Chapter 3: Gamma radiolytic synthesis of Poly(N-vinyl-2-pyrrolidone) capped Au nanoparticles for sensor applications

3.1. Introduction

Metal nanoparticles find application in a wide array of fields including catalysis [101], chemical and bio sensors [102,103], antibacterial substances [104] and drug delivery systems [105]. Noble metal nanoparticles, in particular, such as those of Au and Ag show intense Localized Surface Plasmon Resonance (LSPR) absorption in the visible region [106] and have potential applications as optical biosensors [107,108] or catalytic systems [109,110] for initiating a variety of organic reactions. The high surface area provided by these nanoparticles make them extremely efficient as catalytic materials to carry out reactions that are otherwise difficult to initiate [111].

Although literature provides numerous chemical methods for fabrication of noble metal nanoparticles, most of these methods involve the use of toxic chemical reducing agents or harsh reaction conditions. Therefore, in recent years, radiolytic reduction route for generation of metal nanoparticles has emerged as an attractive alternative technique to these chemical reduction methods. Irradiation by gamma radiation, such as $^{60}\text{Co}$ gamma irradiation, involves not only more convenient and simpler control parameters for engineering nanoparticles of desired morphology but also presents itself as a clean, environment friendly, room temperature technique that does not involve the use of any external reducing agents [112-115]. The reactive species generated through radiolysis can easily reduce metal ions down to zero valent state. Furthermore, because of the ability to fine-tune the radiation dose, dose rate and to selectively generate the required transient reactive radicals, radiation technology offers better control over the size and the size distribution. Thus, nanoparticles fabricated by radiation technology can be employed as highly efficient catalytic or sensing
materials. The choice of capping agent/stabilizer during synthesis of metal nanoparticles is also crucial since it decides the overall stability and morphology of the particles generated, which in turn decides the long term utility of the system as a facile catalytic or sensor system.

One of the most promising and widely used capping agents for metal nanoparticle stabilization is Poly (N-vinyl-2-pyrrolidone) (PVP) (Pimpang et al, 2011; Wang et al, 2005; Borodko et al, 2006). PVP possesses functional groups, such as $\text{C=O}$ and $\text{N}$ and a long polymer chain. These functional groups contain lone pairs of electrons which help in stabilization of metal nanoparticles at their surface through covalent interaction, while the polymer chain restricts aggregation of metal nanoparticles by steric hindrance. Apart from this, PVP is a biocompatible polymer. Hence nanoparticles synthesized in PVP can have potential biological applications.

The estimation of biologically relevant molecules such as Hydrogen peroxide is one of the primary applications that has been targeted using PVP stabilized metal nanoparticles. 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 [116]. It causes irritation to eye, skin and mucous membrane when present in the environment. Hydrogen peroxide is also produced in stoichiometric amounts during 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 [117], spectrophotometry [118], kinetic flow-injection method [119], fluorescence [120], enzymatic method [121], chromatographic techniques [122] and electrochemical methods [123]. However most of these methods suffer from limitations in terms of specificity, selectivity, kinetically instability or incompatibility with aqueous environments. Recently a new method for determination of
hydrogen peroxide based on a peroxidase-catalyzed reaction and using Au nanoparticles has been reported [124].

Similarly, the unique optical properties of metal nanoparticles can also be exploited for the estimation of trace levels of Mercury in the environment. Mercury is a widespread pollutant with distinct toxicological profiles, and it exists in a variety of different forms such as metallic, ionic, and as part of organic and inorganic salts and complexes. The quantification of mercury in aquatic ecosystems in particular is of considerable interest since exposure to high levels of Hg\(^{2+}\) is detrimental to physical health and can cause brain damage and other chronic diseases [125-127]. At the same time routine estimation of Hg\(^{2+}\) is essential for evaluating the safety of aquatically derived food supplies [128]. As per World Health Organization guidelines, the permissible limit of inorganic mercury in drinking water is 6µg/L, while the total body burden of mercury for adult humans should not exceed 20-30mg. The methods currently used for the determination of Hg\(^{2+}\) include atomic absorption spectrometry [129], inductively coupled plasma-mass spectrometry [130], selective cold vapor atomic fluorescence spectrometry [131], organic fluorophores/chromophores [132-135], semiconductor nanocrystals [136,137], cyclic voltammetry [138,139], etc., all of which are rather complicated, time-consuming, and costly as well as inappropriate for point-of-use applications. To overcome these drawbacks, several techniques have been developed for the simple, rapid detection of Hg\(^{2+}\) in aqueous solutions. Colorimetric methods, in particular, are extremely attractive because they normally involve visible changes which can be seen with the naked eye.

