

5

3-APTMS and organic carbonyl moiety role during the synthesis of AuNPs specific to pH- and salt- tolerance

5.1. INTRODUCTION

The use of functional material acting as reducing and stabilizing reagents play major role in controlling the various properties of AuNPs for specific application through control over the dispersibility and functional ability of nanomaterials (Saha et al., 2012). The role of organic amine has been extensively studied by several workers (Pandey and Chauhan, 2012d; Pandey and Singh, 2015g; Pandey and Singh, 2015 h). The precise conversion of 3-Aminopropyltrimethoxysilane (3-APTMS) capped gold ions into respective nanoparticles in the presence of wide range of reducing agents differing in the polarity index (3-glycidoxypropyltrimethoxysilane, cyclohexanone, tetrahydrofuranhydroperoxide) has been documented in previous chapters. The synthesis of functional AuNPs involving the active participation of 3-APTMS and 3-GPTMS is known (Pandey and Chauhan, 2012d). In the second chapter it has been shown that the ratio of 3-GPTMS/3-APTMS control the dispersibility of as synthesized AuNPs in desired solvent of known polarity index, but the use of two functional alkoxy silanes enable auto hydrolysis, condensation and polycondensation of resulting AuNPs and the replacement of one of the silane by other

organic reducing agents has been tried in third and fourth chapters. Accordingly, the use of tetrahydrofuranhydroperoxide and cyclohexanone are demonstrated replacing 3-GPTMS. Depending upon the organic moiety used during the synthesis, nanoparticles differ in properties like dispersibility in both aqueous and organic media, nanogeometry and catalytic ability as described in previous chapters. Although, in the previous chapters the dispersibility of as prepared AuNPs in different solvents has been discussed in detail but the functional ability of the same in different buffering systems remained in question and for many applications, it is desirable to stabilize colloids over a wide range of buffer conditions. Therefore, there is a need to explore the pH sensitivity and salt tolerance of AuNPs made from the precise alternations of organic moiety during nanoparticle synthesis which has been undertaken in the present chapter.

Improvement in dispersibility and catalytic activity of AuNPs has led to the search of some other reducing agents that allow the formation of catalytic material during the process of nanoparticle synthesis. Previous chapter deals with the role of α -hydrogen during 3-APTMS and cyclohexanone mediated synthesis of AuNPs. The use of cyclohexanone during such synthesis forms separate layer under specific conditions and leads to poor dispersibility in aqueous medium. However, the use of hydrophilic reducing agents may facilitate dispersibility in such medium. Since the role of α -hydrogen adjacent to carbonyl group during AuNPs synthesis from cyclohexanone and 3-APTMS is known, the choice of aldehydes and ketones having similar moiety is sought. In addition to that the formation of organic–inorganic hybrid displaying catalytic activity between 3-APTMS and aldehydes or ketones is also known (Wight and Davis, 2002). It is intended to understand the synthesis of AuNPs from 3-APTMS-capped gold ions using the series of organic reducing agents

containing carbonyl functional groups (Aldehydes and Ketones) having gradual increase in hydrocarbon content. The use of aldehydes and ketones, other than cyclohexanone, during the synthesis of gold nanoparticles provided a breakthrough in assessing the salt- and pH-dependent study of 3-APTMS stabilized gold nanoparticles. Formaldehyde, Acetaldehyde, Acetone, t-butyl dimethyl Ketone (t-DMK) are the organic reducing moieties chosen for the conversion of 3-APTMS capped gold ions into AuNPs. The present chapter provides deeper insight on pH and salt sensitivity of as synthesized AuNPs. A typical application justifying homogenous catalysis in 0.1 M phosphate buffer at pH 7 has also been undertaken. The results based on these lines are reported in this chapter.

5.2. EXPERIMENTAL

5.2.1. Materials and Instrumentation

3-Aminopropyltrimethoxysilane is purchased from Aldrich Chem. Co. Tetrachloroauric acid was purchased from HiMedia; Formaldehyde, Acetaldehyde, Acetone, t-butyl dimethyl ketone are obtained from Merck, India. All other chemicals employed were of analytical grade. Aqueous solutions were prepared by using doubly distilled-deionized water (Elga water purification system). Unless mentioned otherwise, all the experiments were performed at room temperature. The absorption spectra of nanoparticles were recorded using a Hitachi U-2900 Spectrophotometer. Transmission electron microscopy studies were performed using Morgagni268D (Fei Electron Optics).

5.2.2. Preparation of 3-APTMS stabilized nanoparticles

In a typical procedure, 1 mL of sol was prepared by mixing 0.3 mL solutions of 0.025 M of HAuCl_4 to 0.3 mL solutions of 3-APTMS (1M). The resulting yellow solution was stirred vigorously over a vortex cyclo mixer for 2 min. The solution was then subjected to reduction by adding 0.4 mL of formaldehyde/acetaldehyde/acetone or t-DMK. The

resulting mixture was again stirred over a cyclo mixer for 2 min. The mixture was left to stand in the dark for 2h. After this, the colour of the sols turned to red indicating the formation of AuNPs. Different color of AuNPs sol for different reducing agents could be prepared by varying the concentration of 3-APTMS.

5.2.3. Electrochemical measurements

All electrochemical measurements were performed in an electrochemical cell equipped with a three electrode configuration having working volume of 3 mL with Electrochemical Workstation Model CHI660B, CH Instruments Inc., TX, USA. The working electrode was graphite paste electrode. Ag/AgCl reference electrode and a platinum counter electrode were used in all electrochemical measurements. An analysis of the effect of scan rates (v) on peak current density (j) was conducted by recording the cyclic voltammograms of same at various scan rates from 0.01 to 0.5 $V s^{-1}$. The behavior of potassium ferricyanide in absence and the presence of AuNPs were studied between a potential range -0.2 to $0.6 V$ vs. Ag/AgCl. The homogenous electrocatalysis of ascorbic acid was recorded based on i-v measurements.

