Research Paper

Role of C-Terminal Methylation and Side Chain Guanidinium Group of Arginine in Stabilization of Gold Nanoparticles

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The methylation of arginine plays a significant role in deciding the gene expression, however, no study is available in which differentiation between amino acids L-arginine and L-arginine methyl ester has been demonstrated on the basis of C-terminal methyl group. Though the interaction between arginine and gold nanoparticle surface has potential biological applications, the impact of arginine binding motif on gold nanoparticle surface remains poorly understood. To fully understand these binding interactions, herein, we have modified the citrate capped gold nanoparticles with L-arginine and L-arginine methyl ester. The optical properties of these particles were examined using UV-vis spectroscopy which showed absorption maxima at 518 nm and the size of the particles was found to be 14 ± 2 nm analyzed using TEM. The results presented here indicated that the stability of gold-amino acid interface is governed by the presence of free carboxyl group and cationic side chain of arginine. The amino acid and nanoparticle interactions can be used for the specific fictionalizations of the amino acid capped nanomaterials. These results may help in the specific functionalization of the amino acid capped nanomaterials that exploit surface-based activity.

Graphical Abstract
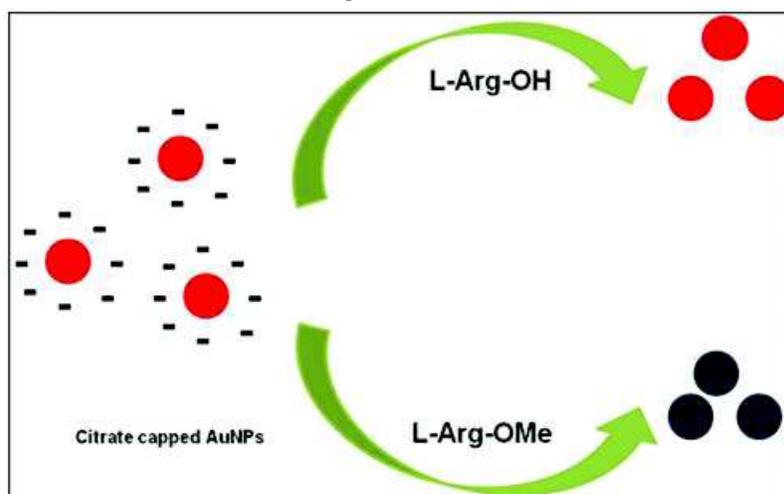
Introduction

The cationic amino acid arginine is an essential part of human body and plays a major role in the processes of metabolism and urea cycle (Morris, 2007; Morris 2002). The abnormal increment in the level of arginine results in various biological disorders such as argininemia which leads to the increase in

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concentration of ammonia making it toxic for the living organisms (Jay *et al*., 2012). Further, the modification of amino acids in histone proteins by various epigenetic changes such as acetylation, methylation and phosphorylation generally results in control of gene expressions (Handy *et al*., 2011). In this context, it has been observed that methylation of amino acid L-arginine plays an important role in deciding the gene expression (Fuhrmann and Thompson, 2015). Further, the binding interactions between nanoparticles and amino acids play a crucial role and the surface of gold nanoparticles (AuNPs) can be stabilized by amino acids. However, the colloidal stability of AuNPs in solution phase is a major challenge. The tendency of AuNPs to undergo aggregation depends on the exposure provided to the Au surface. The aggregation of AuNPs has a large impact on the physical and chemical properties of these nanoparticles in various applications. Thus, the surface modification of the AuNPs must be made in a precise way to prevent nanoparticle aggregation. For this purpose, it is important to understand the interactions between AuNPs and amino acids. Such interactions are desirable to explore the applications of AuNPs in sensing and treating diseases (Rani *et al*., 2016). The colloidal stability of AuNPs can be achieved by modifying the nanoparticle surface using physical and chemical adsorption of amino acids and their derivatives. In this context, the colloidal stability of cysteine capped AuNPs has been improved by substituting methyl group at α position of cysteine (Osante *et al*., 2014). This stability was confirmed by atomistic molecular dynamics simulations study of AuNPs capped with L-cysteine and α-methyl L-cysteine which showed the difference in the organization of amino acids with respect to AuNPs surface. It also revealed the excellent dispersibility of AuNPs in solution phase at different pH values. Also, the long-term colloidal stability of AuNPs has been achieved by capping the AuNPs capped with poly (ethylene glycol) modified amino acid L-aspartic acid (Zhan *et al*., 2015). These composites provide excellent stability in various biological conditions and also improved resistance against NaCN digestion. Further, the functionalization of AuNPs with cysteine and its dimer cystine has been studied which revealed that both cysteine and cystine undergoes different mechanisms (Acres *et al*., 2014). In the first case, the cysteinate produced upon adsorption of cysteine

