

6.1. Introduction:

Metallic nanodendrites are 1D nano structured materials which are attractive due to their high degree of connectivity throughout the structural surface as well as their large surface area to volume ratio. Such properties make it very useful for a number of potential applications in catalysis, plasmonics, sensing, unusual chemical reactivity, opto-electronic etc. These nanostructured materials of different shape having diverse facets which is capable to catalyze the reactions with different efficiencies. Therefore, it is interesting to synthesize metal nanodendrites and challenging too. Depending upon the reducing agent chosen, method of preparation, reduction rate, reaction time, etc final product can take a variety of different shapes [Zhou *et al.*, 1999; Ren *et al.*, 2011; Tao *et al.*, 2007; Yin *et al.*, 2002]. Anisotropic nanomaterials have become particularly attractive because their decreased symmetry often leads to unusual chemical and physical behavior. Thus, more interesting shapes bring complexity to the optical response, which has been shown to be crucial toward important technological developments. The use of light in metal precursor solution viz. UV irradiation as the driving force may provide generation of colloidal metal nanostructures [Jin *et al.*, 2001; Tao *et al.*, 2007; Wang *et al.*, 2003; Chaki *et al.*, 2002]. We have adopted a photochemical assisted formation of silver nanodendrites by phenothiazine (PTZ) without using external reductant, surfactant etc. The light assisted reduction of Ag^+ ions is initiated by the excitation of the phenothiazine. The excitation of phenothiazine produces singlet and triplet excited states, which is capable of undergoing electron-transfer reactions in the presence of electron donors or acceptors. The use of phenothiazine molecule serves dual purpose: to initiate the reduction of silver ions as well as stabilization due to adsorption of phenothiazine and its oxidation product with metal surface to protect the nanostructures. Phenothiazine (PTZ) is a photo chemically active electron donor compound having low ionization potential and can easily form radical-cations as discussed in the previous *chapter 5* under phenothiazine section. Photo chemically generated

electron during the photoionization of phenothiazine can be used for the reduction of Ag (I) to generate silver nanostructures.

Environmentally important analytes such as nitrites have much attracted the attention of analytical chemists and electrochemist in recent times. Nitrite plays key role in biological (final products in protein metabolism), environmental (widely distributed in nature), clinical and many agricultural industrial processes.

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- Nitrite plays key role in environmental (widely distributed in nature, in nitrogen cycle, nitrite ion is an intermediate species, resulting from the oxidation of ammonia or from reduction of nitrate)
- Clinical and many agricultural industrial processes.

Nitrite ion is classified as an environmentally hazardous species because of its toxicity and it combines with blood pigments which produce meta-haemoglobin and causes oxygen depletion in the tissues. Also it reacts with amines and amides and generates carcinogenic N-nitroso-amine in body which may leads to kidney and liver failure [Salimi *et al.*, 2006; Ning *et al.*, 2010]. In nitrogen cycle, nitrite ion is an intermediate species, resulting from the oxidation of ammonia or from reduction of nitrate. Spectrophotometry, chromatography, flow injection analysis, chemiluminescence and various electro-analytical techniques are the common methods for estimation of nitrite [Li *et al.*, 2012; Yu *et al.*, 2010]. However, most of these methods require complex process, rather slow response and some of them are expensive also. Electrochemical determination is based on the electrocatalytic oxidation of nitrite by using suitable electrocatalyst which offers several advantages as compared to other analytical technique, especially no interference from nitrate ion and molecular oxygen, which are usually the major limitation in the determination of nitrite [Yang *et al.*, 2006; Pal *et al.*, 2010]. Thus, there is a need to develop simple, effective and less expensive electrochemical method for quantitative detection of the nitrite. Electrochemical detection of nitrite is difficult at the conventional bare electrode and requires relatively high over-potential potential due to poisoning of electrode surface. Also it gives sluggish response

result in decrease electrode sensitivity and accuracy [Khairy *et al.*, 2010; Azad *et al.*, 2010]. Hence an alternative way is to modify the electrode with suitable electro-catalyst to lower overpotential and poisoning of electrode surface and improve sensitivity. Here in, we have utilized (Ag–PTZH) hybrid nanomaterials for modification of the electrode and developed amperometric technique for electro-sensing of nitrite.

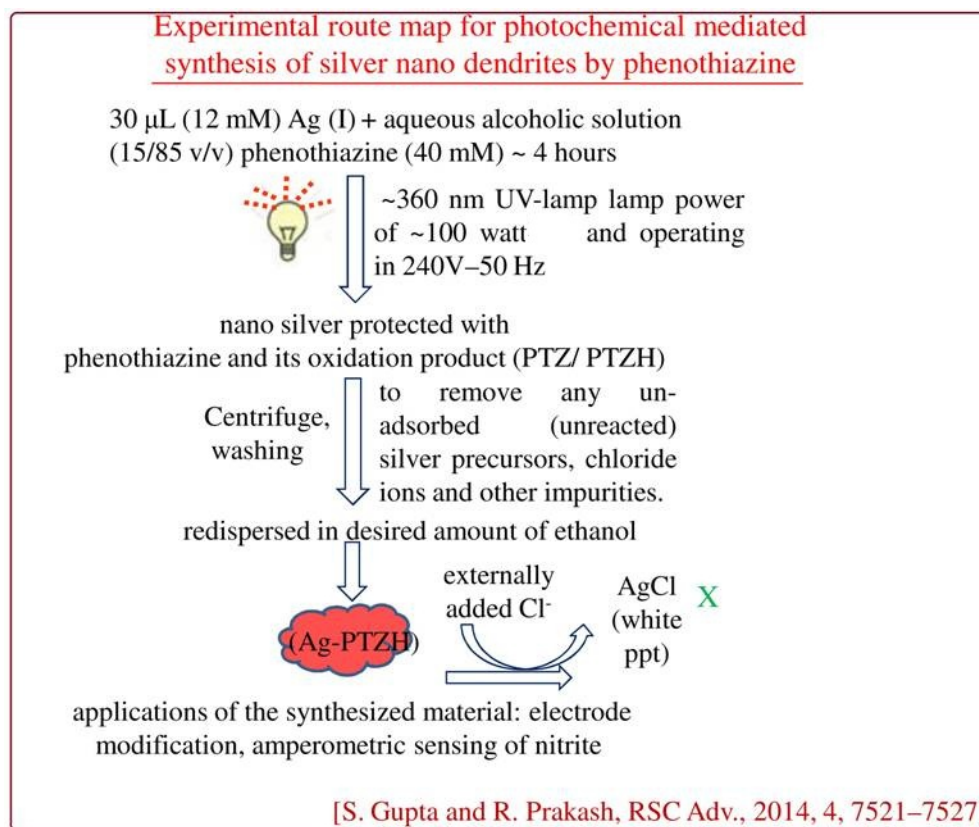
6.2. Experimental Section

6.2.1. Materials:

Silver nitrate and phenothiazine (Sigma-Aldrich, USA), indium tin oxide (ITO) plates (Asahi Beer Optical Ltd, Japan), ethanol (99.5%) and HCl (SRL, India), sodium nitrite, sodium nitrate, ammonium chloride and tris buffer (Merck India) were used as such as received, unless otherwise mentioned. The glassware used in the preparation of silver dendrites nanostructures was cleaned with freshly prepared aqua regia (3:1, HCl/HNO₃) and rinsed comprehensively with ultrapure water (Merck India).