5.3. RESULTS

5.3.1. Organic reducing agents and 3-APTMSmediated synthesis of AuNPs

AuNPs functionalized with primary amine have received great attention (Leff et al., 1996). Previous chapters have demonstrated the synthesis of noble metal nanoparticles involving the role of 3-APTMS with 3-GPTMS, cyclohexanone and THF-HPO. It has been found that the presence of organic reducing agents profoundly affects the properties of as formed AuNPs. The nanoparticles made through 3-APTMS and 3-GPTMS are mostly dispersible in organic solvent with limited dispersibility in water under specific ratio of 3-APTMS/3-GPTMS whereas THF-HPO and 3-APTMS mediated nanoparticles are mostly

dispersible in relatively more polar solvent and are not dispersible in non-polar media. The use of cyclohexanone enables the dispersibility of AuNPs in both organic and aqueous medium. These findings are mainly limited to the dispersibility in pure solvents and directed to study the same in buffering systems at variable pH. The present chapter deals with the use of formaldehyde, acetaldehyde, acetone and t-DMK during the conversion of 3-APTMS capped gold ions. Fortunately, it was found that all these organic reducing agents enable the synthesis of AuNPs within less than 1 h under ambient condition. Fig.5.1-Fig.5.4 show the TEM images of AuNPs made from 3-APTMS capped gold ions in the presence of: Formaldehyde (Fig. 5.1, P), Acetaldehyde (Fig. 5.2, Q), Acetone (Fig. 5.3, R) and t-DMK (Fig. 5.4, S). Placed right to the TEM images are their corresponding diffraction patterns.

Further, the effect of these organic reducing agents on the catalytic ability of the AuNPs has been examined by making a comparative study on the mimetic ability of as synthesized AuNPs (P, Q, R, and S). For this oxidation of o-dianisidine in presence of H_2O_2 using AuNPs as catalyst is studied. The findings based on the results obtained from Fig. 5.5 are recorded in Table 5.1. The finding reveals highest K_m value for P (AuNPs made using 3-APTMS and formaldehyde) and lowest for S (AuNPs made using 3-APTMS and t-DMK). The AuNPs are synthesized in all four cases and the difference lies with the use of organic reducing agent. As the reducing agents themselves are non catalytic but its reaction product with 3-APTMS might be catalytic. It is thus important to understand the role of reaction product of 3-APTMS and aldehyde/ketone on the catalytic activity of AuNPs if any. Accordingly, peroxidase mimetic ability of homogenous suspension (not containing Au^{3+}) made from 3-APTMS and formaldehyde (P'), acetaldehyde (Q'), acetone (R') and t-DMK (S') was monitored as shown in Table 5.2 and Fig. 5.6. Results were very affirmative with

3-APTMS and aldehydes (formaldehyde, Acetaldehyde) solutions being catalytic towards oxidation of o-dianisidine while the other two being non-catalytic or less catalytic.

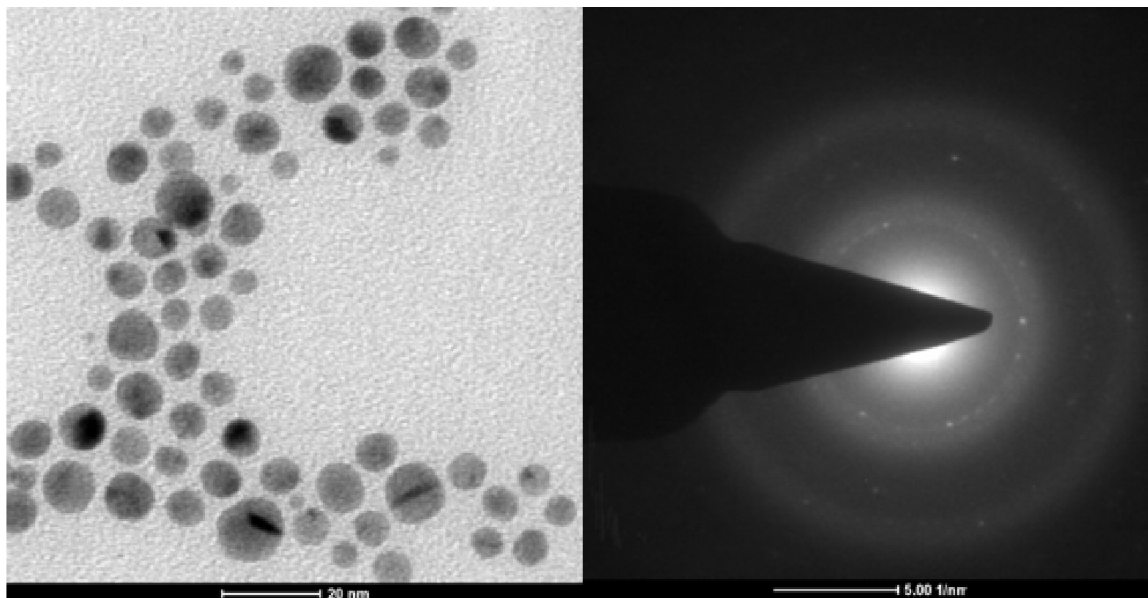


Figure 5.1. TEM image(left) and diffraction pattern(right) of AuNPs made using 3-APTMS and formaldehyde.