on AuNPs caused aggregation of the particles due to lower coverage of cysteinate in the charged state while in the later case particles were quite stable even in the charged state.

Further, the AuNPs can be also be used in the development of a colorimetric sensor due to its exceptional optoelectronic properties (Bala *et al*., 2016). In this context, the detection of amino thiols of amino acids such as methionine, cysteine and homocysteine which are considered as biomarkers for various diseases was done using citrate capped AuNPs (Rajeshwari *et al*., 2017). Similarly, the citrate capped AuNPs have been used as a colorimetric method for the detection of an anti-thyroid drug 4-hydroxy-2-mercapto-6-methylpyrimidine (Hormozi-Nezhada and Ghayyema, 2014). In a recent report, the L-tartaric acid capped AuNPs were used in the visual differentiation between L-mandelic acid and D-mandelic acid forms (Song *et al*., 2015). However, currently, no study is available in literature where differentiation between amino acids L-arginine and L-arginine methyl ester has been demonstrated on the basis of C-terminal methyl group. Considering these facts, herein, we report the synthesis and mechanistic study of L-arginine and L-arginine methyl functionalized AuNPs and their application in differentiating the amino acids L-arginine and L-arginine methyl ester using citrate capped AuNPs.

Materials and Methods

Materials

Tetrachloroauric acid (HAuCl$_4$), tri-sodium citrate (TSC), L-Arg-OH, L-Arg-OMe dihydrochloride, L-Ala-OMe, L-Gly-OMe, diargnininemetyl ester (**4a**), histidinearargininemetyl ester (**4b**), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were obtained from Sigma, India and used as received. The ultrapure water used for the synthesis has a resistivity of 18.2 MΩ-cm. The glassware and magnetic beads was cleaned using aqua regia.

Synthesis of AuNPs

The synthesis of AuNPs was carried out using Turkevich method (Turkevich *et al*., 1951). In a typical protocol, 10 ml of 0.25 mM HAuCl$_4$ was reduced with TSC (0.2 ml, 34 mM) under boiling condition. The reaction mixture was stirred until deep

ruby red colour appeared. The suspension was further subjected to centrifugation at 9000 rpm for 30 min.

Synthesis of Amino Acid Capped AuNPs

Amino acid capped AuNPs were prepared by incubating AuNPs with amino acids (10:1 v/v) in de-ionized water (Bajaj *et al*., 2017). The resulting nanoconjugates were characterized using UV-vis spectroscopy, TEM and FT-IR.

Characterization of the AuNPs and amino acid capped AuNP

UV-vis spectroscopy

UV-visible spectrophotometer (Jasco, V-530) was used to determine the stability of amino acid conjugated AuNPs.

TEM Analysis

TEM analysis was done to determine the shape and size of the amino acid capped AuNPs. Amino acid capped AuNPs were prepared as described above and samples were adsorbed on carbon coated 300 mesh copper grid and left for 1min under air drying. After that the grid was analyzed using TEM (H-7500, Hitachi) operating at an acceleration voltage of 40 kV to 120 kV equipped with CCD camera with a resolution of 0.36 nm.

XRD Analysis

The crystallinity of the amino acid capped AuNPs was checked by X-ray diffraction (XRD; PAN alycitalX'pert Pro.) with Cu-Kα radiation (1.5418 Å) in the range 15-70 °C at 8 °C min-1 scanning speed.

FT-IR Analysis

The solid state FT-IR spectra of amino acid and amino acid capped AuNPs were obtained on a Thermoscientific, Nicolet iS50 FTIR spectrophotometer.

