) Map of Nigeria with arrow showing plant collection site

3.1. Optical properties of Ag/Co BNPs

Uv-vis spectra of the phytosynthesized silver hybrid at room temperature are depicted in Fig. 2. There was a noticeable colour change in the reaction from light brown to deep brown as a result of excitation of surface plasmon vibrations [24]. Appearance of surface plasmon resonance (SPR) was an evidence of nanoparticles formation; but, onset growth and nucleation were delayed till the 48th, 120th and 191st hours of the reaction time from the cluster prepared from 1.0, 2.0 and 3.0 mM precursor solutions respectively. Growth comparison in Ag/Co BNPs prepared at room temperature and 70°C is presented in Fig. 3. It is obvious that temperature increased kinetic energy of the reaction by increasing the rate of collision between metal ions (Ag^+ and Co^{2+}) and the reducing agents (phytochemicals) present in the plant extract, thereby lowering activation energy of the reaction medium. Peak broadening indicated formation of polydispersed particles [25]. The particles were expected to be large in size due to the longer reaction time, as plant-mediated green synthesis is time dependent. Ageing of particles was also predictable.

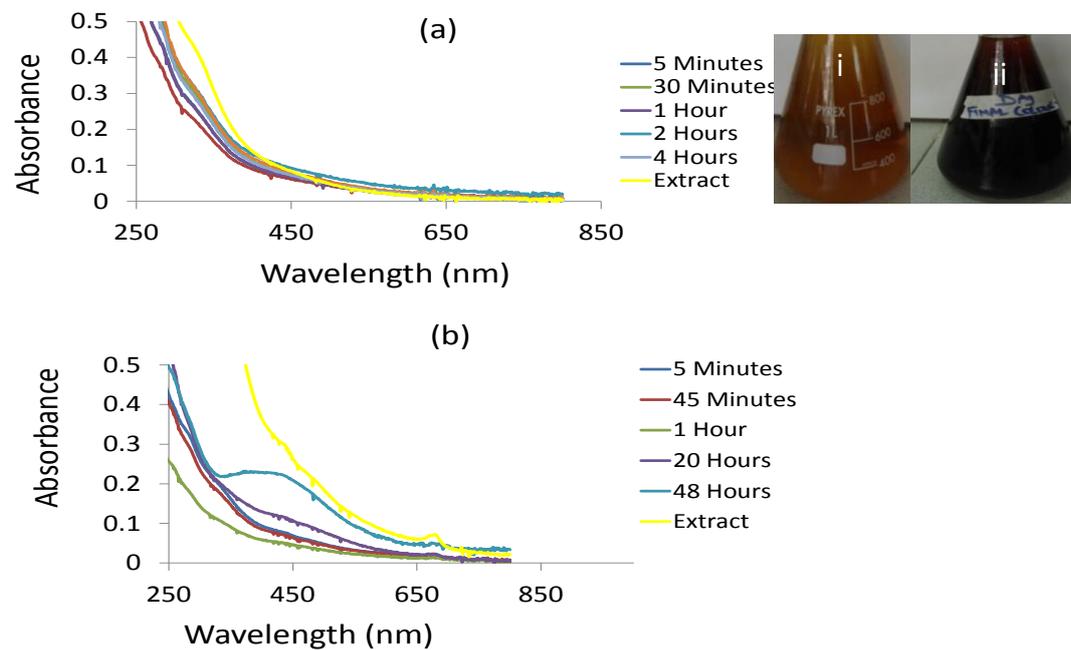
Red shift in the SPR band (450-550 nm) is thought to be the effect of Co in the hybrid nanoparticles. This observation pointed to increase in particle size of $\text{Ag}_{\text{core}}\text{Co}_{\text{shell}}$. According to Maaz (2007) and David (2003), an increase in particle size results in shift of absorption edge to longer wavelength [26-28]. The observed two absorption peaks were in agreement with the results attained from TEM. Maximum absorption wavelength in the Ag/Co BNPs was more shifted than that of single metal Ag NPs (420-480 nm) from previous works [14, 29]. No characteristic plasmon band resonance (PBR) peak was observed in the particles synthesized in 0.5 mM precursor solution. This indicated that the reducing agent at this concentration was not significant for hybrid NPs synthesis [30]. This could also plausibly be that the working concentration was too low for the reduction of metal ion. In addition, PBR was not pronounced in the particles at 3.0 mM precursor solution, possibly because high concentration of metal salt proved to inhibit the synthesis of nanoparticles.

