Chapter 6: Synthesis of gold nanoparticles in aqueous polyvinyl pyrrolidone by radiolytic method and their application for estimation of hydrogen peroxide

6.1. Introduction

Metal nanoparticles are currently being explored for their versatile applications as catalysts [253], chemical and bio sensors [254,255], antibacterial substances [256] and drug delivery systems [257]. Noble metal nanoparticles, such as Au, Ag possess bright color in aqueous solution [104] and are potentially useful as biosensor and for biological imaging. Radiolytic reduction route for generation of metal nanoparticles is being studied by many researchers in recent years [258–260]. The use of ionizing radiation for the synthesis of metal nanoparticles is promising as highly reactive species with high reduction potential are produced in situ by the radiation, which is hard to achieve by other chemical methods. These reactive species can easily reduce metal ions down to zero-valent state. Furthermore, because of its ability to fine-tune the radiation dose, dose rate and to selectively generate the required transient reactive radicals, it may offer better control over the size and the size distribution. Also it is a room temperature process and the reaction system is cleaner as it is devoid of any external chemical reducing agent. Irradiation of aqueous solution by high energy radiation generates transient radicals through water radiolysis. These transient radicals contain both reducing as well as oxidizing species. To create a total reducing condition specific solute is added, which can scavenge oxidizing species. The reducing radicals such as $e_{aq}^-$ and $H^-$ are utilized to reduce metal ion precursor to metal in zero valent state. These metal atoms coalesce to form metal nanoparticles in presence of a capping agent. Poly (N-vinyl-2-pyrrolidone) (PVP) has been reported as a suitable capping agent in preparation of metal nanoparticles [261–263], because it has functional group namely $\geq C=O$ and $\geq N\equiv$ and long polymer chain. The functional groups containing lone pair of electrons help in stabilization of metal nanoparticles at their surface by covalent interaction, where as the polymer chain restricts aggregation of metal nanoparticles by steric hinderance. Apart from this, PVP is a biocompatible polymer. Hence nanoparticles synthesized in PVP can have
potential biological applications. In this communication we report a facile method for synthesis of gold nanoparticles in PVP in presence of small amount of silver ion, isopropanol and acetone by using high energy gamma radiation. Different experimental parameters such as Au$^{III}$ concentration, PVP concentration, Ag$^{+}$ concentration and molecular weight of PVP have been optimized to get Au nps of desirable size and size distribution. Hydrogen peroxide is widely used as an oxidant, a disinfectant and a bleaching agent in various industries, such as textile, paper and pulp, pharmaceutical industries [264]. It causes irritation to eye, skin and mucous membrane when present in the environment. Hydrogen peroxide is produced in stoichiometric amounts during the oxidation of biological analytes (e.g. glucose) by dissolved oxygen in the presence of corresponding oxidase. Hence micro and trace level determination of hydrogen peroxide is considerably important in clinical chemistry, analytical biochemistry and environmental science. Existing methods for the determination of hydrogen peroxide include titrimetry [265], spectrophotometry [266], kinetic flow-injection method [267], fluorescence [268], enzymatic method [269], chromatographic techniques [270] and electrochemical methods [271]. Recently a new method for determination of hydrogen peroxide based on a peroxidase-catalyzed reaction and using Au nanoparticles has been reported [272]. In this work the H$_2$O$_2$ concentration in aqueous solution was determined by monitoring the oxidation of o-phenylene diamine (o-PDA) by H$_2$O$_2$ in presence of enzyme, horse radish peroxidase (HRP). Au nanoparticle solution when added to the reaction system containing o-PDA, H$_2$O$_2$ and HRP the interaction of oxidation product with Au nanoparticles results in an enhanced absorption peak at 427 nm. The absorbance value of this peak at $\lambda_{\text{max}}$ increases linearly with increase in H$_2$O$_2$ concentration in two ranges, i.e., $2.5 \times 10^{-6}$ mol dm$^{-3}$ to $2 \times 10^{-4}$ mol dm$^{-3}$ and $1 \times 10^{-7}$ mol dm$^{-3}$ to $3 \times 10^{-6}$ mol dm$^{-3}$ H$_2$O$_2$ in two separate set of experimental parameters. The detection limit is $1 \times 10^{-7}$ mol dm$^{-3}$. Hence using this system H$_2$O$_2$ concentration can be estimated from $1 \times 10^{-7}$ mol dm$^{-3}$ to $2 \times 10^{-4}$ mol dm$^{-3}$ by choosing appropriate experimental parameters.
Chapter 6