Results

Synthesis and Characterization of Citrate Capped AuNPs

The citrate capped AuNPs were chosen as precursor to synthesize amino acid and amino acid methyl esters

functionalized AuNPs as citrate can act as cross-linker between amino acid and nanoparticle surface (Glusker, 1980). For this purpose, initially, the citrate capped AuNPs were prepared by reducing the chloroauric acid with tri-sodium citrate at boiling temperature (Turkevich *et al*., 1951). The ruby red color suspension was obtained which indicated the formation of AuNPs and particles were purified using repeated cycles of centrifugation followed by re-dispersion in ultra pure water. The UV-vis spectrum of the resulting nanoparticles showed absorption maxima at 518 nm which corresponded to the surface plasmon resonance (SPR) which is defined as the well-known spectroscopic characteristic of metal nanoparticles and gives a sharp and intense absorption band in the visible range of AuNPs. The diameter of the spherical particles is found to be 14 ± 2 nm (Fig. S1).

Synthesis of L-arginine and L-arginine Methyl Ester Stabilized AuNPs

The amino acids, L-arginine and L-arginine methyl ester, owing to their cationic nature and biological significance were selected to study their interactions with negatively charged citrate capped AuNPs (Sethi and Knecht, 2009). Since, L-arginine capped AuNPs find use in wide range of applications as described in the introductory section, therefore, synthesis of arginine stabilized AuNPs is highly desirable. The synthesis of L-arginine and L-arginine methyl ester capped AuNPs was carried out using electrostatic approach (Bajaj *et al*., 2017). In order to achieve this, different concentrations (10-1000 µM) of amino acids were added to colloidal suspension of AuNPs individually and characterized by UV-vis spectroscopy (Fig. 1A). In the UV-vis spectra, the SPR peak observed at 518 nm for L-arginine stabilized AuNPs at all the concentrations indicated that the particles were fairly stable under this concentration range. The extra stability of these AuNPs may be probably due to the affinity of L-arginine towards the gold surface through side chain guanidinium group (Hong *et al*., 2009).

On the other hand, AuNPs prepared at higher concentration range i.e. 50-1000 µM of L-arginine methyl ester led to the formation of larger sized particles which were not stable and showed aggregation with the appearance of additional absorption band in the region of 650-700 nm (Fig.
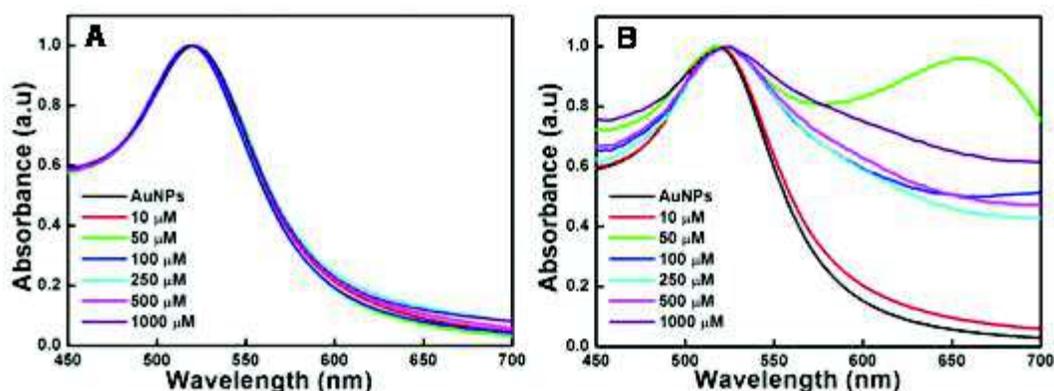
Fig. 1: UV-vis spectra of L-arginine capped AuNPs (A) and L-arginine methyl ester capped AuNPs (B)

1B). However, the particles prepared at 10 µM concentration of L-arginine methyl ester showed an absorption band corresponding to the standard AuNPs. indicating the stability of particles. On comparing the UV-vis spectra of AuNPs stabilized with L-arginine and L-arginine methyl ester, it was observed that the AuNPs were not stable beyond 10 µM concentration with respect to L-arginine methyl ester while they were found to be stable at all the concentrations with respect to L-arginine.