Luminescence properties of Ag/Co BNPs is presented in Figure 4. There was a noticeable strong local field in the nanoparticles as photoluminescence emission peaks was observed at 739 nm after

excitation at 330 nm. This was plausibly due to the surface of the hybrid nanoparticles predominantly occupied by Co atom. Existence of a single emission peak in the longer wavelength band region indicated electron quantum confinement [31]. Moreover, the observed higher emission wavelength suggested stoke's shift. The sturdy peak intensity displayed by the nanohybrid was a consequence of the allowed vibrational transition in the nanocluster [32]. Hence, the optical properties possess by Ag/Co BNPs is an indication for potential plasmonic applications as wideband optical modulators in the visible range of the spectrum [33], also in devices that source, detect and control light.

3.2. Morphology of Ag/Co BNPs

Particle size distribution and representative TEM image of the silver allied nanoparticles are presented in Figs. 5(a) and 4(b) respectively. Another representative micrograph with average size of 97.77 ± 25.53 nm is depicted in Fig. 5(c). The image (Fig. 5b) revealed silver (core) with dark colour and cobalt (shell) which corroborates the two absorption bands observed in the Uv-vis spectra. This is in agreement with previous work done by Shenyet *al.* [34]. Furthermore, this could also be as a result of Ag^+ which got reduced first to form the core because of its higher positive electrochemical potential than cobalt [16]. Average size of the nanocluster was 202.5 ± 62.75 nm. EDX analysis confirmed formation of Ag/Co BNPs which was enriched with plant extract which acted as the capping agent (Fig. 6). Similar finding was reported by Mntungwaet *al.* [35].



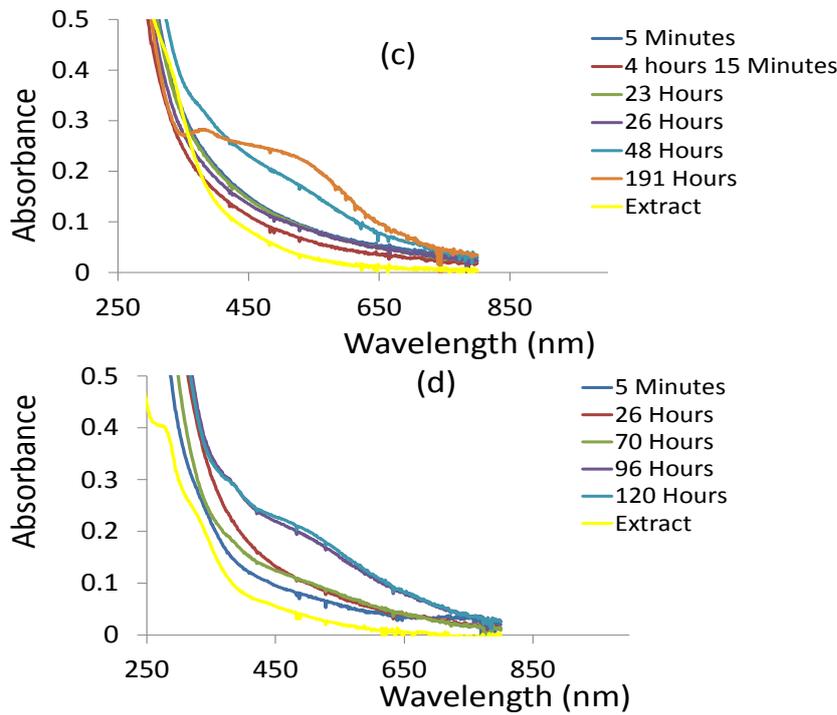


Fig. 2: Room temperature time-resolved Uv-vis spectra of Ag-Co BNPs prepared by reducing (a) 0.5 mM (b) 1.0 mM (c) 2.0 mM (d) 3.0 mM precursor solutions using the extract of *C. indica* leaves. Inset: (i) Precursor mixture with extract before reduction (ii) Final dispersion formed after reduction.

