Review on green synthesis of positively charged biocompatible gold nanoparticles in water: Use of ascorbic acid as reducing agent.

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Abstract

Well-monodispersed AuNPs were prepared by the reduction of $KAuCl_4$ using sodium borohydride and ascorbic acid as reducing agents. Due to using different reducing agent power, we obtained different results for AuNPs that were functionalised with same ligands. The effect of two different reducing agents on size and stability of the colloidal cationic phosphonium AuNPs was investigated. Their formation and stability was monitored by using UV−Vis absorption spectroscopy, Transmission Electron Microscopy (TEM) and dynamic light scattering (DLS). Small sizes with higher stability during a period of time were generated when $NaBH_4$ was used as reducing agent.

AuNPs functionalised by tri(phenyl)phosphoniopropylthiosulfate (4C) (3-thioactylpropyl)-triphenylphosphonium bromide (5) and tri(p-tolyl)phosphoniopropyl- thiosulfate (4) zwitterion *via* using $NaBH_4$ as reducing agent in H2O / DMSO as solvent, the maximum absorption bands centred at 519, 519 and 529 nm respectively. While, when the ascorbic acid is used as reducing agent, the maximum absorption of AuNPs functionalised by these ligands were centred at 542, 575 and 557 nm respectively. For (6-thioacetylhexyl)tris(2,4,6-trimethoxyphenyl)-phosphonium bromide (8B) showed little difference between two methods, whereas the absorbance centred at 522 nm in the case of $NaBH_4$ and at 528 nm in the case using ascorbic acid as a reducing agent.

ATR-FTIR, NMR, ESI-MS techniques can be employed to identify the kinds of functionality of ligands attached to the AuNPs.

In this research, we proposed the bio-reducing agent, ascorbic acid, to synthesise the AuNPs, when compared to AuNPs fabricated by using $NaBH_4$ as reducing agent. We studied the relationship between the reducing agents and the stability / sizes of AuNPs. It was found that, various sizes with different stabilities occurred based on various reducing agent used in this Chapter, where AuNPs of different sizes are known to produce contradictory results sometimes *in vivo* and *in vitro* bio application 00.

All these results in this Chapter, confirmed that $KAuCl_4$ could be reduced during the green synthesis of AuNPs through ascorbic acid, and the same protect ligands used in the case of $NaBH_4$. $KAuCl_4$ – $NaBH_4$ system produced small sizes with small mean deviation and indicated spherical shape. However, they were nearly poly-dispersed and poly-shaped for most process conditions when $KAuCl_4$ – ascorbic acid system was used as reducing agent. Different sizes of AuNPs usually have different bio-applications. Development of more environmentally friendly and biocompatible synthetic ways is one of the main aims to allow their use in biomedical applications. Ascorbic acid has been used as a benign naturally available reducing agent to synthesise AuNPs, due to its high water solubility, low toxicity, and biodegradability. In this review, a green synthesis methodology used in order to produce positively charged biocompatible AuNPs for possibility biomedical applications is outlined.

Keywords: Low toxicity, High water solubility, Biodegradability, Biomedical applications, Nanodevices, Drug delivery.

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Introduction

A considerable amount of gold nanoparticle (AuNP) synthetic methods havebeen reported in the literature in recent years. AuNP surface has shown to interact with various organic thiolate ligands, making them versatile nanodevices for their potential use in drug delivery, imaging and biosensing applications. Therefore, the development of more environmentally friendly and biocompatible synthetic routes is one of the main aims to allow their use in biomedical applications. One of the most used reducing agents for noble metal salt reduction is sodium borohydride, a strong and excellent hydrides source. However, other reducing agents such as sodium citrate, ascorbic acid, and tannic acid are reported to be green alternatives to sodium borohydride [1-4]. Their use represents significant progress in the development of methodologies for the green synthesis of biocompatible AuNPs in their colloidal form.

Malassis recently developed a one-step, green synthesis using biocompatible ascorbic acid. The authors used this compound

as reducing and stabilisingagent and carried out the synthesis at room temperature. They successfully synthesised different size gold (8 – 80 nm) and silver (20 – 175 nm) NPs by controlling the pH of either the metal salt solution or the ascorbic acid solution. Another example of the synthesis of NPs using ascorbic acid is the one reported [4]. They developed another green synthetic method to produce D-penicillamine-template copper NPs *via* ascorbic acid reduction. The authors aimed to use these NPs as mercury ion sensors. Cao [5] reported a methodology to synthesise silver NPs by using tannic acid, another green alternative to sodium borohydride. The authors used the tannic acid as reducing and stabilising agent, carrying out the synthesis in a water bath at a controlled temperature of 30°C. The size of NP in a range of 7 – 66 nm were observed and the samples were monodispersed, and that silver NPs coated with tannic acid showed better colloidal stability when compared to citrate stabilised silver NPs.