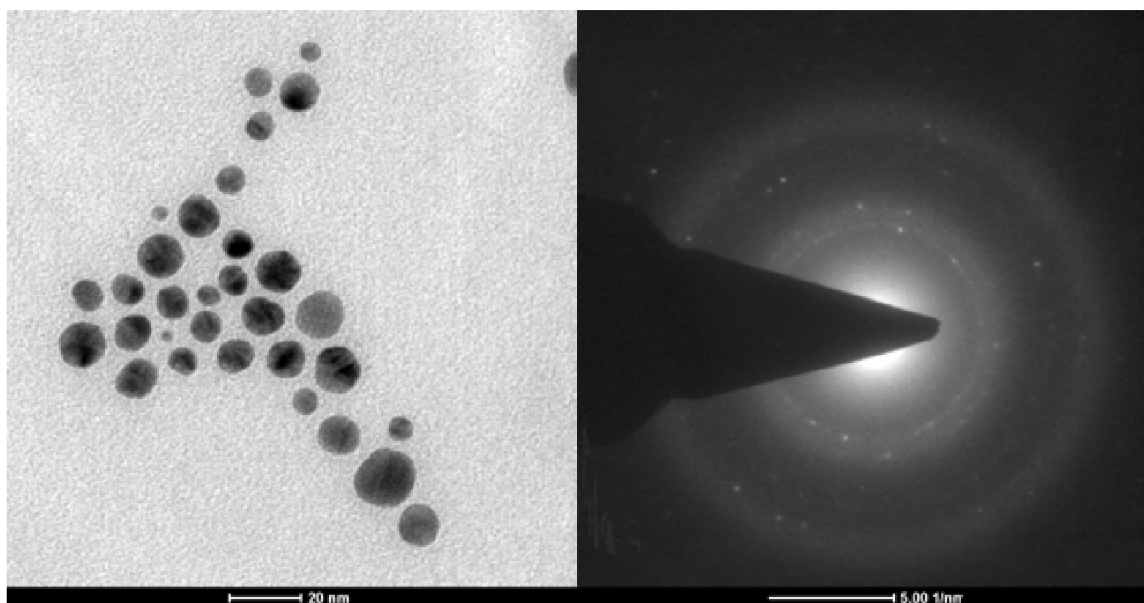


Figure 5.2. TEM image (left) and diffraction pattern(right) of AuNPs made using 3-APTMS and acetaldehyde.

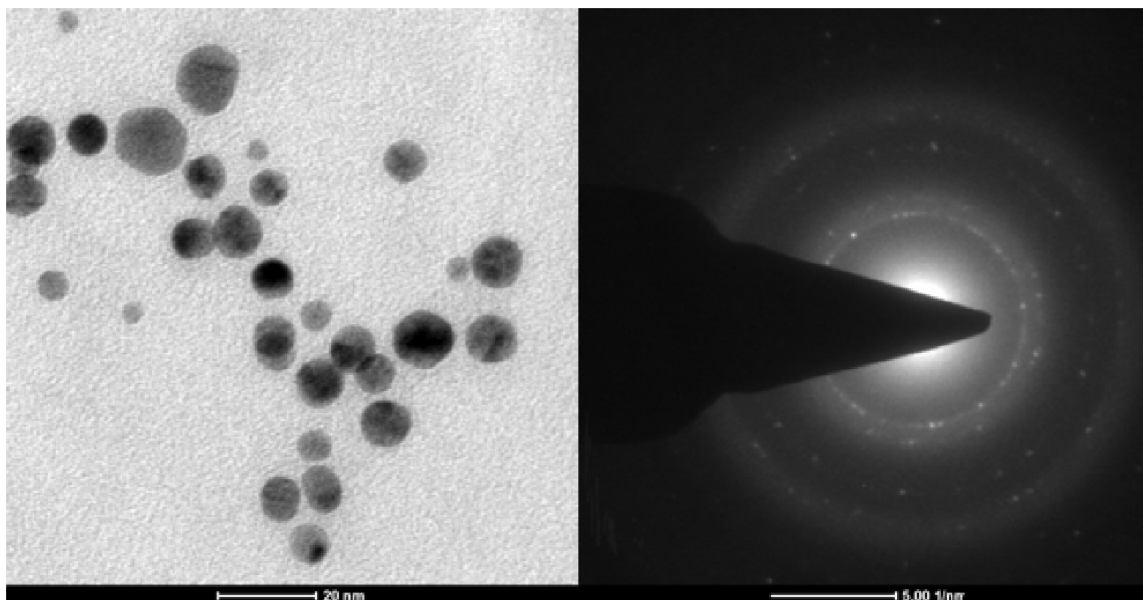


Figure 5.3. TEM image (left) and diffraction pattern (right) of AuNPs made using 3-APTMS and acetone.

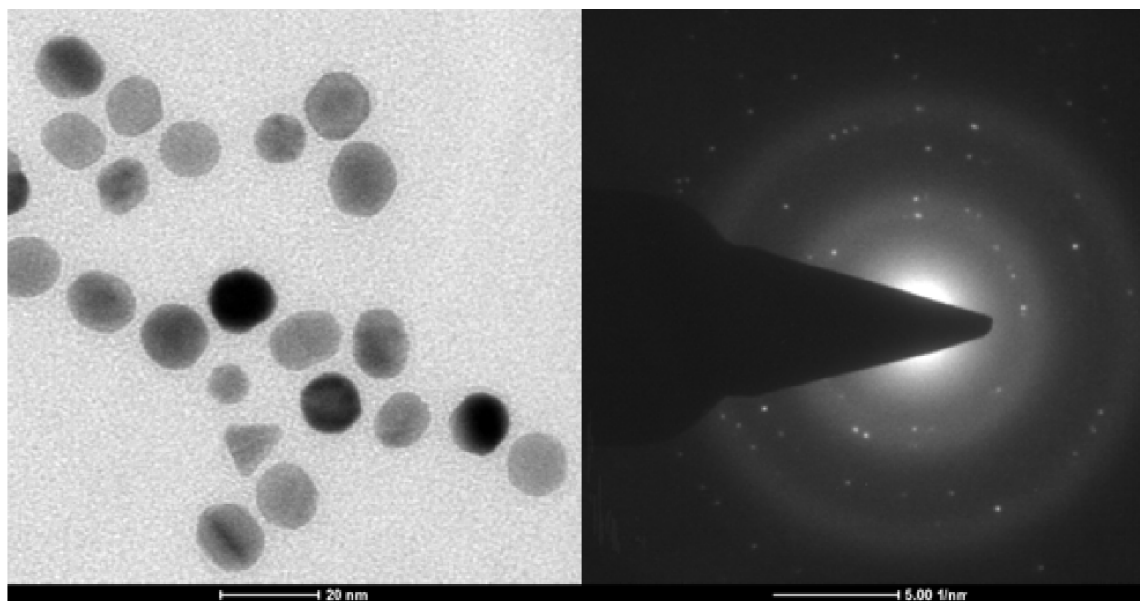


Figure 5.4. TEM image (left) and diffraction pattern (right) of AuNPs made using 3-APTMS and t-butyl methyl ketone.