6.2.2. Photochemical Synthesis of gold nanoparticles by phenothiazine:

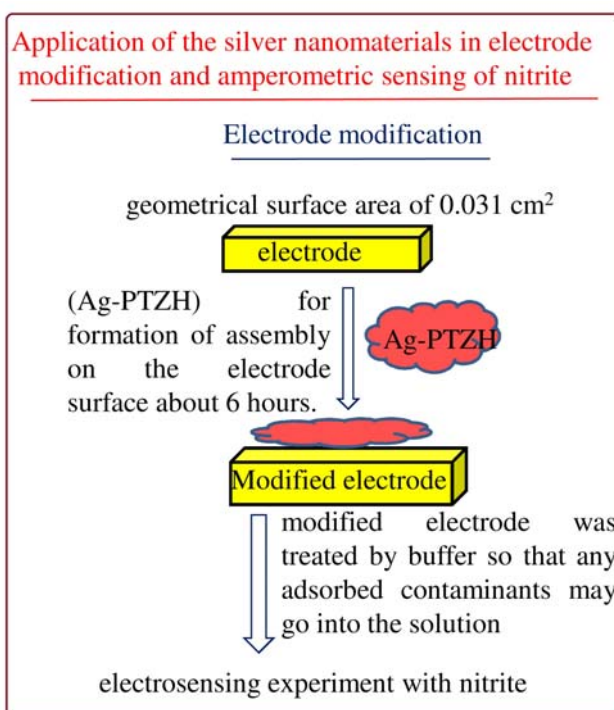
A typical synthesis of the silver dendrite nanostructures as follows: 30 μ L of AgNO₃ (12 mM) was added drop wise in ethanolic solution (Since phenothiazine is insoluble in water and is soluble in ethanol, aqueous ethanolic [15: 85 v/v] solution was made to dissolve the phenothiazine {40mM}) without stirring the content and simultaneously whole reaction content was irradiated using a 100W sun lamp having spectral output of \sim 360 nm to undergo photochemical reduction of Ag(I) for \sim 4 hrs [Gupta *et al.*, 2014]. The glass vial was kept at a distance of 12 cm from the light source to avoid heating of the solution. After completion of reaction, resulting solution was centrifuged at 12000 rpm for 4 min, to obtain the content of silver nanocrystals protected with phenothiazine and its oxidation product (PTZ/PTZH). This process is repeated for twice.



The obtained content was washed several times with mixture of alcohol and milli-Q-water (v/v: 40/60) to remove any un-adsorbed (unreacted) silver precursors and other impurities. Thus, the obtained content is redispersed in desired amount of ethanol so that it can be used for further application/experiments purposes.

6.2.3. Application of silver nanostructures in amperometric sensing of nitrite:

A polycrystalline gold electrode with a geometrical surface area of 0.031 cm^2 was used as a substrate for fabrication of nanostructured platform with prepared hybrid material. Thoroughly cleaned polycrystalline gold electrode was soaked in ethanolic solution of hybrid nanomaterial Ag-PTZH for formation of assembly on the electrode surface for 6 hours [Gupta *et al.*, 2014]. The assembled Ag-PTZH on the gold surface was examined by taking CV and FESEM images.



Oxidation potential, i.e. 0.44 V vs. AgCl/Ag was chosen for amperometric analysis of nitrite ions from CV studies under optimized conditions. A systematic change in the current was noticed after each addition of nitrite into the supporting electrolyte solution. Solutions used for electrochemical measurements were made with ultrapure millipore water (18.3 M Ω cm) and extensively purged with nitrogen gas before use.

6.2.4. Instrumentation:

UV-vis absorption spectrum was recorded using Lambda-25 Perkin Elmer (Germany) spectrophotometer by using a quartz cuvette having optical path length of 1 cm. Microscopic view of sample was examined using transmission electron microscope (HRTEM Tecnai G2, 20 FEI Corporation Netherlands) operating at 200 kV. Few μ L of the colloidal silver was dropped on the carbon-coated copper grids (obtained from Pelco International, USA) and allowed to dry. Fabricated electrode hybrid material Ag- PTZH was examined by FESEM images, recorded with a JEOL JEM 6700F field emission scanning electron microscope (FESEM).

Cyclic voltammogram and amperometric analysis for modified electrode was performed using Autolab Potentiostat-Galvanostat (PG-STAT, 302, Netherlands) using computer controlled GPES software. A conventional three-electrode system consisting modified electrode served as the working electrode, Pt foil as the counter electrode, and Ag/AgCl as the reference electrode, were used for all the electrochemical measurements. Neutral tris buffer (pH 7.2) was used as a supporting electrolyte and the scan rate was kept 50mV/sec for each measurement. Elemental analysis was recorded with X-Ray photoelectron, kratos analytical instrument, shimadzu group company AMICUS XPS UK.

6.3. Results and Discussion

6.3.1. Characterizations of photochemically synthesized silver dendritic nanostructures:

Qualitative and quantitative analytical spectrophotometric determination of gold and other metal salt had been done by using Phenothiazine [Nemcova *et al.*, 1990]. Nemcova and their research group have shown the reaction of some metal ion with phenothiazine which produces colored solution but they did not report the formation of such nanostructures and also did not go for microscopy. To the best of our knowledge there is no report available in scientific literature on the photochemical synthesis of silver dendrites nanostructures by using phenothiazine (PTZ) as a reductant as well as stabilizer. Phenothiazine was selected on the basis of its unique property, as a reducing capability under UV-visible light as well as capability to stabilize the silver nanostructures through the interaction with sulphur and nitrogen sites present in phenothiazine. Few amines and amino acids have capability to reduce Ag (I) as well as stabilize the nanoparticles through weak covalent as well as weak electrostatic interaction [Chaki *et al.*, 2002]. Photo-reduction of Ag⁺ ions is initiated by the excitation of the phenothiazine in the presence of UV irradiation. The reduction of Ag (I) is believed to occur according to the reaction mechanism as shown in figure 6.1. Phenothiazine get photoxidised

and generate the electron during the course of photochemical redox reaction and hence that produced photo electrons are used in reduction of silver salt {Ag (I)} [Gupta *et al.*, 2014].