The chemical reduction of gold salts in the presence of a variety of stabilisers, such as e.g. donor ligands, to produce zerovalent metal colloids in aqueous or organic media is considered one of the most powerful and well known synthetic methods in this field. Ascorbic acid is a vitamin C, which participates in several biochemical reactions. As an influential part of the organism biochemistry, it has an antioxidant effect which is considered one of its best known biological functions. Ascorbic acid has been used as a benign naturally available reducing agent to synthesise AuNPs, because of its high water solubility, low toxicity, and biodegradability.

In addition, metal NPs exhibited good uniform size when using ascorbic acid [6]. On the other hand, it is observed that the absorption peak of the nano-composite without ascorbic acid was quite narrow and symmetrical, indicating a narrow size distribution of NPs. However, after the ascorbic acid is added, a wider size distribution is considered by the broadening of the absorption peak of the blue mixture which confirms the induced growth of the nanoparticles. The ascorbic acid diffuses to the surface of the NPs where electron transfer takes place causing the formation of the atoms and following growth of the clusters to NPs, the colour changed from colourless to blue.

According to Luty-Błocho it is noted that AuNPs processing with the $HAuCl_4$ – $NaBH_4$ system produced small sizes with small mean deviation and indicated spherical shape. However, they were poly-dispersed and poly-shaped for most process conditions when $HAuCl_4$–ascorbic acid system was used as reducing agent [7]. Furthermore, the amount of ascorbic acid plays a significant role in determining the size and shape of the AuNPs. AuNPs have peak wavelength between 530 and 540 nm. Sizes changes between 10 and 23 nm were measured when varied quantities of the ascorbic acid were added.

Some researchers have used sodium hydroxide with ascorbic acid to reduce gold salt, because it's addition to a concentrated aqueous solution of HAuCl4, causing neutralisation of its acidity and that as result to the rapid formation of hydroxyl-Au complexes such as ($AuCl_3(OH)$, $AuCl_2(OH)_2$, $AuCl(OH)_3$ and $Au(OH)_4$ with (pK1=8.3, pK2=7.5, pK3=6.4, and pK4=5.4) respectively as result of the progressive substitution of Cl−. The concentration of these species highly depends on the pH of the reaction mixture. In addition, it is notable that, the $HAuCl_4$ in aqueous solution consists of $[AuClx(OH)_4\text{-}x]$ when (x>2) at low pH but $[AuClx(OH)_4\text{-}x]$when(x<2) at high pH. These influences on the synthesis, structure, and property of AuNPs colloids.

In addition, according to Ziegler, Cand EychmüLler the spherical particles were generated by using of the mixture of ascorbic acid as reducing agent and sodium citrate as stabiliser ligands. Where this method proceeded, nontoxic and easy to remove stabilisers, low poly-dispersity and the large size range, which can be offered an excellent new method that can be applied in many fields of nano science [8].

Jana shown that, AuNPs can be produced by controlling their sizes by using of either strong or weak reducing agent mixture. The nucleation rate was controlled *via* varying the ratio of strong ($NaBH_4$) reducing agents and weak reducing agent (ascorbic acid), where noted $NaBH_4$ predominantly induced nucleation, while ascorbic acid induced growth. For instance, small size (<5 nm) produced as result to high ratios of $NaBH_4$ which induced rapid nucleation. In contrast, when lower ratios of $NaBH_4$ were used, AuNPs with broad size and shape distributions were produced because the nucleation rate was very slow and the may also causing NPs aggregates. The $NaBH_4$ –ascorbic acid method gives 4-10 nm AuNPs with varying concentration of the gold salt.

In this Chapter, a green synthesis methodology to produce positively charged biocompatible AuNPs for potential biomedical applications is outlined. As previously reported, the use of functionalised AuNPs in therapeutic purposes is promising. A synthesis of cationic phosphonium AuNPs was developed, by reducing gold (III) salt using ascorbic acid, a greener alternative to sodium borohydride. We also describe the synthesis of new cationic phosphonium ligand (6-thioacetylhexyl)-tris(2,4,6-trimethoxy- phenyl)phosphonium bromide (8B) and tri(p-tolyl)phosphoniohexylthiosulfate (9A) zwitterion used to functionalise the AuNPs surface in mixture H_2O / DMSO. Two previously reported phosphonium ligands, triphenylphosphoniopropylthiosulfate (4C) zwitterion, (3-thioactylpropyl)triphenylphosphonium bromide (5), tri(p-tolyl)- phosphoniopropylthiosulfate (4) and (6-thioacetylhexyl) tris(2,4,6-trimethoxyphenyl)-phosphonium (8B) were used for this work to produce the cationic phosphonium AuNPs in H_2O / DMSO with $NaBH_4$. Same NPs were also produced using ascorbic acid as reducing agent in aqueous solution.