5.3.2. The salt and pH sensitivity of AuNPs.

Citrate AuNPs are stable due to Charge stabilization which is achieved by means of

electrostatic repulsion (Turkevich, 1985). But, addition of small amount of salt causes agglomeration due to charge screening and nanoparticles approach each other because of London attractive forces (Enustun and Turkevich, 1963; London, 1930). The practical application of AuNPs especially in therapeutics and analysis require insensitivity of the nanogeometry to the working medium including complex biological systems. Control over salt and pH sensitivity of AuNPs requires a precise investigation and many researches have been made in this respect (Yang et al., 2012; Zhang et al., 2012). Use of 3-APTMS along with several other reducing agents can provide an impeccable control over such properties. In previous chapter where 3-APTMS capped AuNPs were made using cyclohexanone as reducing agent, showed dispersibility in water at specific conditions. And the dispersibility is affected if the ionic strength of the water is increased due to the presence of salt. One of the reagent, 3-APTMS, used for AuNPs synthesis is amphiphilic and the other reducing agent depending on the working media (aqueous or organic) can be chosen. Water loving organic reducing agents besides imparting water dispersibility to AuNPs also play important role in imparting salt- and pH- resistant properties to AuNPs. The results on these lines are described herein. Stability of AuNPs made using fixed 3-APTMS concentration and different reducing agents (formaldehyde, acetaldehyde, acetone) in water and four different buffering medium of pH 8, 7, 6 and 5 is recorded as shown in Fig. 5.7 and Table 5.3. AuNPs made using aldehyde reducing agents (P, Q) are comparatively more stable to various pH-changes than the AuNPs made using ketonic reducing agents (R, S). Similarly, Fig. 5.8 shows the effect of 3-APTMS concentration on pH resistant properties of AuNPs.

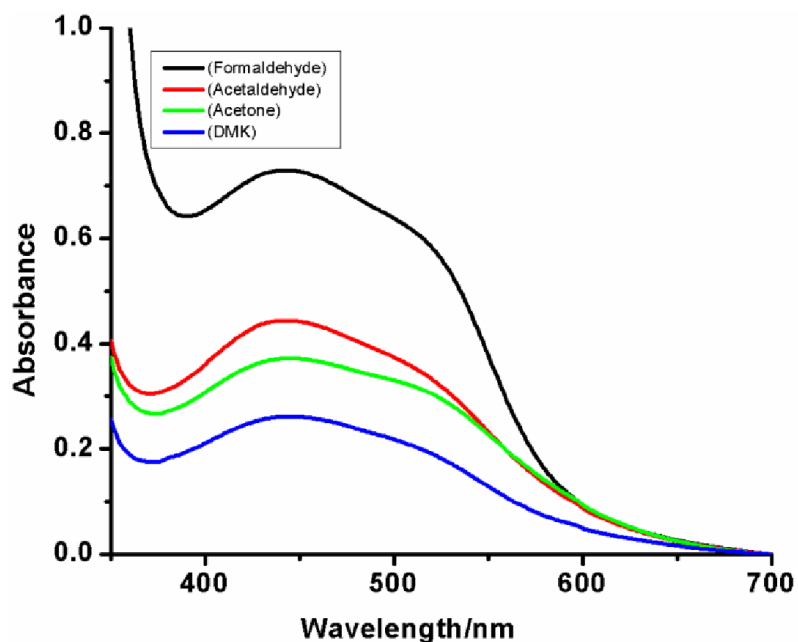


Figure 5.5. UV-Vis spectra displaying a comparison between peroxidase mimetic ability of AuNPs made using 3-APTMS and Formaldehyde (black line), acetaldehyde (red line), acetone (green line) and t-DMK (blue line).

Table 5.1. A comparative study on the peroxidase mimetic ability of AuNPs made from the use of 3-APTMS capped Au³⁺ and various organic reducing agents (P for formaldehyde; Q for acetaldehyde; R for acetone; and S for t-DMK).

3-APTMS Conc.			
Sample name	(M)	Organic reducing agent	Km (mM)
P	0.2	Formaldehyde	7.0
Q	0.2	Acetaldehyde	28.0
R	0.2	Acetone	30.4
S	0.2	t-DMK	37.1

For this AuNPs with formaldehyde as reducing agent at three different concentrations of 3-APTMS was synthesized. As the size of AuNPs increases there is a broadening in the surface Plasmon band and red shifting in the spectral data of AuNPs (Su et al., 2003;

Srivastava et al., 2005). Inset to Fig. 5.7 and Fig. 5.8 shows how the wavelength of maximum absorption changes with change in pH to have an idea of size change.

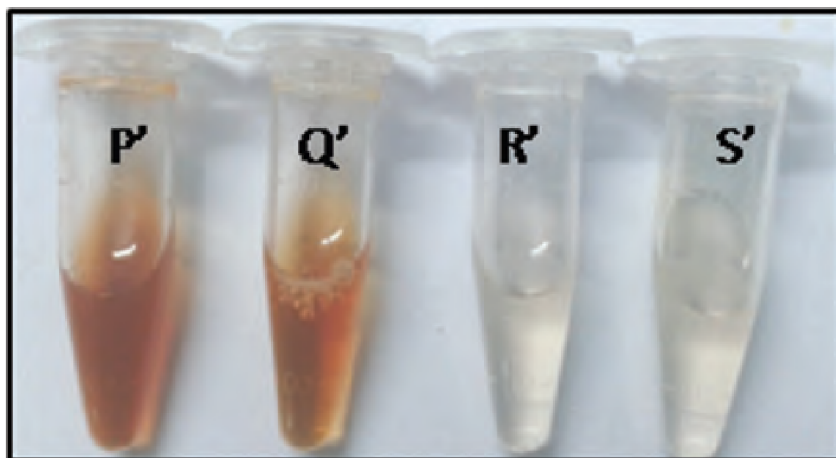


Figure 5.6. Image showing catalytic behavior for solutions made using formaldehyde (P'), acetaldehyde (Q') and non catalytic behavior for the solution made using acetone (R'), t-butyl methyl ketone (S')

Table 5. 2. A comparative study on the peroxidase mimetic ability of solutions made from the use of 3-APTMS and various organic reducing agents (P' for formaldehyde; Q' for acetaldehyde; R' for acetone; and S' for t-butylmethyl ketone) in absence of Au³⁺.