Further, the TEM images of both L-arginine and L-arginine methyl ester capped AuNPs were compared. It was found that L-arginine capped AuNPs (at 50 µM concentrations) showed the formation of stable, mono-dispersed and spherical

particles with a diameter of 15 ± 2 nm (Fig. 2A), whereas the particles came closer in case of L-arginine methyl ester capped AuNPs (at 50 µM concentrations) and resulted in the formation of aggregates (Fig. 2B). These observations lead to an assumption that the presence of free carboxyl group has a larger impact on the stability of AuNPs. This may be attributed to the absence of hydrogen bonding induced by the presence of methyl ester at the C-terminal of L-arginine methyl ester (Fig. 3A-B).

To ascertain the capping of arginine and arginine methyl ester onto the AuNP surface, amino acid capped AuNPs were chararterized using FT-IR. The FT-IR spectrum of L-Arginine displayed two peaks at 3275.51 and 3038.0149 cm^{-1} corresponded to N-H
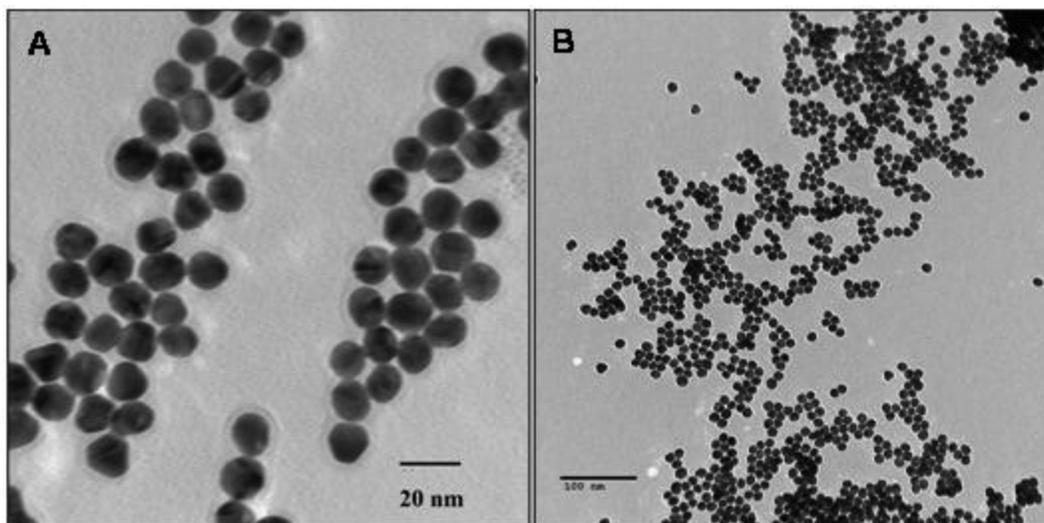

Fig. 2: TEM image of L-arginine (50 µM) capped AuNPs (A) and L-arginine methyl ester (50 µM) capped AuNPs (B)
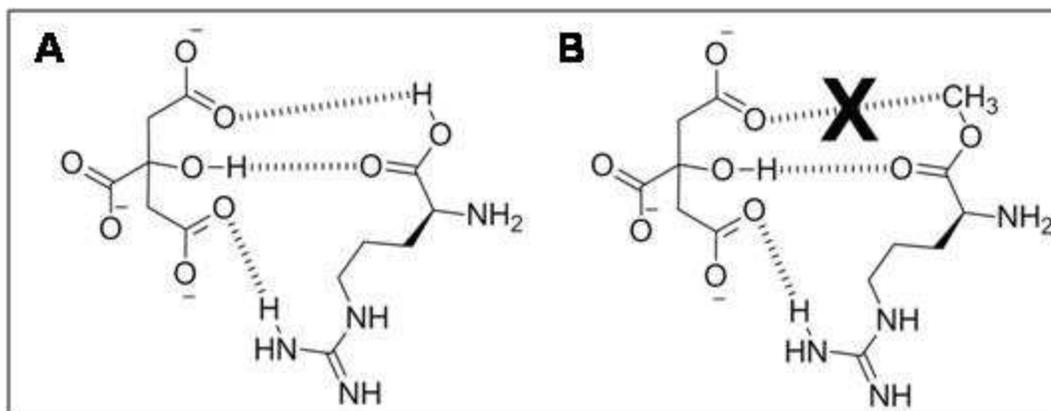
Fig. 3: Depiction of possible interactions of citrate with L-arginine (A) and L-arginine methyl ester (B) with citrate on the surface of AuNPs