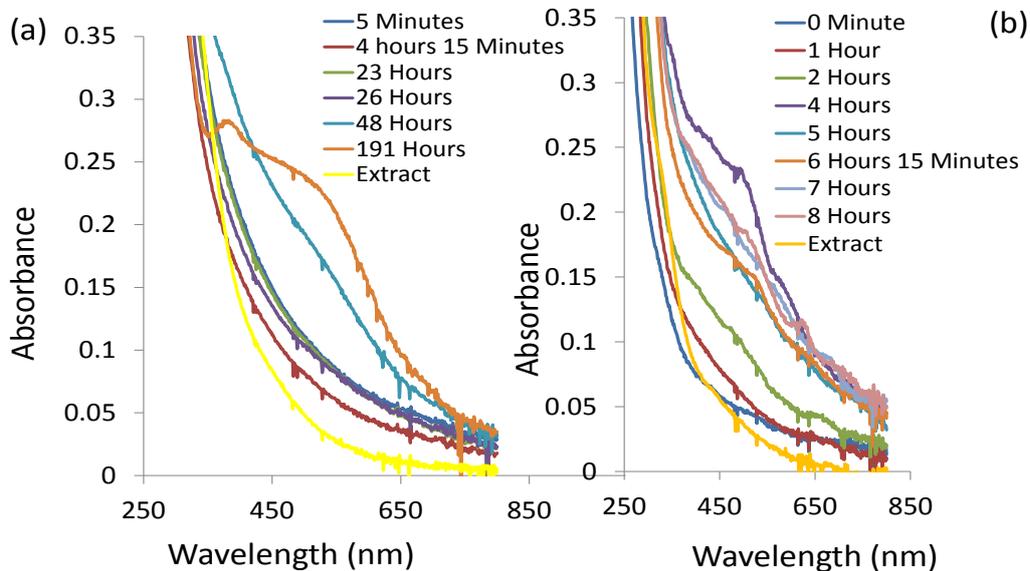


Fig. 3: Growth comparison of Ag/Co BNPs using 2.0 mM precursor (a) Room temperature, (b) 70°C