Sample name	3-APTMS Conc.		Organic reducing agent	Km (mM)
	(M)			
P'	0.2		Formaldehyde	Active
Q'	0.2		Acetaldehyde	Active
R'	0.2		Acetone	Slightly Active
S'	0.2		t-DMK	Inactive

Similarly, there is recorded an increase in salt tolerance of AuNPs with increase in 3-APTMS concentration as shown in Fig. 5.9. Here again the inset shows the change in wavelength of absorption maxima of AuNPs with change in salt concentration. The results

based on the salt- and pH- tolerances of AuNPs as a function of 3-APTMS concentration are given in Table 5.4.

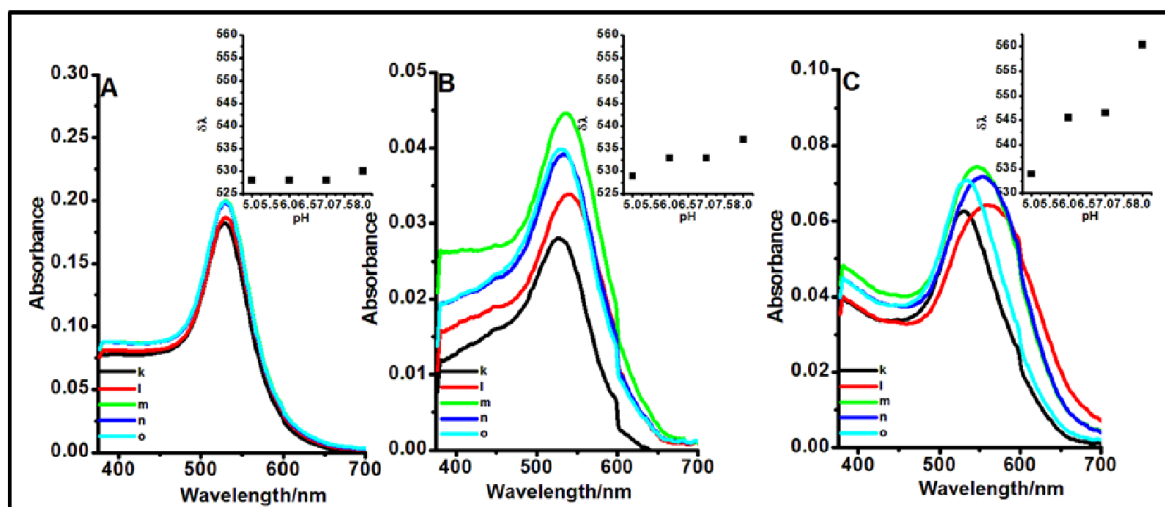


Figure 5.7. UV-Vis spectra displaying the effect of pH on AuNPs made by using 3-APTMS (0.2 M) and (A) formaldehyde (B) acetaldehyde and (C) acetone. Inset shows the change in wavelength (λ_{\max}) with pH of the buffering media (k = water, l = 8, m = 7, n = 6, o = 5).

Table 5.3. pH-Sensitivity of AuNPs, made using 3-APTMS (0.2 M) and formaldehyde, acetaldehyde or acetone.

Sample	3-APTMS	Organic reducing	Sensitivity
Name	Conc. (M)	agent	pH
A	0.2	Formaldehyde	Low
B	0.2	Acetaldehyde	High
C	0.2	Acetone	High

5.3.3. Electrochemical behaviour of potassium ferricyanide

The finding on the salt tolerance and pH sensitivity revealed practical usability of 3-APTMS and formaldehyde mediated AuNPs. Accordingly, it was intended to understand the contribution of AuNPs during charge transport at electrochemical interface and the redox electrochemistry of potassium ferricyanide in buffering system containing high salt

concentration to validate the use of as synthesized AuNPs in bioelectrochemistry. AuNPs are an excellent catalytic nanomaterial (Kumar et al., 2015; Panigarhi et al., 2007). In the present study redox electrochemistry of potassium ferricyanide in absence and the presence of AuNPs is monitored. Fig.5.10 shows cyclic voltammogram of the potassium ferricyanide in absence (Fig.5.10A) and the presence (Fig.5.10B) of AuNPs in 0.1M phosphate buffer containing 0.1MKCl at the scan rate of 10, 20, 35, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 mV s^{-1} between -0.2 to +0.6V vs. Ag/AgCl reference electrode. The plot of scan rate vs current density and $(\text{scan rate})^{1/2}$ vs current density are given in Fig. 5.11. Electrochemical oxidation of Ascorbic Acid using potassium ferricyanide as mediator in the absence and presence of AuNPs is also monitored as given in Fig. 5.12.

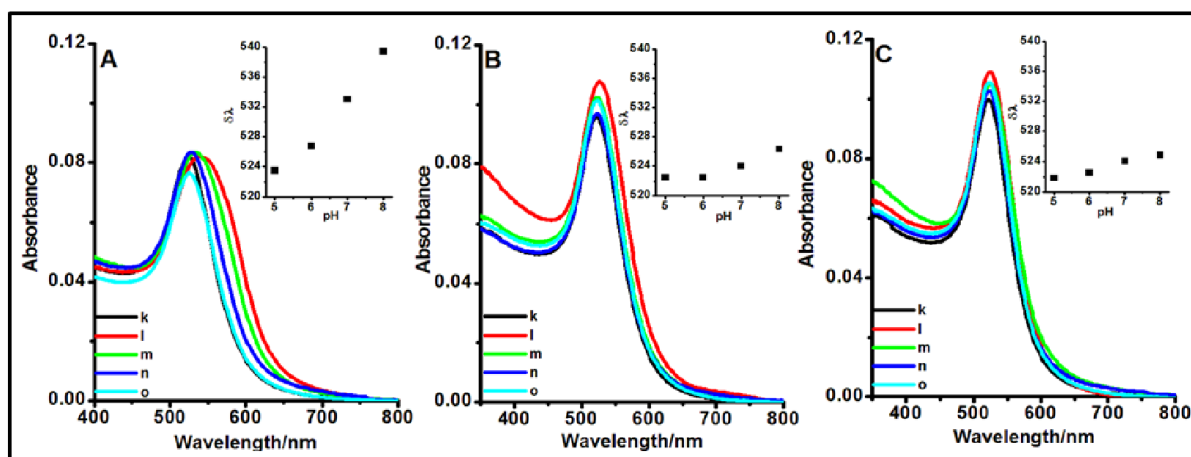


Figure 5.8. UV-Vis spectra displaying the effect of pH on AuNPs made by using formaldehyde and 3-APTMS (A) 0.1 M (B) 0.2 M and (C) 0.4 M. Inset shows the change in wavelength (λ_{max}) with pH of the buffering media (k = water, l = 8, m = 7, n = 6, o = 5).