Materials and Methods

All chemicals used were purchased from Sigma-Aldrich and Fisher Scientific Ltd. All reagents were used as received without further purification. All solutions were prepared with redistilled water. All the glassware was washed using aqua regia solution (HCl / HNO_3, 3:1), followed rinsing thoroughly with distilled water before use.

Cationic phosphonium ligand syntheses

Different techniques have been approved for synthesis various dimensions of nanoparticles and in order to functionalise their surface for improving their applications. The main challenges in developing different strategies are their low poly-dispersity with high purity [9].

Synthesis of tri(phenyl) (4C) and tri(p-tolyl) phosphoniopropylthiosulfate (4) zwitterion: Synthetic method outlined in Chapter 2.

Synthesis of (3-thioacetylpropyl)triphenylphosphonium bromide (5): Synthetic method outlined in Chapter 2.

Syntheses of the (6-thioacetylhexyl)triphenylphosphonium bromide derivatives ligands: The syntheses of (6-thioacetylhexyl) triphenylphosphonium bromide (8D), (6-thioacetylhexyl)tri(p-tolyl)-phosphonium bromide (8A) and (6-thioacetylhexyl)-tris(2,4,6-trimethoxyphenyl-phosphonium bromide (8B) are shown in Scheme 1. The compounds were synthesised *via* reaction of tri(phenyl)phosphine (1D), tri(p-tolyl)phosphine (1A) and tris(2,4,6-trimethoxyphenyl)-phosphine (1B) (3.8mmol) with approximately bromo-hexanol (15 mmol). In a round bottom flask with a reflux condenser were refluxed forfive hours in acetonitrile (0.024 mmol). A Pale yellow, brown oil and brown solid were collected, and recrystallized from diethyl ether (Reaction 1, Scheme1). The yield was 70%, 65%, and 75% respectively [10].

The resulting salts (6D), (6A),(6B) were dissolved in hydrobromic acid (0.18 mmol, 10 ml) (48%)in a round bottom under reflux five hours (Reaction 2, Scheme 1).Thena mixture of bromohexyl-tri(phenylphosphonium bromide (7D), bromohexyl-tri(p-tolyl)phosphonium bromide (7A) and bromohexyl-tri(1,3,5-trimethoxy- phenyphosphonium bromide (7B) (2 mmol) and potassium thioacetate (3 mmol) were stirring overnight at room temperature in aqueous ethanol (1:1, 10ml) (Reaction 3, Scheme 1). The compounds looked like a yellow oil, brown oil and brown precipitate respectively (8D), (8A), (8B). TLC has used to monitor the reactions by using 20% methanol: 80% DCM (dichloromethane) as a mobile phase. The (6-thioacetylalkyl)- triphenylphosphonium bromide ligands (8D), (8A), (8B) were achieved by DCM extraction of the mixture of reaction and purified by diethyl ether. The yield was 65%, 70%, and 75% respectively. The compounds (8D), (8A) and (8B) looked like a yellow oil, brown oil and white precipitate respectively,with a melting point of 8B at 206 - 208°C (Figure 1).

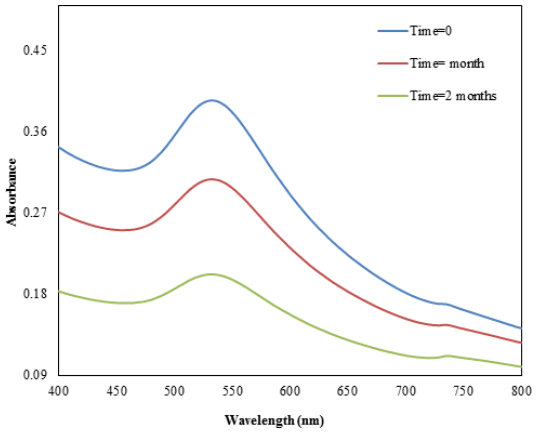

Figure 1. *UV-Vis for the stability of the 8D-AuNPs dispersed in H_2O/ DMSO, using $NaBH_4$ as reducing agentat time=0, 1 and 2 months. Where time=0 is the initial time of the frish 8D-AuNPs synthesed.*