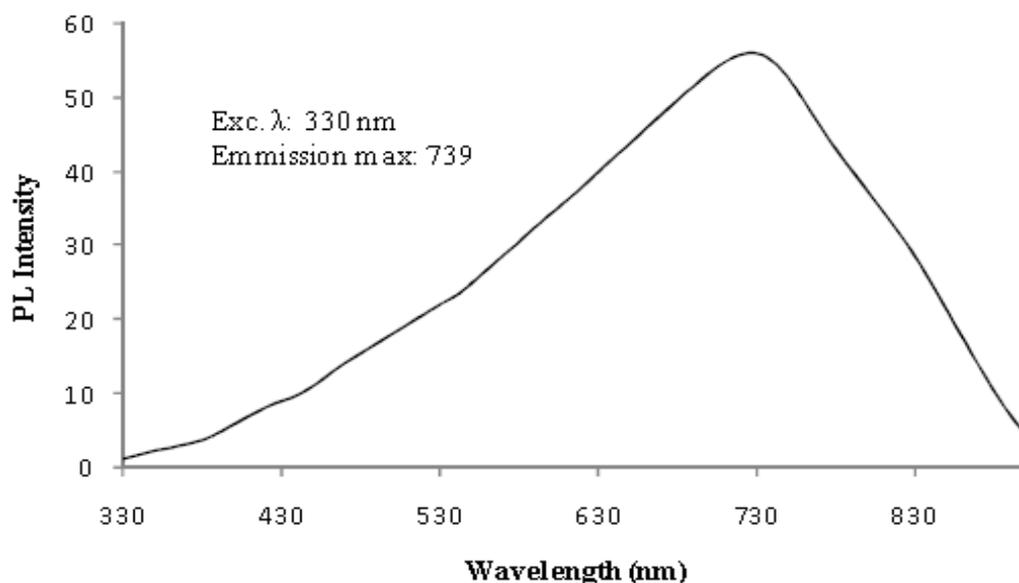


Figure 4: The PL emission spectrum of Ag/Co BNPs synthesized

3.3 FT-IR analysis

Possible functional groups present in the biosynthesized Ag/Co BNPs were identified in the FT-IR spectrum of Ag/Co BNPs (Fig. 7) which displayed bands due to O-H stretching vibrational mode at 3360 cm^{-1} which overshadowed N-H stretching in terpenoid found within this region. C-H stretching at 2938 cm^{-1} (medium absorption) was a sharp peak, C=C stretching at 1659 cm^{-1} , C=N stretching and the C-O deformation at 1557 cm^{-1} and 1065 cm^{-1} bands respectively. Glycosides, alkaloids and terpenoids were detected in our earlier study when leaves of *C. indicawere* extracted in water by cold extraction [16]. These secondary metabolites were responsible for the reduction of metal ions in the precursor, capping and stability of the Ag/Co BNPs as they were formed [36]. Terpenoids and glycosides in the leaf extract were considered to be the adsorbed biochemicals on the surface of Ag/Co BNPs as spotted in the FT-IR analysis.

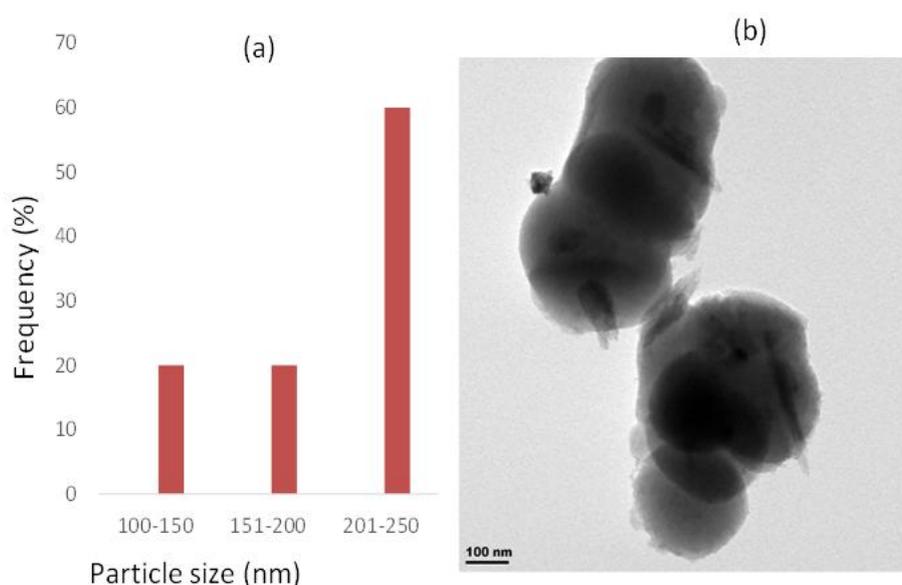
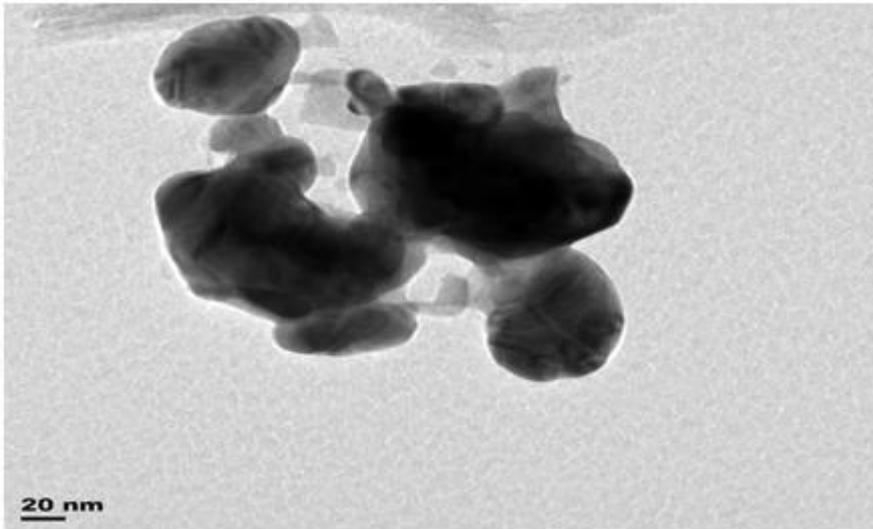