6.2. Results and discussions

6.2.1. Formation of Au nanoparticles and characterization

Irradiations were carried out in $^{60}$Co gamma chamber having a dose rate of 2.2 kGy h$^{-1}$ determined using Fricke dosimetry [148]. An aqueous solution containing $5 \times 10^{-4}$ mol dm$^{-3}$ Au$^{\text{III}}$, 0.5% PVP ($M_w = 3,60,000$ Da), $6 \times 10^{-5}$ mol dm$^{-3}$ AgNO$_3$, 0.2 mol dm$^{-3}$ isopropanol and $5.8 \times 10^{-2}$ mol dm$^{-3}$ acetone was purged with N$_2$ and irradiated for an absorbed dose of 1.7 kGy. The formation of Au nanoparticles and the saturation dose was measured by spectroscopic monitoring.

When an aqueous solution containing Au$^{\text{III}}$, PVP, isopropyl alcohol, AgNO$_3$ and acetone is purged with N$_2$ and subjected to gamma irradiation, radiolysis of water takes place. As a result reactive transient species, viz. $e_{aq}^-$, H$^-$, ‘OH are generated (Equation 6.1). isopropyl alcohol present in the reaction medium reacts with H$^-$ and ‘OH to give isopropyl radical and acetone reacts with $e_{aq}^-$ to give isopropyl radical (Equations 6.2 and 6.3). This isopropyl radical is capable of reducing Au$^{\text{III}}$. Hence the only reducing species present in the system is isopropyl radical, which reduces the metal ion to metal in zero valent state, i.e., Au$^{\text{III}}$ to Au$^0$ as shown in the mechanism (Equations 6.4-6.7) [273].

\[
\begin{align*}
\text{H}_2\text{O} & \xrightarrow{\gamma} e_{aq}^- + \text{H}^- + \text{OH}^- \quad (6.1) \\
\text{‘OH/H}^- & + (\text{CH}_3)_2\text{CH-OH} & \rightarrow & (\text{CH}_3)_2\text{‘C-OH} + \text{H}_2\text{O}/\text{H}_2 \quad (6.2) \\
e_{aq}^- & + (\text{CH}_3)_2\text{C}=\text{O} & \rightarrow & (\text{CH}_3)_2\text{‘C-OH} + \text{OH} \quad (6.3) \\
\text{Au}^{\text{III}} & + (\text{CH}_3)_2\text{‘C-OH} & \rightarrow & \text{Au}^{\text{II}} + (\text{CH}_3)_2\text{C}=\text{O} + \text{H}^+ \quad (6.4) \\
\text{Au}^{\text{II}} & + \text{Au}^{\text{II}} & \rightarrow & (\text{Au}^{\text{II}})_2 \quad (6.5) \\
(\text{Au}^{\text{II}})_2 & \rightarrow & \text{Au}^{\text{III}} + \text{Au}^{\text{I}} \quad (6.6) \\
\text{Au}^{\text{I}} & + (\text{CH}_3)_2\text{‘C-OH} & \rightarrow & \text{Au}^0 + (\text{CH}_3)_2\text{C}=\text{O} + \text{H}^+ \quad (6.7)
\end{align*}
\]

After generation of Au$^0$ coalescence step leads to formation of Au nanoparticles in presence of PVP as a capping agent. PVP contains functional groups like $\text{>C}=\text{O}$ and $\text{>N–}$ [274], which helps in anchoring metal nanoparticles on their surface. These Au nanoparticles show characteristic surface plasmon band at around 522 nm (Figure 6.1). Figure 6.1 shows the yield of Au nanoparticles increases with increase in absorbed dose till all precursor Au$^{\text{III}}$ ions are exhausted.
Figure 6.1: Absorption spectra of aqueous Au nanoparticle solution obtained at dose (a) unirradiated reaction mixture, (b) 0.5 kGy, (c) 0.9 kGy, (d) 1.3 kGy, (e) 1.5 kGy, (f) 1.6 kGy, (g) 1.8 kGy at a dose rate 2.2 kGy h\(^{-1}\)