Synthesis of tri(phenyl)phosphoniohexylthiosulfate derivatives zwitterion: The synthesis of the tri(phenyl) phosphoniohexylthiosulfate zwitterion was carried out following the reactions showed in Scheme 2. In a round bottom flask with a reflux condenser a mixture of bromohexyl-tri(phenyl)phosphonium bromide, bromohexyl-tri(P-tolyl)-phosphonium bromide (7D), (7A) (1 mmol) and sodium thiosulfate (1.5 mmol) were refluxed for five hours in aqueous ethanol (1:1, 10 ml) (Reaction 1, Scheme 2). TLC was usedin order to follow the progress of the reaction by using 10%: 90 % methanol: DCM as a mobile phase. The compounds (9D) and (9A) looked like a white precipitate with a melting point at 64-66°C and a brown oil respectively and were purified diethyl ether. The yield was 62 %, 74 % respectively.

Protecting AuNPs by synthesis of phosphonium-monolayer using protecting ligands including thioacetate and thiosulfate ligands using $NaBH_4$ reduction in (H_2O/DMSO) and comparison with ascorbic acid reduction in water

AuNPs are generally synthesized via the reduction of gold salts, nucleation and growth of metallic particles in solution. An aqueous synthesis is commonly nowadays carried out by the citrate-reduction route, which produces AuNPs with a narrow and mono-modal size distribution. By varying the reaction conditions, the average particle diameter can be controlled between about 2 – 200 nm [11].

The synthesis of functionalised AuNPs was carried out using either $NaBH_4$ or ascorbic acid as the reducing agent to promote the formation of Au0 and colloidal AuNPs.

Synthesis of cationic phosphonium gold nanoparticles by $NaBH_4$ in H_2O / DMSO: AuNPs functionalised by (4C), (5), and (4) zwitterion using $NaBH_4$ in DMSO are shown in experimental section / Chapter 2.

The triphenyl (8D), tri(p-tolyl) (8A), (6-thioacetylhexyl) tris(2,4,6-trimethoxyphenyl)-phosphonium (8B), (9D), and (9A) zwitterions were used to protect AuNPs. All these ligands have the same preparation technique. 8D, 8A, 8B, 9D, and 9A-AuNPs were prepared in DMSO 0.8 mmol, 0.25mmol, 0.3 mmol, 0.4 mmol and 0.21 mmol respectively. The volume of DMSO used for these was 30 mL. A solution of potassium tetrachloroaurate (0.12 mmol) in H_2O (10 mL) was also prepared. Then the ligands and gold salt solutions were mixed, and stirred for five hours. The reduction was carried out by adding 5 mL of a freshly prepared aqueous solution of $NaBH_4$ (2.0 mmol) to the H_2O/ DMSO mixture. In order to remove the excess of ligands in each one of the gold colloidal solutions prepared, liquid-liquid extractions were carried out using diethyl ether.

Syntheses AuNPs using ascorbic acid as reducing agent: The reduction of gold (III) salts using ascorbic acid ($C_6H_8O_6$) was a one-pot reaction using water as a solvent. Functionalised AuNPs were synthesised *via* the following procedure. The phosphonium ligands, 4C, 5, 4 and 8B (0.03, 0.022, 0.026, and 0.024 mmol, respectively)were dissolved in 25 ml deionised water under a stirring speed of 800 rpm in a 60°C water bath for two hours. After the ligands were dissolved, 0.027, 0.020, 0.021, and 0.012 mmol of $KAuCl_4$ salt dissolved in 10 ml of deionised water was added to each respective ligand solution. The reaction solutions were observed to change from colourless to yellow, and the solutions were left to stir for a further four hours until the yellow colour changed to colourless. Solutions of 0.013, 0.0089, 0.0099 and 0.0054 mmol ascorbic acid in 3 ml

of deionised water were freshly prepared and added to each of the colourless gold ligand solutions. Stirring was continued over moderate heat (40°C water bath).

Once the gold-ligand solution had stirred for approximately half an hour, 0.1M NaOH solution (1mL) was added dropwise until a permanent colour change occurred. The final solutions were left to stir overnight to encourage functionalisation of the AuNPs and no extra change in colour took place, indicating the reactions were completed.Samples were taken and analysed using UV-Vis, TEM, and DLS techniques to measure the size of AuNPs and to assess whether agglomeration had occurred between the particles [12].

