CHAPTER - 5

5(A). An Investigation on Fragmentation of Supported Au NPs@Agarose Film by Thiols and the Role of their Synergy in Catalysis of p-Nitrophenol Reduction

5(B). A Property Study on Storage/Release of Silver nanoparticles in Chitosan-Silver Nanocomposite leading to Successful Catalytic Applications
The fate and pre-conceptualised nature of gold nanoparticles (Au NPs) took a new turn after the report of thiol-derivatised gold nanoparticles by Brust et al.\textsuperscript{1} in 1994. They introduced a two-phase liquid-liquid system for the preparation of Au NPs attached with thiol self-assembled monolayers behaving unusually as a simple chemical compound with moderate polydispersity. Consequentially, thiol-capped Au NPs have been extensively studied over the two decades reporting a various number of papers establishing the role of thiols in stabilizing as well as regulating the size of Au NPs in a solution.\textsuperscript{2-9} Due to the persistence of aggregation\textsuperscript{10-13} related problems, scientists next reported a photophysical fragmentation of these thiol-capped Au NPs induced by a laser.\textsuperscript{10} It is believed that this non-conventional perspective of Au NPs fragmentation by thiols is due to the photo-ejection of electrons, which is followed by charging of the nanoparticle surface. Novo et al.\textsuperscript{14} in a report showed charge induced fragmentation of gold nanorods by sodium borohydride. As a follow-up, Wang et al.\textsuperscript{15} showed fragmentation of Au NPs by the cysteine biomolecule. They demonstrated that by mixing gold chloride with cysteine and excess of sodium borohydride, Au NPs of 3-5 nm size are first obtained, which were then converted into ultrasmall nanoparticles sized <1.5 nm after 6 h of aging at room temperature. In other reports, Riaz et al. demonstrated thiol-ubiquinone induced fragmentation,\textsuperscript{16} and thioanisole induced size-dependent fragmentation of Au NPs.\textsuperscript{17} The role of alkanethiol fragmented Au NPs in controlling the fluorescence properties of Au NPs for the sensitive and selective
detection of Hg(II) was shown by Huang et al.\textsuperscript{18} and Ke et al.\textsuperscript{19} operated the core etching of lysozyme type VI (Lys VI)-stabilized luminescent Au\textsubscript{8} clusters using thiol. However, the feasibility of such non-conventional thiol-mediated fragmentation of Au NPs when supported on a solid platform is still an unexplored area of research.

### 5.1. The Objective: To Investigate the Fragmentation of Supported Gold Nanoparticles by Selective Thiols and its Applicability

In this chapter, we investigated the feasible role of thiols in fragmenting Au NPs supported on an agarose (Agr) hydrogel film. Addressing within the limits of our knowledge, it is the first report to show the non-conventional thiol-mediated fragmentation of Au NPs studded into an Agr hydrogel film solid support as a hybrid material. The Au NPs, unlike other reported methods,\textsuperscript{20-23} were generated inside the agarose hydrogel film through adsorption of 5 mM tetrachloroauration salt (HAuCl\textsubscript{4}) solution by a preformed Agr hydrogel film, which was subsequently reduced by only 10 min exposure to 365 and 254 nm wavelengths UV radiation. The role of agarose hydrogel as a solid support in facile and quick generation of Au NPs was also considered. This hybrid Au@Agr film was then investigated to study the feasibility of such a system to undergo the recently popularized non-conventional fragmentation of Au NPs by a group of thiols (viz., thioglycolic acid (TGA), cysteine (CS), 2-mercaptoethanol (ME), L-methionine (MET), thiourea (TU), and 1,3-propanedithiol (PDT)). The surface plasmon resonance (SPR) peak of Au NPs detected by UV-visible spectrometry strongly depends on its particle size, and any change in the size of Au NPs is reflected by a shift in the SPR peak wavelength either towards lower (blue shift) or higher (red shift) wavelength values.\textsuperscript{24,25} Accordingly, to study the fragmentation of
Au@Agr by thiols such a blue/red shift in the SPR peak of Au NPs was closely monitored by UV-visible spectrometry. Among all the six thiols, TGA, CS, ME and MET were found to be capable of showing fragmentation of Au@Agr displaying a considerable 6 to 11 nm blue shift in the SPR peak of Au@Agr. Other thiols of the group, i.e., TU and PDT, did not show any sign of fragmentation, instead they have been found to cause red shifts in the SPR peak of Au NPs.

The application of these films in rapid catalysis of an industrially important reduction reaction of p-nitrophenol (p-NP) to p-aminophenol (p-AP) is also demonstrated in the work. p-NP is a known pollutant which is very noxious and sluggish while p-AP is of great importance serving as an intermediate for the synthesis of many analgesic, antipyretic drugs, and polymers. Moreover, p-NP reduction to p-AP is one of the most ideal reactions studied for establishing catalytic efficiency of a catalyst due to its easy mode of monitoring with UV-visible spectroscopy and occurrence of no side reactions. The films (especially, TGA-Au@Agr & CS-Au@Agr) were successfully used as catalysts in the reduction of p-NP to p-AP in the presence of excess sodium borohydride (NaBH₄). The calculated rate constant was found to be 1.6 x 10⁻¹ s⁻¹ and 1.1 x 10⁻¹ s⁻¹ for TGA-Au@Agr and CS-Au@Agr catalysed reduction reactions, respectively, following pseudo first-order kinetics. This rate constant value of the order 10⁻¹ s⁻¹ is one of the fastest catalytic rates observed for any solid supported catalyst, to the best of our knowledge. A thriving synergism between TGA or CS and Au@Agr stimulating a smooth relay of electrons from borohydride ions (BH₄⁻) to p-NP could be credited for such a rapid catalysis shown by TGA-Au@Agr and CS-Au@Agr films.

Hence, this work is an endeavour to investigate and establish an intriguing feasibility for the non-conventional behaviour of a group of thiols to fragment Au NPs supported
in a solid Agr hydrogel film in the absence of any auxiliary applied energy. The study also explored the synergy functioning inside the film to make it an efficient catalytic material for reduction of p-NP to p-AP.

5.2. Materials
Agarose low gelling temperature (SRL, India), tetrachloroauric acid (Sigma Aldrich), thioglycolic acid (Sigma Aldrich), L-cysteine hydrochloride monohydrate (Merck), L-methionine (Sigma Aldrich), 2-mercaptoethanol (Himedia), thiourea (Merck), 1,3-propanedithiol (Sigma Aldrich), p-nitrophenol (Loba Chemie), and sodium borohydride (Merck) were used as received. All other reagents used were of analytical grade.

5.3. Experimental
5.3.1. Methodical step-by-step protocol to investigate thiol-mediated fragmentation of gold nanoparticles (Au NPs) studded into agarose hydrogel film
5.3.1.1. Agarose hydrogel film (Agr). Agarose hydrogel of 2.5% (w/v) was prepared following the conventional method of mixing 0.25 g agarose in 10 mL of Milli Q water and allowing microwave dissolution for 25 s. The film casting of the microwaved solution was done by pouring it into a petri dish (10 cm diameter) and cooling to room temperature, which was then kept for 4 hours (at room temperature) to obtain a thin Agr hydrogel film.$^{38}$
5.3.1.2. Au NPs studded agarose hydrogel film (Au@Agr). To the petri dish containing the prepared Agr hydrogel film, 10 mL of 5 mM HAuCl$_4$ solution was poured and kept as such for 1 h 15 min. After this, the HAuCl$_4$ solution was poured out from the petri
dish, and the film was washed thoroughly with plenty of Milli-Q water to remove any unreacted HAuCl₄, if present. Then, the HAuCl₄ treated Agr film was exposed to 365 nm and 254 nm wavelength UV light for 10 min. The ruby red coloration of the Agr film after UV exposure indicated the formation of Au nanoparticles studded into the agarose hydrogel film, i.e., Au@Agr.

Scheme 5.1 Schematic of the preparation of gold nanoparticles studded into an agarose film (Au@Agr) and the subsequent fragmentation of Au@Agr mediated by a group of thiols. Schematic of each film is also supported by the image of the original films prepared in the laboratory.

