Chapter 4: A comparative study of the synthesis of Poly(n-vinyl-2-pyrrolidone) capped Ag nanoparticles by different radiation sources: application for estimation of uric acid

4.1. Introduction

The formation of metallic nanoparticles in solution initiated by $\gamma$-ray [146,147], electron beam [148,149] and X-ray [150-157] irradiation has been proved to be an alternative route for obtaining dispersed nanoparticles. Though irradiation using high energy radiation sources like gamma and electron beam is a well established technique, synchrotron radiation X-rays as an ionizing radiation source have been little explored for fabrication of metal nanoparticles. The possibility of synchrotron X-ray source for the direct reduction of gold precursor solutions was first explored by Rosenberg and co-workers [158]. The high flux and high energy of synchrotron X-rays can be utilized for reduction of metal ion precursor to generate metal nanoparticles in solution. The interaction of X-rays with aqueous medium is similar to that of $\gamma$ -rays. The primary radicals generated via radiolysis of water by X-rays are eaq$^-$, H· and ·OH. The first two are reducing in nature while ·OH is an oxidizing radical. X-ray irradiation method too offers the advantages of being a clean reaction system devoid of chemical reducing agents, better control over reaction parameters, rapid synthesis and high reduction yields due to the high X-ray flux.

In recent years, the use of metal nanoparticles as analytical and bioanalytical sensors has been receiving significant attention because of their unusual optical, electronic, and chemical properties [159-161]. Large numbers of methods have been developed for the fabrication of metal nanoparticles. However, the major disadvantages in most of the methods include use of toxic reducing chemicals, poor nanoparticle size distribution, and the poor dispersion of the nanoparticles in the polymer host [162]. Radiolytic methods, thus, have
emerged as an efficient alternative technique for fast and one step synthesis of uniformly dispersed metal nanoparticles [112-114]. The reducing radicals generated in-situ during radiolysis of water, such as $e_{\text{aq}}^-$ and H·, are utilized to reduce metal ion precursors to metal in zero valent state. These metal atoms coalesce to form metal nanoparticles in presence of a capping agent. These nanoparticles can therefore be effectively employed as biosensors with minimum probability of interference from external additives. The use of a biocompatible polymer like Poly (N-vinyl-2-pyrrolidone) (PVP) as the capping agent [163,164] further enhances their viability to detect biologically relevant molecules without disturbing the natural environment of the biological samples in which the estimation is usually done.

Localized surface Plasmon Resonance (LSPR)-based optical chemical sensors and biosensors are an appropriate, simplified, cheap and rapid alternative to more sophisticated detection techniques. The LSPR wavelength is extremely sensitive to the local environment around the nanoparticles, which facilitates their use as sensing devices [165]. In particular, this property can be utilized for highly sensitive detection of target molecules in medical applications. One such important application of nanoparticles has been made for the estimation of a biologically relevant molecule Uric acid. Uric acid represents the major catabolite of purine breakdown in humans. The normal concentration of uric acid in blood samples is reported to be in the range 150–420µM [166]. High levels of uric acid in the blood (hyperuricemia or Lesch-Nyhan syndrome) are linked with gout and other conditions including increased alcohol consumption, obesity, diabetes, high cholesterol, high blood pressure, kidney disease, and heart disease [167,168]. On the other hand, abnormally low uric acid levels are symptoms of diseases, such as multiple sclerosis [166]. Hence estimation of uric acid in blood can be used as a diagnostic tool for monitoring a large number of diseases. Furthermore, uric acid is an antioxidant in human adult plasma and is involved in various pathological changes [169]. In view of this, numerous techniques have been developed over
the years for detection and estimation of uric acid levels, which include enzymatic methods [170], spectrophotometry [171,172] electroanalysis [173], high performance liquid chromatography [174], fluorimetry [175,176] and chemiluminescence [177]. However, most of these methods suffer from limitations in terms of detection limits, temperature constraints or requirement of additional reagents. Radiolytically synthesized metal nanoparticles based sensors offer a simple, attractive and low cost alternative to these conventional estimation techniques.