Results and Discussion

AuNPs were synthesised by varying experimental conditions in this chapter. It verified their sizes combining two different methods. Both $NaBH_4$ and ascorbic acid were used as reducing agents, and different sizes were noted by using three complementary techniques including DLS, UV-Vis, and TEM. This tended to give comparable results in the case of mono-dispersed particle distributions.

Characterisation of the colloidal solutions of AuNPs using $NaBH_4$ in comparison with ascorbic acid as reducing agent via using UV-Visible and DLS studies

It is known that shape of the UV–visible spectra give different information about the size of the AuNPs [13].

By using different reducing agents, AuNPs with different diameter were prepared. UV-Vis and DLS were regularly used to measure the size and size diameter, and to see the stability of AuNPs solutions through periods of time. Comparison between two methods is discussed in this chapter and clearly explains the effect of reducing agents on the size of AuNPs prepared, and also on their stabilities. When a reducing agent such as sodium borohydride was used, smaller AuNPs with a diameter from 3-5 nm have been prepared. AuNPs of different sizes are known to produce different results which might find application *in vivo* and *in vitro* bio application.

From the previous study, metal NPs can be soluble in many solvents such as (ethanol, DCM, toluene) depending on the polarity of the capping agent such as thiol ligands, which have a great affinity for noble metal surfaces, whereas the highly stable NPs were generated. Capped NPs which can be repeatedly isolated and re-dissolved in common organic solvents without showing any signs of decomposition, such as particle growth or loss of stability.

Syntheses of AuNPs were carried out using all these ligands in this Chapter with varying results. Synthesis 4C-AuNPs, 4-AuNPs and 5-AuNPs in H_2O/DMSO by using $NaBH_4$ as reducing agent are explained in detail in our paper. The synthesis of AuNPs using 4C, 4, 5 and 8B ligands were successful with both directions, in the case of $NaBH_4$ and ascorbic acid as reducing agents. The rest of ligands were unable to dissolve in an aqueous solvent and there are unsuitable for the synthesis route using an ascorbic acid as reducing agent in order to functionalised AuNPs.

The unsuccessful syntheses formed a black bulk material solution that was unchanged by continuous stirring to the red colour. Multiple repeat syntheses were carried out for each ligand with changing condition of the experiment. Including the concentration of reactants, temperature and time of reaction. In order to eliminate the possibility of experimental error. Each synthesis was unsuccessful and resulted in the same black bulk material. This could be due to poor the solubility of the ligands in the water. AuNPs solutions were stored in the dark under ambient atmosphere and temperature and were reducingly taken for analysis using UV-Vis and DLS to determine the formation and stability of the AuNPs manufactured.

AuNPs functionalised by 8D, and 8A were produced *via* using $NaBH_4$ as reducing agent. Similar results were obtained and the peaks of absorbance were centred at 532, 523, 516 and 535 nm respectively (Figure 1).

These results were confirmed by DLS as shown in Table 1. Whereas, 8D, 8A, 9D and 9A-AuNPs sizes diameter were in the range of 32 ± 2 nm, 8.8 ± 1.6 nm, 6.9 ± 1.3 nm and 33 ± 2.2 respectively. Figures 1 displays the UV-Visible spectra of fresh AuNPs solutions (time = 0) and the same solutions after 1 and 2 months for 8D-AuNPs, also 9D-AuNPs stayed stable for 2 months . It shows 8A-AuNP stay stable for 0, 1, 2, 3, 4, 5, 6 months respectively, and 9A-AuNP stays stable for 8 weeks as well. With no sign of degradation or aggregation through DLS or UV-Visible absorption spectroscopy analysis for all these AuNPs solutions, this indicates that the particle sizes were not changed over this period.

Table 1. *8D, 8A, 9D and 9A-AuNPs solutions in H_2O/DSMO using $NaBH_4$ with the UV-Vis and DLS studies. All these types of AuNPs prepared as described in experimental section.*

Name of AuNPs	Peak wavelength (nm)	Diameter size (nm)
8D-AuNP	532 nm	32 ± 2 nm
8A-AuNP	523 nm	8.8 ± 1.6 nm
9D-AuNP	516 nm	6.9 ± 1.3 nm
9A-AuNP	535 nm	33 ± 2.2 nm

It was expected that the $NaBH_4$ synthesised AuNPs would be smaller than the ascorbic acid synthesised nanoparticles owing to $NaBH_4$ being a stronger reducing agent.

From our last work, it can be seen that, AuNPs functionalised by 4C, 5 and 4 ligands using $NaBH_4$ as reducing agent have bands centred at 519.

In contrast, as shown in the UV–Vis spectra, when the ascorbic acid is used as reducing agent, the maximum absorptions of 4C, 5,4-AuNPs are centred at 542, 575 and 557 nm respectively.