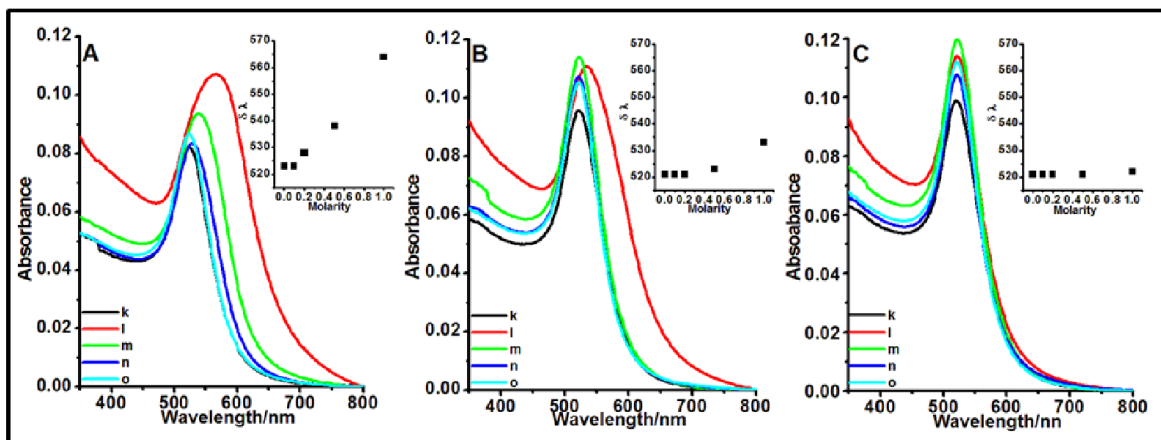


Figure 5.9. UV-Vis spectra displaying the effect of salt concentration on AuNPs made by using formaldehyde and 3-APTMS (A) 0.1 M (B) 0.2 M and (C) 0.4 M. Insets show the change in wavelength (λ_{\max}) with salt concentration (k = water, l = 0.1 M, m = 0.2 M, n = 0.5 M, o = 1.0 M).

Table 5.4. Salt and pH sensitivity of AuNPs made using formaldehyde and varying 3-APTMS concentration.

Sample Name	3-APTMS	Organic reducing agent	Sensitivity	
			Salt	pH
A.	0.1	Formaldehyde	High	Low
B.	0.2	Formaldehyde	High	Low
C.	0.4	Formaldehyde	High	Low

5.4. DISCUSSION

5.4.1. Structural analysis of AuNPs

AuNPs have been fabricated using 3-APTMS and four different reducing agents. TEM images of 3-APTMS mediated AuNPs made using different reducing agents as shown in Fig. 5.1-Fig. 5.4 reveal spherical shape of the AuNPs with the size ranging from 7-10nm. Diffraction pattern of the respective TEM images are given on the right side of each image.

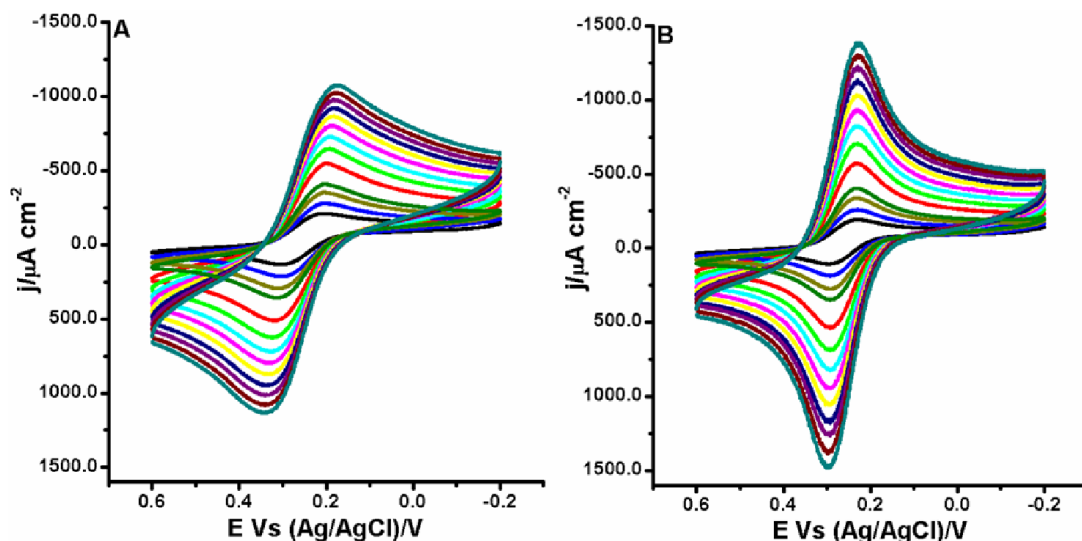


Figure 5.10. Cyclic voltammogram of potassium ferricyanide in absence (A) and the presence (B) of AuNPs in 0.1 M phosphate buffer of pH 7 containing 0.1 M KCl at the scan rate of 10, 20, 35, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 V s^{-1} .