The chapter highlights the synthesis of silver nanoparticles (Ag NPs) using high energy radiation sources, namely synchrotron X-ray, γ-ray and electron beam (EB) in presence of PVP as a stabilizing agent. The silver nanoparticles formed were characterized by UV-visible spectroscopy and TEM analysis. Effect of various reaction parameters, such as radiation dose, dose rate, concentration and molecular weight of the stabilizer and precursor ion concentration, on the morphology of the nanoparticles was also investigated. Ag NPs fabricated via gamma irradiation technique were subsequently employed as a LSPR based optical sensor system for estimation of uric acid. The technique is based on the catalytic oxidation of silver nanoparticles by hydrogen peroxide, which is generated in-situ during enzymatic degradation of uric acid in presence of enzyme uricase [178-182]. The method was successfully employed for estimation of uric acid concentration in bovine and human serum samples.

4.2. Results and discussion

4.2.1. Synthesis of polymer capped Ag nanoparticles by gamma, electron beam and synchrotron radiation sources and their characterization

The interaction of ionizing radiation, such as γ-ray, X-ray and accelerated electron beam with aqueous solution is fully understood and well established. Water, being the major
component of an aqueous solution, absorbs most of the radiation energy and undergoes radiolysis. The radiolytic transient species formed, namely hydrated electron (eaq\(^-\)), hydrogen atom radical (H\(\dot{\cdot}\)) and hydroxyl radical (\(\cdot\)OH) are highly reactive in nature. Eaq\(^-\) and H\(\dot{\cdot}\) possess high reduction potentials and can reduce metal ions to lower valences or to atoms in the zero valent state. These metal atoms subsequently coalesce to form metal nanoparticles in presence of a capping agent, such as polymers, ligands, surfactants etc. The \(\cdot\)OH radical, being oxidizing in nature can oxidize the metal atoms back to ions. Therefore, isopropyl alcohol is added to the system to scavenge the \(\cdot\)OH radicals. Isopropyl alcohol reacts with \(\cdot\)OH radical to generate isopropyl radical, which is mildly reducing in nature and capable of reducing Ag\(^+\) as well. Thus, the overall atmosphere is turned into a reducing one by the addition of isopropyl alcohol to the system.

Briefly, an aqueous solution containing 2X10\(^{-4}\) mol.dm\(^{-3}\) Ag\(^+\), 0.5% PVP (w/v) (Mw = 40,000 Da), 2 X10\(^{-1}\) mol.dm\(^{-3}\) isopropanol was irradiated by exposing the solution to X-ray beam for an absorbed dose of 2 kGy (time of irradiation=63s). The formation of Ag NPs was indicated by development of yellow color in the solution. The Ag NPs formed were characterized by measuring their UV-visible spectra after appropriate dilution. Fig. 4.1 presents the evolution of the LSPR band of Ag NPs with increasing duration of irradiation. The yield of Ag NPs formed increased with the increase in radiation dose, manifested by an increase in intensity of the LSPR band. The absorption maxima (\(\gamma_{\text{max}}\)) was centred at \(~411\text{nm}\) and the saturation dose required to achieve near complete reduction of 2X10\(^{-4}\) mol.dm\(^{-3}\)Ag\(^+\) in aqueous PVP solution was estimated to be 2kGy (irradiation time=63s).

The Ag NPs formed were further characterized by TEM analysis. Fig.4.2 highlights the TEM image of Ag NPs prepared by X-ray irradiation. The particles were observed to have a broad size distribution with average size in the range 10-15nm.
The precursor solutions for $\gamma$ and EB-irradiations were maintained at the same concentration; however, prior to $\gamma$-radiation, the precursor solution was deaerated by $N_2$ purging. The UV-visible spectra of Ag NPs fabricated via gamma irradiation under similar experimental conditions are presented in Fig. 4.3. The saturation dose was determined to be 1.7 kGy, which is close to that obtained in X-ray irradiation method. The yield of the Ag NPs, manifested by the intensity of characteristic LSPR band of Ag NPs, increased with increase in absorbed radiation dose till all precursor Ag\textsuperscript{I} ions were exhausted. However, the LSPR peak in this case was observed to be narrower with $\gamma_{\text{max}}$ at \(~401$nm. This indicated formation of smaller sized particles with narrow size distribution in case of $\gamma$-radiolysis, compared to those obtained by X-ray radiolysis. This observation was further substantiated by the TEM analysis of Ag NPs synthesized by $\gamma$-irradiation. Fig. 4.4 shows the TEM image of the freshly prepared PVP-capped-Ag nanoparticles. Ag NPs formed in PVP are mainly spherical in nature and exhibit narrow size distribution with average particle size in the range of 8-10 nm. Particle size analysis also revealed the particles obtained by radiolytic process to be of uniform size distribution with average hydrodynamic diameter of around 11nm. Fig. 4.16a represents the AFM image of the PVP-capped-Ag nanoparticles, which also verifies the spherical nature of the nanoparticles generated. The results obtained in case of EB irradiation method were observed to be similar to those obtained in the case of $\gamma$-irradiation.