For 8B-AuNP, there was not a lot of difference between the two methods. The absorbance centred at 522 nm using $NaBH_4$ and at 528 nm using ascorbic acid as reducing agent.

The stabilities of AuNPs used ascorbic acid in this Chapter were low compared with AuNPs used $NaBH_4$. AuNPs prepared by ascorbic acid appear to have higher wavelength than for $NaBH_4$.This was also confirmed by DLS results with the AuNPs diameter being smaller when $NaBH_4$ was used as reducing agent. The most commonly weak reducing agent used to reduce many metal ions in the solution phase is L-ascorbic acid, which forms complexes with comparatively low stability constants.

It is having been identified that the reducing potential of the ascorbic acid depends on the pH of the solution. The ascorbic acid exists in the protonated form, ($AscH_2$) at pH below 4.1. With increasing of the pH, it transforms into ascorbate form, $AscH^-$. However, at pH above 11.6, it is completely deprotonated to

form, (Asc_2−).

Therefore, the ascorbic acid has different reducing power with regard pH. Spherical AuNPs can be produced with high mono-dispersity when initial pH of the ascorbic acid solution is indeed controlled. Au NPs with averages diameter of 18, 10 and 7 nm, were generated from a $HAuCl_4$ / ascorbic acid mixture with low poly-dispersity indices of 0.4, 0.3 and 0.2 at a pH of 10.2, 10.7, and 11.1 respectively. Furthermore, AuNPs were generated by using ascorbic acid as reducing agent with different size. UV-vis showed different absorbance at 540, 542, 545, 547, and 550 nm, relating to different sizes of indicating AuNPs diameter. The goal of adding NaOH into the ascorbic reaction system in our experimental was to change the acidity of AuNPs solution because AuNPs are not stable at low pH as mentioned in the literature, also study reports have clearly indicated the influence of pH on the growth of the nanoparticles.

Sau illustrated that, ascorbic acid was not enough to reduce of Au(III) ions to AuNPs and for this reason NaOH combined ascorbic as mixture reducing agent.In addition, it is noted that, ascorbic acid was a weak reducing agent and unable to reduce the silver ion in order to produce AgNPs. However, with increased the pH of the solution via adding NaOH, which increase the reducing power of ascorbate. Where this result confirmed that Ag ions are not reduced under (lower pH when using weaker reducing agent) reaction conditions.

It was proposed that in comparison between ascorbic acid and ascorbate, where the latter is a stronger reducing agent than ascorbic acid. For instance, ascorbic acid's pka is 4.1. Also, at pH higher than 4.1, most of ascorbic acid, turn into ascorbate. More ascorbate in the solution indicates faster reduction of Au ion to atomic Au on the tip facets. Usually having a pH higher than 4.1 lead to form longer Au nanorods.

It was not possible to achieve a synthesis of phenyl- and tolyl-phosphine with six carbons functionalised AuNPs in comparison with tris(2,4,6-trimethoxyphenyl) containing ligands. Using ascorbic acid became this reducing agent is not as strong as NaBH4, which was used previously. The lack of success could also be due to other factors such as alkyl chain length, monolayer quality and the nature of the substrate.

The only ligands containing tris (2,4,6-trimethoxyphenyl) phosphine derivatives were able to functionalise AuNPs. According to Fevre, MV., et al., tris(2,4,6-trimethoxyphenyl) phosphine was used as an organic catalyst for the Group Transfer Polymerization (GTP) of methyl methacrylate (MMA) and tert-butyl acrylate (tBA). Only tris(2,4,6-trimethoxyphenyl)-phosphine (TTMPP) was able to bring about the controlled GTP of both monomers at room temperature in contrast with trialkylphosphines and tributyl-phosphine. In addition, the GTP of MMA could also be performed in bulk, whilst maintaining good control over molar masses and dispersity. This is because of the moderate activity of the TTMPP catalyst compared to other nucleophiles, such as N-heterocyclic carbenes, ligands containing this group have previously been confirmed as successful.

Moreover, TTMPP is considered are good catalyst precursor in important reactions such as hydrogenation, hydroformylation, and polymerization became both oxygen and phosphorus atoms in its structure which are good donors. TTMPP is a relatively strongly basic phosphine (pKa = 11.2) compared, for instance to triphenyl- phosphine, Ph3P (pKa in CH_3NO_2=2.73). As well as, it was given an order of decreasing basicity of P(4-CH3OC6H4), > P(4-CH3C6H4), > P(4-C1C6H4)3> P(C6H5)3. Because donate groups such as a (OCH3) raising the apparent basicity's of the phosphines, and causing large changes in the basicities of these phosphines. Furthermore, modification ligands such as thiol ligands contain (methoxy-benzene groups) modified AuNPs found to be most stable compared to other thiol compounds contain (Benzene or Toluene).