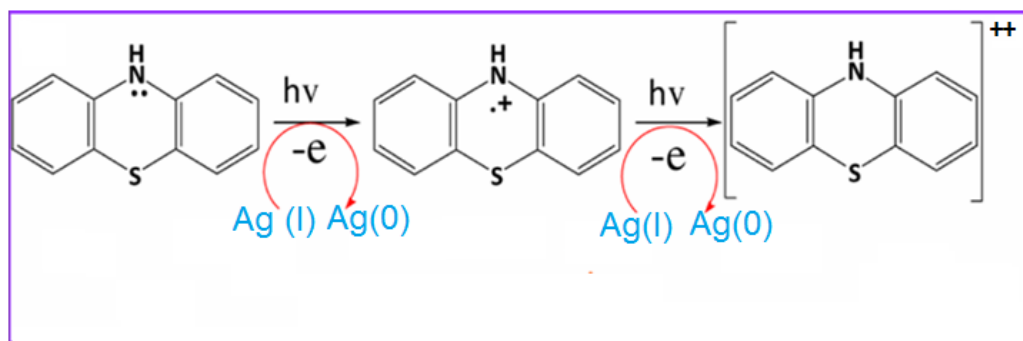


Figure 6.1: Reaction scheme for the photochemical assisted formation of nano silver by phenothiazine.

Dendrite structure of nano silver exhibited a *very-very broad absorption band* ~ 436 nm appeared in UV-vis as shown in figure 6.2. Pure dendritic silver nano structures do not exhibit SPR band in UV-visible spectrum. UV-visible spectra of silver nanostructures strongly depended upon the morphology.

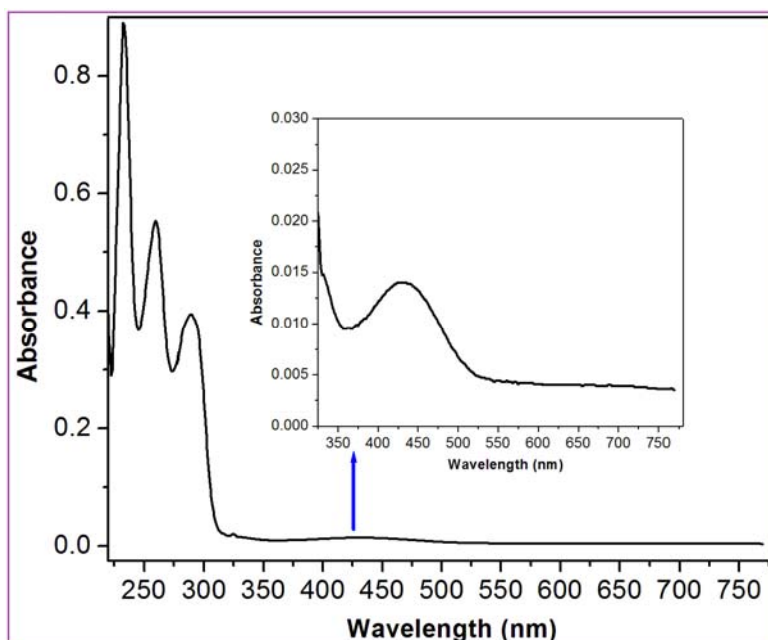


Figure 6.2: UV-vis spectrum of nanosilver, Inset to the figure show zoom view of band of nano silver.

Although at the end surface of silver nano dendrites, the shape and the size of the silver nano dendrites are different, also consist with some plates like nano crystal morphology which have diverse size and branches (figure 6.6, TEM image). Hence Ag dendrite exhibits the SPR properties depending on the morphology of the branches with different shapes and size [Ren *et al.*, 2011]. Nano silver can also be characterized by electrochemical cyclic voltammogram. The cyclic voltammogram of Ag-PTZH on ITO electrode surface showing characteristic Ag surface oxidation and the corresponding oxide reduction confirmed that the ITO surfaces were modified with nano silver as shown in figure 6.3 [Singh *et al.*, 2012].

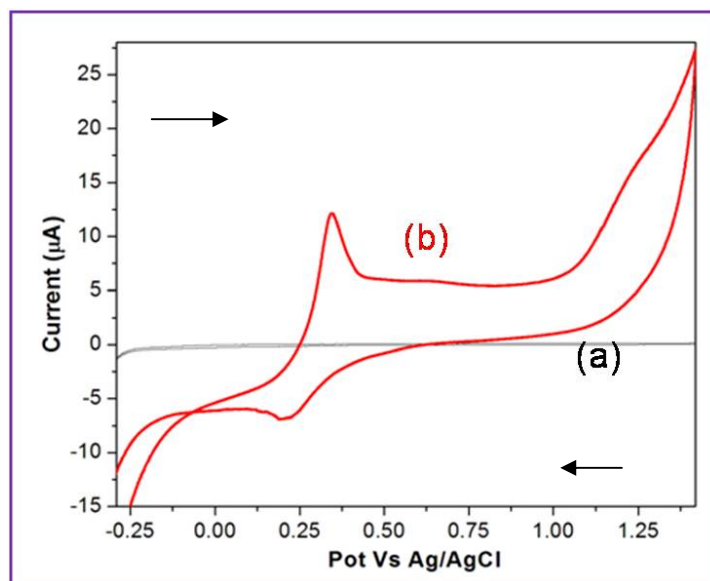


Figure 6.3: Bare unmodified electrode (a) and Characteristic CV of nano silver in Tris HCl buffer (b)

In FT-IR spectrum (figure 6.4), we focus on the regions where we can see some major changes of assign band of PTZ and Ag-PTZH and thus highlighted the spectra in the region $\sim 400\text{--}1150\text{ cm}^{-1}$. The FT-IR spectrum of phenothiazine was assigned from the literature [Palafox *et al.*, 2002]. This shows angle bending of δ_{as} (C-S-C) at 502 cm^{-1} , angle bending of δ_s (C-S) 15% and δ_s (C-N) 30% at 601 cm^{-1} , angle bending δ_s (C-N-C) 25% and δ_s (N-H) 30% at 659 cm^{-1} , angle bending of δ_s (N-H) 30% and δ_s (C-C-C) 30% at 880 cm^{-1} was appeared respectively. In the FT-

IR spectrum of Ag-PTZH these peaks were observed at 462, 585, 649 and 830 cm^{-1} , respectively.