The smaller size of Ag NPs obtained via gamma irradiation method can be attributed to the isotropic irradiation offered by the cylindrical design of the gamma chamber, which leads to uniform irradiation of the precursor solution. This results in the formation of a large number of seeds at any given instant, thereby limiting the precursor ion supply and subsequently restricting the growth of the nuclei to yield small, uniform sized particles. A similar trend is observed in case of EB irradiation technique. However, in the case of X-ray irradiation, the linear geometry of the beam and its restricted size leads to non homogeneous
energy deposition along the beam-solution interception. This culminates in an inhomogeneous rate of precursor ion reduction along the beam. Thus, the quantity of seeds formed in this irradiation method is substantially lower, resulting in the formation of larger sized particles.

**Fig. 4.1** Absorption spectra of aqueous Ag nanoparticles in PVP prepared by X-ray irradiation for different irradiation time: (a) 12s, (b) 23sec, (c) 41s, (d) 63s; $[\text{Ag}^+]$=2X10$^{-4}$ mol.dm$^{-3}$, $[\text{PVP}]$= 0.5%, $[\text{Isopropanol}]$=2X10$^{-1}$ mol.dm$^{-3}$, dose rate= 113.7kGy.h$^{-1}$.

**Fig. 4.2** TEM image of Ag nanoparticles in PVP prepared by X-ray irradiation
**Fig. 4.3** Absorption spectra of aqueous Ag nanoparticles in PVP prepared by γ-irradiation for different absorption dose: (a) 1kGy, (b) 1.3kGy, (c) 1.6kGy; [Ag$^+$]=2X10$^{-4}$ mol.dm$^{-3}$, [PVP]= 0.5%, [Isopropanol]=2X10$^{-1}$ mol.dm$^{-3}$, dose rate= 4.0kGy.h$^{-1}$

**Fig. 4.4** TEM image of Ag nanoparticles in PVP prepared by γ-irradiation
4.2.2. Effect of variation of precursor ion concentration

Fig. 4.5, Fig. 4.6 and Fig. 4.7 present the UV-visible spectra of Ag NPs, synthesized by X-ray, γ-ray and EB irradiation methods respectively, for Ag⁺ concentration variation within the range of 1X10⁻⁴ mol.dm⁻³ to 4X10⁻⁴ mol.dm⁻³. The concentrations of all other reactants were kept undisturbed. The λ_max was observed to be red shifted with increase in Ag⁺ concentration in case of X-ray (Fig. 4.5) and γ-irradiation (Fig. 4.6) methods, whereas it underwent a blue shift in case of EB-irradiation method (Fig. 4.7). This implied that the Ag NPs size increased with increase in Ag⁺ concentration in case of X-ray and γ-irradiation, whereas it decreased in the case of EB irradiation. The increase in particle size at higher precursor concentration has been reported earlier. However, decrease in size in case of EB-irradiation method can be explained on the basis of the very high dose rates delivered by E-beam. This results in the formation of a large number of nuclei within a short time span. With limited precursor ion concentration, the size of the clusters formed also gets restricted, resulting in the formation of smaller sized particles.