The present chapter reports a facile method for synthesis of gold nanoparticles (Au NPs) in PVP using high energy gamma radiation. Various experimental parameters such as Au\(^{3+}\) precursor ion concentration, PVP concentration, molecular weight of PVP have been optimized to obtain Au NPs of desired size and size distribution. 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 peroxidise (HRP). Radiolytically synthesized PVP-Au-NPs have also been employed as a LSPR based optical sensor for the estimation of trace levels of Hg ions in aqueous environments. The estimation of Hg$^{2+}$ is based on the selective interaction of the metal ion with PVP, which gets manifested as a change in the LSPR band of PVP-Au NPs.

3.2. Synthesis of Au nanoparticles

3.2.1. Formation of Au nanoparticles and their characterization

The radiolytic reduction has been proven to be a powerful tool for producing uniform and highly dispersed metallic clusters. When a N$_2$ purged aqueous solution containing Au$^{3+}$ precursor ions, PVP and 2-propyl alcohol is subjected to gamma irradiation, radiolysis of water takes place, generating reactive transient species, such as e$_{aq}^-$, H, OH (Eq. 1). Radiolytic species e$_{aq}^-$ and H are reducing in nature, whereas OH acts as an oxidizing agent. Therefore, 2-propyl alcohol is used to transform the entire reaction medium into a reducing one; 2-propyl alcohol reacts with H and OH to generate 2-propyl radical (Eq. 2), which is a mild reducing agent capable of reducing Au$^{3+}$ to metal in zero valent state, i.e., Au$^{3+}$ to Au$^{0}$ (Eq. 3). The overall reduction in the medium is therefore carried out by the two reducing species, namely (CH$_3$)$_2$C-OH and e$_{aq}^-$ (Eq. 4)

$$\gamma\text{-radiation}$$

$$\begin{align*}
H_2O & \xrightarrow{\gamma\text{-radiation}} e_{aq}^- \text{, } H \text{, } OH \text{, etc.} & (1) \\
OH/H & + (CH_3)_2CH-OH \rightarrow (CH_3)_2C-OH + H_2O/H_2 & (2) \\
Au^{3+} & + 3e_{aq}^- \rightarrow Au^0 & (3) \\
Au^{3+} & + 3(CH_3)_2C-OH \rightarrow Au^0 + 3(CH_3)_2C=O + 3H^+ & (4)
\end{align*}$$
The formation of \( \text{Au}^0 \) is followed by coalescence step leading to formation of Au clusters in presence of PVP as a capping and stabilizing agent. PVP contains functional groups like >C=O and >N \([140]\), which facilitate anchorage of metal nanoparticles and help in regulating the size of the Au-nanoparticles. These PVP-Au NPs show characteristic surface plasmon band at around 522nm. Figure 3.1 shows that the yield of PVP-Au NPs increases with increase in absorbed radiation dose till all precursor \( \text{Au}^{3+} \) ions are exhausted. The saturation dose for reduction of \( 5 \times 10^{-4} \) mol.dm\(^{-3}\) \( \text{Au}^{3+} \) was determined to be 1.8 kGy. The TEM image (Figure 3.2) reveals PVP-Au NPs fabricated using PVP of molecular weight 3,60,000 Da as the stabilizer are spherical in nature with average particle size ranging between 6-13nm.