stretching vibrations (Fig. 4A). However, when L-Arginine was conjugated with AuNPs, the N-H stretching peak gets brodened and gave only one band at ~3292.81 cm^{-1} (Fig. 4A) indicated successful capping of L-Arginine. On the other hand, L-Arginine methyl ester capped AuNPs did not show any band corresponding to L-Arginine methyl ester indicated that L-Arginine methyl ester was not able to stabilize AuNP surface. Furthermore, the crystalline behavior of L-arginine capped AuNPs (as representative) was analyzed using XRD in the 2θ range of 30-80º. It was observed that L-arginine capped gave peaks at 38.07º, 44.16º, 64.45º and 77.46º corresponding to different planes of AuNPs (Fig. S2) thereby confirming the crystallinity of the particles.
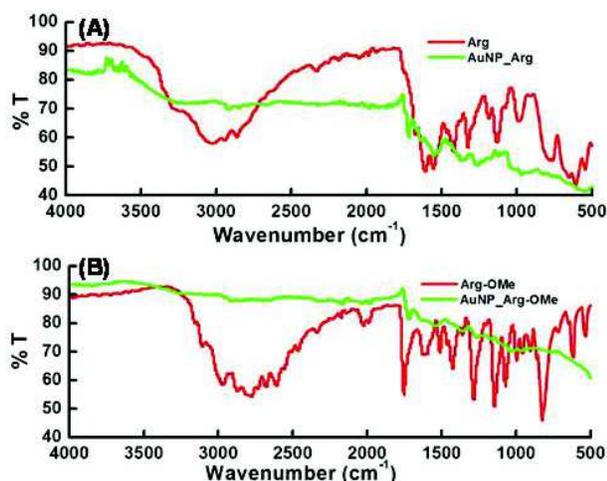
Further, to confirm the role of guanidinium group

Fig. 4: FT-IR spectra of L-arginine (A) and L-arginine methyl ester (B) capped AuNPs

of L-arginine methyl ester in stabilization of AuNPs, the investigation was carried out on amino acids i.e. L-glycine methyl ester and L-alanine methyl ester stabilized AuNPs. The synthesis of L-glycine methyl ester and L-alanine methyl ester capped AuNPs were done by varying the concentration of amino acids. The SPR peak was observed at 518 nm for both L-glycine methyl ester and L-alanine methyl ester stabilized AuNPs at all the concentrations indicating that the particles were fairly stable under this concentration range (Fig. 5A-B). These results suggested that the stability of AuNPs is dependent not only on free carboxyl group but also depend on the guanidinium group of L-arginine methyl ester.

Further to check the accuracy of this method, diarginine methyl ester (**4A**) stabilized AuNPs were synthesized in the range 10-1000 µM concentrations (Bajaj *et al*., 2017). Interestingly, it was observed that particles were stable at all the concentration which is clearly depicted from the absorption maxima corresponding to standard AuNPs (Fig. 6). These results indicate that diarginine methyl ester capped AuNPs shows high stability as compared to mono argninine methyl ester capped AuNPs. This is probably due to the fact that gold surface has strong affinity towards the guanidinium group (Hong *et al*., 2009) and diargninine methyl ester possesses two guanidinium groups for stabilization of AuNPs. These observations further underline the importance of guanidinium group in the stabilization of AuNPs.