Characterisation of AuNPs using transmission electron microscopy

The TEM is considered the perfect device for the characterisation of structural and chemical at the nanoscale, which can be easily producing information about nanostructured materials. The colloidal solutions of AuNPs functionalised using 8B when NaBH4was used as reducing agent. While, show 4, 8B as protecting ligands for AuNPs in case ascorbic acid used as reducing agent, whereas all analysed by TEM. Micrographs of all three AuNPs samples showed spherical or semi-spherical shaped particles. Then, particle sizes for samples TEM 1.1,TEM 1.2 and TEM 1.3 were obtained by analysing at least 150 particles per sample from several images taken [13].

It is known that, in order to determine the larger particles is formed due to the aggregation of small particles or as result to the growth of separated particles, TEM used for this reason according to the literature.

In this paper, TEM shows that most of the AuNPs are spherical in shapeand a mono-dispersional state without obvious aggregations. Furthermore, TEM image of 8B-AuNP analysis results are presented in which shows size and size distributions for 8B-AuNP when $NaBH_4$ used and were approximately 7 ± 1.2 nmin size and the shape is almost spherical as mention in the previous study. Similarly, the size of 8B-AuNP was detected by DLS 9.2 ± 0.9 nm. The average size of 8B-AuNP increased to 8 ± 1.6 nm according to TEM results when pH of the solution was changed *via* using ascorbic acid as reducing agent. The size became bigger compare in the case of $NaBH_4$ with less stability as mentioned above.

According to Sun the AuNPs prepared in aqueous solution using an ascorbic acid as reducing agent was nearly spherical and the average sizes were about 7 – 8 nm. Which also confirmed by using UV-Vis, where a strong absorption peak occurred at roughly 540 nm indicates the formation of AuNPs [8]. While, according to Khan Z monodispersed spherical gold particles prepared (shape ranging from 80 nm to 5 mm) *via* reducing Au(III) with iso-ascorbic acid at 20 °C.

It is well known that, the reduction by iso-ascorbic acid is equivalent in reducing properties with ascorbic acid but biologically is considered inactive. Furthermore, iso-ascorbic acid (an isomer) has also utilised instead of ascorbic acid. However, reports of stabilisation without any other surface functionalisation are scarce.

Similar results were collected of 8B-AuNP by DLS, where give 13 ± 1.2 nm in the case of ascorbic acid. The size was observed to increase slightly with time as illustrated in UV–Vis spectrophotometer results. However, remained almost unchanged a lot when $NaBH_4$was used. While some aggregation to larger particles occurs when ascorbic acid was used, which demonstrated big size with less stability.This may be explained due to reduce the stability of a weak reducing agent, which is not efficient enough to reduce efficiently lead to low pH as illustrated in the literature. As well as, 4-AuNPs illustrated small size when $NaBH_4$ used in comparison in the case of ascorbic acid, as observed by TEM where size was 10 ± 1 nm in the case of $NaBH_4$, and DLS also confirmed the size which was 9.6 ± 2 nm. It is found that AuNPs when produced using ascorbic acid have a narrow size distribution (31 ± 5, 36 ± 6, and 40 ± 5 nm) and can be readily stabilized *via* adjusting the initial pH of the reaction solutions.

Same situation in 8B-AuNP was noted when ascorbic acid used in case 4-AuNP, size increase to 11 ± 1.8 nm and DLS was 36.3 ± 2 nm. Aggregates, which observed after many weeks in DLS and UV results of samples taken during early stages of the preparation and weekly.

Many experiments carried out to understand effect of ascorbic acid as reducing agent through AuNPs preparation. For example, AuNPs synthesised by using ascorbic acid as a reducing agent occurred spectrum consist a single SRP band at 550 nm in the whole UV–visible region (350 – 800 nm), where the peak at 550 nm is shifted to higher wavelength nearly to 600 nm when a higher ascorbic acid concentration used. The intensity of SRP band also declines with increase in ascorbic acid concentration [14]. Kim illustrated that, ascorbic acid / $HAuCl_4$ ratio play a significant role during the growth of AuNPs [14].