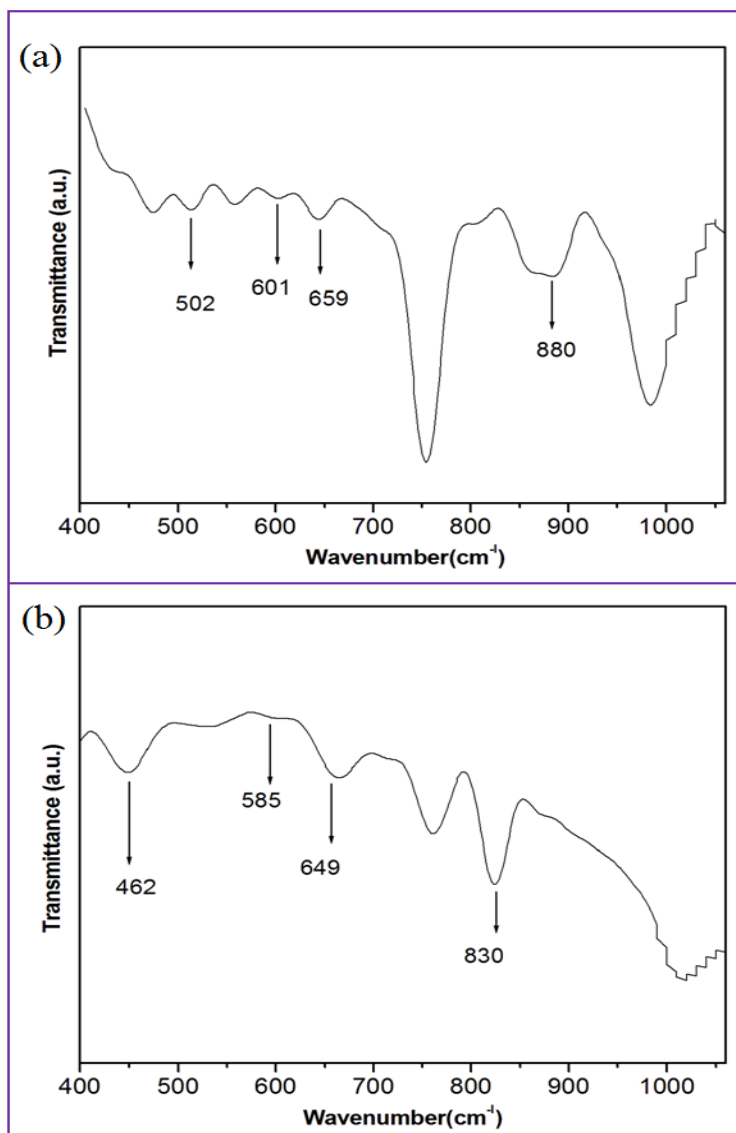


Figure 6.4: (a) FT-IR of PTZ (b) FT-IR of Ag-PTZH

Silver having affinity towards nitrogen and sulphur hence if interaction takes place in C–N–Ag, C–S–Ag, then there might be shift in IR stretching of these frequencies. The decrease in assigned stretching frequency is due to the transfer of electron density from the S and N atoms to silver atom, resulting in the electrostatic interaction with sulphur and nitrogen atom with silver which provides stability to nanostructures [Chaki *et al.*, 2002].

XPS spectra of photo reduced nano silver gives peaks at 374.1 and 368.1 eV correspond to $\text{Ag } 3d_{3/2}$ and $\text{Ag } 3d_{5/2}$ respectively [Sun *et al.*, 2004]. Also, with this result it is reasonable to assume that phenothiazine and its oxidation product were co adsorbed onto the surface of nano silver through electrostatic interaction between sulphur and nitrogen atoms, supported by XPS studies as shown in figure 6.5 and consistent with FT-IR result.

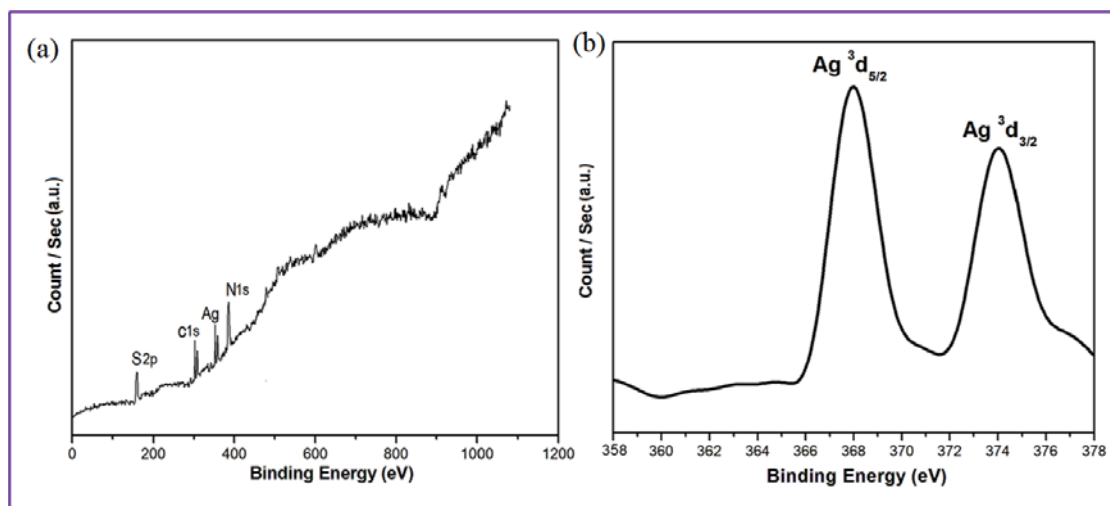


Figure 6.5: (a) XPS survey spectrum of Ag-PTZH in full range (b) X-ray photoelectron spectra of Ag (3d).