5.3.1.3. Thioglycolic acid (TGA) mediated fragmentation of Au nanoparticles studded into Agarose film (TGA-Au@Agr). Au@Agr film was treated with thioglycolic acid (TGA) to investigate any fragmentation of Au NPs studded inside the Agr film. 2% (v/v) of TGA in Milli-Q water was prepared, 10 mL of which was poured into a petri dish containing the Au NPs studded agarose (Au@Agr) film and kept for 1 h at room temperature. The 2% TGA-Au@Agr film was then investigated with UV-visible analysis to ensure any fragmentation of Au NPs by TGA. The UV-visible spectrum was taken in reflectance mode, which was then converted instrumentally to absorbance through the Kubelka–Munk equation. Likewise, cysteine (CS), 2-mercaptoethanol (ME), L-methionine (MET), thiourea (TU), and 1,3-propanedithiol (PDT) were also used to prepare other thiol fragmented Au@Agr films, and named as 2%CS-Au@Agr, 2%ME-
Au@Agr, 2%MET-Au@Agr, 2%TU-Au@Agr, and 2%PDTAu@Agr, respectively. The complete step-by-step methodical formulation of thiol-Au@Agr film is presented in a pictorial form in Scheme 5.1. The images of Agr, Au@Agr, and thiol-Au@Agr films prepared in the laboratory are also included in Scheme 5.1.

5.3.2. Investigation of thiol-Au@Agr film as a catalyst for p-NP reduction

All the thiol-Au@Agr films were investigated for catalytic reduction of p-nitrophenol (p-NP) to p-aminophenol (p-AP) in the presence of sodium borohydride (NaBH$_4$). For the process, 20 mL of 1 mM p-NP was mixed with 100 mL of 5 mM NaBH$_4$ solution. The resulting solution showing $\lambda_{\text{max}}$ at 400 nm due to the formation of p-nitrophenolate ion (p-NP(r)) was then monitored for the complete catalysis study. A small piece of thiol-Au@Agr film (1x1 cm, thickness~0.2 mm) was immersed in 2 mL of p-NP(r) followed by easy stirring, and then the UV-visible spectrum of the p-NP(r) monitoring its 400 nm peak was taken in regular interval of time, viz., 2.5 s, 5 s, 10 s, 15 s up to 60 s.

5.4. Characterization

The hydrogel films were characterized by UV-visible spectroscopy (Shimadzu-UV 2600) to study changes in the absorbance pattern of Agr, Au@Agr and thiol-Au@Agr films (each films strip used sized 1x1 cm, thickness~0.2 mm). The spectra were first obtained in the reflectance mode in the 800-190 nm range, which were then converted into their absorbance values instrumentally through the Kubelka-Munk equation. The surface morphology and fragmentation of Au@Agr were investigated using a scanning electron microscope (SEM) from Carl Zeiss (Sigma VP). The energy dispersive X-ray spectroscopy (EDX) connected to SEM was used to ascertain the elements present in all
the thiol-Au@Agr films. For the SEM and EDX analysis, a 1x1 cm strip was placed on a stub, dried at 50 °C for 1 h and used for imaging. The particle size distribution analyses of freshly prepared Au NPs solution and all thiol-Au solutions were done with a Malvern Zetasizer NanoZS 90.

5.5. Results and Discussion

5.5.1. Fragmentation of Au@Agr Films by Selective Thiols

An investigation of the proficiency of a group of thiols to mediate a non-conventional fragmentation of Au NPs, which were studded into agarose hydrogel films, is presented here. The peculiar role of agarose in assisting a facile fragmentation of Au NPs and the candidature of such a films in rapid reduction catalysis of p-nitrophenol (p-NP) is also demonstrated. For the study, Au NPs were first studded into the Agr films, as detailed in the experimental section (Scheme 5.1). Briefly, a 2.5% (w/v) Agr films was treated with 10 mL of 5 mM HAuCl₄ solution, and then Au³⁺ was reduced to Au⁰ by exposing the films to 365 and 254 nm wavelength UV radiation for only 10 min. The completion of the Au³⁺→Au⁰ reduction process was indicated by ruby red coloration of the Agr film resulting in the formation of an Au NPs studded Agr film (Au@Agr). The formation of the Au@Agr film was experimentally proved by UV-visible spectrophotometry analysis. In the UV-vis spectrum of Au@Agr film, a sharp peak at a wavelength of 540 nm was distinguishably observed suggesting the successful formation of Au NPs of size 40-60 nm with UV exposure of Au³⁺ loaded Agr (Figure 5.1 (A)). A distinct variance in the spectral patterns of the Agr and Au@Agr films were also observed through UV-visible analysis. Furthermore, the actual loading of Au³⁺ into the Agr film was checked and found to be 45% (Figure 5.1 (B)). An excess loading of Au³⁺ above 45% resulted in
the formation of aggregated Au NPs of sized >60 nm when subjected to UV treatment. Therefore, this loading capacity was taken as the standard for the complete study.

Figure 5.1 (A) The UV-visible spectrum of agarose (Agr) and Au@agarose (Au@Agr) hydrogel film, and (B) The UV-Visible analysis to determine the loading of Au$^{3+}$ into the agarose film.

Figure 5.2 The UV-visible spectra showing the blue/red shift in SPR peak of Au NPs studded into agarose film (Au@Agr). (A) 2%TGA-Au@Agr, (B) 2%CS-Au@Agr, (C) 2%ME-Au@Agr, (D) 2%TU-Au@Agr, (E) 2%PDT-Au@Agr, and (F) 2%MET-Au@Agr.

After ascertaining the successful formation of Au NPs studded into the Agr film (Au@Agr) through UV-visible analysis, the feasibility of interaction of thiols towards such Agr supported Au NPs (Au@Agr) was studied. A group of thiols selected for the study included thioglycolic acid (TGA), cysteine (CS), 2-mercaptoethanol (ME), L-methionine (MET), thiourea (TU), and 1,3- propanedithiol (PDT). Each thiol was allowed to interact with Au@Agr film for 1 h at room temperature after which the film
was analysed by UV-visible spectroscopy to check any perceivable changes in the spectral pattern of Au@Agr. Interestingly, it was observed that after interaction with thiol, the Au@Agr film showed a blue shift in the SPR peak of Au NPs observed earlier in its UV-visible spectrum. However, such kind of blue shift was found only in case of Au@Agr treated with TGA, CS, ME and MET. In TGA-Au@Agr, the blue shift observed in the SPR peak of Au NPs was 7 nm from 540 nm→533 nm. In CS-Au@Agr, this shift was from 545 nm→539 nm, a 6 nm shift. Moreover, in ME-Au@Agr and MET-Au@Agr, an 11 nm blue shift in both was observed showing 540 nm→529 nm & 545 nm→534 nm shifts, respectively. The PDT and TU treated Au@Agr, i.e., PDT-Au@Agr and TU-Au@Agr, respectively, failed to show any such blue shift in the SPR peak of Au NPs. Instead, a 14 nm red shift (534 nm→548 nm) was observed in PDT-Au@Agr, and in TU-Au@Agr, no shift was evident. This peculiar behaviour of different thiols towards Au@Agr and also the selectivity of thiols to show blue or red shifts of the SPR peak of Au NPs studded into Agr (Au@Agr) detected by a UV-visible spectrophotometer is shown in Figure 5.2. It is well known that a blue/red shift in the SPR peak of a metal nanoparticle is an optical manifestation of a significant change in its particle size.\textsuperscript{24,25} In order to further examine this change in the particle size of Au@Agr on binding with thiols, a systematic scanning electron microscopy (SEM) analysis was done before and after treating Au@Agr with thiols. As illustrated in the SEM images (Figure 5.3), the change in particle size of Au@Agr (50-60 nm) on treatment with thiols could be vividly perceived in accordance with the blue shifts of the SPR peak in the UV-visible spectrum of Au@Agr. In the SEM images of TGA-Au@Agr and CS-Au@Agr, a clear dense distribution of Au NPs having a particle
size ≤ 30 nm was observed, contrary to the sparsely distributed Au NPs of size ≤ 30 nm in ME-Au@Agr as well as in MET-Au@Agr.

Figure 5.3 The scanning electron microscopy (SEM) images of Au NPs studded into the agarose film (Au@Agr), 2% TGA-Au@Agr, 2% CS-Au@Agr, 2% ME-Au@Agr and 2% MET-Au@Agr showing successful fragmentation of Au NPs particle size after thiol treatment.