![Absorption spectra of aqueous Au nanoparticle solution obtained at radiation dose](image)

**Fig. 3.1** Absorption spectra of aqueous Au nanoparticle solution obtained at radiation dose (a) unirradiated reaction mixture, (b) 0.5kGy, (c) 0.9kGy, (d) 1.3kGy, (e) 1.5kGy, (f) 1.6kGy, (g) 1.8kGy
3.2.2. Effect of variation of Au\(^{3+}\) concentration

Figure 3.3 presents the UV-visible spectra of PVP-Au NPs as a function of radiation dose. It was observed that as the Au\(^{3+}\) concentration increased from \(1 \times 10^{-4}\) mol dm\(^{-3}\) to \(1 \times 10^{-3}\) mol dm\(^{-3}\) the intensity of the LSPR band also increased indicating increase in yield of PVP-Au NPs. An initial red shift of 27nm was recorded as the precursor ion concentration increased from \(1 \times 10^{-4}\) mol dm\(^{-3}\) to \(4 \times 10^{-4}\) mol dm\(^{-3}\). This can be attributed to the fact that at lower precursor concentrations smaller nuclei are generated because of lower local concentration and beyond a certain size the growth is arrested with the exhaustion of Au\(^{3+}\) ions, leading to formation of smaller particles, which absorb at lower wavelength. Beyond \(4 \times 10^{-4}\) mol dm\(^{-3}\) of Au\(^{3+}\) concentration, there was not much shift in spectral peak as well as FWHM of the spectra.

Fig. 3.2 TEM image of Au nanoparticles prepared using PVP of molecular weight 3.60 kDa
3.2.3. Reduction under milder condition

The presence of acetone was observed to affect the spectral behaviour of PVP-Au NPs (figure 3.4). In the absence of acetone, the two prominent reducing species are $e_{aq}^{-}$ and 2-propyl radical. $e_{aq}^{-}$ ($E^0 = -2.9 \text{ V}_{\text{NHE}}$) is a stronger reducing agent than 2-propyl radical. Acetone scavenges the aqueous electrons generated via radiolysis to produce 2-propyl radical ($E^0 = -1.8 \text{ V}_{\text{NHE}}$) (Eq. 2), which are relatively weak reducing agents. Therefore, in presence of acetone and 2-propyl alcohol, the only reducing agent in the system is 2-propyl radical [141], thereby resulting in slower reduction rate of the precursor ions. The slower reduction rate, in turn, yields a narrower particle size distribution, which gets manifested as a narrower LSPR band for the PVP-Au NPs.

![Absorption spectra of aqueous Au nanoparticle solution obtained for Au$^{3+}$ concentration](image)

Fig. 3.3 Absorption spectra of aqueous Au nanoparticle solution obtained for Au$^{3+}$ concentration (a) $1 \times 10^{-4}$, (b) $2.5 \times 10^{-4}$, (c) $4 \times 10^{-4}$, (d) $8 \times 10^{-4}$, (e) $1 \times 10^{-3} \text{ mol.dm}^{-3}$
Fig. 3.4 Absorption spectra of $4 \times 10^{-4}$ mol.dm$^{-3}$ aqueous Au nanoparticle solution (a) in presence of acetone and (b) in absence of acetone

3.2.4. Role of AgNO$_3$ in nanoparticle formation

The role of addition of trace quantities of Ag$^+$ ions during Au NPs formation was also investigated. It was observed that the spectrum was narrower in presence of Ag$^+$ ions (figure 3.5), indicating more uniform size distribution. Ag$^+$ in minute concentrations ($6 \times 10^{-5}$ mol.dm$^{-3}$) is known to adsorb upon certain faces of the Au crystal, leading to controlled growth. However, there is not much change in the spectral pattern of the PVP-Au NPs for Ag$^+$ in the concentration range of $6 \times 10^{-5}$ mol dm$^{-3}$ to $1.5 \times 10^{-4}$ mol dm$^{-3}$. Beyond this concentration, a blue shift was observed in the spectra, which can be attributed to the formation of an Au-Ag alloy nanostructure [142]
Fig. 3.5 Absorption spectra of aqueous Au nanoparticle solution (4×10^{-4} \text{ mol.dm}^{-3}) obtained in presence of (a) 0, (b) 6×10^{-5}, (c) 1×10^{-4}, (d) 1.5×10^{-4}, (e) 2×10^{-4}, (f) 3×10^{-4}, (g) 4×10^{-4} \text{ mol.dm}^{-3} \text{ Ag}^+ \text{ ions}

