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Novel Carbidopa Functionalised Silver Nanoparticles a Selective Detection for Lead and Levodopa

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Abstract. Novel approaches to engineer nanoparticles with desired chemical characteristics open new opportunities to utilize such materials for assorted applications. In this context, various methods have been developed to prepare metal nanoparticles. In the present work, a single-step synthesis method was reported for the preparation of silver nanoparticles by using carbidopa which is useful in the treatment of Parkinson's disease to increase the dopamine level of the brain. Here we used the carbidopa drug as a capping agent. Nanoparticles were characterized by UV-Visible spectroscopy, Particle Size Analyzer(PSA), Dynamic Light Scattering (DLS), Powder X-ray Diffractometry (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Transmission Electron Microscopy (TEM). Then amino acid detection study was performed with all 24 amino acids, which provides the successful data for sensing the amino acid L-dopa. These prepared nanoparticles were further applied for metal analyte studies which reveal that lead can be sensed successfully by using these nanoparticles. Nanoparticle also shows radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Introduction

Nanoscience has recently been introduced as modern interdisciplinary research, definable as total information on nano-size fundamental properties items [1]. Nanotechnology provides materials processing, manipulation and uses below 100 nm level. Because of the large surface area to volume ratio, nanoparticles offer novel properties which lead to use in various fields of application [2]. The size and shape of nanomaterials offer additional effective control over many of their physical and chemical properties and their potential application in optoelectronics, recording media, sensing devices, medicines and catalysis [3, 4].

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. Metal nanoparticles are often used in various fields in a wide variety of applications. In particular, as the shapes, sizes and compositions of metallic nanoparticles are closely related to their physical, chemical and optical properties, technologies based on metallic nanoparticles have been used in a range of fields, from chemistry to medicine [1-3].

Due to the unique properties of silver nanoparticles like size and shape depending on optical, electrical and magnetic properties, it can be integrated into composite fibers, antimicrobial applications, biosensor materials, cryogenic superconductive materials, cosmetic products, and electronic components [4]. Silver nanoparticles have the highest antimicrobial performance compared to other metal nanoparticles due to their huge surface area diameters of 100– 15000 [5-10]. Quite a lot of physical and chemical methods for the synthesis and stabilization of silver nanoparticles have been used previously [11-14]. The most frequently used method is the chemical reduction method, which involves the reduction of metal ions using various reduction agents, such as citric acid, borohydride (Lee and Meisel method) [15], NH_2OH [16], N_2H_4 [17], ethylene glycol [18], ethanol

[19]. Many organic compounds are used as capping or stabilizing agents to prevent agglomeration like poly vinyl alcohol [20], poly vinyl pyrrolidone [21] and cetyl trimethylammonium bromide [22]. Organic molecule-coated silver nanoparticles attract the interest of researchers due to their versatility to produce novel materials such as heavy metals removal, catalysis, surface plasmon resonance, pharmaceuticals and colourimetric sensors [23-27].

Silver nanoparticles have been of great importance for the detection of toxic metal ions in the chemosensor area over the past few years, due to their unique optical properties, sharp surface nanostructures and strong surface interface to improve host-guest interaction capability. The Metal Enhanced Fluorescence (MEF) effect is favoured for the functionalized nanoparticles. Bharat et al. reported calix[4]resorcinarene polyhydrazide (CPH) protected fluorescent silver nanoparticles (AgNps) for a selective sensor of Fe(III) [8], Keyur et al. reported calix[4]pyrrole octahydrazide (CPOH) capped silver nanoparticles for a selective sensor of Hg(II) [28], Bharat et al. prepared calix[4]resorcinarene polyhydrazide (CPH) capped silver and gold nanoparticles which can be used as a selective sensor for bio-molecules like L-Dopa and histidine accordingly [29]. Octamethoxy resorcin[4]arene tetrahydrazide (OMRTH) capped silver nanoparticles also synthesize which act as a sensor for cadmium by Bharat et al [30]. Recently researcher's focuses on drug-capped metal nanoparticles to enhance their activity like anti-bacterial, anti-oxidant and fluorescence. Sharif et al. reported Isoniazid capped silver nanoparticles for selective sensors of chromium [31].

Transition metal cations have serious human health issues, above their certain limit. Thus, the colourimetric sensory system needs two important features for its quick, responsive and selective detection. (i) Robust coating agent on the surface of nanoparticles, for better stability and avoids aggregation [32, 33]. (ii) Specific binding property for selective cations [34].

The creation of highly sensitive and selective analytical methods for the identification of ions such as Hg^{+2}, Pb^{2+}, Mg^{+2}, Zn^{+2}, Ba^{+2}, Cd^{+2}, Li^{+1} etc are essential. Many analytical techniques such as fluorescence spectroscopy, atomic absorption spectrometry, inductively coupled plasma mass spectrometry, and inductively coupled plasma emission spectrometry was used for this purpose. These methods need expensive and sophisticated instruments for detection, so there is a need to develop a simple, easy and cheap method for the detection of such kinds of ions.

Silver Nps have sensitive optical properties, It becomes excellent colourimetric sensors for these ions. The molar extinction coefficient of silver Nps is 3-5 orders higher compared to others due to their Localized Surface Plasmon Resonance (LSPR). Such shifting positions of the LSPR band, affected by the shift in the inter-particle gap, can be easily measured by UV-Vis-spectroscopy and even clearly seen with a naked eye [35]. Due to its sensitive optical properties, it can be used as a sensor for metals also [36, 37].

Carbidopa, known to be a peripheral decarboxylase inhibitor prevents the instant breakdown of Levodopa allowing it to enter the brain and increase dopamine levels. It inhibits the conversion of L-dopa [38, 39]. Levodopa and Carbidopa are used in combination to treat the symptoms of parkinson's disease or parkinson-like symptoms (e.g., shakiness, stiffness, difficulty moving)[40]. Parkinson's disease is thought to be caused by too little of a naturally occurring substance (dopamine) in the brain. Levodopa transforms the brain into dopamine, thus helping to control motion. Carbidopa prevents levodopa from breaking down in the bloodstream, so that more levodopa may enter the brain. Carbidopa may also reduce some of the side effects of Levodopa, such as nausea and vomiting [41]. In this paper, the synthesis of carbidopa capped silver metal nanoparticles and their effective use for the selective sensor of L-dopa amino acid and lead metal ions have been reported. These scaffoldings also act as naked eyes detection of both analytes so it's used as a colourimetric sensor also.

Materials and Methods

All the reagents and metal salts of AR grade were purchased from Sigma-Aldrich and used without further purification. Solvents used for spectroscopic studies were purified and dried by standard procedures before use. All aqueous solutions were prepared from quartz distilled deionized water which was further purified by a Millipore Milli-Q water purification system (Millipack 20, Pack name: Simpak 1, Synergy). Metal salts such as Silver Nitrate, Magnesium Nitrate, Barium

Nitrate, Cadmium Nitrate, Lithium Carbonate, Zinc Nitrate, Mercury Sulphate and Lead Acetate were purchased from Sigma-Aldrich. Carbidopa API was purchased from Divi's Laboratories Limited, Andhra Pradesh. Amino Acids Reference Standard Kit was purchased from the SRL (46832). Antioxidant activity of the nanoparticles is performed by 2,2- diphenyl-1-picrylhydrazyl (DPPH) which is purchased from Sigma Aldrich. Other reagents and solvents of analytical grade were purchased from commercial sources and used without further purification. All aqueous solutions were prepared from Millipore water (resistivity, 18 ΩX; Millipore Systems). Nanoparticles are prepared by vigorous stirring at room temperature in a magnetic hot plate stirrer REMI 2MLH. Absorption spectra were studied on an SYSTRONICS DOUBLE BEAM UV-Vis spectrophotometer: 2201. XRD measurements were done by Bruker axs-D8. FTIR spectra were recorded in Bruker FTIR Spectrometer ALPHA II. The particle size and zeta potential were determined by using the Malvern Zeta sizer (Model; ZEN3600) as such without dilution. TEM images were recorded in MACK/model JEOL, JEM 2100 at an accelerated voltage of 200 kV. A drop of a dilute solution of a sample in water on carbon-coated copper grids was dried in a vacuum and directly observed in the TEM.