Figure 5.4 The particle size distribution plot assessed from SEM images (Figure 5.3) of Au@Agr, 2% TGA-Au@Agr, 2% CS-Au@Agr, 2% ME-Au@Agr and 2% MET-Au@Agr.
It would be informative to have a clear picture of Au NPs size distribution in all the films simply by assessing their SEM images. As shown in figure 4, in Au@Agr, a very broad particle size distribution with Au NPs ranging from 30 to 60 nm was obtained. In TGA-Au@Agr, the observation of very small and densely distributed fragmented Au NPs depicted the highest density of <10 nm Au NPs with fewer numbers of <20 nm AuNPs. And, in CS-Au@Agr, the Au NPs density was highest in the particle size range of 11-20 nm with comparatively lesser density in the particle size range of 1-10 nm. On the other hand, the sparse distribution of Au NPs in ME-Au@Agr showed particles in 11-20 nm size range with very few in 21-30 nm size range. The Au NPs in this similar size range was also determined in MET-Au@Agr, but the number of particles was further less in support of their sparser distribution observed in the SEM image. For PDT-Au@Agr and TU-Au@Agr, the other thiols of the group, no sign of fragmentation was evident in their SEM images (Figure 5.5). In fact, bigger sized Au NPs (60–70 nm) were seen in PDT-Au@Agr, supplementing the 14 nm red shift in SPR peak of Au NPs observed in its UV-visible spectrum. Moreover, a distribution of 50-60 nm sized Au NPs in the SEM image of TU-Au@Agr was observed confirming no shift in the SPR peak of Au@Agr upon binding with TU, which was detected in their UV-visible spectra.

The peculiar and intriguing behaviour of thiols to successfully fragment supported Au NPs (Au@Agr) could be credited to electron ejection on Au NPs by chemisorbed thiols. A shift in the SPR band wavelength caused by an electron donor or acceptor
in proximity can be comprehended due to alterations in the density of free electrons available on the nanoparticle. These alterations in turn cause a difference in SPR energy making it spectroscopically perceptible. Thiol being a strong nucleophile can easily donate electron density to Au NPs, which accumulates at their surface.\textsuperscript{15,40,41} The stabilisation of a nanoparticle strongly depends on the surface charge density which upon reaching a critical value causes excess surface tension on the nanoparticle.\textsuperscript{14,15,42} In TGA-Au@Agr, CS-Au@Agr, ME-Au@Agr and MET-Au@Agr, when the surface charge density exceeded this critical value, increased disturbance led to fragmentation of the nanoparticle. A lesser density of Au NPs in ME-Au@Agr and MET-Au@Agr could be attributed to the presence of terminal -OH groups in ME and -S-CH\textsubscript{3} groups in MET. It is reported that presence of -SH as well as -COOH groups together is the crucial factor for effective fragmentation of Au NPs, where -SH group plays the role of an electron donor and -COOH group aids in hydrogen bonding between adsorbed thiols and free thiols contributing to local concentration around the Au NPs.\textsuperscript{15} Due to the absence of one favouring factor among these two in ME and MET, a lower degree of fragmentation process resulted in the reduced density of fragmented Au@Agr. The contradictory behaviour of no fragmentation observed in TU-Au@Agr and PDT-Au@Agr could also be accounted for by the lack of suitable functional groups. TU does not have a terminal S-atom making the donation of free electrons to Au NPs difficult, and also the presence of the NH\textsubscript{2} group at both ends made interaction further difficult.\textsuperscript{15} Thus, TU-Au@Agr did not show any shift of the Au@Agr SPR peak. PDT-Au@Agr, on the other hand, showed a 14 nm red shift of SPR peak of Au@Agr which might be due to lowering of electron density on Au NPs by PDT resulting in aggregation and a red shift.\textsuperscript{40} Therefore, the experimental analyses by UV-visible spectroscopy and SEM
imaging provided significant legitimate observations confirming the fact that thiols (TGA, CS, ME and MET) were capable of playing the chemistry with supported Au NPs (Au@Agr) in a non-conventional way, resulting in successful fragmentation of these supported Au NPs.

![Dynamic Light Scattering (DLS) Analysis](image)

**Figure 5.6** The dynamic light scattering (DLS) analysis showing particle size of freshly prepared Au NPs solution, and fragmentation of Au NPs by TGA, CS, ME and MET in solution.

The role of agarose (Agr) as a support to Au NPs, and a favourable condition for fragmentation as well was inquired about. The reduction of Au$^{3+}$→Au$^{0}$ by UV exposure in only 10 min was made possible by Agr without adding any reducing agent. However, a UV exposure to a solution of Au$^{3+}$ ions could not reduce it to Au$^{0}$ even after 5 h. This clearly indicated the role of Agr as a stimulating condition for obtaining Au NPs simply by quick UV exposure. In addition, to check whether the fragmentation feasibility of thiols works for Au NPs in a solution also (i.e., when Au NPs are not supported by Agr), another investigation based on dynamic light scattering (DLS) was done (Figure 5.6). Herein, the fragmentation of freshly prepared Au NPs in solution by the group of thiols (TGA, CS, ME, MET) was investigated. For the analysis, a 0.1% solution of each
thiol was prepared, and then mixed with freshly prepared Au NPs (2 mL of 0.1% thiol solution + 40 mL of Au NPs solution) followed by DLS investigation for all the samples. Excitingly, the thiol solutions could fragment the 60 nm sized Au NPs up to a maximum of 35 nm sized particles only, which was observed in case of CS-Au. For ME-Au and MET-Au, the Au NPs sizes observed were 38 nm and 40 nm, with TGA-Au showing minimum fragmentation reporting 57 nm sized Au NPs. Clearly, the fragmentation by thiols is less feasible if the Au NPs were present in the solution as the UV-visible and SEM studies (discussed before) clearly show that fragmentation of Au NPs were efficient only when Agr acted as a solid support. This could probably due to restriction of movement of thiol molecules as well as Au NPs supported on Agr, thereby empowering an easy and effective electron density transfer leading to their successful fragmentation into much smaller sizes.

5.5.2. Synergistic Role of Thiols and Au@Agr for Catalytic Reduction of p-Nitrophenol

Understanding of non-conventional chemistry occurring between the thiols and Au@Agr led to another exploration of the viability of the catalytic property of Au NPs. Even after fragmentation of Au NPs, the residence of excess electron density on their surface is quite logical. Therefore, utilization of this electron density for effective and rapid reduction of one of the most refractory pollutant, p-nitrophenol (p-NP), to p-aminophenol (p-AP) was the next goal in this study.

An aqueous solution of p-NP has a characteristic maximum absorption peak appearing sharply at 320 nm in its UV-visible spectrum. Upon addition of freshly prepared NaBH₄ solution in large excess, the 320 nm peak red shifts to 400 nm due to the formation of p-nitrophenolate ion in the alkaline surrounding triggered by the
addition of NaBH₄. When unattended by a catalyst, this 400 nm peak of p-nitrophenolate ion stays stable with no sign of thermodynamically favoured reduction to p-AP (the reported standard reduction potential of p-nitrophenol/ p-aminophenol is -0.76 V and that of H₃BO₃/BH₄ is -1.33 V). As mentioned in various reports,²⁵⁻²⁹ this p-nitrophenolate ion peak does not alter even after several days, indicating that the conversion of p-nitrophenolate to p-AP is a very sluggish process.

Figure 5.7 The UV-visible absorptio

n spectral analysis showing catalysis of the reduction reaction of p-nitrophenol (p-NP) to p-aminophenol (p-AP) using 2% TGA-Au@Agr, 2% CS-Au@Agr, 2% ME-Au@Agr and 2% MET-Au@Agr as catalysts. p-NP(r) is p-nitrophenol ions produced by adding freshly prepared excess solution of NaBH₄ to p-NP solution.