3.2.5. Effect of variation of PVP concentration

The effect of variation of PVP concentration on Au nanoparticle formation was also monitored. Figure 3.6 shows the absorption spectra for 4×10^{-4} \text{ mol.dm}^{-3} \text{ Au nanoparticle solution at various concentrations of PVP (M}_w = 3,60,000\text{Da}). With increase in concentration of PVP from 0.1\% to 2\% (w/v) irregular spectral broadening was observed. This result is contrary to results that were previously observed for other capping agents [143]. With increase in PVP concentration from 0.1\% (w/v) to 0.5\% (w/v), the intensity of the LSPR band also increased. But further increase in PVP concentration to 1\% and 2\% resulted in a decrease in intensity of the LSPR band. Hence an optimum concentration of PVP is essential to achieve maximum concentration of stable Au nanoparticles.
3.2.6 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 3.7). Au nanoparticles stabilized by PVP having molecular weight 40,000 Da show a single intense peak at 533nm and PVP having molecular weight 1,60,000 Da and 3,60,000 Da show similar broad peaks at 513nm and 520nm respectively. For higher molecular weight PVP, only spherical Au NPs with a little variation in size were generated, as confirmed from the TEM analysis. This observation can be explained on the basis of the fact that higher molecular weight PVP has longer polymeric chains which stabilize the metal colloids in a more efficient manner, thereby restricting anisotropic growth of the particles and confining them to spherical dimensions.
3.3. Estimation of Hydrogen Peroxide (H\textsubscript{2}O\textsubscript{2})

3.3.1. Detection principle

The estimation of hydrogen peroxide using PVP-Au NPs is based on the enzymatic oxidation of o-PDA by H\textsubscript{2}O\textsubscript{2} in presence of enzyme Horse radish peroxidase (HRP) [124]. In aqueous solutions 2,3-diaminophenazine (DAP) is the main product [fig. 3.8]. DAP has two Ns forming part of the central ring and two NH\textsubscript{2} groups. The lone pairs of these Ns interact with the Au NPs, thereby leading to a change in the external environment which gets manifested as a change in the intensity of DAP, which absorbs at ~427nm.

Briefly, for H\textsubscript{2}O\textsubscript{2} estimation in the concentration range of 2.5X10\textsuperscript{-6} mol.dm\textsuperscript{-3} to 2 X10\textsuperscript{-4} mol.dm\textsuperscript{-3}, a reaction mixture comprising of 1X10\textsuperscript{-4} mol.dm\textsuperscript{-3} o-PDA, 125 ml of 0.125mg/10 ml of HRP and a pre determined concentration of H\textsubscript{2}O\textsubscript{2} was diluted to 20mL.
using 0.01mL citrate buffer solution and incubated at room temperature for 30 min to allow
the completion of the enzymatic oxidation of o-PDA. To this reaction mixture was added 5ml
of a5X10^-4 mol dm^-3 PVP-Au NPs solution. The resulting solution was allowed to stand for
another 30 min and subsequently the absorption spectra were recorded in the wavelength
range of 250–800nm. For estimation of H_2O_2 at lower concentration range, the concentrate of
the substrate i.e. o-PDA was decreased to 5X10^-5 mol dm^-3. The concentration of all the other
reagents was kept unchanged and an identical protocol was adopted.