HR-TEM investigation was carried out in order to study the microscopic views of the nano silver which reveals that its average dendrites branches width are 25-40 nm in diameter as shown in figure 6.6 (a). Selected area electron diffraction (SAED) pattern displays discontinuous concentric rings characteristic of the fcc cubic structure of silver and higher planes were also observed with very blurred dots. The Scherrer ring of the selected area electron diffraction (SAED) pattern and interplanar fringe spacing obtained from the same structure indicates were indexed 0.24 nm and 0.20 nm to the (111) and (200) plane of fcc silver respectively as shown in figure 6.6 (b) and 6.6 (c) respectively.

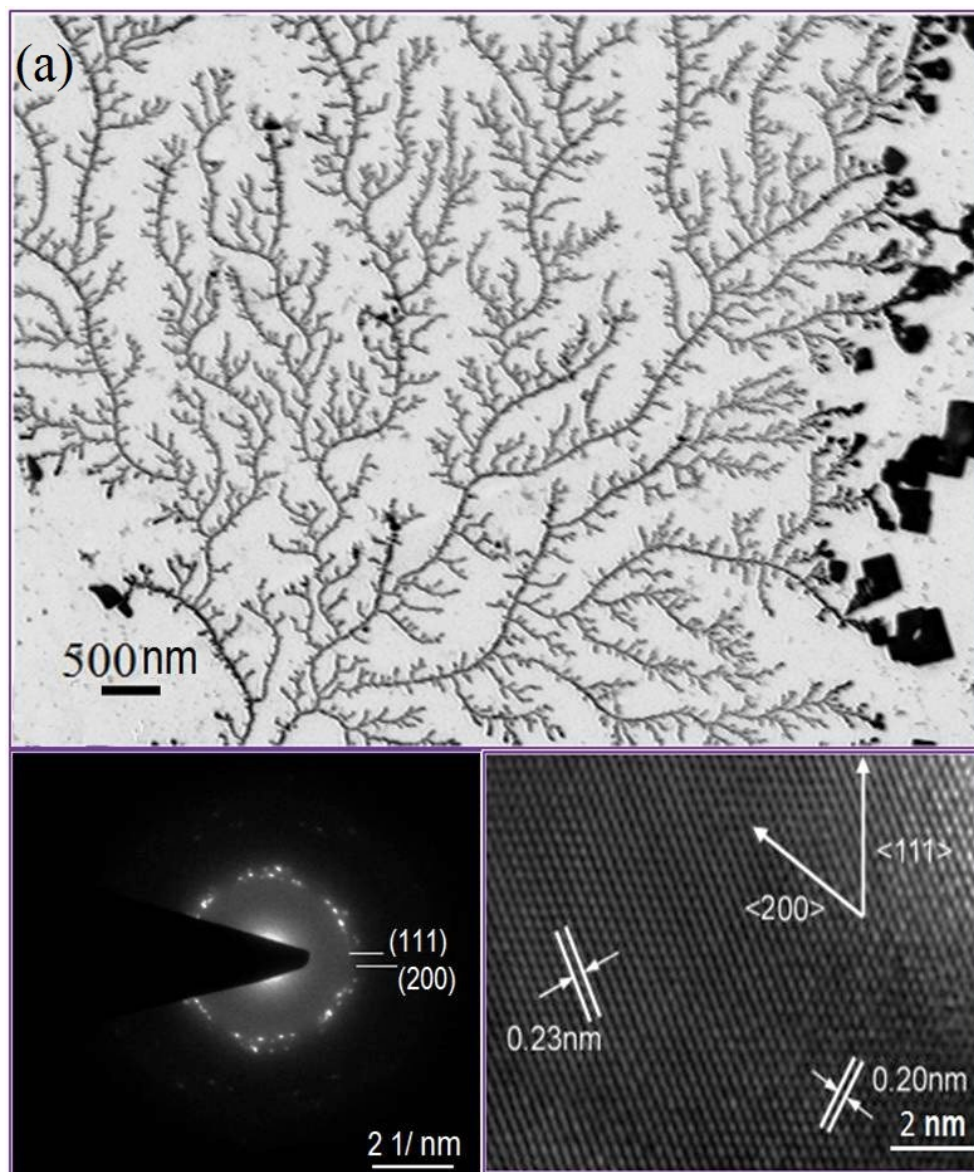


Figure 6.6: (a) TEM image (b) SAED pattern (c) HRTEM images showing lattice fringes spacing of silver nanodendrites.

Photolytic and radiolytic methods often provide a convenient means to produce size and shape controlled metallic nanostructures. The growth of particles depends on the nature of the reductant and the medium in which reduction is being carried out. Growth of dendritic structure generally occurs in non-equilibrium growth condition. Specifically, phenothiazine and its oxidation product not only serves as a capping agent to selectively bind nano silver facets, but also coordinates with

Ag^+ ions to form complexes. Firstly phenothiazine form complex with Ag(I) , secondly this complex promotes Ag(0) nucleations in the presence of light, thirdly diffusion and limited dispersion leads to formation of dendritic nanosilver [Zhou *et. al.*, 1999]. This coordination effect can significantly reduce the concentration of free Ag^+ ions, thus slowing down the reduction and enabling a kinetic control that favors dendrites formation [Mandke *et. al.*, 2012]. When the reaction time was prolonged upto ~ 4 hours, well-defined dendritic nano silver was formed but as soon as reaction time was prolonged for more than 4 hours, some gelatinous appearance in the reaction solution appear, i.e. dendritic pattern agglomerate into some bigger nanoparticle as shown in TEM image figure 6.7.

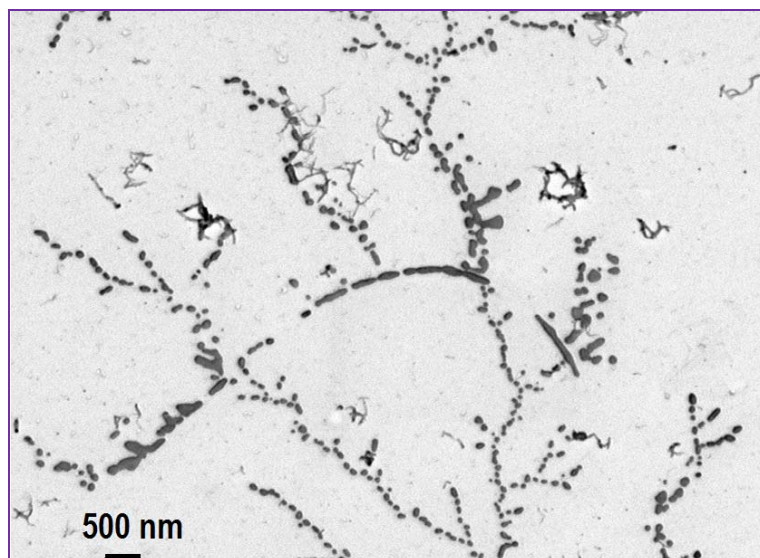


Figure 6.7: TEM images of Ag-PTZH when the reaction is carried out for longer time.