Synthesis of Carbidopa Capped Silver Nanoparticles

4 mL (1 mM) solution of $AgNO_3$ was added to a conical flask containing 89 mL of Millipore water and then 2 mL (1 mM) aqueous solution of carbidopa as capping and stabilizing agent with 2 mL (0.5 N) aqueous solution of $NaBH_4$ (Sodium Borohydride) as reducing agent was added rapidly drop by drop under vigorous stirring. Carbidopa capped silver colloids (CARBI-AgNps) were found immediately but vigorous stirring was continued for 20 minutes to ensure complete homogenization. The colour change of the solution from colourless to yellow indicates the successful development of silver nanoparticles Fig. 1 and Fig 2.

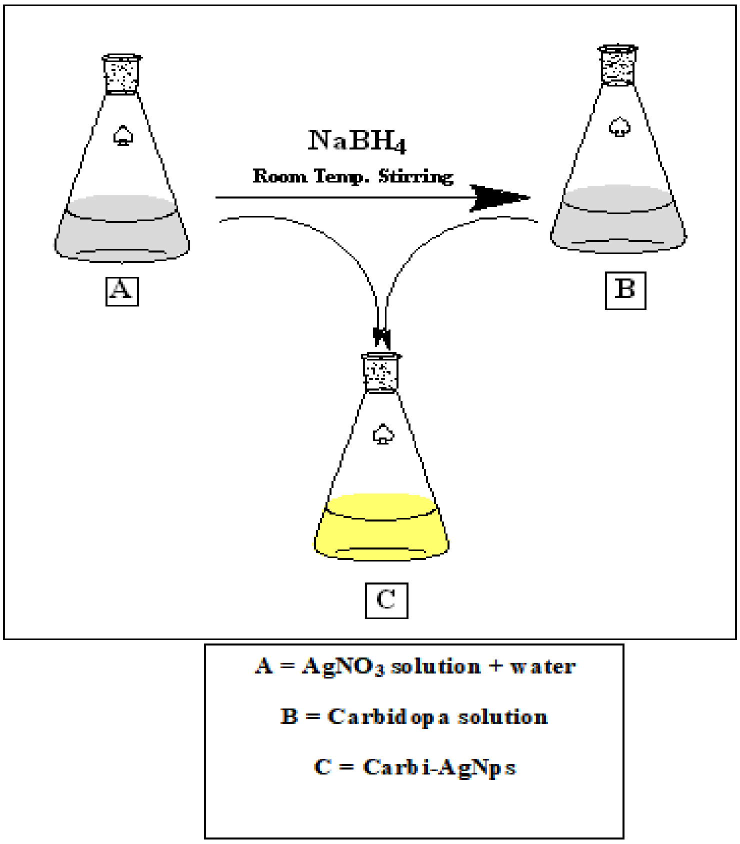

Fig.1: One-pot Preparation of Silver Nanoparticles

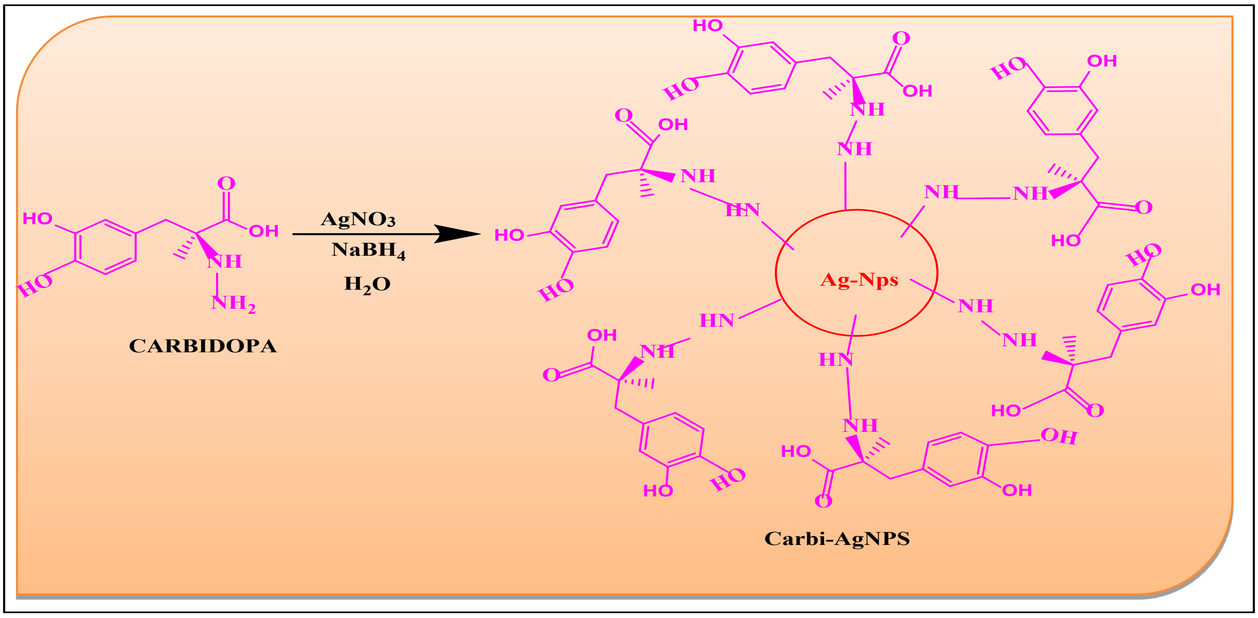

Fig.2: Formation of silver nanoparticles

Interaction of Amino Acids With CARBI-AgNps

Interaction of various amino acids with CARBI-AgNps by UV-Visible spectroscopy measurements

1 mL (1 mM) aqueous solution of all essential 24 amino acids like leucine, glutamic acid, glycine, tryptophan, aspartic acid, proline, cysteine, etc were introduced to 1mL CARBI-AgNps individually. CARBI-AgNps showed a hyper shift in surface plasmon resonance successfully only in presence of L- dopa. Due to higher extinction coefficients of some nanoparticles, reported as colourimetric sensors. They have an affinity to agglomerate in the presence of the analyte, which can lead to colour change. To investigate the colourimetric response of CARBI-AgNps, different amino acids like leucine, glutamic acid, glycine, tryptophan, aspartic acid, L-dopa, proline, cysteine etc concentration were added to these nanoparticles. No visible colour change was observed with any of the amino acids except L-dopa, which exhibits a sharp change in colour from yellow to purple and finally to blue which can be easily judged by the naked eye.

Antioxidant Study

The antioxidant property of silver nanoparticles was evaluated by in vitro antioxidant assay using (1-1-diphenyl-2-picrylhydrazyl) DPPH radical quenching method [42-44]. The reaction mixture containing 3 mL of 1 mM DPPH was mixed with a solution containing (0.5, 1, 1.5, 2, 2.5, 3 ml) amount of nanoparticles and the total volume is make-up of 10 ml with methanol. Put the reaction mixture in dark for 30 minutes and the colour change was measured spectrophotometrically at 517 nm. The radical-scavenging activity (RSA) was evaluated by using the following equation

$$\text{(Percentage of inhibition)} \quad \%\,\text{RSA} = \frac{(Adpph - As)}{Adpph} * 100. \qquad \text{Eq. (1);}$$

Results and Discussion

Spectroscopic Study of Silver Nanoparticles

The Carbidopa wrapped metal nanoparticles (silver) were characterized by UV-Visible spectroscopy. For spherical nanoparticles, only a single Surface plasmon resonance (SPR) band is expected whereas two or more SPR bands are observed for anisotropic particles depending on the shape of the particles. There is a well-defined relationship between nanoparticles size and SPR band position i.e., with an increase in the size of the particle, the surface plasmon resonance band shifts to the red and vice versa. The synthesized metal nanoparticles (AgNps) showed a single surface plasmon band at 404nm Fig. 3 and it remained the same for more than three months (90 days) indicating the presence of highly stable and spherical metal nanoparticles Fig. 4

Characterization of nanoparticles is significant to evaluate the nature of nanoparticles. The characterization of nanoparticles can be performed by using Transmission Electron Microscopy, Scanning Electron Microscopy (TEM, SEM), UV–Vis Spectroscopy, X-ray Photoelectron Spectroscopy (XPS), Dynamic Light Scattering (DLS), Powder X-ray Diffractometry (XRD), and Fourier Transform Infrared Spectroscopy (FTIR) [45].