Fig. 4.5 Absorption spectra of aqueous Ag nanoparticles in PVP prepared by X-ray irradiation for different Ag⁺ concentration: (a) 1X10⁻⁴ mol.dm⁻³, (b) 2X10⁻⁴ mol.dm⁻³, (c) 3X10⁻⁴ mol.dm⁻³, (d) 4X10⁻⁴ mol.dm⁻³; dose = 1.0kGy, 2.0kGy, 3.0kGy, 4.0kGy respectively; [PVP]= 0.5%, [Isopropanol]=2X10⁻¹ mol.dm⁻³, dose rate= 113.7kGy.h⁻¹
Fig. 4.6 Absorption spectra of aqueous Ag nanoparticles in PVP prepared by $\gamma$-irradiation for different Ag$^+$ concentration: (a) $1 \times 10^{-4}$ mol.dm$^{-3}$, (b) $2 \times 10^{-4}$ mol.dm$^{-3}$, (c) $3 \times 10^{-4}$ mol.dm$^{-3}$, (d) $4 \times 10^{-4}$ mol.dm$^{-3}$; dose=1.0kGy, 2.0kGy, 3.0kGy, 4.0kGy respectively; [PVP]= 0.5%, [Isopropanol]=2X10$^{-1}$ mol.dm$^{-3}$, dose rate= 4.0kGy.h$^{-1}$

Fig. 4.7 Absorption spectra of aqueous Ag nanoparticles in PVP prepared by EB- irradiation for different Ag$^+$ concentration: (a) $1 \times 10^{-4}$ mol.dm$^{-3}$, (b) $2 \times 10^{-4}$ mol.dm$^{-3}$, (c) $3 \times 10^{-4}$ mol.dm$^{-3}$, (d) $4 \times 10^{-4}$ mol.dm$^{-3}$; dose=1.0kGy, 2.0kGy, 3.0kGy, 4.0kGy respectively; [PVP]= 0.5%, [Isopropanol]=2X10$^{-1}$ mol.dm$^{-3}$, dose rate=2.0 kGy/pass
4.2.3. Effect of PVP concentration

In order to investigate the role of PVP in stabilization of Ag NPs, the Ag NPs were synthesized with different concentrations of PVP as stabilizers. The PVP concentration was varied from 0.1% to 2% (w/v) in X-ray, γ-ray and EB irradiation methods, results are shown in Fig. 4.8, Fig. 4.9 and Fig. 4.10 respectively. The concentrations of all other reactants were kept identical. There was no appreciable shift observed in the $\lambda_{\text{max}}$ position with increase in PVP concentration in case of X-ray (Fig. 4.8) and γ-ray irradiation methods (Fig. 4.9), whereas it was red shifted in EB-irradiation method (Fig. 4.10). This inferred a marginal increase in average particle size at higher PVP concentrations in case of EB-irradiation method. However, in all the three cases, the FWHM decreased with increase in PVP concentration, which implied formation of Ag NPs with narrow size distribution at higher PVP concentrations in all three cases.

![Absorption spectra of aqueous Ag nanoparticles in PVP prepared by X-ray irradiation for different PVP concentration: (a) 0.1%, (b) 0.5%, (c) 1%, (d) 2% (w/v); $[\text{Ag}^+]$=2X10$^{-4}$ mol. dm$^{-3}$, [Isopropanol]= 2X10$^{-1}$ mol.dm$^{-3}$, dose= 2.0kGy, dose rate= 113.7kGy.h$^{-1}$](image)

**Fig. 4.8** Absorption spectra of aqueous Ag nanoparticles in PVP prepared by X-ray irradiation for different PVP concentration: (a) 0.1%, (b) 0.5%, (c) 1%, (d) 2% (w/v); $[\text{Ag}^+]$=2X10$^{-4}$ mol. dm$^{-3}$, [Isopropanol]= 2X10$^{-1}$ mol.dm$^{-3}$, dose= 2.0kGy, dose rate= 113.7kGy.h$^{-1}$
**Fig. 4.9** Absorption spectra of aqueous Ag nanoparticles in PVP prepared by γ-irradiation for different PVP concentration: (a) 0.1%, (b) 0.5%, (c) 1%, (d) 2% (w/v); [Ag⁺]=2X10⁻⁴ mol.dm⁻³, [Isopropanol]= 2X10⁻¹ mol.dm⁻³, dose=2.0kGy, dose rate= 4.0kGy.h⁻¹

**Fig. 4.10** Absorption spectra of aqueous Ag nanoparticles in PVP prepared by EB-irradiation for different PVP concentration: (a) 0.1%, (b) 0.5%, (c) 1%, (d) 2% (w/v); [Ag⁺]=2X10⁻⁴ mol.dm⁻³, [Isopropanol]= 2X10⁻¹ mol.dm⁻³, dose=2.0kGy, dose rate= 2.0 kGy/pass
4.2.4. Effect of dose rate on nanoparticle formation in EB-irradiation

The effect of variation of dose rate on the NPs morphology was studied in case of EB-irradiation method by irradiating the precursor solution under two dose rate conditions, i.e., 2.0 kGy/pass and 1.0 kGy/pass. The absorption spectra of Ag NPs solution at the two dose rates are shown in Fig. 4.11. There was no shift in peak position with change in dose rate. In other words, the size of NPs produced was independent of the dose rate in case of EB-irradiation method under high dose rate conditions.