With an intent to catalyse this reduction reaction of p-NP, a small strip of 2% TGA-Au@Agr film (size =1x1 cm, thickness~0.2 mm) was inserted into a 2 mL solution of p-NP(r) (p-NP(r)=a stock solution prepared by mixing freshly prepared 20 mL of 1 mM p-NP and 100 mL of 5 mM NaBH₄ solutions showing λₘₐₓ=400 nm). Amazingly, an instant decolorization of the p-NP(r) solution within a few seconds of time was noted as
the first observation. Therefore, to observe precisely, another fresh strip of 2% TGA-Au@Agr film was inserted again, and the noted change in the colour of p-NP(r) solution was then examined every 5 s by the UV-visible spectroscopy (Figure 5.7). It was very exciting to observe that in the presence of 2% TGA-Au@Agr, the sluggish peak at 400 nm corresponding to p-nitrophenolate ion showed a complete decrease in only 20 s of time with an emerging peak appearing around 300 nm substantiating the formation of p-AP. Moreover, since the reaction was monitored every 5 s, the presence of any Au NPs peak would have been seen in the UV-visible spectrum of p-NP(r), if any leaching of Au NPs from the film to the p-NP(r) solution would have occurred. This, in turn validated that the catalytic reduction reaction progressed only on the surface of the 2% TGA-Au@Agr film. The same catalytic reduction analysis of p-NP(r) solution was examined again using other films, viz., 2% CS-Au@Agr, 2% ME-Au@Agr, and 2% METAu@Agr (2% TU-Au@Agr and 2% PDT-Au@Agr were discarded as they did not show any fragmentation of Au@Agr). An instant catalysis for complete reduction of p-NP(r) to p-AP was observed in the presence of 2% CS-Au@Agr in only 30 s of time, as shown in figure 5, featuring degradation of 400 nm peak of p-NP(r) accompanied by the occurrence of a new 300 nm peak for p-AP. Contradictorily, even after the presence of fragmented Au@Agr, 2% ME-Au@Agr and 2% MET-Au@Agr were found to be incapable of showing effective reduction reaction catalysis for p-NP(r) to p-AP even after 10 min (Figure 5.7). This discrepancy was expected because of the presence of a much lower number of Au NPs in 2% ME-Au@Agr as well as in 2% MET-Au@Agr, as is clearly evident from their SEM images shown in Figure 5.3.
The reduction reaction of p-NP to p-AP is considered to be a pseudo-first order reaction owing to presence of NaBH₄ in large excess. As shown in Figure 5.8, a graph of ln (Cᵣ/C₀) was plotted against time to study the reaction when catalysed by all the different catalytic films, viz., 2%TGA-Au@Agr and 2%CS-Au@Agr. Herein, Cᵣ denotes the concentration of p-NP(r) at time ‘t’ seconds in the presence of the catalytic film, and C₀ is the concentration of p-NP(r) at time ‘zero’ seconds. The value of each concentration was calculated from the observed values of absorbance at λₘₐₓ=400 nm after every 5 s during p-NP(r) catalytic cycling. Upon plotting ln (Cᵣ/C₀) values vs. time, a linear relationship was observed for both the p-NP catalytic reactions catalysed by 2%TGA-Au@Agr and 2%CS-Au@Agr. The rate constant obtained from linearly fitted curve reported a value of 1.6 x 10⁻¹ s⁻¹ and 1.1 x 10⁻¹ s⁻¹ for 2%TGA-Au@Agr and 2%CS-Au@Agr catalysed p-NP reduction reactions, respectively. This rate constant value of the order of 10⁻¹ s⁻¹, to the best of our knowledge, is one of the fastest rate constant shown by a solid supported catalyst for the p-NP reduction reaction to date. For a comparative study, the p-NP reduction catalytic reaction was also studied using Agr and Au@Agr film as catalysts. As shown in Figure 5.9, the catalytic behaviour of Agr and Au@Agr was insignificant with the stagnant rate constant values of 0.01 x 10⁻¹ s⁻¹ and 0.05 x 10⁻¹ s⁻¹, when compared to 2%TGAAu@Agr and 2%CS-Au@Agr.
Figure 5.9 (A) The UV-visible spectra showing comparative study of p-nitrophenol (p-NP) reduction by Agr, Au@Agr, TGA-Au@Agr, and CS-Au@Agr films. (B) A comparative plot of ln (C_t/C_0) vs time showing a pseudo-first order rate of reduction reaction of p-NP catalysed by Agr, Au@Agr, TGA-Au@Agr, and CS-Au@Agr films.

Figure 5.10 The UV-visible absorption spectral analysis showing the catalysis of reduction reaction of p-nitrophenol (p-NP) to p-aminophenol (p-AP) using 4% TGA-Au@Agr and 4% CS-Au@Agr as the efficient catalysts. p-NP(r) is the p-nitrophenolate ions produced by adding freshly prepared excess solution of NaBH_4 to p-NP solution. And, the plot of ln (C_t/C_0) vs. time showing the pseudo-first order rate of the reduction reaction of p-NP to p-AP catalysed by 4% TGA-Au@Agr and 4% CS-Au@Agr films.

With an idea to check whether the concentration of thiol while preparing the films has anything to do with the catalysis rate, next catalytic films using a higher concentration of thiol were prepared (which is 4%, earlier it was 2%). From Figure 5.10, it is quite clear that 4% TGA-Au@Agr and 4% CS-Au@Agr films showed a relatively faster catalysis of p-NP reduction reaction with the respective rate constant.
values of $6.4 \times 10^{-1}$ s$^{-1}$ (an approximately four times higher value) and $2.2 \times 10^{-1}$ s$^{-1}$ (an approximately two times higher value). Thus, an increase in thiol concentration did show a faster catalytic rate for the p-NP reduction reaction. A comparison table of different rate constant values obtained for the p-NP reduction to p-AP when catalysed by different films prepared is shown in Table 5.1. Another table showing the comparison between the rate constant values of reduction reaction of p-NP to p-AP when catalysed by different materials reported in the literature and our proposed film is also included in Table 5.2.

Table 5.1 Comparison of rate constant values of p-NP reduction reaction catalysed by all the different films as catalysts.

<table>
<thead>
<tr>
<th>Film</th>
<th>Thiol</th>
<th>% of Thiol</th>
<th>Rate constant (k)</th>
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<tbody>
<tr>
<td>Agr</td>
<td>None</td>
<td>0</td>
<td>$0.01 \times 10^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>Au@Agr</td>
<td>None</td>
<td>0</td>
<td>$0.05 \times 10^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>2% TGA-Au@Agr</td>
<td>Thiglycolic acid</td>
<td>2</td>
<td>$1.6 \times 10^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>4% TGA-Au@Agr</td>
<td>Thiglycolic acid</td>
<td>4</td>
<td>$6.4 \times 10^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>2% CS-Au@Agr</td>
<td>L-Cysteine</td>
<td>2</td>
<td>$1.1 \times 10^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>4% CS-Au@Agr</td>
<td>L-Cysteine</td>
<td>4</td>
<td>$2.2 \times 10^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>2% ME-Au@Agr</td>
<td>2-Mercaptoethanol</td>
<td>2</td>
<td>$3.3 \times 10^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>2% MET-Au@Agr</td>
<td>L-Methionine</td>
<td>2</td>
<td>$1.6 \times 10^{-1}$ s$^{-1}$</td>
</tr>
</tbody>
</table>

Followed by this, a reusability test on 2% TGA-Au@Agr, 4% TGA-Au@Agr, 2% CS-Au@Agr, and 4% CS-Au@Agr was also conducted (Figure 5.11). For the study, a fresh strip of thiol-Au@Agr film (1x1 cm, thickness~0.2 mm) was taken, and the 1st catalytic cycle of the reduction reaction of p-NP was carried out. After the completion of the 1st catalytic cycle, the film strip was recovered from the solution, washed several times with Milli-Q water, dried on a filter paper and then used again for the 2nd catalytic cycle. It was observed that the activity of 2% TGA-Au@Agr and 2% CS-Au@Agr decreased after its first use, but 4% TGA-Au@Agr was equally effective as a catalyst in its second use; whereas, 4% CS-Au@Agr was found to show relatively lower activity in its second use. A SEM image (Figure 5.12) of the used catalytic film was taken, which showed the presence of assemblies of aggregated Au NPs in the used film, which was otherwise
absent in the fresh thiol-Au@Agr film. The aggregation of Au NPs in the film after its first catalytic cycle could be due to the presence of excess NaBH₄ in the p-NP(r) catalytic reaction solution. This aggregation of Au NPs probably caused a decrease in the catalytic activity of the film after the 1st use.