Figure 5: (a) Particle size distribution histogram of Ag/Co determined from TEM image
(b) Representative TEM image of Ag/Co BNPs



(c) Representative TEM image of Ag/Co BNPs

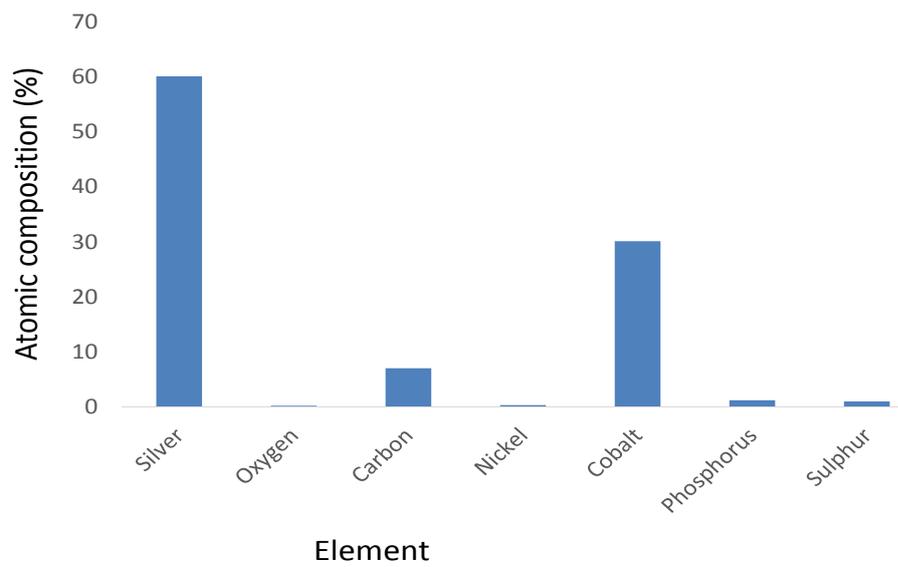


Fig. 6: EDX showing atomic composition of elements present in Ag/Co BNPs

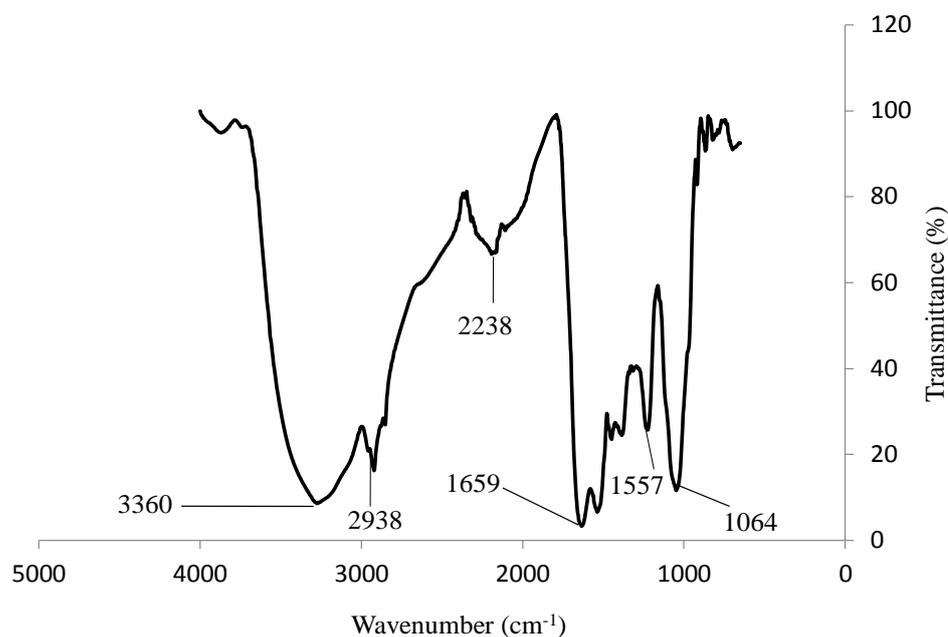


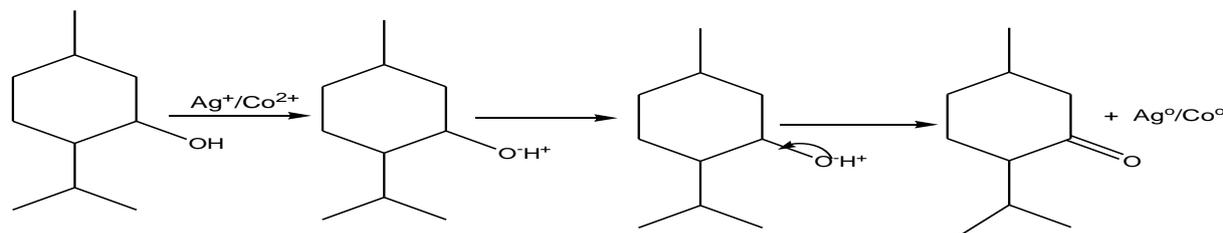
Fig. 7: FT-IR spectrum of Ag/Co BNPs

3.4. XRD analysis

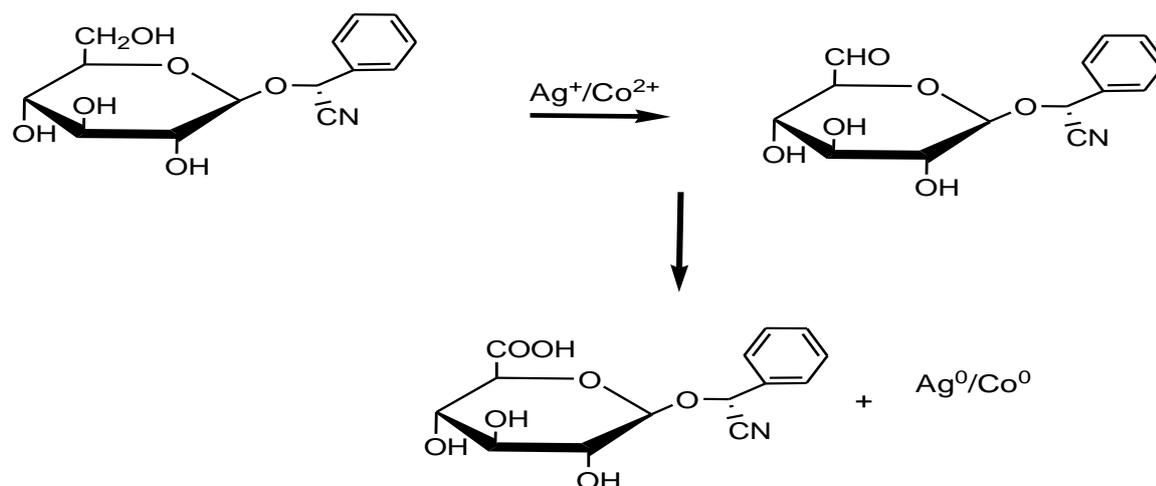
XRD patterns of the synthesized Ag/Co BNPs are depicted in Fig. 8. Diffraction peaks at 2θ values = (38.15°) , (44.37°) , (64.57°) , (77.39°) and (81.08°) corresponding to (111), (200), (220), (311) and (222) diffraction planes respectively were attributed to the crystal planes of cubic Ag structure [37]. Moreover, the spectrum also shows 2θ values = (41.78°) , (44.37°) , (44.37°) , (52.64°) and (58.29°) equivalent to (100), (111) and (001) hcp respectively, also 52.64° and 58.29° . Core-shell structural morphology of Ag/Co are depicted in the diffraction patterns.