**Fig. 4.11** Absorption spectra of aqueous Ag nanoparticles in PVP prepared by EB-irradiation for different absorption dose rates: (a) 2.0 kGy/pass, (b) 1.0 kGy/pass; $[\text{Ag}^+] = 2 \times 10^{-4}$ mol.dm$^{-3}$, [PVP] = 0.5%, [Isopropanol] = 2X10$^{-1}$ mol.dm$^{-3}$

4.2.5. Estimation of uric acid

Uric acid undergoes enzymatic degradation in presence of enzyme Uricase under optimum assay conditions of 37$^\circ$C and pH 7.4 (Fig. 4.12). This pH is also relevant because it represents the physiological pH of biological fluids, i.e., urine or blood serum. Hydrogen
peroxide is generated as one of the reaction products, which is known to be a strong oxidizing agent. This, in turn, causes oxidation of silver nanoparticles resulting in a decrease in intensity of the LSPR band.

\[
\text{H}_{\text{2}O_2} + \text{O}_2 \xrightarrow{\text{Uricase}} \text{H}_{\text{2}}\text{O}_2 + \text{CO}_2 + \text{H}_2\text{O}_2
\]

**Fig. 4.12** Enzymatic degradation of uric acid in presence of Uricase.

Fig. 4.13 shows the changes in the LSPR optical characteristics of Silver nanoparticles with time due to introduction of uric acid and uricase into the system. It was observed that with increasing time, the bright yellow color of silver nanoparticles gradually faded and the solution turned almost transparent. The relationship between absorbance strength change and time is illustrated in fig. 4.13 inset. In order to confirm that the LSPR absorbance change was caused only in the presence of uric acid, phosphate buffer and deionized water alone were separately introduced into the nanoparticle solution and the spectra monitored with time; no spectral change was observed in either case.
For the evaluation of Ag-PVP solution as a uric acid biosensor, different concentrations of uric acid (10 to 100 µM) were taken in 25mL conical flasks and 0.2 mL of 0.137mg/mL Uricase stock solution was added to each of them. The resultant mixtures were diluted to 6.25mL with 20mM phosphate buffer and incubated at 37°C for 20 minutes. The course of the enzymatic reaction was followed by monitoring the absorbance peak of uric acid at 293nm, whose intensity decreases with time (Fig. 4.14). Subsequently, 3.75mL of $4 \times 10^{-4}$ mol dm$^{-3}$ Ag nanoparticle solution (final concentration being $1.5 \times 10^{-4}$ mol dm$^{-3}$) was added to each of the flasks and the mixtures allowed to stand at room temperature for another 60 minutes. The UV-Visible spectra were recorded thereafter in the wavelength range 250-650nm.

![Absorption spectra of 150 µM Ag nanoparticle solution in presence of 50µM uric acid after (a) 0 min, (b) 5 min, (c) 20 min, (d) 45 min, (e) 60 min. Inset: absorbance strength of Ag nanoparticles solution containing uric acid as a function of time.](image)

Fig. 4.13 Absorption spectra of 150 µM Ag nanoparticle solution in presence of 50µM uric acid after (a) 0 min, (b) 5 min, (c) 20 min, (d) 45 min, (e) 60 min. Inset: absorbance strength of Ag nanoparticles solution containing uric acid as a function of time.
Fig. 4.14 Absorption spectra of uric acid solution in presence of Uricase after (a) 0 min, (b) 5 min, (c) 10 min, (d) 15 min, (e) 20 min