**Table 5.2** The comparison table between the rate constant values of reduction reaction of p-NP to p-AP when catalysed by different materials reported in the literature and our proposed film (4% TGA-Au@Agr and 4% CS-Au@Agr).

<table>
<thead>
<tr>
<th>S. N</th>
<th>System</th>
<th>Rate Constant (k)</th>
<th>Time taken</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>4% TGA-Au@Agr film (in solid phase)</td>
<td>6.4 x 10⁻¹ s⁻¹</td>
<td>5 s</td>
<td>Our proposed material</td>
</tr>
<tr>
<td>2.</td>
<td>4% CS-Au@Agr film (in solid phase)</td>
<td>2.2 x 10⁻¹ s⁻¹</td>
<td>15 s</td>
<td>Our proposed material</td>
</tr>
<tr>
<td>5.</td>
<td>Ellipsoid-like MCM-41 supported Ag nanocomposite (in solution)</td>
<td>4.3 x 10⁻³ s⁻¹</td>
<td>180 s</td>
<td>N. Hao, L. Li and F. Tang, <em>J. Mater. Chem. A</em>, 2014, 2, 11565–11568.</td>
</tr>
<tr>
<td>7.</td>
<td>Fe₃O₄@TiO₂/Au@Pd@TiO₂ (in solution)</td>
<td>1.06 min⁻¹</td>
<td>4 min</td>
<td>W. Hu, B. Liu, Q. Wang, Y. Liu, Y. Liu, P. Jing, S. Yu, L. Liua and J. Zhang, <em>Chem. Commun.</em>, 2013, 49, 7596-7598.</td>
</tr>
<tr>
<td>9.</td>
<td>Ag(0.7)-SBA-15 composites (in solution)</td>
<td>9.0 x 10⁻³ s⁻¹</td>
<td>360 s</td>
<td>J. Han, P. Fang, W. Jiang, L. Li and R. Guo, <em>Langmuir</em>, 2012, 28, 4768-4775.</td>
</tr>
</tbody>
</table>
Figure 5.11 The UV-visible spectra showing reusability study of 2%TGA-Au@Agr, 4%TGA-Au@Agr, 2%CS-Au@Agr and 4%CS-Au@Agr films catalysed p-NP reduction reaction.

An explicit insight into the mechanistic behaviour for the catalysis reaction by the films was done (Scheme 5.2). The reduction catalysis started only after adsorption of p-NP and NaBH₄ on the surface of Au NPs. In the present case, each component Agr, Au@Agr and TGA-Au@Agr or CS-Au@Agr played a specific and favouring role in aiding a faster catalytic reduction of p-NP. Agr: (i) the process of adsorption of reactants made easy using an Agr hydrogel film as a solid support (ii) once adsorbed, the solid support also limited the movement of the adsorbed reactants making the electron transfer process swift. Au@Agr: (i) it provided a perfect and aligned surface with extra electrons for the catalysis reaction to play on with a swift relay of electrons from BH₄⁻ to p-NP; (ii) supported Au NPs also diluted the

Figure 5.12 The SEM image of 2%TGA-Au@Agr film after its 1st catalytic cycle of p-NP reduction reaction.
chance for aggregation, which is well known to be harmful for a catalysis reaction. TGA-Au@Agr or CS-Au@Agr: (i) first and foremost, TGA and CS prosecuted the fragmentation of Au NPs delivering much smaller nanoparticles on a solid support for catalysis; (ii) second, the presence of TGA or CS stimulated adsorption of p-NP much easily due to the probability of forming hydrogen bonding between them; (iii) third, such interactions in conjunction with the agarose hydrogel nature made maximum and quick adsorption of p-NP for effective and swift catalysis. Hence, all these insights gave a clear picture of the synergistic roles of Agr, Au@Agr and TGA/CS for such an efficient catalytic activity towards p-NP to p-AP reduction reaction in just ~20 to 30 seconds.

Scheme 5.2 Schematic of the fragmentation of Au@Agr by thiols (TGA), and the synergistic role of TGA and Au@Agr to catalyse the reduction reaction of p-nitrophenol (p-NP).

5.6. Conclusion

In the chapter, we presented an investigation involving the physical experimentation and demonstration of the capability of a group of thiols to fragment an Au@Agarose film in the absence of any auxiliary applied energy, an unexplored non-conventional chemistry between thiols and supported Au NPs. A group of thiols (TGA, CS, ME and MET) were found to be very proficient in fragmenting 50-60 nm sized Au@Agr into
well dispersed ≤30 nm sized nanoparticles. The fragmentation process could be easily explained by the swift transfer of electron density from thiols to Au@Agr causing an excess of charge on Au NPs, thereby undergoing fragmentation to relieve the surface tension. The TGA-Au@Agr and CS-Au@Agr films studded with such fragmented Au NPs were proven to be an efficient catalytic material for rapid reduction of p-NP to p-AP in just ~20 to 30 second of time. The pseudo first order rate constants for 2% TGA-Au@Agr and 2% CS-Au@Agr catalysed p-NP reduction reactions were found to be $1.6 \times 10^{-1}$ s$^{-1}$ and $1.1 \times 10^{-1}$ s$^{-1}$, respectively. A faster catalytic rate for the p-NP reduction reaction was evident on increasing the concentration of TGA as well as CS with two reuses of the same film strip. Such a fast catalysis by TGA-Au@Agr and CS-Au@Agr could be accounted for by a synergistic role of Agr, Au@Agr and TGA or CS complementing each other’s role, and contributing towards an efficient and rapid catalytic behaviour. On all these notes, it can be concluded that this study opens up new insights into the chemistry of thiols towards supported Au NPs as well as the effectiveness of such a system to serve as an amazing catalyst for industrial noxious and lethargic pollutants.

**References**


CHAPTER - 5 (B)

A Property Study on the Storage/Release of Silver nanoparticles in Chitosan-Silver Nanocomposite leading to Successful Catalytic applications

Among many materials that have been studied in the last few years, the biopolymers have been investigated as the soft matrices which can accommodate a variety of metal nanoparticles (NPs). The most characteristic feature of such bio-nanocomposites is that they can offer intrinsic functionalities of both metal NPs and the biointerfaces offered by the polymers. Out of many application fields, these bio-nanocomposites have gained increasing attention in the catalytic activities. The polymers not only provide the support to the catalytic metal nanoparticles, but their structure also induces special properties such as the enhanced reaction rate and the stereo-selectivity. Thus, a proper selection of the polymer and the metal nanoparticle can offer quite large opportunities and applications.

In the previous chapters, we have mainly discussed three such polysaccharide based polymers, viz., chitosan, alginate and agarose. Chitosan, as mentioned in chapter II (a), is a linear cationic polysaccharide bearing the amino and hydroxyl functionalities which can be tuned to develop stimuli-responsive hydrogels. There are also many reports of chitosan based composites and its derivatives. Baek et al. reported the synthesis of highly ordered chitosan-Au core-shell nanostructures. Various chitosan composites include iodine stabilized Cu nanoparticle chitosan composite, CdS QDs/chitosan composite, CaCO₃ and chitosan to produce well defined inorganic-organic nanoboxes and nanoframes, chitosan/lactic acid nanofibre, chitosan/silk hydrogel, and chitosan/glycine chloride. Chitosan has also found to be effective in adsorbing humic
acid, mercuric ions, acid dyes, toluene from aqueous solution, etc. Also, there are growing number of papers on chitosan for its application in the supported catalysis due to its high sorption capabilities, stability of metal NPs on chitosan and the physical versatility of the biopolymer. In the huge family of metal nanoparticles, the silver nanoparticles (Ag NPs) have attracted a great deal of attention in the biomedical and the catalytic applications. Thus, aminopolysaccharide chitosan can uniquely equips hierarchical assembly of nanoscale silver particles with important applications especially when synthesised benignly.