The oxidation products of o-PDA have a weak absorption peak at 427nm. The PVP-Au NPs were observed to significantly enhance the intensity of this weak absorption peak.
With varying concentration of H_2O_2 there is a systematic change in the absorption peak at
427nm, as highlighted in figure 3.9. The detection efficiency of the PVP-Au NPs system was
found to be independent of the molecular weight of the stabilizing agent i.e. PVP, and
displayed similar results when PVPs of molecular weights 40kDa and 160kDa were used as
stabilizing agents in addition to PVP of molecular weight 360kDa.(Figure 3.9 inset).

![Fig. 3.8 Enzymatic oxidation of OPD](image)

Fig. 3.8 Enzymatic oxidation of OPD
Fig. 3.9 Absorption spectra of reaction medium containing OPD, HRP, H$_2$O$_2$ and Au nanoparticles (in PVP, $M_w = 3,60,000$) in citrate buffer with varying H$_2$O$_2$ concentration (higher range of H$_2$O$_2$ concentration) (a) 0, (b) 2.5×10$^{-6}$, (c) 5×10$^{-6}$, (d) 1×10$^{-5}$, (e) 2.5×10$^{-5}$, (f) 5×10$^{-5}$, (g) 7.5×10$^{-5}$, (h) 1×10$^{-4}$, (i) 1.3×10$^{-4}$, (j) 1.6×10$^{-4}$, (k) 2×10$^{-4}$ mol.dm$^{-3}$ (Inset: Linear plot of absorbance at 427nm vs H$_2$O$_2$ concentration incase Au nanoparticle used was in PVP of molecular weight (a) 3,60,000Da (R=0.9986), (b) 1,60,000Da (R=0.9981), (c) 40,000Da (R=0.9981): H$_2$O$_2$ concentration range= 2.5×10$^{-6}$ to 2×10$^{-4}$ mol.dm$^{-3}$)

3.3.2. Detection limits

The response of the PVP-Au NPs based detection system was observed to be linear in the H$_2$O$_2$ concentration range of 2.5×10$^{-6}$ mol dm$^{-3}$ to 2×10$^{-4}$ mol dm$^{-3}$ with correlation factor R > 0.998 irrespective of PVP molecular weight. To estimate lower concentrations of H$_2$O$_2$, 5×10$^{-5}$ mol dm$^{-3}$ o-PDA was taken and all other reagents and experimental procedure kept same as above. The spectral change with change in concentration of H$_2$O$_2$ is shown in
The response was found to be linear in the range of $1 \times 10^{-7}$ mol dm$^{-3}$ to $3 \times 10^{-6}$ mol dm$^{-3}$ H$_2$O$_2$ concentration with correlation factor $R = 0.995$ (figure 3.9 inset). The detection limit in this case was observed to be $1 \times 10^{-7}$ mol dm$^{-3}$. The proposed method therefore can be used for estimation of H$_2$O$_2$ over a wide concentration range of $1 \times 10^{-7}$ mol dm$^{-3}$ to $2 \times 10^{-4}$ mol dm$^{-3}$ by tuning a single reaction parameter as per requirement.

**Fig. 3.10** Absorption spectra of reaction medium containing OPD, HRP, H$_2$O$_2$ and Au nanoparticles (in PVP, $M_w = 3,60,000$) in citrate buffer with varying H$_2$O$_2$ concentration (lower range of H$_2$O$_2$ concentration) (a) 0, (b) $1 \times 10^{-7}$, (c) $3 \times 10^{-7}$, (d) $6 \times 10^{-7}$, (e) $1.2 \times 10^{-6}$, (f) $1.8 \times 10^{-6}$, (g) $2.5 \times 10^{-6}$, (h) $3 \times 10^{-6}$, (i) $5 \times 10^{-6}$ mol dm$^{-3}$ (Inset: Linear plot of absorbance at 427nm vs H$_2$O$_2$ concentration incase Au nanoparticle used was in PVP of $M_w = 3,60,000$ Da(R=0.9954); H$_2$O$_2$ concentration range= $1 \times 10^{-7}$ to $3 \times 10^{-6}$ mol dm$^{-3}$)
3.4 Estimation of Mercury ions