Fig. 4.15 shows the variation in UV-Visible spectra of the silver nanoparticles with increasing concentration of uric acid. It was observed that intensity of the LSPR band decreases gradually with increase in uric acid concentration. The decrease was accompanied by a slight red shift in the absorption maxima of the band, which is attributed to the partial oxidation of nano-Ag [183-186]. The shift in $\lambda_{\text{max}}$ might also be due to the slight aggregation caused by the destruction of the PVP shell stabilizing the nanoparticles, followed by decrease in the distance between the nanoparticles [187]. This is evident from the TEM images of Ag-NPs recorded before and after addition of uric acid (fig. 4.16a and 4.16d). While the control (Ag-NPs) exhibited an average particle size of 5.1 ± 1.6 nm, those in presence of uric acid were found to have bigger particle size of 15.4 ± 4.6 nm. Particle size analyzer data also reveals an increase in average hydrodynamic diameter of the Ag nanoparticles after addition of 20µM uric acid in presence of uricase. AFM analysis was also carried out to study the effect of addition of uric acid-uricase system to the PVP-Ag-NPs. Fig. 4.17a and 4.17b present the AFM images of PVP-Ag-NPs before and after addition of uric acid-uricase.
system. Introduction of uric acid and subsequent generation of hydrogen peroxide in the medium resulted in partial decomposition of the polymer layer leading to the aggregation of the Ag colloids, which is evident from bigger particle size and the blurring of the boundaries existing between the individual particles.

Fig. 4.15 Absorption spectra of Ag nanoparticle solution (PVP, Mol wt.= 40kD) in presence of different concentration of uric acid, after 60 min: (a) 0 µM, (b) 10 µM, (c) 20 µM, (d) 30 µM, (e) 40 µM, (f) 50 µM. Inset: Linear plot of absorbance of Ag nanoparticle vs uric acid concentration ($R^2=0.9964$), uric acid concentration range= 0 to $5\times10^{-5}$ mol.dm$^{-3}$. 
Fig. 4.16 TEM micrograph of Ag nanoparticles prepared using PVP of molecular weight 40kD (a) before addition of uric acid (b) after addition of uric acid
Fig. 4.17 AFM images of Ag nanoparticles prepared using PVP (Mol wt.= 40kD) (a) before addition of uric acid (b) after addition of uric acid

The PVP-Ag-NPs were further characterized by zeta potential measurement. The magnitude of the zeta potential gives an indication of the stability of the nanoparticle suspension system. If all the particles in suspension have a large negative or positive zeta potential, they tend to repel each other and there will be less tendency for the particles to come together to form agglomerates. Therefore, the decrease in the magnitude of zeta potential indicates the tendency of the particles to form agglomerates [188]. In addition, zeta potential also indicates the presence of an oxidized surface layer. Zeta potential measurements for the samples were therefore carried out to further confirm the partial oxidation of the silver nanoparticles by in situ generated hydrogen peroxide. The zeta potential for the control, i.e., PVP-Ag-NPs solution was found to be $-29.6$ mV at pH = 7.4 (Fig. 4.18 inset). However, with increase in uric acid concentration, the zeta potential values were found to become less negative. This is probably due to partial neutralization of the negative charge by Ag$^+$ ions generated via partial oxidation of Ag NP caused by H$_2$O$_2$ generated in situ in uric acid–uricase reaction system (Fig. 4.18). It has been well established that electrostatic stabilization of nanoparticles would typically require a zeta potential above
30 mV or below $\sim$30 mV [189]. Therefore, in the present study the values of zeta potentials suggested that the stability of the PVP-Ag-NPs suspension was based on steric stabilization by the PVP polymer. Unlike electrostatic stabilization, steric stabilization with nonionic polymers is independent of pH and electrolyte concentration. Accordingly, steric stabilization is useful for prevention of agglomeration of nanoparticles in physiological media.

**Fig. 4.18** Zeta potential of Ag nanoparticle as a function of uric acid. Inset: Zeta potential curve for Ag nanoparticle solution without uric acid.

### 4.2.6. Generation of calibration curve

The calibration curve for estimation of uric acid by PVP-Ag-NPs sensor system was established by plotting the absorbance (OD) of SPR band of Ag nanoparticles as a function of uric acid concentration (Fig. 4.15 inset). The response was found to be linear ($R^2 = 0.9964$) in the concentration range of 0–50$\mu$M uric acid and can be represented by Eq. (5)

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\[
\text{Absorbance} = \text{Constant} \times [\text{Uric Acid}]^{\text{power}}
\]

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$\sim \Omega \Omega \sim$
\[ \text{OD} = 1.480 - 0.012[\text{uric acid}] \] (5)

The detection limit of the system was found to be 5µM of uric acid. The calibration curve was further used for the determination of unknown concentrations of uric acid in bovine and human serum samples.