5.7. The Objective: A Property Study on the Storage/Release Behaviour of Chitosan-Ag Nanocomposite and its Applicability

Here, a property study on the storage/release of Ag NPs in chitosan-silver nanocomposite when prepared via two different routes, in-situ and ex-situ incorporation of Ag NPs in chitosan gel (as detailed in chapter II (a)) was done. Although there are methods available for such chitosan-Ag nanocomposite, and its cytotoxic efficacy, molecular relaxation, catalytic properties is also studied but, the property study on the unique storage/release of Ag NPs from chitosan-Ag nanocomposite gel was not considered before. We thoroughly investigated the property variance between these chitosan-Ag-in-situ (CH-Ag-I) and chitosan-Ag-ex-situ (CH-Ag-E) nanocomposite gels. The CH-Ag-E gel showed superior properties in comparison to CH-Ag-I, such as higher swelling ratio (SR) and thermal stability. The study on the storage/release property of the nanocomposite gels showed that CH-Ag-E was capable of showing release of Ag NPs simply by the addition of sodium borohydride (NaBH₄), but such Ag NPs release was not observed in CH-Ag-I. This disparity could be explained from the different
electrostatic interactions of the Ag NPs and the three-dimensional networking in chitosan gel happening in CH-Ag-E and CH-Ag-I. These unique properties of CH-Ag-E was found to be very effective in catalysing the reduction reaction of p-NP, a stagnant industrial pollutant. The pseudo-first order rate constant of the p-NP reduction reaction was found to be 10-fold higher when CH-Ag-E was used as the catalyst instead of CH-Ag-I. The more number of Ag NPs present on the surface of CH-Ag-E resulted in this higher rate constant value by stimulating a fast transfer of electrons necessary of the reduction reaction of p-NP.

5.8. Materials

Low molecular weight chitosan (Sigma Aldrich), glycerol (about 98% purified) (Merck), sodium borohydride (Merck), trisodium citrate-2-hydrate (Merck), silver nitrate (Merck), sodium hydroxide pellets (purified) (Merck), glacial acetic acid (Qualigens Fine Chemicals), p-Nitrophenol (Lobal Chemie) were used as received. All other materials used were of analytical reagent grade from commercial sources.

5.9. Experimental

5.9.1. Preparation of Chitosan-silver nanocomposite gel: The chitosan-silver nanocomposite gels were synthesized following two different routes, viz., in-situ and ex-situ (as previously detailed in chapter 2 (A)). Briefly, the in-situ incorporation of Ag NPs into the CH gel led to the formation of chitosan-Ag-in situ (CH-Ag-I) nanocomposite gel, and the ex-situ embedding of the silver nanoparticles into the CH gel resulted in the formation of chitosan-Ag-ex situ (CH-Ag-I) nanocomposite gel. The
pictorial representation of these two routes are shown as scheme 5.3 and scheme 5.4 below.

**Scheme 5.3** Schematic of the strategic preparation of Chitosan-Ag-in-situ nanocomposite gel (CH-Ag-I).

**Scheme 5.4** Schematic of the strategic preparation of Chitosan-Ag-ex-situ nanocomposite gel (CH-Ag-E).

5.9.2. *The study on the storage/release of silver nanoparticles in CH-Ag-I and CH-Ag-E nanocomposites:* For the analysis, a small piece of CH gel was taken and kept immersed in the freshly prepared Ag NPs solution for 60 mins (as done in the preparation of CH-Ag-E). The Ag NPs loaded gel (i.e., CH-Ag-E) was then recovered from Ag NPs solution, washed thoroughly with water and dried in between the folds of filter paper. Next, to check the possibility to get back the stored Ag NPs, the dried piece of gel was immersed in Milli-Q water followed by the dropwise addition of freshly prepared 5 mM solution of NaBH₄ in it. The used film was then recovered and investigated for the reusability study. The same release study was also tested on CH-Ag-I nanocomposite gel. This complete storage/release study (scheme 5.5) was monitored step-by-step through the UV-visible spectrophotometry.
5.9.3. Applicability of CH-Ag-I and CH-Ag-E nanocomposites in the catalytic reduction of p-Nitrophenol (p-NP) to p-aminophenol (p-AP): The reduction of p-Nitrophenol (p-NP) to p-Aminophenol (4-AP) by sodium borohydride (NaBH₄) catalysed by CH-Ag-I and CH-Ag-E nanocomposite gels was monitored through UV-visible spectrophotometry. Approximately, 2 mL of 5 mM NaBH₄ and 20 µL of 0.05 mM p-NP were mixed together in a 3 mL cuvette. The sharp peak at $\lambda_{\text{max}}=400$ nm of the resulting solution indicating the formation of p-nitophenolate ions (p-NP(r)) was then monitored for the complete study. Initially, a 0.05 g of CH-Ag-E nanocomposite gel was immersed into the cuvette containing p-NP(r) solution and its UV-visible spectrum was then recorded in every 10 mins up until the completion of 60 mins. Also, the whole procedure was repeated with CH-Ag-I nanocomposite gel keeping the reaction conditions and gel amount (0.05 g) identical.

5.10. Characterization

The UV-Visible spectra were taken using Shimadzu (UV1601PC) spectrophotometer. The scanning electron microscopy (SEM) was carried out using Carl Zeiss Σigma VP
using EHT-5 kV. The energy dispersive X-ray spectroscopy (EDX) was done using EDX detector from Oxford Inc. The transmission electron microscopy (TEM) was carried out in JEOL JEM 2100 with operating voltage at 200kV. The Fourier transform infer-red (FTIR) spectroscopic measurements of gels were recorded in Bruker FT-IR spectrometer. The samples for FTIR measurements were prepared in the form of pellets by mixing 20 mg of IR spectroscopic grade potassium bromide (KBr) with 2 mg of dried samples. The spectra were recorded in the transmission mode over 256 scans with resolution of 4 cm\(^{-1}\). The thermogravimetric analysis (TGA) was carried out in Perkin Elmer TGA 4000 from temperature 35 \(^{0}\)C to 500 \(^{0}\)C at 10 \(^{0}\)C heating rate and 20 mL nitrogen flow rate per minute.

**Chapter 5 (B)**

5.11. Results and Discussion

5.11.1. Analysis of Property Variance in Chitosan-Ag-in-situ (CH-Ag-I) and Chitosan-Ag-ex-situ (CH-Ag-E) Nanocomposite Gels

Chitosan of low molecular weight having DDA =77.7% was used to prepare chitosan-Ag nanocomposites gel via two different strategic routes leading to the formation of chitosan-Ag-in-situ (CH-Ag-I) and chitosan-Ag-ex-situ (CH-Ag-E) nanocomposite. In order to systematically study the property variance between CH, CH-Ag-I and CH-Ag-E, the UV-vis spectra of the gels (figure 5.13 (A)) were taken to start with. It is evident from the spectra that CH-Ag-I showed characteristic Ag NPs plasmon peak centered at 409 nm, which was missing in both CH and CH-Ag-E nanocomposite gels. A temperature dependant degradation study of the gels was assessed by TGA thermogram (figure 5.13 (B)). The thermal degradation profile was found to be similar for CH and CH-Ag-I, except the amount of adsorbed water was more in CH-Ag-I. The thermal
stability of CH-Ag-E nanocomposite gel showed vast improvement as compared to CH-Ag-I. The degradation also started at a much higher temperature ~250 °C. In the thermogram shown in figure 5.14, the first degradation range (50 °C -130°C) associated with loss of adsorbed water from the gel. The second range ~220 °C corresponded to the degradation and deacetylation of chitosan. Followed by this, a SEM imaging analysis was done (figure 5.14). A thin layer of each gel was placed on a silicon wafer and coated with Au/Pd alloy before SEM imaging. A characteristic morphology difference was witnessed in CH, CH-Ag-I and CH-Ag-E gels. The CH gel showed fibrous network structure which became more evident on the in-situ incorporation of Ag NPs in CH-Ag-I. In the SEM images of CH-Ag-E, on the other hand, Ag NPs were seemed to be beautifully adsorbed more on the surface of gel in comparison to CH-Ag-I gel.

**Figure 5.13** (A) The UV-vis spectra of chitosan gel (CH), chitosan-Ag-in-situ (CH-Ag-I) and chitosan-Ag-ex-situ (CH-Ag-E) nanocomposite gels. (B) The thermogravimetric analysis (TGA) of chitosan gel (CH), chitosan-Ag-in-situ (CH-Ag-I) and chitosan-Ag-ex-situ (CH-Ag-E) nanocomposites.