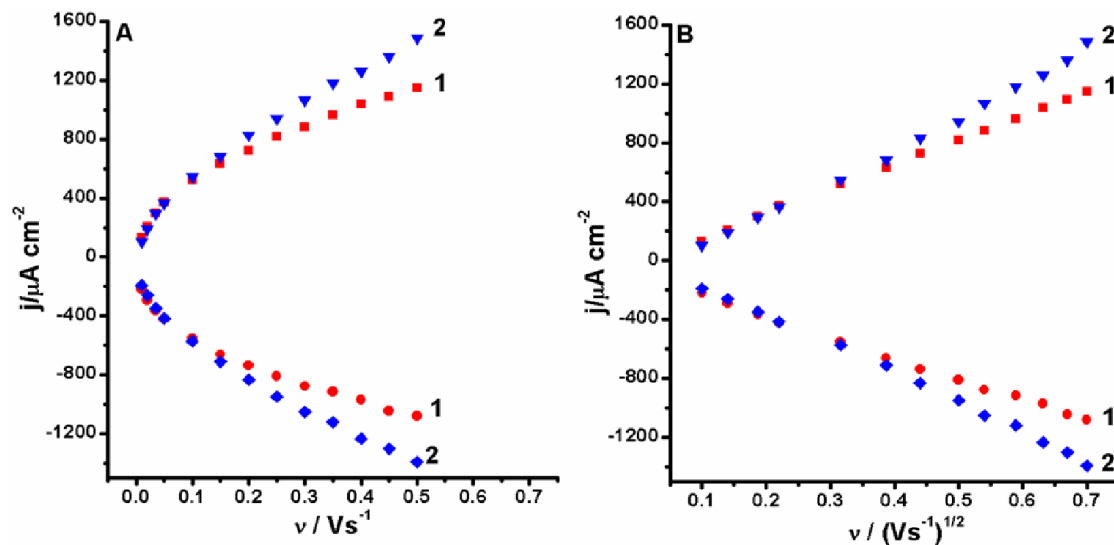


Figure 5.11. (A) Shows the plot of anodic and cathodic current density versus scan rate in absence (1) and the presence (2) of AuNPs. (B) shows the plot of anodic and cathodic current density versus square root of scan rate in absence (1) and the presence (2) of AuNPs.

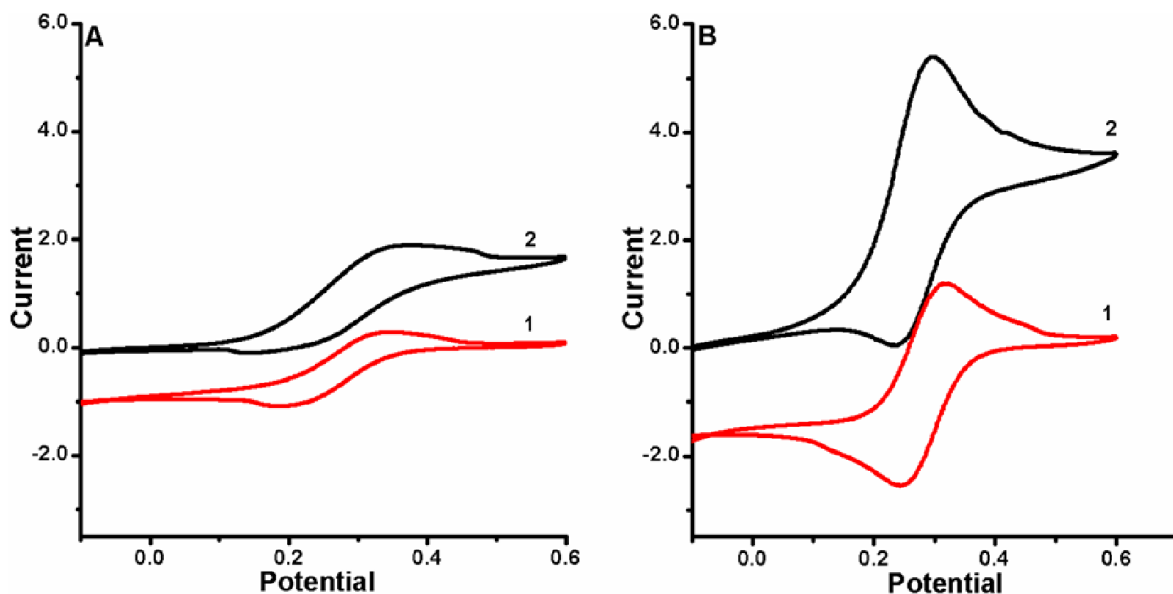


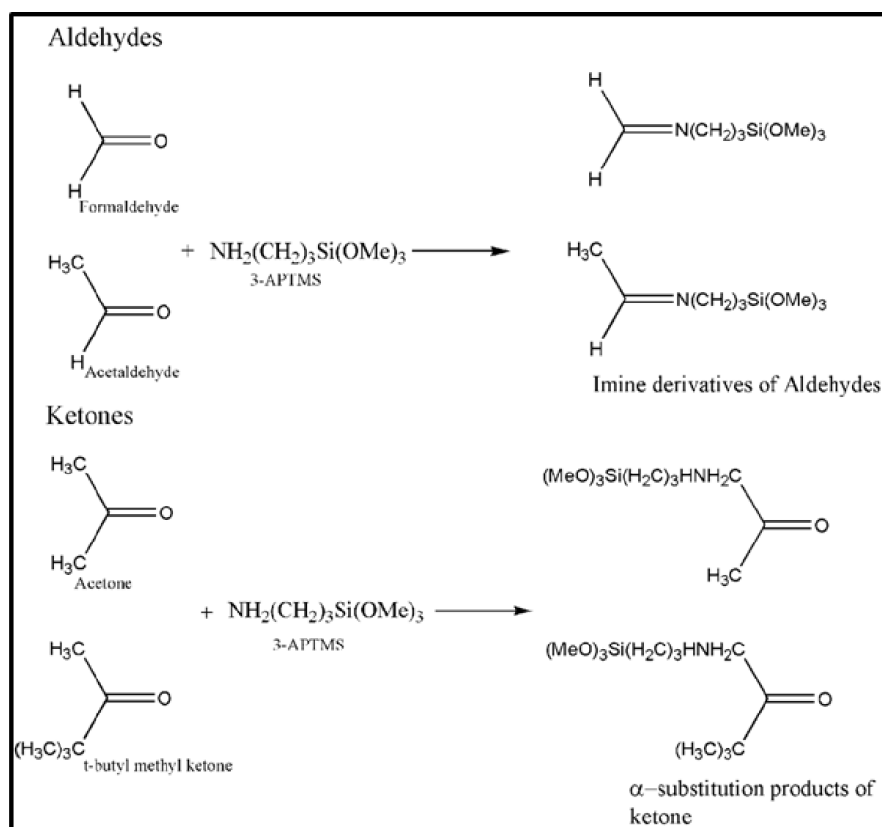
Figure 5.12. Show the oxidation of ascorbic acid using potassium ferricyanide as mediator in absence (A) and the presence (B) of AuNPs in 0.1 M phosphate buffer containing 0.1 M KCl at the scan rate of 10mVs^{-1} .