3.4.1 Detection principle

The presence of Mercury ions in water as a toxic pollutant necessitates the detection of these ions up to extremely low concentrations. The estimation of Hg\textsuperscript{2+} ions in aqueous media by PVP-capped Au nanoparticles is based on the selective complexation of Hg\textsuperscript{2+} ions with PVP. [144,145]. Guner et al [145] reported that compared to anions, cations such as Hg\textsuperscript{2+}, Cd\textsuperscript{2+}, Co\textsuperscript{2+} etc. exhibit high degree of intramolecular complexation with PVP. The complexation occurs between one cation and two or more carbonyl groups of the lactam rings. The process of covalent complexation can be best explained in terms of the HSAB (Hard Soft Acid Base) principle. Since Hg\textsuperscript{2+} is a soft acid owing to its large ionic radius and high polarizability, the probability of its complexation with the carbonyl groups (soft base with large radius) is maximum compared to the relatively harder metal ions. This results in the selective interaction of Hg\textsuperscript{2+} with PVP, which culminates in the shifting of the SPR band of PVP-capped Au NPs.

In this method, a 50µM (final concentration) of PVP capped Au NPs system was used for estimation of Hg\textsuperscript{2+} ions in aqueous medium. Different volumes of a 2 µM stock solution of Hg\textsuperscript{2+} ions prepared in TRIS buffer of pH 10.0 were added to a fixed volume of PVP-Au NPs solution also maintained at pH=10.0. Addition of the metal ion resulted in an instantaneous color change from blight yellow to near colourless. The UV-visible spectra of the reaction mixtures were taken and the $\lambda_{\text{max}}$ plotted as a function of Hg\textsuperscript{2+} concentration. A linear relation was obtained for Hg\textsuperscript{2+} concentrations from 0 to 500nM with $R^2=0.991$ and a minimum detection limit of 40nM.

The effect of addition of Hg\textsuperscript{2+} on the morphology of PVP-Au NPs was monitored by TEM. As evident from fig. 3.11a, control Au NPs are uniformly dispersed spherical particles with an average diameter of 6.4 nm. However, addition of 500nM Hg\textsuperscript{2+} induces rapid change
in the morphology of the PVP-Au-NPs from spherical to larger (mean diameter of 8.3 nm), irregular sized particles (Fig 3.11b) with broader size dispersion compared to the control sample. This can be explained on the basis of preferential interaction of Hg$^{2+}$ with capping/stabilizer molecules (PVP), which leads to reduced interaction of the PVP molecules with Au atoms. This subsequently results in decrease in the stabilization of Au clusters and enhances their tendency to agglomerate. Fig.3.11c depicts the TEM image of the PVP-Au-NPs system five minutes after addition of Hg$^{2+}$ ions. The process of agglomeration of smaller clusters undergoes near completion within the short time span to give rise to bigger Au agglomerates (with the particle size ranging between 19 and 40 nm).

The hydrodynamic diameter of the PVP-Au-NPs was determined using a particle size analyzer. Particle size analysis also revealed the particles obtained by radiolytic process to be of uniform size distribution with average hydrodynamic diameter of around 52.16 nm. (fig. 3.12a). Addition of Hg$^{2+}$ ion solution to the PVP-Au NPs system resulted in an increase in the particle size distribution accompanied by an increase in the average particle size (fig.3.12b). This further corroborates the results obtained through TEM analysis.
Fig. 3.11 TEM images of PVP-Au-NPs in (a) absence of Hg$^{2+}$ ions (b) immediately after addition of 500nM Hg$^{2+}$ (c) 5 minutes after addition of Hg$^{2+}$
Fig. 3.12 Particle size analysis of Au nps in (a) absence of Hg$^{2+}$ ions and (b) immediately after addition of 500nM Hg$^{2+}$