These techniques are used for determining various properties such as size, geometry, shape, crystallinity, and surface area. For example, the morphology shape and particle size of nanoparticles could be specified by TEM.

The size distribution of the carbidopa capped silver nanoparticles was characterized by three different techniques like Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and Particle Size Analyzer (PSA). TEM is used to visualize the shape as well as to measure the diameter of nanoparticles. Whereas, PSA provides information regarding the size of aggregates rather than the diameter of individual particles. TEM image of (AgNps) as shown in Fig.5a depicts that the synthesized silver nanoparticles are more or less mono dispersed with spherical or roughly spherical shape and had an average diameter of 10 nm with a majority of particles within the size range of 3 to 7nm. Also, no aggregation or formation of large particles was observed by the TEM image.

The size distribution of the silver nanoparticles was also measured by Malvern's particle size analyzer (PSA). As shown in Fig. 5b the particles are homogeneously monodispersed with an average particle size in the range of 30-40 nm. The statistical graph and size distribution Vs intensity has been shown in Fig. 5c the overall particles charge in a particular medium is denoted as their zeta potential value which is responsible for deciding the fate of stability. The particle size image is shown in Fig. 6 and synthesized silver nanoparticles had a 23 ± 2 MeV zeta potential value Fig. 7, which is sufficient to keep the particles away from aggregation and maintained stability. DLS analysis is a well-established technique for measuring the size of molecules and particles typically in the submicron region, and with the latest technology lower than 1 nm. DLS study was performed to know about the size distribution profile of green silver nanoparticles produced through a biological route. However, the DLS study shows that the synthesized nanoparticle is in the range of 30-40 nm in small proportions Fig. 8.

Depiction in Fig. 9, A representative of the crystalline structure of carbidopa synthesized AgNps were analyzed by XRD measurements. AgNps solution drop-coated on glass was done throughout the experimental work. a Bruker axs- D8 Advance instrument operating at a voltage of 40 kV and current of 20 mA with Cu Kα radiation. The XRD pattern showed three intense peaks at 38°, 47°, and 77° corresponding to 111, 200 and 311 planes in the whole spectrum of 2θ. XRD profile of the silver nanoparticle displaying the structural information and crystalline nature of the silver nanoparticle synthesized from carbidopa, The average particle size of the silver nanoparticle was identified by using the following Debye-Scherrer's equation

The calculation of average grain size was attained by using Scherer's Eq. (2).

$$D = K\lambda \,/\, \beta Cos\theta \text{ Å} \qquad \text{Eq. (2);}$$

Where D is the average crystallite size in Å, K is the shape factor, λ is the wavelength of X-ray (1.5406 Å) Cu -Kα radiation, θ is the Bragg angle, and β is the corrected line broadening of the Nps. FTIR measurement was carried out through the wavenumber range from 400 to 4000 cm^{-1} using the KBr pellet method at room temperature. FTIR was used to identify the functional groups that are involved in the nanoparticle reduction and apparent oxidative stability with other nanoparticle syntheses. The FT-IR spectrum of AgNps synthesized using carbidopa showed absorption bands indicating the presence of active functional groups in the synthesized silver Nanoparticles. The intensity peaks are slightly increased for the period of silver nanoparticle synthesis like 3554, 3456, 3358, 3229 and 3192 cm^{-1} as well as some intensity peaks decreased like 1650, 1618, and 2872, 2360 cm^{-1}. Fig 10 shows the band at 3554, 3456, 3358, 3229 and 3192 correspond to -COOH, N-H, O-H stretching vibrations of carboxylic, amide, alcohol functional group. The peak at 1650 and 1618 indicate C=C and C=O stretching vibrations and its related to the C=C of aromatic rings overlapped with the carbonyl group and C–OH stretching vibrations, respectively.

The peak at 1557 represents N=O. The band at 2872, 2360 corresponds to C=C stretching vibration. The weak band at 1922 indicates C-N stretching vibrations and it corresponds to the presence of alcohols, carboxylic, acids and amines in the carbidopa. The presence of active functional groups in carbidopa results in the swift reduction of silver ions to silver nanoparticles. To obtain a good signal-to-noise ratio silver nanoparticles were taken in the range of 500–4000 cm^{-1}.
The variety of N- and O-containing functional groups as well as the possible C−C and C−H result in several possible assignments for the bands observed. The broad bands observed near 3400 cm^{-1} are likely due to −O−H stretch from hydroxyl groups expected to be present in the carbidopa. The band may also be at least partly due to incorporated water. The bands appearing between 3132 and 2872 cm^{-1} are C−H stretching modes from hydrocarbon chains. Bands due to C−O−H bending vibrations appear near 1459 cm^{-1}. The amide band from protein carbonyl stretch appears at 1618 cm^{-1} and has been noted elsewhere in similar nanoparticle analyses in Fig.10.