Figure 6.2: TEM image of Au nanoparticles prepared using PVP of molecular weight 3,60,000 Da

The TEM image (Figure 6.2) shows that Au nanoparticles formed in PVP of molecular weight 3,60,000 Da is spherical in nature with two types of size distribution having average particle size 13 nm and 6 nm.
6.2.2. Reduction under milder condition

It was observed that the presence of acetone affects the spectral behaviour of Au nanoparticles (Figure 6.3). In absence of acetone the reducing species are $e_{aq}^{-}$ and isopropyl radical. $e_{aq}^{-}$ ($E^0 = -2.9$ V$_{NHE}$) is a stronger reducing agent than isopropyl radical. Acetone is known to scavenge aqueous electron produced by water radiolysis to give isopropyl radical ($E^0 = -1.8$ V$_{NHE}$) (Equation 6.3). In presence of acetone and isopropyl alcohol the only reducing agent in the system is isopropyl radical (Section 5.3.3.1 in Chapter 5).

![Figure 6.3: Absorption spectra of aqueous Au nanoparticle solution obtained (a) with acetone and (b) without acetone for 1.7 kGy of absorbed dose](image)

The isopropyl radical is a milder reducing agent, so that reduction by this radical is slower than that by $e_{aq}^{-}$. Slower reduction rate is suitable for achieving narrower size distribution of Au nanoparticles as shown in Figure 6.3, also indicated by decrease in FWHM. Full width at half maximum (FWHM) can be useful to find size distribution of particles in a solution. The broader is the peak, the broader is the size distribution of the particles [275]. In case of smaller particles absorption is the prominent process. With increase in particle size the scattering of light becomes more pronounced. The scattering leads to broadening of plasmon band.
6.2.3. Role of AgNO₃ in nanoparticle formation

Comparing spectra of Au nanoparticles prepared in the presence and absence of a small amount of AgNO₃, the spectrum was found to be narrower in presence of AgNO₃ (Figure 6.4) indicating more uniform size distribution in this condition. Ag⁺ at this concentration, i.e., $6 \times 10^{-5}$ mol dm⁻³ is known to adsorb upon certain faces of Au crystal, leading to controlled growth. There is not much change in the spectral pattern in the AgNO₃ concentration range $6 \times 10^{-5}$ mol dm⁻³ to $1.5 \times 10^{-4}$ mol dm⁻³ (Figure 6.4).

![Figure 6.4: Absorption spectra of aqueous Au nanoparticle solution obtained for Ag⁺ concentration (a) 0, (b) $6 \times 10^{-5}$, (c) $1 \times 10^{-4}$, (d) $1.5 \times 10^{-4}$, (e) $2 \times 10^{-4}$, (f) $3 \times 10^{-4}$, (g) $4 \times 10^{-4}$ mol dm⁻³ for 1.7 kGy of absorbed dose]

Beyond this concentration there is a blue shift in spectra, which may be due to AuAg alloy nanoparticle formation [276]. Hence in all the experiments $6 \times 10^{-5}$ mol dm⁻³ AgNO₃ has been used.

6.2.4. Effect of variation of PVP concentration

The effect of variation of PVP concentration on Au nanoparticle formation was investigated. Figure 6.5 shows the absorption spectra for $4 \times 10^{-4}$ mol dm⁻³ Au nanoparticle solution at various concentrations of PVP ($M_w = 3,60,000$Da). With increase in concentration of PVP from 0.1% to 2% (w/v) irregular spectral broadening was observed. This result is contrary to the earlier observed in case of Ag/guar gum system (Section 3.3.3, Chapter 3). As
the PVP concentration is increased from 0.1% (w/v) to 0.5%, the intensity of the peak increases. But further increase in PVP concentration to 1% and 2% resulted in a decrease in intensity of the surface plasmon band. Hence an optimum concentration of PVP is essential to achieve maximum concentration of stable Au nanoparticles. In all other experiments 0.5% PVP concentration is maintained.