**Figure 5.14** The scanning electron microscope (SEM) images of chitosan gel (CH), chitosan-Ag-in-situ (CH-Ag-I) and chitosan-Ag-ex-situ (CH-Ag-E) nanocomposites.
Next, a swelling behaviour study of the gels in water as the swelling medium at room temperature was investigated. A pre-weighed amount of the gels (approximately 0.0018 g) was immersed into the deionised water for 48 hours, and their wet weights were determined after blotting in between the folds of the filter paper to remove the excess surface water. The swelling ratio (SR) was calculated using the equation shown below:

\[
\text{Swelling Ratio (SR)} = \frac{\text{Weight of swollen gel (}W_s\text{)}}{\text{Weight of gel in dry state (}W_d\text{)}}
\]

The SR value for CH and CH-Ag-I gels was found to be 1.11. But, the SR for CH-Ag-E was determined to be 2.44, a value almost double than that for CH and CH-Ag-I. This high SR value for CH-Ag-E could be due to the presence of more number of free amine groups in CH-Ag-E in comparison to that in CH-Ag-I.

**Figure 5.15** The Fourier-Transform Infra-Red (FT-IR) spectra of chitosan gel (CH), chitosan-Ag-in-situ (CH-Ag-I) and chitosan-Ag-ex-situ (CH-Ag-E) nanocomposite gels.

The FTIR spectra of the CH, CH-Ag-I and CH-Ag-E nanocomposite gels are shown in figure 5.15. In CH gel, 1651 cm\(^{-1}\), 1568 cm\(^{-1}\), 1375 cm\(^{-1}\) were assigned to amide I, amide II and amide III, respectively. 1152 cm\(^{-1}\) designated to the saccharide structure. 1320 cm\(^{-1}\) indicated -CN bond stretching. The CH-Ag-I and CH-Ag-E nanocomposite gel showed similar peak positions. 3400 cm\(^{-1}\) peak was attributed to -OH stretching vibration, 2930 cm\(^{-1}\) and 2868 cm\(^{-1}\) represented the presence of -CH aliphatic chain. The CH-Ag-E showed the -CH aliphatic peak at 2930 cm\(^{-1}\) and 2879 cm\(^{-1}\). The
characteristics amide I, amide II and amide III peaks at 1651 cm⁻¹, 1568 cm⁻¹, 1375 cm⁻¹, respectively were present in CH-Ag-I and CH-Ag-E. The 1153 cm⁻¹ peak of saccharide structure and 1320 cm⁻¹ peak of -CN bond stretching vibration were present in both CH-Ag-I and CH-Ag-E.

5.11.2. The Storage and Release of Ag NPs from CH Gel Matrix

A property study to analyse the capability of CH gel to store as well as release the Ag NPs, thereby acting as a storage container of Ag NPs was inspected here. The reversible binding of Ag NPs to the CH gel matrix was monitored by the UV-visible spectrophotometry (figure 5.16) depicting the strategic storage of Ag NPs inside chitosan gel matrix (CH-Ag-E) and the subsequent release of the Ag NPs.

![UV-vis spectra](image)

**Figure 5.16** The UV-vis spectra of the prepared Ag NPs (black), Ag NPs solution after placing the CH into it 60 minutes (red), Ag NPs released from the CH by adding sodium borohydride (green).

The UV-vis spectrum of the prepared Ag NPs is shown in figure 5.16 (black line) having the plasmon resonance peak centered at 407 nm. After placing the CH into this Ag NPs solution for 60 minutes, the UV-vis absorption spectrum of the solution was again recorded (figure 5.16 (red line)). A significant decrease in the absorption value of Ag NPs was observed indicating the successful loading of Ag NPs in the chitosan gel matrix. The subsequent release of Ag NPs on addition of freshly prepared NaBH₄
solution resulted in the emergence of the plasmon resonance peak at 386 nm as shown in figure 5.16 (green line).

To further investigate the UV-vis analysis, the transmission electron microscope (TEM) imaging was done. Figure 5.17 shows the representative TEM image of the freshly prepared Ag NPs loaded into CH gel and the subsequently released Ag NPs accompanied by the comparative particle size distribution plot. As apparent from the particle size distribution assessed from TEM images the size of the prepared Ag NPs were in the range of 15-20 nm, while the released Ag NPs were smaller and the majority of the particles were less than 5 nm in size. This, in turn explained the blue shift to 386 nm observed in the plasmon resonance peak of Ag NPs in its UV-vis spectrum.

![Figure 5.17](image_url)  
*Figure 5.17* The transmission electron microscope (TEM) images of the loaded Ag NPs (freshly prepared), the released Ag NPs from CH, and their comparative size distribution plot indicating particle density.

The scanning electron microscopy (SEM) was also used to scrutinize the storage of Ag NPs and its subsequent release on addition of NaBH₄. Figure 5.18 shows the SEM images of chitosan gel. On putting a small piece of chitosan gel into a solution of Ag NPs (CH-Ag-E), it showed deposition of Ag NPs all over in the gel matrix as depicted in its SEM image, which was also confirmed by energy-dispersive X-ray spectroscopic (EDX) analysis (figure 5.18). Subsequently, after the release of Ag NPs by addition of sodium borohydride, SEM image showed that the Ag NPs got completely removed from
the gel matrix. The EDX analysis also confirmed that there were no Ag present in the chitosan gel matrix. The structural changes due to the storage of Ag NPs inside chitosan gel matrix, i.e., chitosan-Ag-ex-situ (CH-Ag-E) nanocomposite gel and the subsequent release of the Ag NPs was also monitored by FTIR and depicted in figure 5.19. While the CH didn’t show well resolved -OH and -CH aliphatic stretching vibrations, after loading of Ag NPs the -OH stretching peak at 3400 cm\(^{-1}\) and -CH stretching peaks at 2930 cm\(^{-1}\) and 2879 cm\(^{-1}\) were distinctly visible. All other peaks were found to be present even after loading of Ag NPs. After release of Ag NPs, a slight shift in the amide-I, amide-II and amide-III peaks were obtained at 1651, 1594, 1375 cm\(^{-1}\), respectively.

![Figure 5.18](image.png)

**Figure 5.18** The representative scanning electron microscope (SEM) images showing the storage and release of Ag NPs in CH gel. The SEM images include CH (chitosan gel), the Ag NPs loaded CH, i.e., CH-Ag-E (chitosan-Ag-ex-situ nanocomposite gel), and CH-Ag-E after the subsequent release of Ag NPs on addition of NaBH4, CH-Ag-E after release. The corresponding energy-dispersive X-ray spectroscopic (EDX) analysis of the gel in each step of the study to check the presence of Ag in the gel surface along with its elemental analysis is also included in the figure.
Moreover, the extracted Ag NPs could be again loaded in the chitosan hydrogel and re-extracted. To investigate this, a small piece of CH gel was first loaded with Ag NPs for 1 hour followed by its subsequent release on addition of NaBH₄. This extracted solution containing smaller sized Ag NPs was then re-loaded into a fresh piece of CH gel. As observed, it was possible to re-load the extracted Ag NPs into the gel and to extract it back simply by adding NaBH₄ (figure 5.20). The quantitative analysis of the amount of loading of Ag NPs was determined to be 0.0164 M/g of CH gel. It is worth mentioning here that CH-Ag-I could not show this typical Ag NPs storage/release behaviour at all.

**Figure 5.19** The Fourier-Transform Infra-Red (FT-IR) spectra showing the storage and release of Ag NPs in CH gel. The FT-IR spectra include CH (chitosan gel), the Ag NPs loaded CH, i.e., CH-Ag-E (chitosan-Ag-ex-situ nanocomposite gel), and CH-Ag-E after the subsequent release of Ag NPs on addition of NaBH₄, CH-Ag-E after release.

**Figure 5.20** The UV-vis spectrum of Ag NPs showing re-loading and re-extraction from the chitosan (CH) gel.
In order to check the feasibility of CH gel to show such storage/release behaviour towards other metal nanoparticles, gold nanoparticles (Au NPs) were chosen and the similar study was carried. Interestingly, the loading of Au NPs into the CH gel took more time unlike Ag NPs. In figure 5.21, the UV-vis spectrum of the freshly prepared Au NPs centred at 546 nm is shown by blue curve. On putting a small piece of CH gel into this Au NPs solution, the absorption of Au NPs was not observed even after 60 min. The absorption of Au NPs was observed on keeping the CH gel for a longer time, 120 min. This is completely contradictory to Ag NPs, where a considerable absorption started after 10 mins only. Also, it was not possible to recover the loaded Au NPs from CH gel on addition of NaBH₄.