4.2.7. Estimation of uric acid in bovine and human serum samples

The concentration of uric acid in serum samples is normally found in the micromolar range. Therefore, the method described earlier was effectively applied for determination of uric acid concentration in serum samples. To minimize interference from proteins present in the serum sample, 5mL of bovine serum sample was initially heated at 90°C to initiate denaturation of the proteins. After cooling down to room temperature, the resultant mixture was centrifuged at 4000rpm for 5 minutes to separate denatured proteins. The supernatant was removed and filtered using a 45micron syringe filter. The volume of the filtrate was made up to the original volume using 20mM phosphate buffer and divided into two parts of 1 mL each. The first part of the filtrate was subjected to the uric acid estimation protocol using PVP-Ag biosensor system, as described earlier. The second part of the filtrate was diluted ten times and its UV-Visible spectrum was recorded for background correction of the SPR band of the sample. The same procedure was adopted in case of bovine serum sample. The concentrations of uric acid in both human and bovine serum samples were estimated from the calibration plot (Fig. 4.15 inset). The interference of ions, such as chlorides, sulphates, chlorides and sulphates in the uric acid estimation process may be ignored in this case where the silver nanoparticles have been sterically stabilized using PVP, as the effect of the ions on the LSPR band of Ag-NPs becomes prominent only in case of electrostatically stabilized systems, such as citrate stabilized Ag nanoparticles [190,191]. For comparison purpose, a commercial colorimetric uric acid assay kit (Quantichrom Uric Acid Assay Kit-DIUA-250) was used to estimate the uric acid in the samples under similar reaction
conditions. Table 4.1 presents the results obtained from the proposed PVP-Ag-NPs biosensor and those obtained from the comparison method for three different human serum samples (HS-1, HS-2 and HS-3). A one sample T-test was performed to test the null hypothesis and the results are presented in Table 4.1. The absolute values of the estimated t were lower than the critical t value (α = 0.05, df = 4) of 2.776, which suggested that the results obtained using the pro-posed method, were not significantly different from those obtained by the comparison procedure at a 95% confidence level. A one-way ANOVA F-test was also performed on the results obtained from proposed method and comparison method for three human serum samples in order to check the null hypothesis (Table 4.1). In this case, F_{crit}(1,4) = 7.71 at α = 0.05. The p and F values obtained from the ANOVA F-test were 0.97 and 0.001, respectively. Since p > , and F < F_{crit}, it can be concluded that the uric acid concentration estimated by our proposed method is not significantly different from that obtained with the comparison method at 95% level of significance. The concentration of uric acid in bovine serum sample was estimated to be 181 ± 3.7µM (average ± standard deviation) by using the proposed method. This was also found to be in reasonably good agreement with the measured value of 190µM determined using the commercial kit method.

Table 4.1 Determination of uric acid in human serum samples using proposed method and comparison method; results of one sample T-test and one way ANOVA F-test.

<table>
<thead>
<tr>
<th>Sample (Human serum)</th>
<th>Uric acid concentration (µM)</th>
<th>One sample t-test</th>
<th>One way ANOVA F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Other Method(^a)</td>
<td>Proposed method(^b)</td>
<td>t(α=0.05, n=5)</td>
</tr>
<tr>
<td>HS-1</td>
<td>185</td>
<td>180±4.4</td>
<td>-2.54</td>
</tr>
<tr>
<td>HS-2</td>
<td>153</td>
<td>149±4.1</td>
<td>-2.18</td>
</tr>
<tr>
<td>HS-3</td>
<td>243</td>
<td>248±4.9</td>
<td>+2.28</td>
</tr>
</tbody>
</table>

\(^a\) Other method: QuantiChrom Uric Acid Assay Kit (DIUA-250)

\(^b\) Average ± standard deviation (for 5 determinations)
4.2.8. Effect of PVP molecular weight on sensor properties: Detection principle