Hence on the basis of previous literature report cited, we conclude that these dendritic patterns was formed in non-equilibrium growth, so when the reaction system gradually transformed from non-equilibrium to equilibrium with an increase of the reaction time, the dendritic pattern disappeared which is consistent with TEM images observed. It was found that the appropriate molar ratio between phenothiazine and silver ions, reaction time play very important roles in dendritic morphology [Mandke *et al.*, 2012; Gupta *et al.*, 2014]. Elementary dendritic

mechanism in which, at the tip surface growth rate is much faster than whole surface [Bockris *et al.*, 2001]. The growth of the well-defined silver dendrite nanostructures might proceed in the interspaces of the resulting crystals of phenothiazine and its oxidation product. Hence we propose the scheme which illustrates the growth of silver nanodendrites structure as shown in figure 6.8. At the tip surface of the growing nano silver nuclei, cultivate at much faster speed and this effect multiples continuously, ultimately generates the silver dendritic nano structures [Gupta *et al.*, 2014].

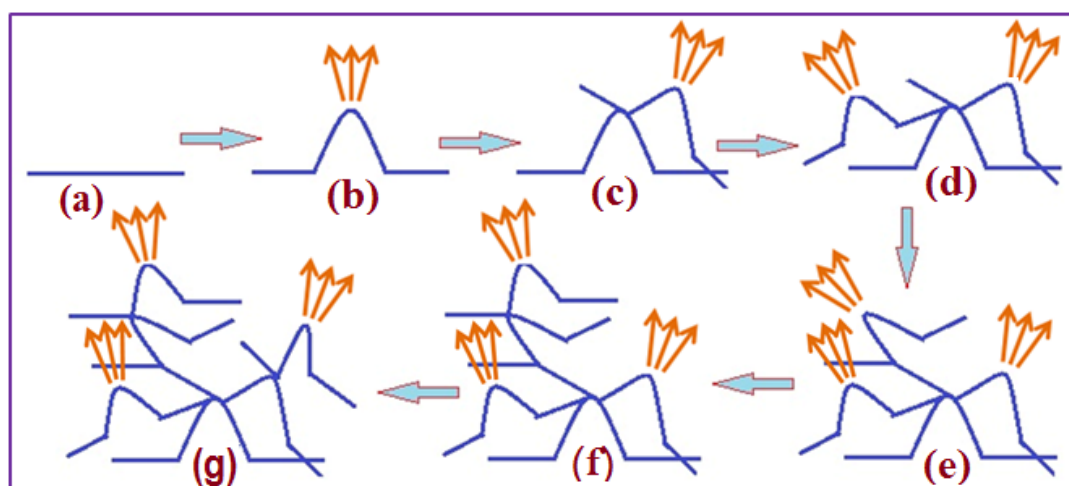


Figure 6.8: Scheme illustrating for formation of silver nanodendrites (a-g).

6.3.2. Application of the silver dendritic nanostructures in amperometric sensing of nitrite:

Deliberate tailoring of electrochemical interfaces with nanostructured hybrid electrode material and semiconductor particles has gained enormous interest with respect to the development of electrochemical nanoscale devices [Sun *et al.*, 2002]. One of the objectives of the present finding was to utilize the nanoparticles based platform for the electrocatalytic sensing of nitrite ions. Micro/nano electrode ensembles have been widely used for the development of electro-sensing of analyte. They show several advantages over conventional macro electrodes due to higher ratio between the faradaic and capacitive currents, increase in mass

transport, decrease influence of solution resistance, less electrode fouling problem and better detection limit [Manivannan *et al.*, 2011].

Ag-PTZH can be used as modifiers of electrode surfaces due to some of their characteristics: it is fairly soluble in water so that leaching of material from electrode surface can be avoided and can be easily adsorbed on gold electrode surface through interaction with sulphur and nitrogen. The majority of modified interfaces are based on the affinity between thiols and gold (S-Au) interaction or the affinity between amines and gold (N-Au) interaction [Wen *et al.*, 2006]. This gives us an opportunity to immobilize Ag-PTZH molecules on electrode surface thereby resulting Ag-PTZH might be further linked to the electrode by virtue of bridging to polycrystalline gold electrode via nitrogen and sulphur linkage. FESEM images and EDAX of modified electrode with Ag-PTZH as shown in figure 6.9.

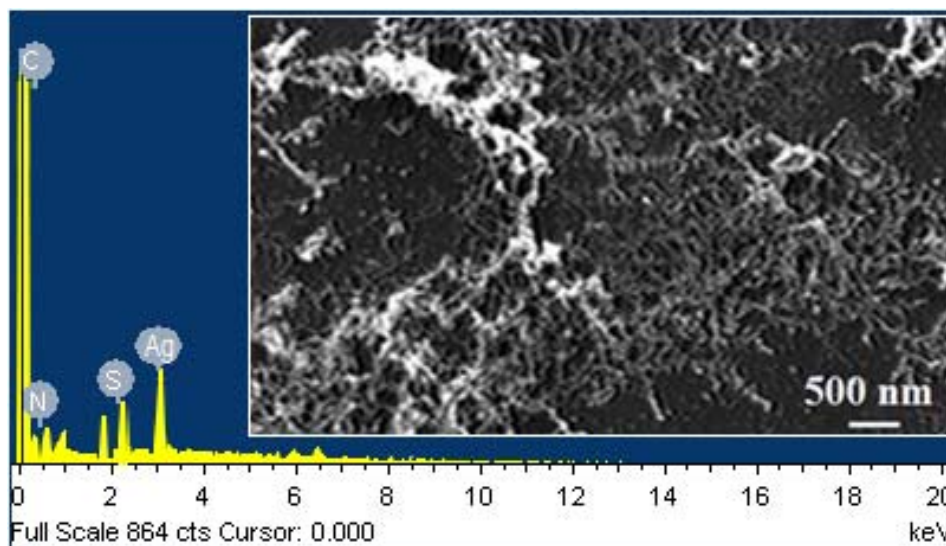


Figure 6.9: FESEM and EDAX image of the modified electrode surface with silver nano dendrites.