![Absorption spectra of aqueous Au nanoparticle solution obtained for PVP concentration (a) 0.1%, (b) 0.5%, (c) 1%, (d) 2% for 1.7 kGy of absorbed dose](image)

**Figure 6.5:** Absorption spectra of aqueous Au nanoparticle solution obtained for PVP concentration (a) 0.1%, (b) 0.5%, (c) 1%, (d) 2% for 1.7 kGy of absorbed dose

### 6.2.5. Effect of variation of Au\(^{\text{III}}\) concentration

From Figure 6.6 it can be observed that as Au\(^{\text{III}}\) concentration increases from \(1 \times 10^{-4}\) mol dm\(^{-3}\) to \(1 \times 10^{-3}\) mol dm\(^{-3}\) there is a steady increase in absorbance indicating increase in yield of Au nanoparticles. Initially there is a 27 nm redshift in the peak position as the concentration changes from \(1 \times 10^{-4}\) mol dm\(^{-3}\) to \(4 \times 10^{-4}\) mol dm\(^{-3}\). At lower precursor concentration small nuclei are formed because of lower local concentration and beyond a certain size the growth is arrested because there is no further supply of Au\(^{\text{III}}\), leading to formation of smaller particles. These smaller particles absorb at lower wavelength. Above \(4 \times 10^{-4}\) mol dm\(^{-3}\) of Au\(^{\text{III}}\) concentration there is not much shift in spectral peak as well as FWHM of the spectra. The
narrow spectrum even at $1 \times 10^{-3}$ mol dm$^{-3}$ Au$^{III}$ indicates good particle size distribution even at this concentration.

![Absorbance vs Wavelength](image)

**Figure 6.6: Absorption spectra of aqueous Au nanoparticle solution obtained for Au$^{III}$ concentration (a) $1 \times 10^{-4}$, (b) $2.5 \times 10^{-4}$, (c) $4 \times 10^{-4}$, (d) $8 \times 10^{-4}$, (e) $1 \times 10^{-3}$ mol dm$^{-3}$ for absorbed dose of 0.4, 0.9, 1.5, 3.0 and 3.5 kGy respectively**

It is reported in the literature that at higher precursor ion concentration the spectrum deforms and broadens due to large particle size distribution. But in this case, PVP appears to be an efficient stabilizer in which particle size with narrow distribution can be obtained even for $1 \times 10^{-3}$ mol dm$^{-3}$ precursor concentration.

### 6.2.6. Effect of PVP molecular weight on Au nanoparticle formation

In this study PVP of molecular weights 40,000; 1,60,000 and 3,60,000 Da have been used to find the effect of PVP molecular weight on Au nanoparticle formation. The molecular weight of PVP plays a very important role in controlling the shape and size of Au nanoparticles (Figure 6.7). Au nanoparticles stabilized by PVP having molecular weight 40,000 Da show a single intense peak at 533 nm and PVP having molecular weight 1,60,000 Da and 3,60,000 Da show similar broad peaks at 513 nm and 520 nm respectively. For higher molecular weight PVP only spherical Au nanoparticles with a little variation in size are generated as confirmed from the TEM image (Figure 6.2), whereas lower molecular weight PVP can stabilize
different shaped Au nanoparticles other than spheres [277] by selectively blocking certain crystallographic faces of Au nanocrystal. Larger particle size is obtained in case of lower molecular weight PVP, because less steric effect is imparted by shorter carbon chain [278].