In addition, as mentioned early, smaller AuNPs are normally produced by using stronger reducing agents such as $NaBH_4$, phosphorus, tetrakis(hydroxymethyl)phosphonium chloride. While, larger size particles (diameter 25–110 nm) were produced by using ascorbic acid as reductant which confirm our results in this Chapter.

According to DLS results, 4-AuNPs changed from 36.3 ± 2 nm to 50 ± 2.2 after 10 weeks in the case of ascorbic acid. 8B-AuNP changed from 13 ± 1.2 nm to 31 ± 2 after 10 weeks. However, 8B-AuNP changed from 9.2 ± 0.9 nm to 20.4 ± 1.7 nm after 11 weeks when $NaBH_4$ used. In addition, 4C-AuNP and 5-AuNP with ascorbic acid were changed from 17 ± 1.5 nm and 74 ± 3.9 nm to 39 ± 2.1 nm (after 7 weeks) and 101 ± 12 nm (after 8 weeks) respectively. AuNPs have shown larger sizes based aggregates are present after several weeks from the synthesis of AuNPs, thus confirming a low stabilising potential in ascorbic acid solution. While AuNPs are often stable for long periods of time in the case of $NaBH_4$. According to the previous study, DLS results can be less accurate in case poly-disperse or agglomerated NPs where DLS is more sensitive to bigger particles. In addition, a single TEM image may not be totally representative of the entire sample.

Moreover, literature data showed that, it is observed a very good agreement between some analysis technic used to characterise AuNPs such as the electron microscopy and disc centrifuging data. While, DLS was slightly larger diameters compared with mentioned technic, which explained to that, DLS measures the hydrodynamic diameter rather than the natural one of the AuNPs [15]. In general, DLS data presented in this Chapter were in agreement with TEM images to some extent.

Conclusion

In summary, ascorbic acid is an essential nutrient for humans and some other animal species also having antioxidant properties. It is well to know that redox chemistry of ascorbic acid plays a significant role in human nutrition as well as in the synthesis of advanced nanomaterials of noble metal. For this reason, the studies on the mechanism of redox reactions of ascorbic acid in biological systems has become important to bio-, inorganic-, and surface-chemists nowadays.

In this work, we present the size-controlled synthesis of AuNPs by chemical reduction method using ascorbic acid and $NaBH_4$ as reducing agent. The AuNPs were produced directly and simply by the reduction of Au (III) with two different reducing agents, including sodium borohydride and ascorbic acid.All of these methods can successfully produce AuNPs regardless of a type of reducing agents. This study shows that there are fundamental differences between two methodswith several different properties of AuNPs.Based on these observations, clear evidence of the reduction of gold (III) to gold (0) and formation of colloidal AuNPs were obtained, as a maximum absorbance at 519, 519 and 529 nm was observed in the corresponding UV-Visible spectra for 4C, 5 and 4-AuNPs respectively when $NaBH_4$was used.

The stability study carried out over time. According to the UV and DLS results, with a sample of the functionalised AuNPs showed that, the AuNPs synthesised by using $NaBH_4$ was stable for a long time (nearly up to 6 months), compared to 4C, 5 and 4-AuNPs used ascorbic acid as reducing agent, which stay stable maximum for 10 weeks at 542 nm, 575 and 557 nm respectively. DLS was in the range of 17 ± 1.5 nm, 74 ± 3.9 and 36.3 ± 2 respectively. With no sign of degradation or aggregation through DLS or UV-Visible absorption spectroscopy analysis where. The particles show good shape and size uniformity in addition to good long-term stability. The two reducing agents lead to the low poly-dispersity and the formation of only spherical particles.

It summarises all the results of varying the reducing agent in this Chapter. By using three complementary techniques (TEM, DLS and UV-Vis), AuNPs over a size range of 7.3 ± 2 to 9.6 ± 2 nm (diameter) were prepared by $NaBH_4$ as reducing agent. The AuNPs were associated with a larger size in the case when a weak reducing agent (ascorbic acid) was used, where size range was 13 ± 1.2 nm to 74 ± 3.9 nm.

Different preparation of colloidal AuNPs is essential to produce different sizes and offer different stabilities, because biocompatible AuNPs have increased significant attention in current years for potential applications in bio-diagnostics, and nano-medicine as result of their interesting size-dependent.

Development of more environmentally friendly and biocompatible synthetic methods is one of the main goals to contribute in biomedical applications.Green methods used to

synthesis AuNPs by using ascorbic acid as bio-reducing agent was important nowadays, especially within the biological cells due to its low toxicity.

In final, TEM, DLS, and UV characterisation suggest that AuNPs can be obtained *via* using bio-reducing agent (ascorbic acid) and $NaBH_4$ as well.

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