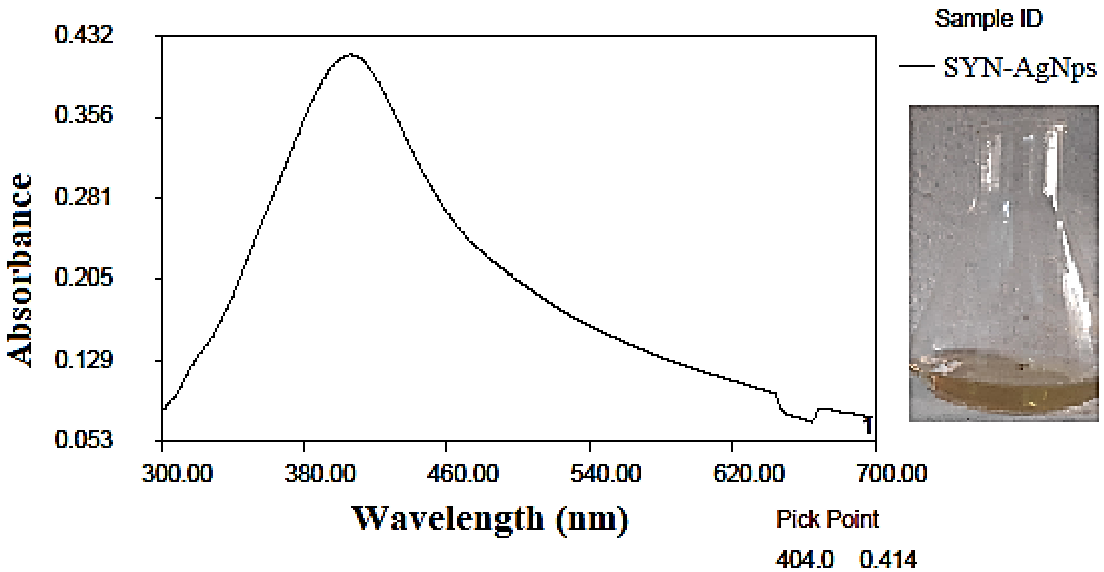

Fig. 3: Characterization of CARBI-AgNps by UV-Vis Spectroscopy

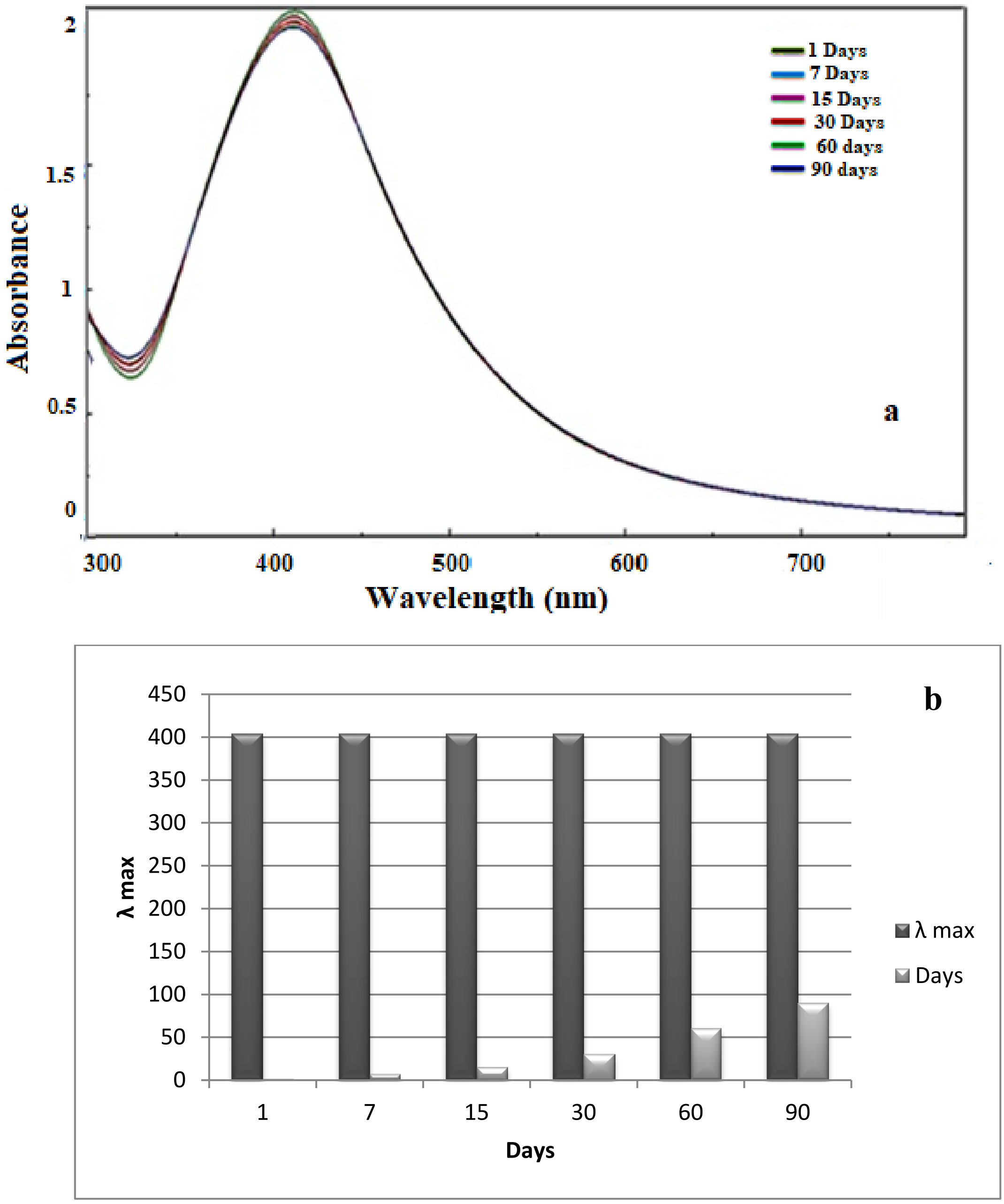

Fig. 4: (a) Stability study of CARBI-AgNps up to 90 days and its SPR band (b) Time-dependent stability of CARBI-AgNps

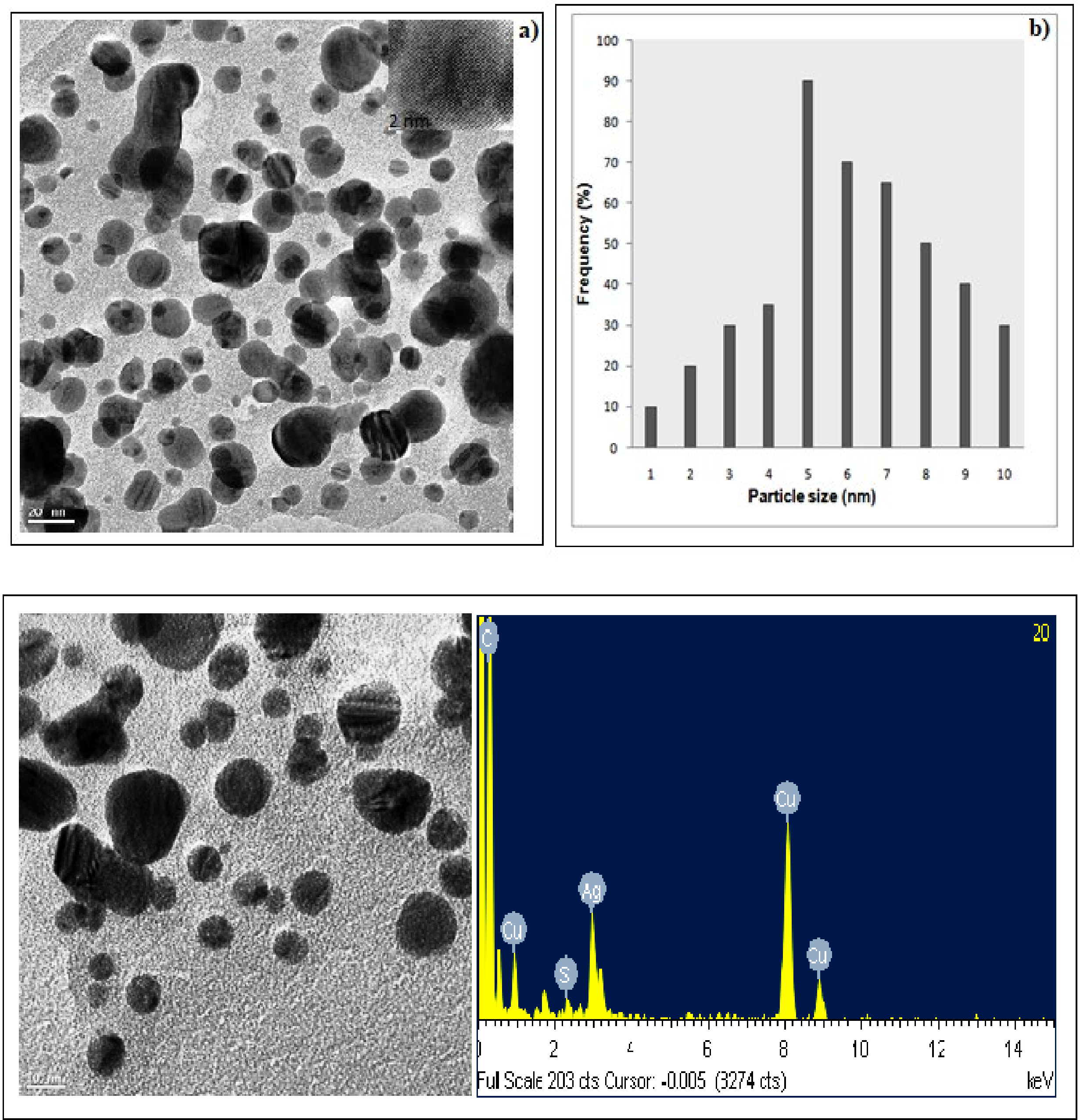

Fig. 5: a) TEM image of Carbidopa capped AgNps b) Particle size distribution graph of CARBI-AgNps c) EDX spectrum of CARBI-AgNps