The voltammetric responses of the polycrystalline gold electrode, and modified electrode with Ag-PTZH were examined by taking the 0.1mM $\text{Fe}(\text{CN})_6^{4-/3-}$ in tris buffer solution. Significant increase in the peak current was observed for the same amount of $\text{Fe}(\text{CN})_6^{4-/3-}$ for modified electrode as compared to unmodified

polycrystalline electrode as shown in a figure 6.10, indicating that the modified electrode with Ag-PTZH increase in the surface area which favors faster electron-transfer reaction.

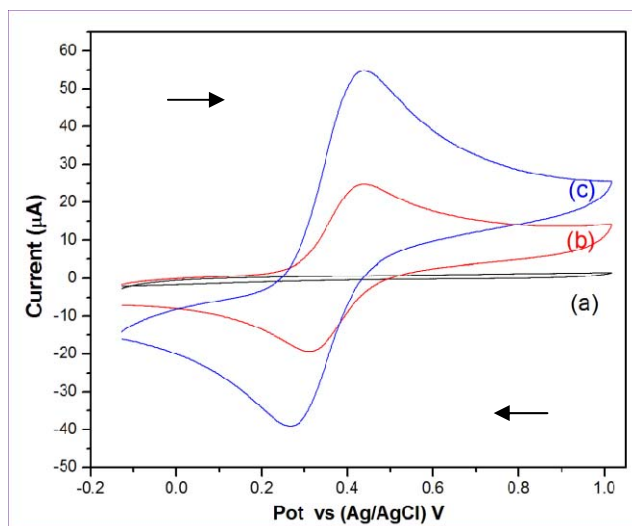


Figure 6.10: CV Response for (a) Bare electrode (b) CV of $\text{Fe}(\text{CN})_6^{4-/3-}$ redox couple on without modified electrode (c) CV of $\text{Fe}(\text{CN})_6^{4-/3-}$ redox couple on Ag-PTZH modified electrode.

Modified electrode did not show any redox peak in the potential window 0.0 V to 0.5 V vs. Ag/AgCl in blank. However, it can be seen well-defined voltammetric responses were obtained for successive additions of nitrite as shown in figure 6.11.

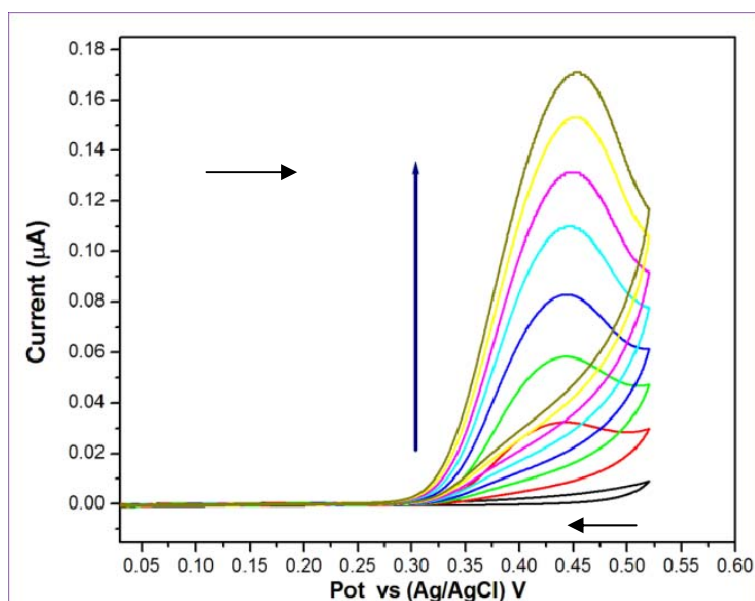


Figure 6.11: Cyclic voltammogram response of successive addition of nitrite in 0.1 M tris buffer at pH 7.2.

On successive addition of nitrite substantial increase in the peak current reflects fast electron-transfer reaction on modified electrode owing to the high catalytic effect of the nanostructured platform [Gupta *et al.*, 2014]. Proposed schematic representation of the nanostructured platform of the Ag-PTZH as a modified electrode has been shown in figure 6.12.

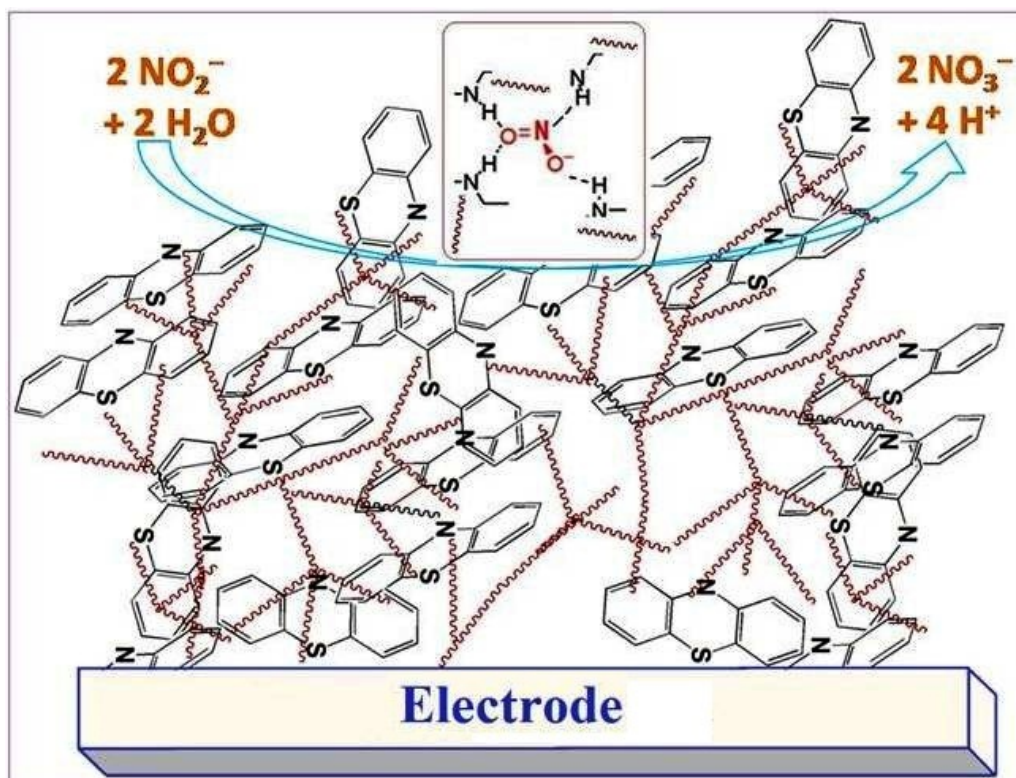


Figure 6.12: Schematic representation of the nano integrated platform of the Ag-PTZH as a modified electrode for electro-sensing of nitrite.