Figure 6.7: Absorption spectra of aqueous Au nanoparticle solution obtained for PVP of molecular weight (M_w) (a) 40,000 Da, (b) 1,60,000 Da, (c) 3,60,000 Da for 1.7 kGy of absorbed dose

6.2.7. Estimation of Hydrogen peroxide

6.2.7.1. Estimation of H_2O_2 in the range of 2.5×10^{-6} mol dm^{-3} to 2×10^{-4} mol dm^{-3}

An aqueous solution of horseradish peroxidase (HRP) was prepared by dissolving 0.125 mg HRP in 10ml nanopure water for further use. For estimation of H_2O_2 in the higher concentration range, 1 × 10^{-2} mol dm^{-3} citrate buffer (Citric acid/ Sodium citrate) solution, 1 × 10^{-4} mol dm^{-3} o-PDA, 125 µl of 0.125 mg/10ml of HRP and a certain quantity of H_2O_2 were successively added to a conical flask and the total volume was diluted to 20 ml with nanopure water. The reaction mixture was kept at room temperature for 30 minutes to allow the completion of the reaction between H_2O_2 and o-PDA. 5ml of a 5 × 10^{-4} mol dm^{-3} gold nanoparticle solution (in terms of Au^{III}) was added to this reaction mixture. The resulting solution was allowed to stand for another 30 minutes and subsequently the absorption spectra were recorded in the wavelength range of 250-800 nm.
6.2.7.2. Estimation of H\textsubscript{2}O\textsubscript{2} in the range of 1×10\textsuperscript{-7} mol dm\textsuperscript{-3} to 3×10\textsuperscript{-6} mol dm\textsuperscript{-3}

For estimation of H\textsubscript{2}O\textsubscript{2} at lower concentration range, 5×10\textsuperscript{-5} mol dm\textsuperscript{-3} o-PDA was taken. The concentrations of all the other reagents and the order of addition of these reagents remain same. Same procedure was followed as in section 6.3.1.

o-PDA undergoes catalytic oxidation by H\textsubscript{2}O\textsubscript{2} in presence of HRP [272]. The oxidation product of o-PDA has a weak absorption peak at 427 nm. As mentioned in section 6.3.1. Au nanoparticle solution was added to the reaction mixture after the product formation was over, i.e., after 30 minutes.

![Figure 6.8: Enzymatic oxidation of o-PDA with H\textsubscript{2}O\textsubscript{2}](image)

The Au nanoparticles generated through radiolytic method were found to enhance the absorption peak of the oxidation product of o-PDA. This may be due to the interaction of Au nanoparticles with 2, 3- diaminophenazine, which is the final oxidation product of o-PDA [279,280] (Figure 6.8). With varying concentration of H\textsubscript{2}O\textsubscript{2} there is a systematic change in the absorption peak at 427 nm for 1×10\textsuperscript{-4} mol dm\textsuperscript{-3} Au nanoparticles (in terms of Au\textsuperscript{III}) stabilized by PVP of molecular weight 3,60,000 Da and for 1×10\textsuperscript{-4} mol dm\textsuperscript{-3} o-PDA as shown in Figure 6.9. When Au nanoparticles stabilized by PVP of different molecular weight, such as 40,000 Da and 1,60,000 Da were used for H\textsubscript{2}O\textsubscript{2} estimation, similar results were observed for same range of H\textsubscript{2}O\textsubscript{2} concentration. The absorbance value at \lambda\textsubscript{max} (427 nm) for reaction mixture containing Au nanoparticles stabilized by different molecular weight PVP has been plotted against H\textsubscript{2}O\textsubscript{2} concentration in Figure 6.9 (inset). The response is linear in the range of 2.5×10\textsuperscript{-6} mol dm\textsuperscript{-3} to 2×10\textsuperscript{-4} mol dm\textsuperscript{-3} H\textsubscript{2}O\textsubscript{2} concentration with correlation factor R > 0.998 irrespective of PVP molecular weight. To estimate lower concentrations of H\textsubscript{2}O\textsubscript{2}, 5×10\textsuperscript{-5} mol dm\textsuperscript{-3} o-PDA was taken and all other reagents and experimental procedure kept same as above. The spectral change with change in concentration of H\textsubscript{2}O\textsubscript{2} has been shown in Figure 6.10. The response is linear in the range of 1×10\textsuperscript{-7} mol dm\textsuperscript{-3} to 3×10\textsuperscript{-6} mol dm\textsuperscript{-3} H\textsubscript{2}O\textsubscript{2} concentration with correlation factor R = 0.995 Figure 6.10 (inset).
Figure 6.9: Absorption spectra of reaction medium containing OPD, HRP, H$_2$O$_2$ and Au nanoparticles (in PVP, $M_w = 3,60,000$ Da) in citrate buffer with varying H$_2$O$_2$ concentration (higher range of H$_2$O$_2$ concentration) (a) 0, (b) 2.5 x 10^{-6}, (c) 5 x 10^{-6}, (d) 1 x 10^{-5}, (e) 2.5 x 10^{-5}, (f) 5 x 10^{-5}, (g) 7.5 x 10^{-5}, (h) 1 x 10^{-4}, (i) 1.3 x 10^{-4}, (j) 1.6 x 10^{-4}, (k) 2 x 10^{-4} mol dm$^{-3}$