To validate this hypothesis histidine arginine methyl ester (**4B**) capped AuNPs were synthesized in the range 10-1000 µM (Bajaj *et al*., 2017). It was
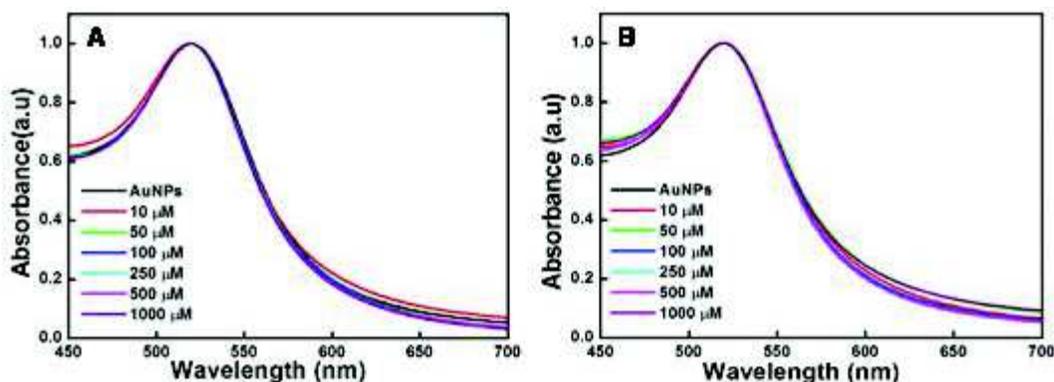
Fig. 5: UV-vis spectra of L-glycine methyl ester (A) and L-alanine methyl ester (B) capped AuNPs
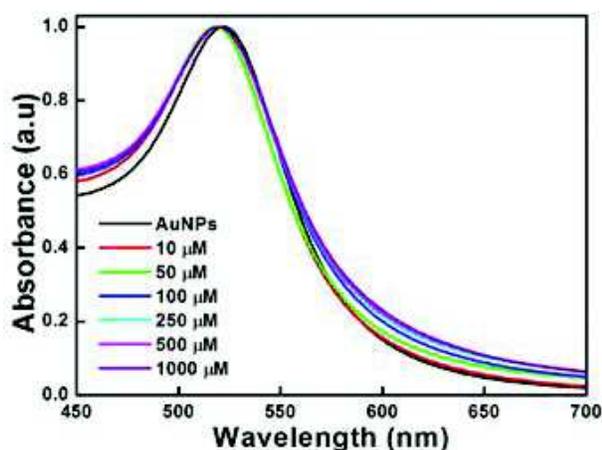

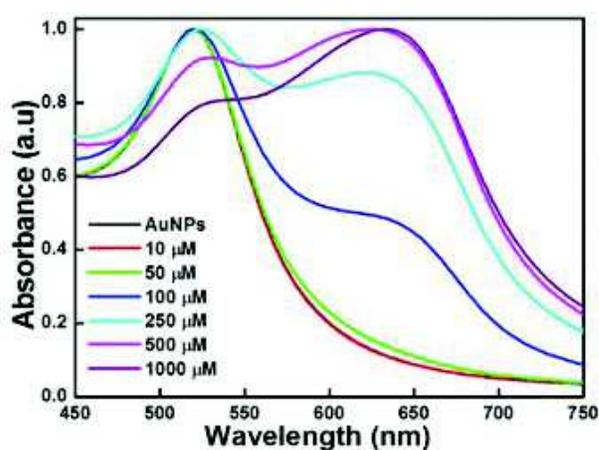Fig. 6: UV-vis spectra of diarginine methyl ester capped
AuNPs

Fig. 7: UV-vis spectra of histidinearginine methyl ester
capped AuNPs

found that particles were not stable beyond 10 μM concentrations as depicted from the additional absorption band observed at higher concentrations (Fig. 7). These results confirm the role of guanidinium group in the stabilization of AuNPs. Thus, it can be concluded that the L-arginine methyl ester induced aggregation of AuNPs depends on both C-terminal methyl group and guanidinium group.

Discussion

The abnormal increment in the levels of arginine has become a major issue and the epigenetic changes such as methylation of the amino acids in histone proteins results play a key role in control of gene expressions. Currently, no method is available in which differentiation between amino acids L-arginine and L-arginine methyl ester was done on the basis of C-terminal methyl group. So, there is growing need to

discover a tool to overcome this issue. Considering the unique optoelectronic properties of AuNPs, we proposed a citrate capped AuNPs based system that was used to differentiate between L-arginine and L-arginine methyl ester.