For amperometric study a fixed reduction potential 0.44 volts Vs AgCl/Ag was chosen from cyclic voltammetry studies and aliquots of nitrite were injected into a stirred supporting electrolyte solution. A systematic change in the current was noticed after each addition of nitrite into the supporting electrolyte solution, and a steady state response was attained within a few seconds. Electrochemistry behind this sensing of nitrite to such a low detection limit is due to high connectivity

throughout silver dendrites nano structure capped with phenothiazine and its oxidation product which exhibit high electron transfer kinetics via interaction of Ag-PTZH with oxygen atom of NO_2^- as depicted in figure 6.12 of the proposed schematics [Gupta *et al.*, 2014].

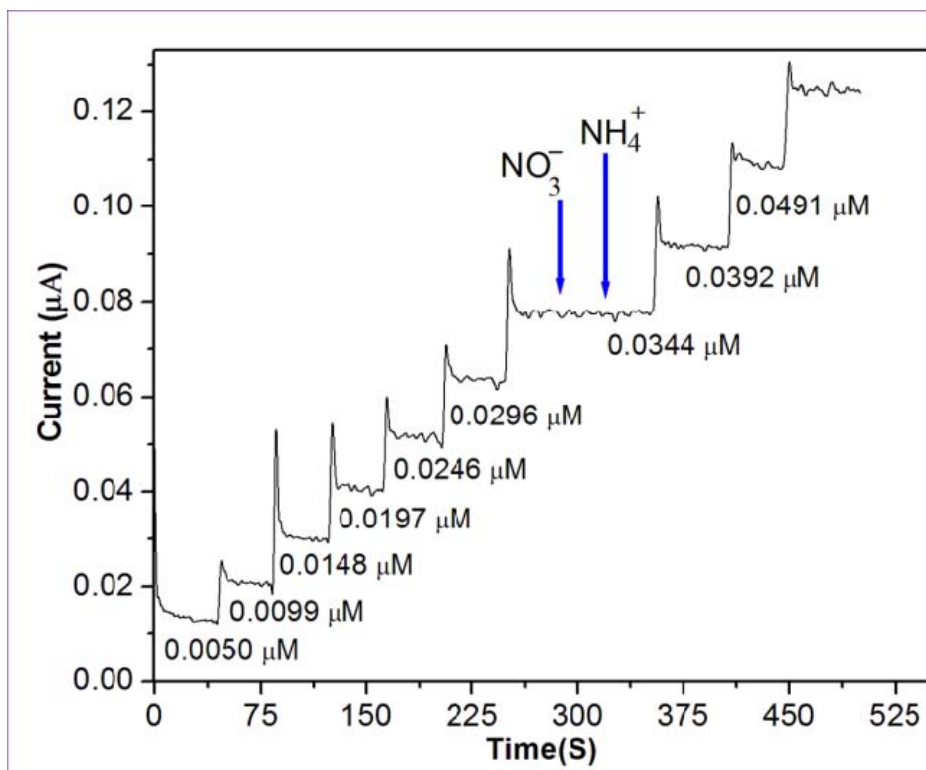


Figure 6.13: Amperometric response at an applied potential of 0.44 volts Vs. Ag/AgCl for successive addition of nitrite in 0.1 M tris buffer at pH 7.2 under stirred condition.

After the sixth standard addition of nitrite, equal quantities of 0.05 mM of nitrate and ammonium ion were added in the sequence to study the interference in the analysis of nitrite ion and their influence on the current response was noted. However, it can be seen from the figure 6.13 the added possible interfering anions (nitrate and ammonium) had no effect on the chronoamperometric current. The sensing platform is highly sensitive and show linear response toward nitrite additions with a sensitivity and limit of detection $2.4 \mu\text{A}/\mu\text{M}$ and $(2.3 \pm 0.05) \text{ nM}$

respectively at S/N (signal-to-noise ratio): 3 as shown in chronoamperometric calibration plot figure 6.14.

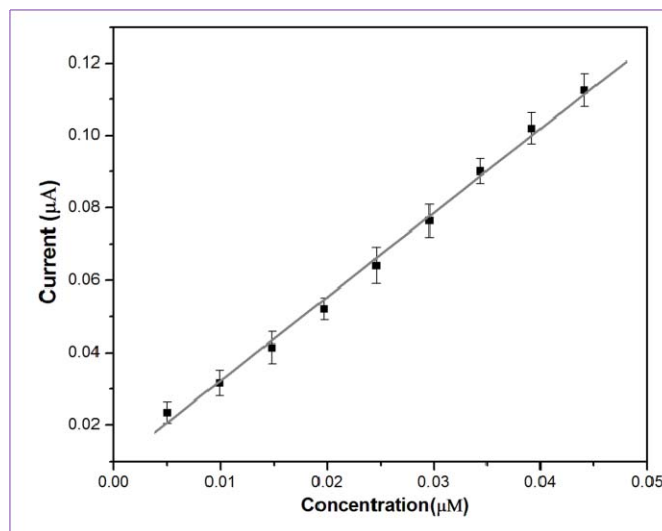


Figure 6.14: Corresponding standard addition calibration plot of chronoamperometric current Vs nitrite concentration.

Amperometric experiments were repeated four times with modified electrode and experiments were highly reproducible. The mean deviation of the current values was within ~6 % for repeated chronoamperometric experiment.

6.4. Concluding remarks of the chapter:

Photochemical assisted synthesis of silver dendrites nanostructure using phenothiazine is outlined and its application in the amperometric sensing of nitrite ions is demonstrated. It was found that appropriate molar ratio between phenothiazine & silver ions and reaction time play very important roles in dendritic morphology. Method described in this work offers a selective, sensitive, enzyme less electroanalytical determination of nitrite and field deployable onsite measurements where one cannot afford to carry costly equipments like ICP-OES or spectrometers. The sensing platform is highly sensitive and showed linear response toward nitrite additions with a sensitivity and limit of detection $2.4 \mu\text{A}/\mu\text{M}$ and $(2.3 \pm 0.05) \text{ nM}$ respectively at S/N (signal-to-noise ratio): 3. It is believed that

present work will strongly promote the fast development of the photochemical assisted formation of other noble metallic nanomaterials by phenothiazine and its application in electrosensing devices/other electrochemical electron transfer studies.