5.11.3. Efficient Catalytic Reduction of p-NP to p-AP by Nanocomposite Gels

The superior catalytic property of CH-Ag-E & CH-Ag-I nanocomposite gels compared with those of CH gel was demonstrated by the reduction of p-nitrophenol (p-NP) to p-Aminophenol (p-AP) with sodium borohydride as the model reaction. The catalysis was studied spectrophotometrically by monitoring the fate of the p-nitrophenolate (p-NP (r))
peak ($\lambda_{\text{max}}$=400 nm) as done in the section (A) of this chapter.

**Figure 5.22** The UV-visible the catalysis of the p-nitrophenol (p-NP) reduction reaction by CH-Ag-E and CH-Ag-I nanocomposite gels. A comparative plot of ln (C_t/C_0) vs time showing a pseudo-first order rate of reduction reaction of p-NP catalysed by CH-Ag-E and CH-Ag-I nanocomposite gels.

As evident from the figure 5.22, in the presence of the CH-Ag-E, the 400 nm p-NP (r) absorbance peak gradually degraded confirming the successful reduction of p-NP in 45 min of time. On the other, this degradation of the p-NP (r) peak was found to be relatively very slow on using CH-Ag-I as the catalyst. The plot clearly showed that the rate of the reduction was faster when CH-Ag-E nanocomposite gel was used as catalyst than CH-Ag-I nanocomposite gel. The rate constant of both these pseudo-first order catalytic reactions were then calculated by plotting the ln (C_t/C_0) values against time, where C_t denotes the concentration of p-NP (r) at time ‘t’ seconds in the presence of the catalytic film, and C_0 is the concentration of p-NP(r) at time ‘zero’ seconds. The CH-Ag-E catalysed p-NP reduction reaction showed the rate constant of 3.62 x 10^{-5} s^{-1}, a value 10-fold higher than what obtained from the CH-Ag-I catalysed reaction, 0.62 x 10^{-6} s^{-1} (figure 5.22).

The understanding of the actual mechanism of the study was then dealt with. The binding of Ag NPs to chitosan was due to the electrostatic adsorption of the negatively charged citrate stabilized Ag NPs into the cationic chitosan main chain. Chitosan in the aqueous solution is positively charged in the form of R-NH$_3^+$. The Ag NPs can have
affinity towards R-NH$_2^+$ group of the chitosan through van-der Waals forces. Similar mechanism is also valid for Au NPs, although the adsorption of Au NPs onto chitosan hydrogel was slow and it took more than 1 hour for substantial adsorption of Au NPs (discussed earlier). However, the reason for this observation is not clear. Another interesting observation was that while it was possible to extract the Ag NPs from the CH-Ag-E nanocomposite gel by the addition of NaBH$_4$, in the case with CH-Ag-I nanocomposite gel, it was not possible to extract the loaded Ag NPs. The reason could be explained by the fact that in CH-Ag-I, Ag NPs was incorporated while making the chitosan gel as a consequence the Ag NPs electrostatically absorbed forms a 3-dimensional gel structure which were thus quite stable. Therefore, in such a structure it was not easy to remove the Ag NPs. On the other hand, CH-Ag-E nanocomposite gel which was formed by placing the chitosan gel in Ag NPs solution resulted in the electrostatic adsorption of Ag NPs onto the already formed gel structure. The electrostatically adsorbed Ag NPs were loosely bound and thus, could be extracted by addition of NaBH$_4$. This also explained why CH-Ag-E was effective in catalysing reduction of p-NP to p-AP in comparison to CH-Ag-I. The CH-Ag-E nanocomposite gel with loosely bound Ag NPs helped to smoothly transfer electrons from BH$_4^-$ to nitrophenolate, and thus acting as a better catalyst for the purpose. We also want to add here that although in CH-Ag-E the Ag NPs were electrostatically adsorbed on chitosan hydrogel, the sonication of the CH-Ag-E could not remove the loaded Ag NPs showing the interactions between Ag NPs and chitosan hydrogel were quite strong.

Effect of size of the loaded Ag NPs solution and the amount of gel on the catalytic reaction: The role of size of the Ag NPs loaded in CH-Ag-E nanocomposite gel was
also investigated. For the study, three different Ag NPs solutions were freshly prepared and their particle sizes as determined by the dynamic light scattering (DLS) analysis (figure 5.23) were found to be 26 nm ($\lambda_{\text{max}}=410$ nm), 56 nm ($\lambda_{\text{max}}=421$ nm), and 67 nm ($\lambda_{\text{max}}=431$ nm). These Ag NPs solutions were then individually loaded into three different pieces of CH gel and monitored by the UV-visible spectrometry (figure 5.24 (A)). The successful loading of the Ag NPs into the CH gel resulted in the formation of three different gels, named as CH-Ag-E28, CH-Ag-E56 and CH-Ag-E67, and were used as the catalysts for the p-NP reduction reaction. As observed in the figure 5.24 (B), in the presence of CH-Ag-E28 maximum degradation in the p-NP (r) peak was obtained. The other two, CH-Ag-E56 and CH-Ag-E67 were found to be almost ineffective in the catalysis process. Therefore, a better catalytic activity could be obtained by decreasing the size of the loaded Ag NPs.

Figure 5.23 The dynamic light scattering (DLS) graphs showing the particle size of the prepared Ag NPs.

Figure 5.24 (A) The UV-visible spectra showing loading of Ag NPs of three different sizes, and (B) The UV-visible spectra showing the catalysis of p-NP by CH-Ag-E28, CH-Ag-E56 and CH-Ag-E67.
It was also observed as expectation that larger the amount of the Ag NPs loaded chitosan gel (CH-Ag-E), the faster would be the catalysis of the reduction of p-NP (figure 5.25 (A)). It is quite obvious that increasing the amount of Ag loaded chitosan gel (CH-Ag-E) will increase the surface area, thus increasing the catalytic process. Next, we checked the reusability of the CH-Ag-E nanocomposite gel as the reduction catalyst. In this study, the same piece of CH-Ag-E nanocomposite gel was used several times for the catalytic reduction of p-NP to p-AP. The UV-visible spectra showed representative three such cycle of the use of the same CH-Ag-E gel as the catalyst (figure 5.25 (B)). This indicated the reusability potential of CH-Ag-E nanocomposite gel and will find industrial applications.

![Figure 5.25](image)

**Figure 5.25** (A) The UV-visible spectra showing the catalysis of the reduction of p-NP by different amounts of CH-Ag-E gel, and (B) The reusability study of CH-Ag-E gel for the catalysis of the reduction of p-NP.

**5.12. Conclusion**

In the chapter, a systematic study on the storage/release property of chitosan-silver nanocomposite gel was done. The chitosan-Ag nanocomposite gel, if prepared through two different routes (ex-situ and in-situ) showed few interesting property variances. The chitosan-Ag-ex-situ (CH-Ag-E) gel could act a store house of silver nanoparticles, which could be stored into the gel and released when required simply by adding NaBH₄.
The released Ag NPs were very small in size (~5-10 nm) when compared to the loaded Ag NPs (50-60 nm). Contrary, the chitosan-Ag-in-situ (CH-Ag-I) gel could not show such interesting storage/release property. Such property variance could be attributed to the different electrostatic interaction between Ag NPs and chitosan gel network. In CH-Ag-I, the absorbed Ag NPs formed a stable 3-dimensional gel structure making the removal of Ag NPs difficult. However, in CH-Ag-E the Ag NPs were electrostatically adsorbed onto the already formed gel network, and thus these loosely bound Ag NPs could be extracted simply by addition of NaBH₄. This storage/release property of CH-Ag-E was further employed in the catalysis of reduction reaction of p-Nitrophenol (p-NP) to p-Aminophenol (p-AP). The catalytic reduction rate was found to be dependent on the size of the Ag NPs loaded into the chitosan gel and the amount of the gel taken as well. Moreover, the reusability of the CH-Ag-E gel was found to be four cycle of the catalytic reduction reaction. Therefore, such kind of storage/release properties of CH-Ag-E gel made it a ready-to-use material to obtain Ag NPs of very small sizes leading to the wide area of its application.

References


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