3.5 Mechanisms of reactions

Proposed reaction mechanisms are shown in Schemes 1 and 2 below:



Scheme 1: Bioreduction of silver/cobalt ions to silver/cobalt BNPs by terpenoids



Scheme 2: Bioreduction of silver/cobalt ions to silver/cobalt BNP by glycosides

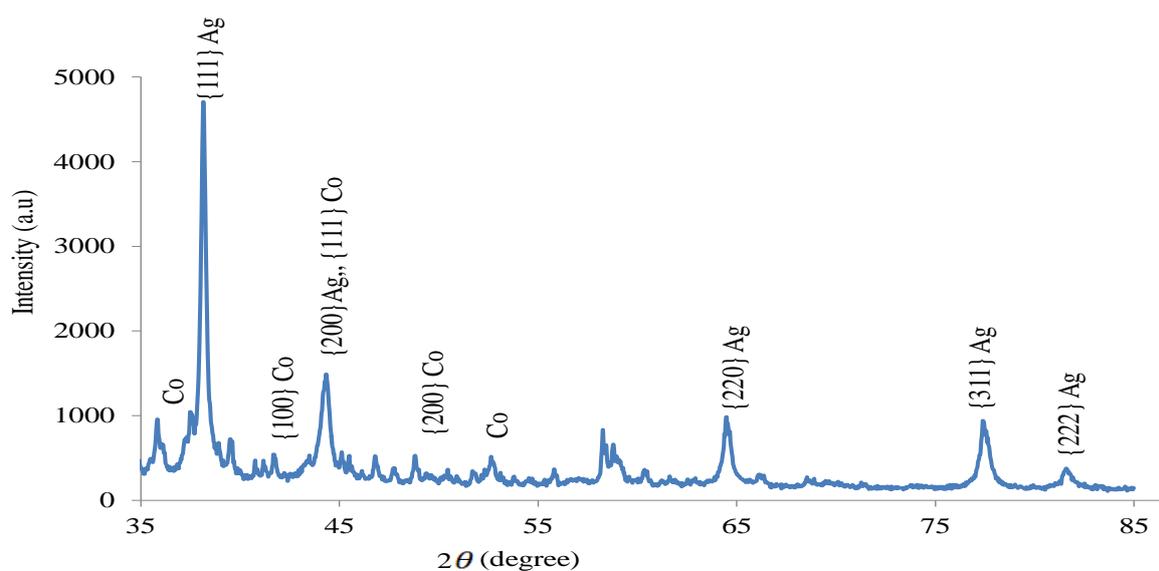


Fig. 8: XRD patterns of Ag/Co BNPs

4. Conclusion

In summary, core-shell/alloy structured Ag/Co BNPs were produced via phyto-reduction process of *Canna indica* aqueous leaf extract. The nanoparticles were crystalline. The FT-IR pointed the moieties of the terpenoids and glycosides adsorbed on the nanocluster surface, which promoted capping and stability. Red shift in the SPR band was in the region 450-550 nm. Optical properties of the nanoparticles have indicated them as potential optical materials. This study can also take advantage of the surface plasmon absorption for therapeutic and diagnostic uses.

5. Acknowledgements

Authors are grateful to Mr. Olusola Rotimi of the University of Western Cape in South Africa for the FT-IR, XRD, TEM and EDX characterizations. The authors also acknowledge Covenant University.

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