The diffraction patterns with concentric circles are characteristic of FCC geometry. Some irregular shapes (triangular) appear on moving from formaldehyde to t-DMK.

5.4.2. Chemistry behind 3-APTMS and organic reducing agent mediated synthesis of AuNPs.

The finding on the catalytic activity of AuNPs (P, Q, R, S) and the reaction product (P', Q', R', S'), as shown in Fig. 5.5 and Fig. 5.6 along with the known chemistry of imine formation reveals the following conclusions: (1) imines are typically formed by the condensation of primary amines and aldehydes, (2) imine are formed less readily with ketones than with aldehydes, (3) Catalytic nature of the linkage between 3-APTMS formaldehyde/acetaldehyde, (4) A solution of 3-APTMS and acetone or 3-APTMS and t-butyl methyl ketone is non catalytic in nature and (5) Imines (made from 3-APTMS)

are catalytic in nature (Wight and Davis, 2002). These findings predict that aldehydes (formaldehyde or acetaldehyde) used during 3-APTMS mediated synthesis of AuNPs is accompanied (major product) with imine formation while the use of ketones doesn't enable the formation of the same (major product) under prevailing experimental conditions. In previous chapter the α -substitution reaction during cyclohexanone mediated conversion of AuNPs was proposed. Based on these findings we propose separate reaction mechanism for the interaction between 3-APTMS/aldehyde and 3-APTMS/ketone (Scheme 5.1).



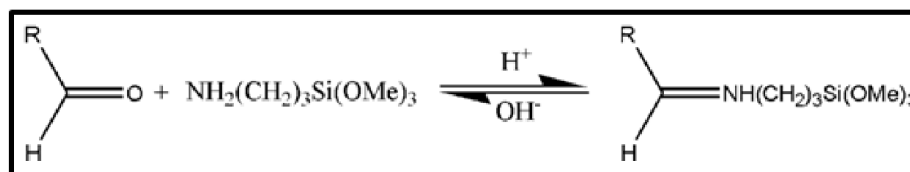
Scheme 5.1 Mechanism proposed for 3-APTMS interaction with Aldehydes and ketones.

The mechanism is also confirmed from the fact that chloride ion is a good leaving group and $-\text{NH}_2$ is a good nucleophile, resulting in α -amino substitution. From the experimental

findings we also know that as we move from formaldehyde to t-butyl methyl ketone there is decreased formation of catalytic imine linkage. Thus ketones mediated synthesis of 3-APTMS capped Au³⁺ mostly end up in amino substitution at α - position.

5.4.3. Mechanism operating in Salt- and pH- tolerances of AuNPs

Stability of AuNPs to different conditions of pH- and salt concentration is necessary for its use in various applications. Thus, the AuNPs synthesized in the present chapter have been checked for their stability to different conditions of working media. The results on the pH- and salt- tolerance of AuNPs have been given in Fig. 5.7, Fig. 5.8 and Fig. 5.9. The results clearly indicate that formaldehyde and increased 3-APTMS concentrations favor the stability or pH- and salt- tolerance ability of AuNPs. Scheme 5.2 shows the equilibrium involved during imine formation from 3-APTMS and aldehydes. As can be seen there exists an equilibrium between imine and the reactant, forward step being catalyzed by H⁺ while the backward step by OH⁻. This explains why a small change in pH does not affect the stability of AuNPs. With an increase in concentration of the 3-APTMS there is increased formation of imine thus imparting greater stability towards change of pH. In addition to that an increase in 3-APTMS concentration causes increase in micellar activity that allows high salt tolerance during specific application.



Scheme 5.2. Equilibrium displaying involvement of H⁺ and OH⁻ during imine formation.

5.4.4. Redox behavior of potassium ferricyanide in the presence of AuNPs

The redox behaviour of potassium ferricyanide in the presence of AuNPs significantly improved, compared to the behavior of the same in absence of AuNPs, the redox (anodic and cathodic peak) separation revealing the contribution of AuNPs during interfacial charge transport is shown in Fig. 5.10. The scan rate and (scan rate)^{1/2} dependent variation of current density in the absence and presence of AuNPs has also been recorded to justify the faster charge dynamics as shown in Fig.5.11A and Fig.5.11B respectively. These findings clearly demonstrate the significance of as synthesized AuNPs in electroanalytical chemistry. The results shown in Fig. 5.12A (in absence of AuNPs) and Fig. 5.12B (in the presence of AuNPs) for the oxidation of ascorbic acid in the absence and presence of AuNPs confirm the introduction of electrocatalysis of as synthesized gold nanoparticles.

5.5. CONCLUSION

The present article describes that the 3-APTMS capped Au³⁺ can be precisely converted into respective nanoparticle of controlled pH and salt tolerance in the presence of formaldehyde, acetaldehyde, acetone and t-butylmethylketone. An increase in alkyl content significantly influence the dispersibility in various working medium. With a decrease in the hydrocarbon content of the organic reducing agent nanoparticles compatibility in water increases and that in organic media decreases. Besides imparting desired compatibility to the AuNPs, the organic reducing agents also affect the pH sensitivity of the AuNPs with aldehydic AuNPs being more pH-tolerant compared to ketonic AuNPs. Also, an increase in 3-APTMS concentration increases the salt- and pH-tolerance of the AuNPs. These findings have been exploited in justifying charge transport dynamics at electrochemical interface

resulting from the electrochemical behavior of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{2-}$ redox system. The presence of AuNPs facilitates the charge transport process and introduces electrocatalysis in reacting system.