Inset: Linear plot of absorbance at 427 nm vs H$_2$O$_2$ concentration in case Au nanoparticle used was in PVP of molecular weight (a) 3,60,000 Da (R=0.9986), (b) 1,60,000 Da (R=0.9981), (c) 40,000 Da (R=0.9981): H$_2$O$_2$ concentration range= 2.5 x 10^{-6} to 2 x 10^{-4} mol dm$^{-3}$

The detection limit in this system is 1 x 10^{-7} mol dm$^{-3}$. The colorimetric detection method reported by Wu et al. [272] is useful for detection of H$_2$O$_2$ in the range 1.3 x 10^{-6} mol dm$^{-3}$ to 4.1 x 10^{-5} mol dm$^{-3}$ with a detection limit 6 x 10^{-7} mol dm$^{-3}$. Also, they have reported a shift in the spectra of the chemically synthesized Au nanoparticles with change in H$_2$O$_2$ concentration, which was explained due to aggregation of Au nps. In our work, it was observed that there is no shift in the Au nanoparticle spectra with change in H$_2$O$_2$ concentration. The method reported in this communication is a better method for estimation of H$_2$O$_2$ because it can cover wider range and it has a lower detection limit, i.e., 1 x 10^{-7} mol dm$^{-3}$ to 2 x 10^{-4} mol dm$^{-3}$ and 1 x 10^{-7} mol dm$^{-3}$ respectively.
Figure 6.10: Absorption spectra of reaction medium containing OPD, HRP, H₂O₂ and Au nanoparticles (in PVP, M_w = 3,60,000) in citrate buffer with varying H₂O₂ concentration (lower range of H₂O₂ concentration) (a) 0, (b) 1 x 10⁻⁷, (c) 3 x 10⁻⁷, (d) 6 x 10⁻⁷, (e) 1.2 x 10⁻⁶, (f) 1.8 x 10⁻⁶, (g) 2.5 x 10⁻⁶, (h) 3 x 10⁻⁶, (i) 5 x 10⁻⁶ mol dm⁻³
Inset: Linear plot of absorbance at 427 nm vs H₂O₂ concentration incase Au nanoparticle used was in PVP of M_w = 3,60,000 Da(R=0.9954):H₂O₂ concentration range= 1 x 10⁻⁷ to 3 x 10⁻⁶ mol dm⁻³

6.3. Conclusions

Au nanoparticles have been synthesized in PVP by radiolytic route and characterized by uv-visible spectroscopy and TEM. Different experimental parameters were standardized to generate uniform size distributed Au nanoparticles. No external reducing agent has been used in this method. A colorimetric method based on interaction of the oxidation product of o-PDA with radiolytically synthesized Au nanoparticles has been developed for estimation of H₂O₂. H₂O₂ in the concentration range of 1 x 10⁻⁷ mol dm⁻³ to 2 x 10⁻⁴ mol dm⁻³ can be estimated by this method by correctly choosing the reaction parameters as mentioned in the text. The detection limit for this method was found to be 1 x 10⁻⁷ mol dm⁻³. The estimation of H₂O₂ using this particular system is independent of molecular weight of PVP in a wide range.