The change in particle morphology on addition of Hg$^{2+}$ can also be deduced from the changes observed in the LSPR band of PVP-Au NPs (fig. 3.13). Control PVP-Au NPs yielded a single, narrow peak at ~530nm, indicative of uniform sized particles. On the other hand, addition of Hg$^{2+}$ resulted not only in a decrease in the SPR band intensity but also in a slight red shift in the peak at 530nm. Simultaneously, for Hg$^{2+}$ concentrations of 300nm and beyond, a prominent second broad peak appeared at ~740nm, indicative of the agglomeration and loss of structure of the PVP-Au NPs caused by addition of Hg$^{2+}$ ions.
Figure 3.13 Absorption spectra of Au nanoparticle solution in presence of (a) 0 (b) 100 (c) 200 (d) 300 (e) 400 and (f) 500 nM Hg$^{2+}$ ion. (Inset: Calibration plot of Hg$^{2+}$ ion concentration.)

3.4.2 Effect of pH

The pH of the reaction medium was found to significantly influence the detection efficiency of the system. In order to determine the effect of pH variation, the reaction was carried out under different pH values- 3.0, 4.5, 6.0, 8.0, 10.0 and 11.0. It was observed that while acidic pH (3.0, 4.5 and 6.0) and pH 8.0 did not induce any change in the SPR band of Au NPs, alkaline pH (10.0 and 11.0) were found to produce significant decrease in the SPR band intensity on addition of Hg$^{2+}$ ions while keeping all other reaction conditions same. This is probably because highly alkaline conditions favor displacement of the PVP molecules from the surface of Au NPs. In the process, carbonyl groups of the lactam rings of PVP, that were earlier interacting with the Au metal atoms, get exposed and instead bind with the Hg$^{2+}$
ions present in the solution. This results in a drastic change in the external environment of the NPs, which gets manifested as a decrease in the band intensity (fig. 3.14)

![Absorption spectra of Au nps-500nM Hg²⁺ reaction mixture at pH (a) 3.0 (b) 6.0 (c) 8.0 (d) 10.0 and (e) 11.0](image)

**Figure 3.14** Absorption spectra of Au nps-500nM Hg²⁺ reaction mixture at pH (a) 3.0 (b) 6.0 (c) 8.0 (d) 10.0 and (e) 11.0

### 3.4.3. Effect of PVP concentration

The effect of stabilizer concentration on the detection efficiency of the sensor system was also investigated. It was observed that high concentrations of PVP reduced the sensitivity of the Au NPs towards Hg ions, which was manifested in a marginal change in LSPR band intensity on addition of Hg ions to the Au NPs [fig 3.15]. High concentrations of PVP probably led to complete encapsulation of the Au NPs within the polymer shell, thereby inhibiting any changes in the LSPR of nanoparticles that might be induced in presence of Hg ions. The selective interaction of PVP molecules with Hg ions, which is responsible for inducing the LSPR changes in Au NPs, is rendered insignificant under very high...
concentrations of the stabilizer. Hence, PVP concentration was optimized at 0.05% (w/v), which was sufficient not only to provide stability to the Au NPs but also induced maximum shifts in LSPR band intensity of Au NPs on addition of the analyte.

3.4.4. Effect of PVP molecular weight

The stabilizer molecular weight was also found to play a crucial role in determining the sensor efficiency. Au NPs synthesized in higher molecular weight PVPs (160kDa and 360kDa) as stabilizers were observed to have a lower sensitivity towards the same analyte concentration as compared to Au NPs synthesized using PVP of molecular weight 40kDa as the stabilizer [fig. 3.15 inset]. This can be attributed to the fact that higher molecular weight PVPs have longer polymeric chains which wrap around the Au NPs in a more effective manner and thereby make the local environment of the nanoparticles less susceptible to any changes in LSPR characteristics that might be induced in presence of Hg$^{2+}$ ions. Hence, for all our estimation purposes, PVP of molecular weight 40kDa was used as the stabilizer since it was found to least interfere in the detection process, while at the same time provide sufficient stability to the nanoparticle system.
Figure 3.15 Relative change (%) in OD of reaction mixture containing PVP-Au-NPs and 500nM Hg$^{2+}$ ions as a function of PVP % (w/v) (Inset: Relative change (%) in OD of reaction mixture containing PVP-Au-NPs and 500nM Hg$^{2+}$ ions as a function of PVP molecular weight).