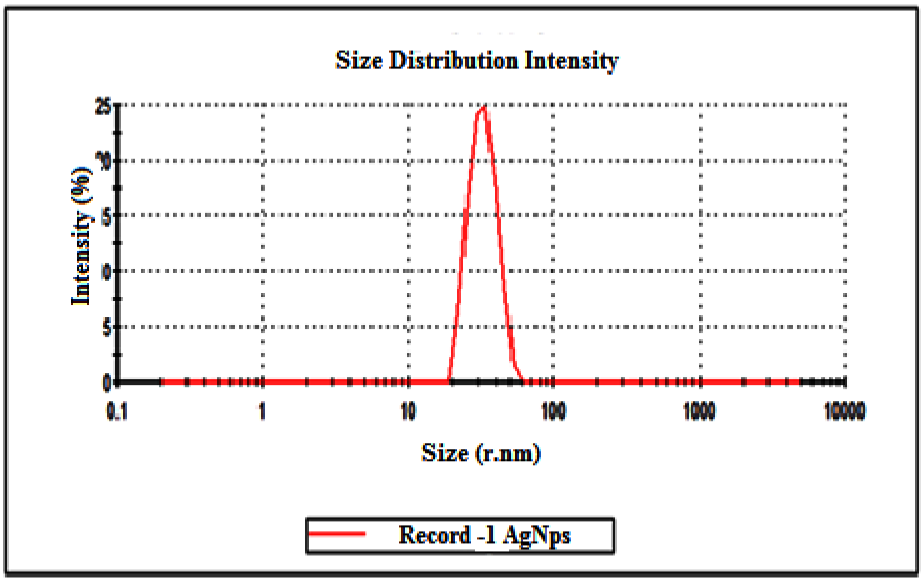

Fig. 6: Particle size Analyzer (PSA) image of carbidopa capped AgNps

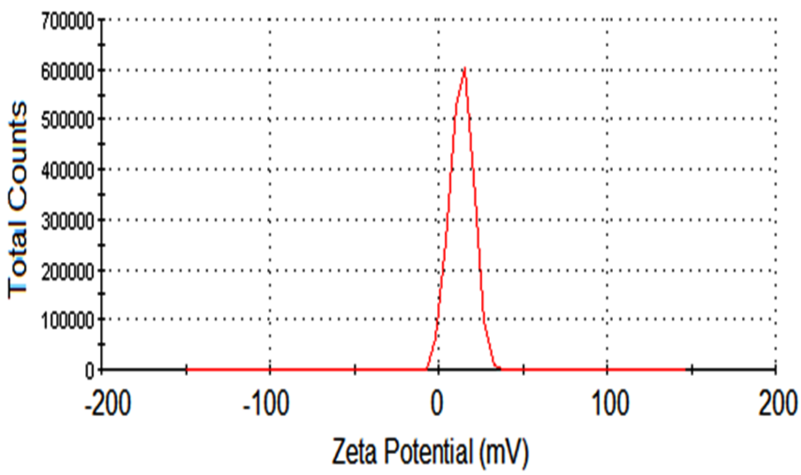

Fig. 7: Zeta potential image of carbidopa capped AgNps

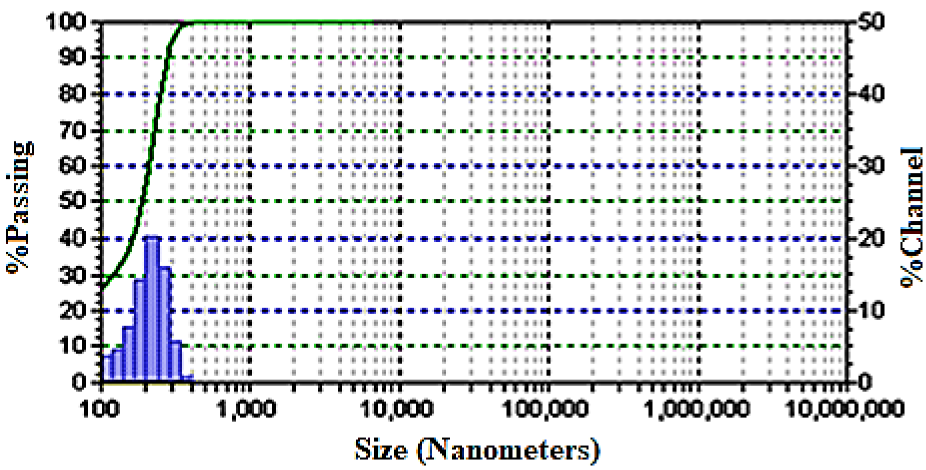

Fig. 8: DLS image of carbidopa capped AgNps

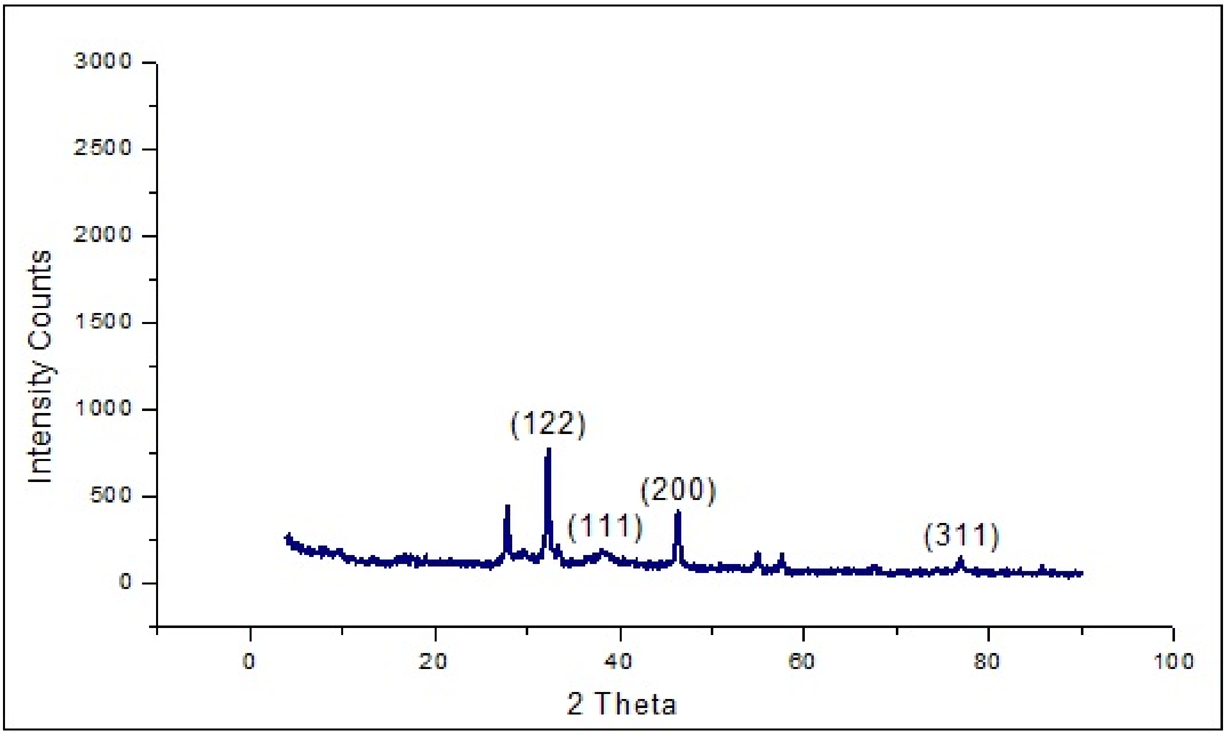

Fig. 9: XRD patterns of carbidopa capped AgNps

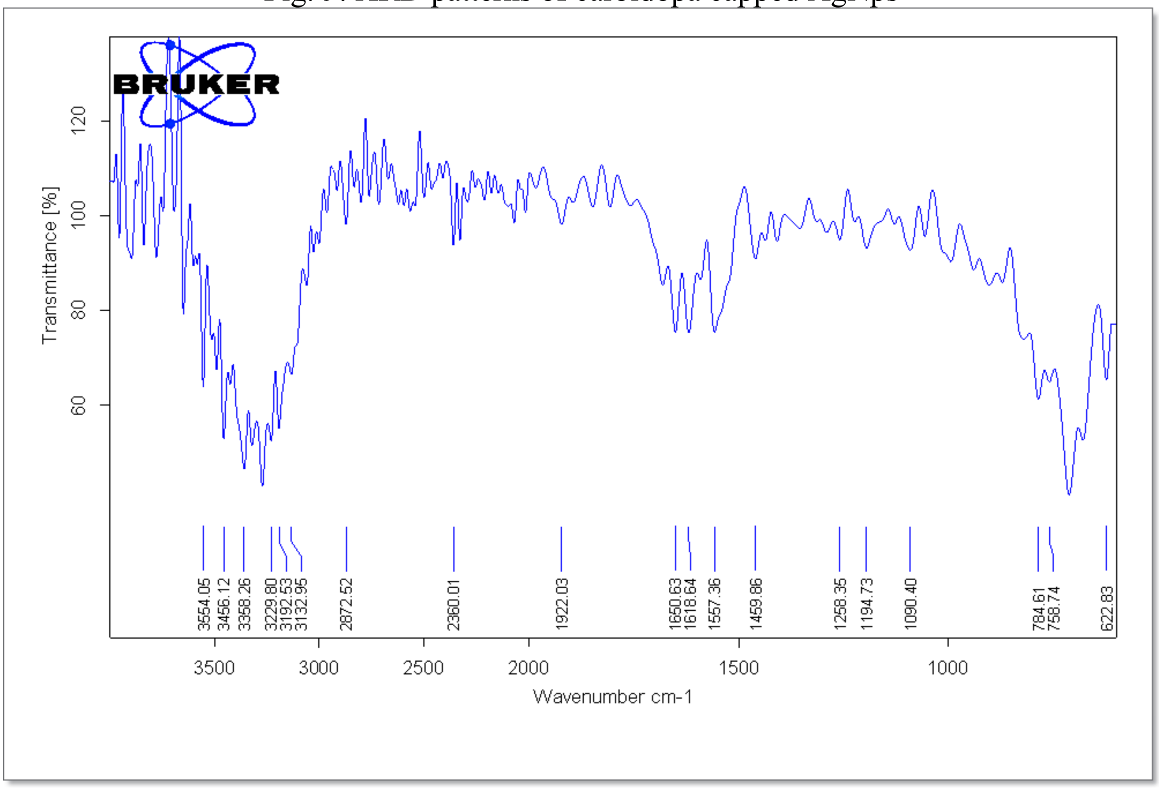

Fig.10: FT-IR spectrum of carbidopa capped AgNps

3.2 Applications of CARBI-AgNps

3.2.1 Study of metal salts with CARBI-AgNps

It can be observed from Fig. 11 that, in presence of Pb^{2+} ion can also show by colour change sensitive to the naked eye. Fig. 12 illustrates the UV-Visible absorption spectra of CARBI- AgNps in presence of Pb^{2+} ion. The UV- Visible absorption spectra of CARBI- AgNps do not change in the presence of Mg^{+2}, Ba^{+2}, Cd^{+2}, Li^{+1}, Zn^{+2} and Hg^{+2} ions. Significant changes in the UV–Vis spectra of the CARBI-AgNps, AgNps and lead acetate were observed. The observations as seen in the UV-Visible absorption spectra of CARBI-AgNps + Pb^{2+} ion, give the best result at the lowest concentration of Pb^{2+} ions