The experiments discussed earlier had been carried out using silver nanoparticles synthesized using PVP of molecular weight 40kD as the capping agent. In order to study the effect of PVP molecular weight on the sensor properties of Ag nanoparticles, similar experiments were conducted using PVP of molecular weights 160kD and 360kD. For higher molecular weight PVPs, decrease in absorbance with increase in uric acid concentration was found to be very low for low uric acid concentrations, when the spectra were recorded after 60 min (Fig. 4.19). However, on recording the spectra again after 8 h, significant decrease in absorbance was observed for the system where PVP of molecular weight 160 kD was used as the capping agent (Fig. 4.20); though no change in spectral characteristics was observed after 8 h in case of PVP-Ag system with PVP of molecular weight 360 kD as the capping agent (data not shown). The calibration plots for PVP-Ag sensor systems with higher molecular weight PVP were found to exhibit poor linearity as compared to that with PVP of molecular weight 40 kD (Fig. 4.17 inset, Fig. 4.19 inset). $R^2$ values were found to be 0.9964, 0.8826 and 0.7730 for PVP with molecular weights 40 kD, 160 kD and 360 kD, respectively. This implies that the oxidation and degradation of silver nanoparticles became progressively slower when PVP of higher molecular weights was used as capping agents. High molecular weight PVPs (160 kD and 360 kD) have longer polymeric chains, which wrap around the Ag clusters and effectively shield them from external oxidizing agents. However, lower molecular weight PVP (40 kDa) has comparatively shorter polymeric chains and hence its ability to stabilize Ag clusters is also less. Therefore, Ag in such cases is more accessible to hydrogen peroxide, which causes significant oxidation of the Ag atoms present on the surface of the clusters, resulting in a decrease in the absorbance of the SPR band.
Fig. 4.19 Absorption spectra of Ag nanoparticle solution (PVP, Mol wt.= 160kD) in presence of different concentration of uric acid, after 60 min: (a) 0 µM, (b) 10 µM, (c) 20 µM, (d) 30 µM, (e) 40 µM, (f) 50 µM. Inset: Linear plot of absorbance of Ag nanoparticle vs uric acid concentration ($R^2=0.8826$), uric acid concentration range= 0 to $5\times 10^{-5}$ mol.dm$^{-3}$.

Fig. 4.20 Absorption spectra of Ag nanoparticle solution (PVP, Mol wt.= 160kD) in presence of different concentration of uric acid, after 8 hrs: (a) 0 µM, (b) 10 µM, (c) 20 µM, (d) 30 µM, (e) 40 µM, (f) 50 µM. Inset: Linear plot of absorbance of Ag nanoparticle vs uric acid concentration ($R^2=0.9679$), uric acid concentration range= 0 to $5\times 10^{-5}$ mol.dm$^{-3}$.
4.3. Conclusions

The application of X-ray irradiation induced reduction of aqueous metal ions was explored as a probable tool for fabrication of metal nanoparticles in addition to gamma and E-beam irradiation techniques. A comparative study of the three techniques revealed a more poly-dispersed particle size distribution for Ag NPs synthesized using X-ray irradiation compared to those obtained using gamma ray irradiation and EB-irradiation methods. Parameters such as precursor ion concentration, stabilizer molecular weight and concentration were found to play a crucial role in deciding the morphology of the particles formed. A novel biosensor was subsequently designed using PVP-Ag-NPs synthesized via gamma radiolytic method, and demonstrated for estimation of uric acid in biological samples. No external reducing agent has been used in this method. A colorimetric method based on oxidation of PVP stabilized Ag nanoparticles by in situ generated hydrogen peroxide has been developed for estimation of uric acid. Linear range of detection of uric acid by this technique was found to be 0 to $5 \times 10^{-5}$ mol.dm$^{-3}$ with minimum detection limit of $5 \times 10^{-6}$ mol.dm$^{-3}$. The molecular weight of the capping agent PVP was found to play a crucial role in the efficacy of the PVP-Ag-NPs system as a sensor for uric acid estimation; lower the molecular weight of PVP, faster and better the response towards sensing the uric acid. Further, this method was also effectively employed to determine concentration of uric acid in actual biological fluids, namely bovine and human serum samples. T-tests and F-test carried out on the analysis results demonstrated that the proposed method offers reasonably good accuracy and precision in case of real samples. The PVP-Ag-NPs system was also observed to possess a shelf life of more than six months when stored at 4°C. This novel detection method using nanomaterials has potential applications in medical and environmental monitoring as a simplified, low cost biosensor for uric acid estimation in the micro-molar range.