3.4.5. Interference study and selectivity

One of the primary factors deciding the efficiency of any sensor system is its selectivity and the extent to which it remains free from interference due to the presence of additional species in the analyte mixture. We checked for the interference of other metal ions in the detection system by subjecting different metal ions such as Ca$^{2+}$, Cu$^{2+}$, Cd$^{2+}$ and Fe$^{2+}$ to the same estimation protocol individually as well as in combination. 500nM solutions of each of the metal ions were prepared and added to the PVP-Au NP system. While Ca$^{2+}$, Cu$^{2+}$ and Fe$^{2+}$ showed no change in the LSPR band of Au NPs, addition of Cd$^{2+}$ resulted in a slight decrease in intensity, but no shift in peak position or change in colour was observed. Addition
of a mixture of all the ions (final concentration of each being 500nM) to the Au NP system yielded a similar negligible decrease in band intensity (fig. 3.17). This can be explained based on the fact that Cd$^{2+}$ is a relatively soft base compared to the other metal ions, though comparatively harder than Hg$^{2+}$, and therefore can complex to some extent with PVP resulting in a minute decrease in LSPR band intensity. However, the effect is visible only at Cd$^{2+}$ concentration as high as 500nM, which is the upper detection limit of our AuPVP based estimation technique.

![Figure 3.16](image)

**Figure 3.16** Interference study: Absorption spectra of Au nps in (a) absence of metal ions (b) 500nM Ca$^{2+}$ (c) 500nM Cu$^{2+}$ (d) 500nM Fe$^{2+}$ (e) 500nM Cd$^{2+}$ and (f) mixture of ions (500nM each)

### 3.5. Conclusions

The chapter describes the green synthesis of PVP capped Au nanoparticles via a clean, environment friendly gamma radiolytic route. The fabricated PVP-Au NPs were characterized by uv-visible spectroscopy and TEM techniques. Various experimental parameters were optimized to obtain Au NPs of desired morphology. The sensing properties

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of the PVP-Au NPs were investigated for two biologically relevant species, namely hydrogen peroxide and Hg$^{2+}$ in aqueous solutions. A colorimetric method based on interaction of the oxidation product of o-PDA with radiolytically synthesized Au nanoparticles was developed for estimation of H$_2$O$_2$. H$_2$O$_2$ in the concentration range of $1 \times 10^{-7}$ mol dm$^{-3}$ to $2 \times 10^{-4}$ mol dm$^{-3}$ can be estimated by this method by suitably tuning the reaction parameters. The detection limit for the proposed method was determined to be $1 \times 10^{-7}$ mol. dm$^{-3}$. The estimation of H$_2$O$_2$ using this particular system was found to be independent of the molecular weight of PVP. The PVP-Au-NPs system was also successfully employed as a highly sensitive and selective LSPR based optical sensor for estimation of nanomolar concentrations of Hg$^{2+}$ ions in aqueous solutions. Parameters, such as pH of solution, molecular weight and concentration of stabilizer were found to influence the detection efficiency of the system. The system showed optimum efficiency under the reaction conditions: pH$>10.0$ and 0.1% PVP (40kDa) as the stabilizer for Au-NPs. The presence of different metal ions, such as Ca$^{2+}$, Cu$^{2+}$, Cd$^{2+}$ and Fe$^{2+}$ did not interfere with the detection efficiency of the system within its detection range of 0-500nM Hg$^{2+}$ ion concentration. The LSPR based optical sensor system offers an easy, convenient and sensitive technique for estimation of Hg$^{2+}$ ions in aqueous solutions, which has potential environmental applications.