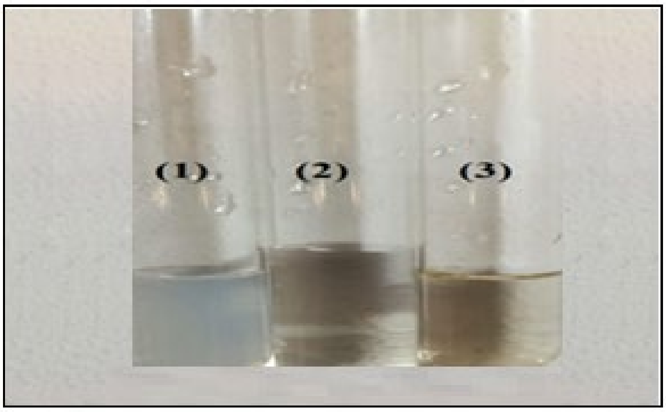

Fig. 11: Colour change due to sensing of Pb^{+2} ion (1) Pb – AgNps, (2) Pb^{2+}, (3) CARBI-AgNps

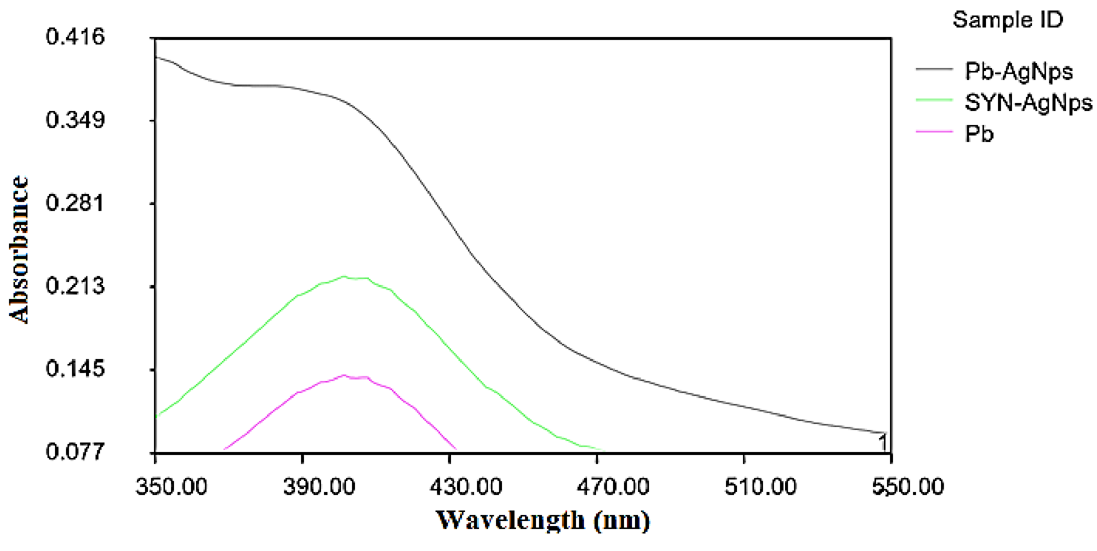

Fig. 12: UV-Vis spectra of Pb-AgNps, CARBI-AgNps and Pb^{+2} Solution

3.3 Study of Amino acids with CARBI-AgNps

The prepared colloidal AgNps were subjected to UV–Vis spectroscopy. The particles gave a strong plasmon resonance band at 404 nm. On interacting with L-dopa in the presence of CARBI-AgNps the particles immediately aggregated with an intense colour change from yellow to purple. The result was reliable with the red shift of UV–Vis spectra. The results obtained clearly indicate that except for L-dopa no other amino acids exhibited a colour change Fig. 13. In addition, UV–Vis data did not reveal the presence of a peak at around 404 nm in the other amino acids except L-dopa [46, 47] Fig. 14.