Initially, the citrate capped AuNPs were synthesized using Turkevich method. After that, these particles were conjugated with L-arginine and L-arginine methyl ester amino acids. For this purpose, different concentrations of amino acids were investigated for effective conjugation to AuNPs. As precise conjugation of AuNPs to amino acids needs to be ascertained, techniques such as UV-*vis* and TEM were used. It is quite evident from the UV-*vis* observation that methylation of arginine markedly affects the stability of citrate capped AuNPs (Fig. 1A-B). The stability of the nanoconjugates was also analyzed using TEM which showed the formation of

stable particles for L-arginine and aggregates in case of L-arginine methyl ester (Fig. 2A-B).This inference underlines the fact that the stability of AuNPs depends on the presence of free carboxyl group of arginine. Based on these observations, the possible binding interactions between free carboxylic group of amino acid and citrate capped AuNPs was proposed (Fig. 3A-B). It was hypothesized that the stability of AuNPs might be ascribed to the presence or absence of hydrogen bonding induced by C-terminal of L-arginine (Fig. 3A-B). The interaction of L-arginine and L-arginine methyl ester on AuNP surface was examined using FT-IR which indicated the successful capping of L-arginine on AuNPs in comparison to that of L-arginine methyl ester (Fig. 4). Further, the crystalline behaviour of the amino acid capped AuNPs remains intact which was confirmed by XRD (Fig. S2).

Further, the role of side chain of the arginine cannot be excluded. For this purpose, L-glycine methyl ester (with no side chain) stabilized AuNPs were synthesized which showed formation of stable particles suggesting that the stability of AuNPs is dependent on the side chain of arginine (Fig. 5A). To further explore the role of charged side chain, L-alanine methyl ester (with uncharged side chain) stabilized AuNPs was examined (Fig. 5B). It was found that the particles were stable at all tested concentrations thereby confirming the role of cationic side chain in the stability of AuNPs (Fig. 5B). These observations led us to assumption that stability of AuNPs depends not only on the free carboxyl group but also on the guanidinium group of L-arginine methyl ester.

Furthermore, the stability of AuNPs was investigated using diarginine methyl ester and histidine-arginine methyl ester (Fig. 6-7). It was observed that the presence of additional arginine in diarginine methyl ester enhances the stability of AuNPs (Fig. 6) as compared to arginine methyl ester (Fig. 1B). This might be due to the affinity of guanidinium group towards gold surface (Sethi and Knecht, 2009). On the other hand,when one of arginine was replaced by histidine in histidine-arginine methyl ester, the stability of AuNPs decreases which further confirmed the role of guanidinium group of arginine in stabilization of AuNPs (Fig. 7). Thus, it is safe to interpret that stabilization of AuNPs is governed by both C-terminal methyl group and guanidinium group of arginine. Thus, this methodologycan serve as a tool for the differentiation between L-arginine and L-arginine methyl ester.

Conclusion

In summary, the synthesis of L-arginine and L-arginine methyl ester capped AuNPs was demonstrated using a simple electrostatic approach. It provides a general synthetic route to differentiate between L-arginine and L-arginine methyl ester based upon the binding affinity towards gold nanoparticle surface. The L-arginine coated gold nanoparticles shows high stability at tested concentration range without any aggregation whereas L-arginine methyl ester was not stable beyond 10 µM concentrations. It indicates that the stability of gold-arginine interface is dependent on the presence of free carboxyl group of arginine. Further, to examine the role of cationic side chain of L-arginine, L-glycine methyl ester and L-alanine methyl ester capped AuNPs were prepared. These experiments suggested that the stability of AuNPs is dependent not only on free carboxyl group but also depend on the guanidinium group of L-arginine methyl ester.

This is the first report where differentiation between L-arginine and L-arginine methyl ester was done (on the basis of C-terminal methyl group), whereas most of the work done in this direction involves the differentiation between L-arginine and N-methyl-L-arginine (on the basis of side chain N-terminal methyl group). Hence, this method can be used as a tool for differentiating L-arginine and L-arginine methyl ester. This methodology was further used to exploit the surface-based activity of amino acid/peptide capped nanomaterials using specific functionalization.

Acknowledgements

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