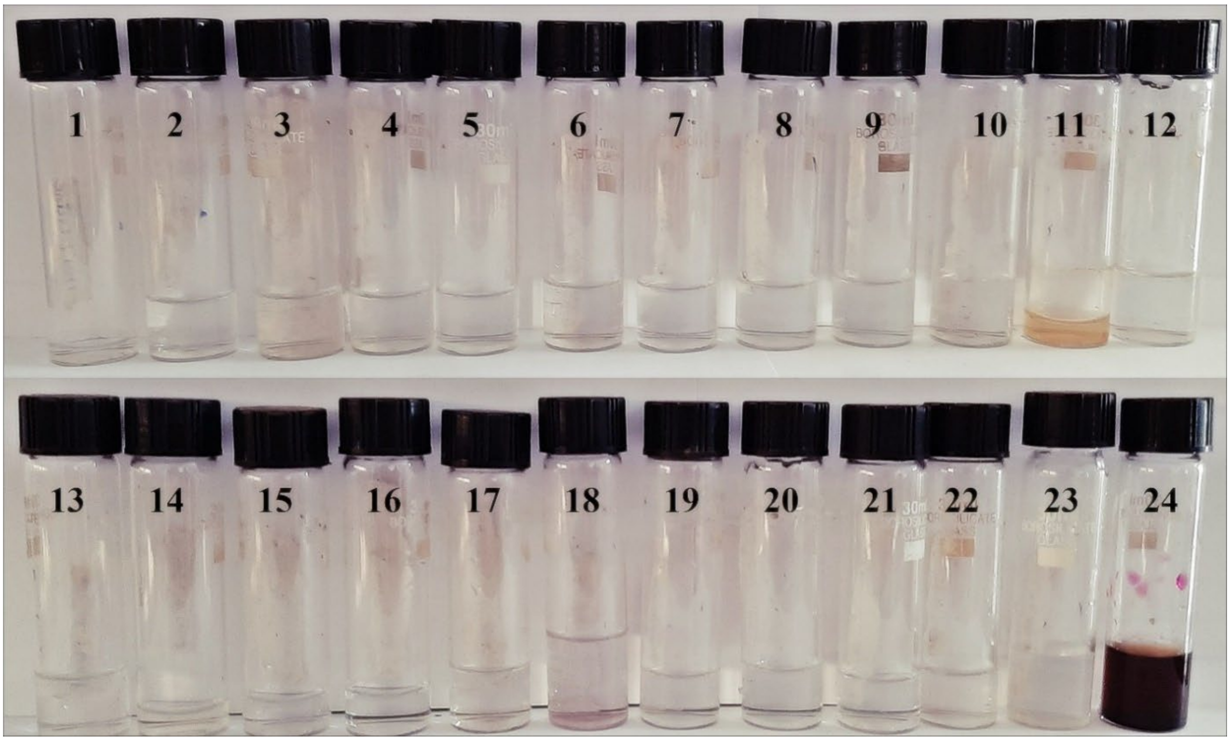

Fig. 13: Interaction of CARBI-AgNps with various amino acids (1. Cysteine, 2. Tyrosine, 3. Lysine, 4. Methionine, 5. Methionine, 6. Glutamic acid, 7. Leucine, 8. Threonine, 9. Aspartic Acid, 10. Hydroxyproline, 11. Valine, 12. Cystine 13. Proline, 14. Serine, 15. Histidine, 16. Glycine, 17. Alanine, 18. Arginine 19. Leucine, 20. Isoleucine, 21. Phenylalanine, 22. L-Ornithine monohydrochloride, 23. Tryptophan 24. L-dopa,)

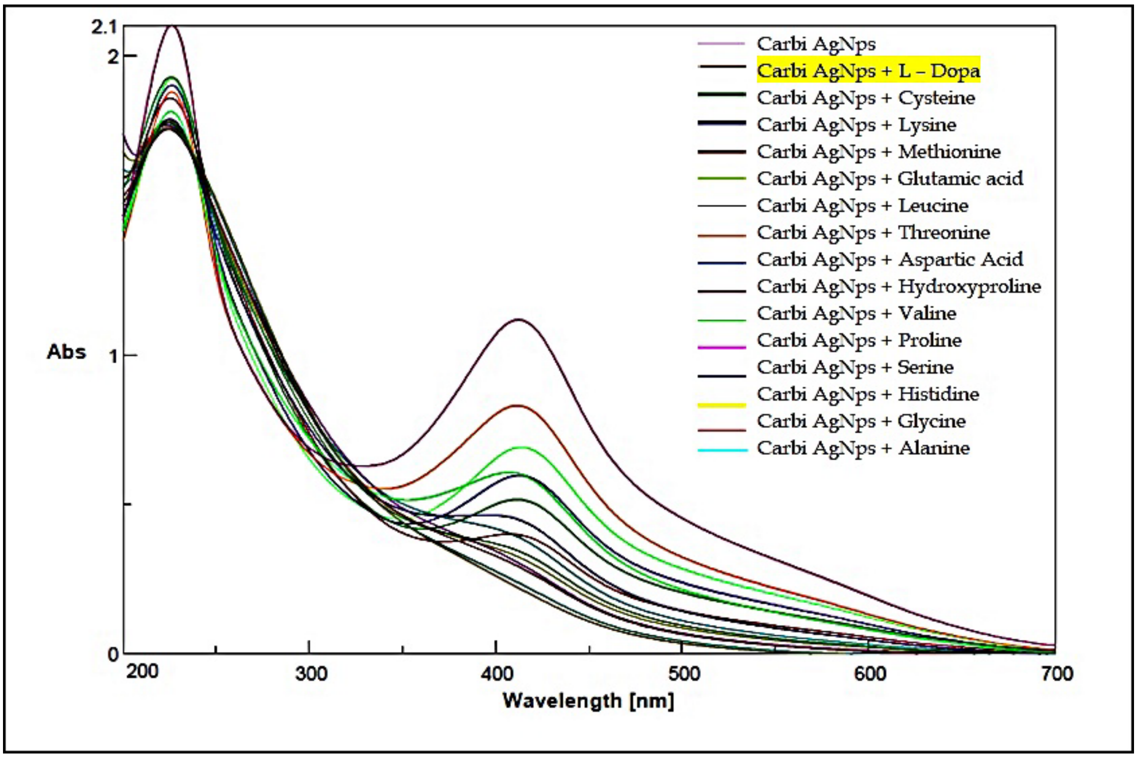

Fig.14: UV-Vis Spectra of Carbidopa capped AgNps with various amino Acids and Selective interaction with L-Dopa amino Acids.

3.4 Antioxidant Study with CARBI-AgNps

In vitro antioxidant assay by (1-1-diphenyl-2-picrylhydrazyl) DPPH radical quenching method

Free radical has the most unpaired outer electron so they are extremely reactive and unstable. The free radicals, by obtaining or losing an unpaired electron, often seek to form a stable bond. In need of reaction conditions, silver nanoparticles can accept/donate an electron to quench radicals [44, 48].

A comparative study of antioxidant activity with standard antioxidant reference materials ascorbic acid (AA) and free radical (DPPH) were conducted shown in Fig. 15. and Table 1 shows the UV data of Anti-oxidant activity. Although the antioxidant activity of CARBI-AgNps is slightly lower than that of standard antioxidant Ascorbic Acid (AA) which is shown in the graph of Absorbance Vs Concentration Fig.16, it is, therefore reasonable to propose that CARBI-AgNps hold the potential of their use as a good antioxidant agent Fig.17 shows maximum % RSA (Radical Scavenging Activity) at Lowest concentration [49].

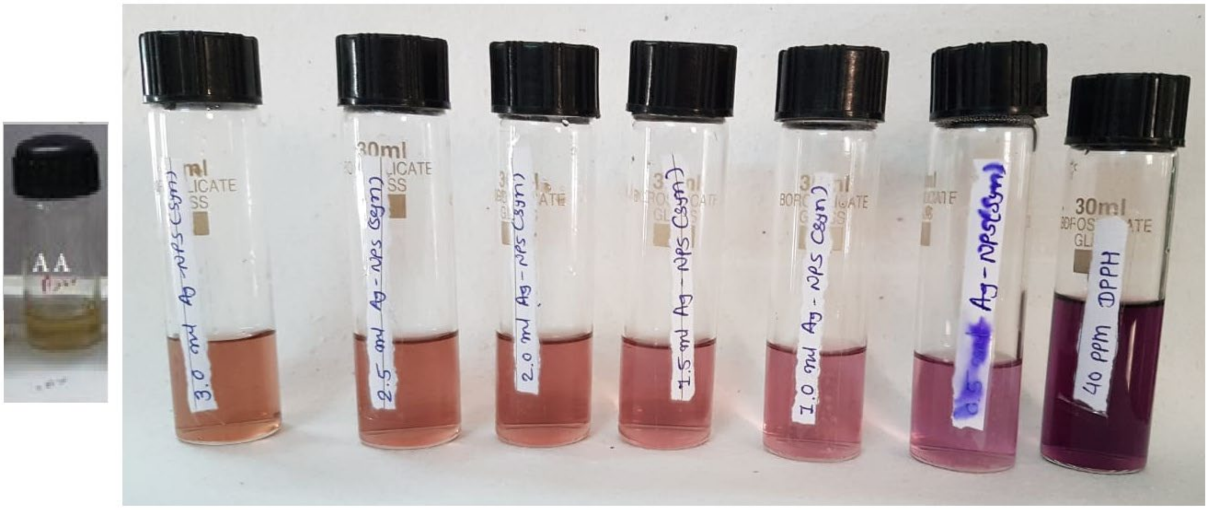

Fig. 15: Antioxidant assay of DPPH with standard Ascorbic Acid and different concentrations of CARBI-AgNps

Table 1: UV data of Anti-oxidant activity

Sr. No.	Wavelength	Concentration	Absorbance
1	517	0.5	0.269
2	517	1	0.307
3	517	1.5	0.325
4	517	2.0	0.354
5	517	2.5	0.361
6	517	3	0.366
7	517	AA	0.998
8	517	DPPH	1.109

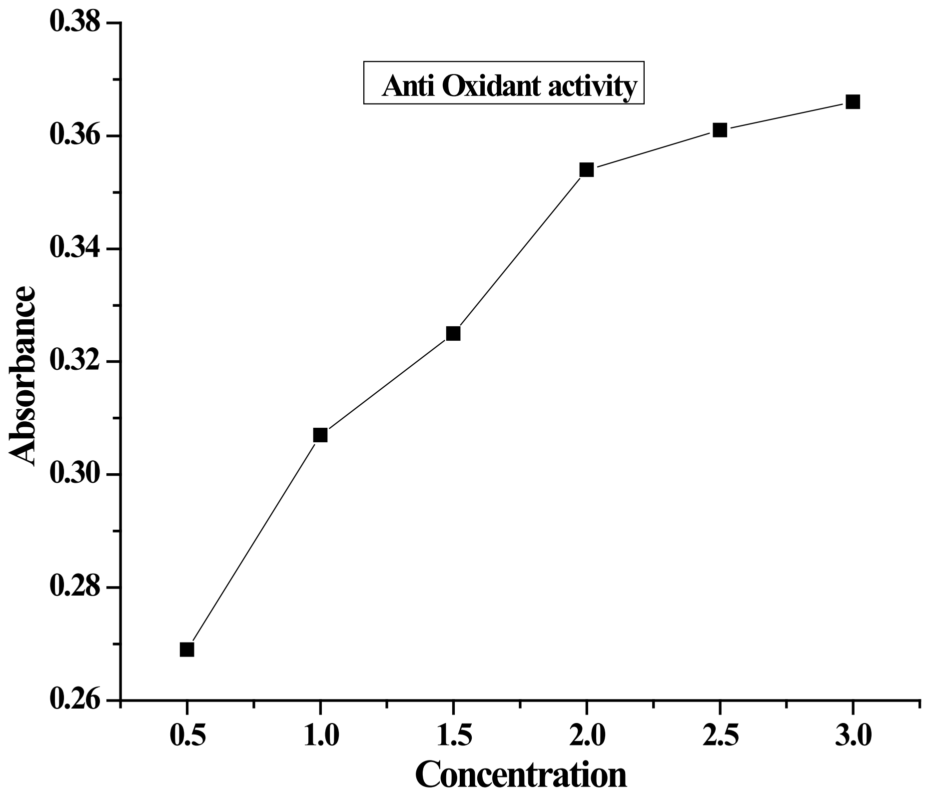

Fig. 16: DPPH Antioxidant Assay at λmax = 517 nm between the different concentrations of CARBI-AgNps and standard Ascorbic Acid (AA)

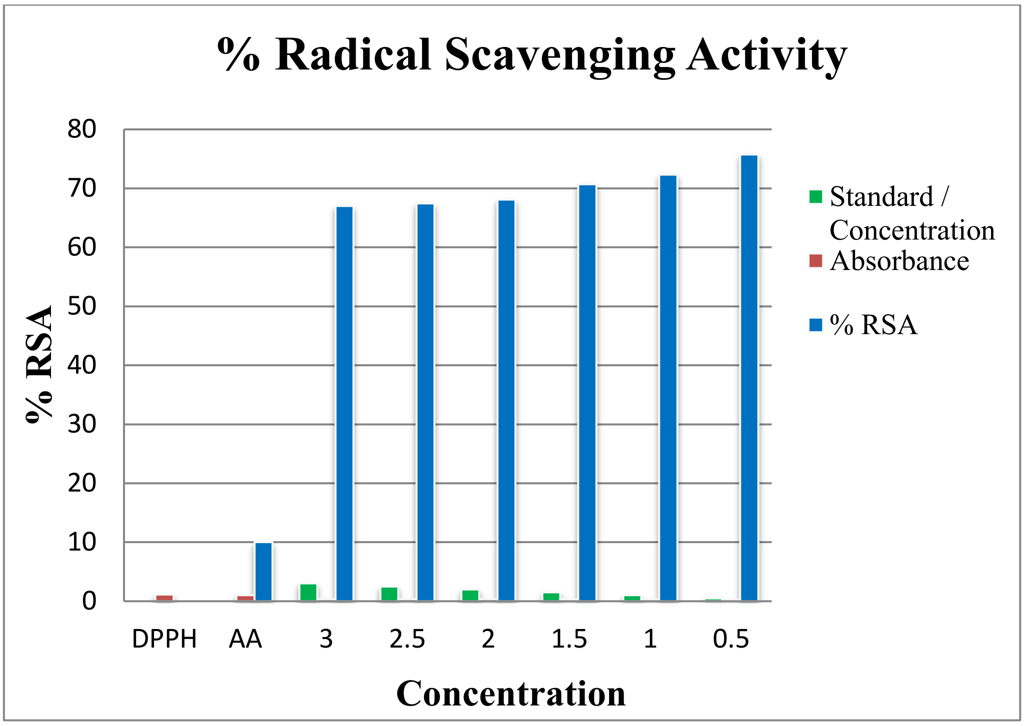

Fig. 17: % RSA with Absorbance and Concentration

Conclusions

A rapid, simple one-pot synthesis method for AgNps has been developed by using carbidopa as a capping agent. Drug-capped nanoparticles found stability for long periods.

From the interaction study with all 24 amino acids like Glycine, DL-Aspartic Acid, Alanine, Phenylalanine, L-Cysteine Hydrochloride monohydrate, DL-Methionine, L-dopa, L-Glutamic Acid, L-Histidine Hydrochloride, L-Hydroxyproline, L-Isoleucine, DL-2 Aminobutyric Acid, DL-Nor-Leucine, L-Leucine, L-Ornithine Monohydrochloride, L-Tryptophan, L-Arginine Hydrochloride, L-Cystine, L-Proline, DL-Serine, L-Lysine Monohydrochloride, L-Tyrosine, DL-Valine, and DL-Threonine it can conclude that prepared nanoparticles are selective and sensitive probe for L-dopa, which have been confirmed with UV- Visible spectroscopy.

Synthesized nanoparticles are also selective and sensitive probes for lead metal, which have been confirmed with UV- Visible spectroscopy after the interaction study of various metal salts with CARBI-AgNps.

In addition, carbidopa capped AgNps (CARBI-AgNps) was also successfully evaluated to show radical scavenging activity against DPPH in various amounts like it shows up to 75.74 % RSA in a minimum concentration of AgNps and up to 66.99 % RSA in the maximum concentration of AgNps in 35 minutes.

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Conflicts of Interest

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