

Preparation of isoniazid-coupled gold and silver nano-systems: Investigation on antibiotic corona and metal core reliant biological events

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ABSTRACT

In the present work, we have established a single step distinctive synthesis method to prepare metal nanoparticles by using isoniazid, wherein isoniazid works as reducing and stabilizing agent to produce monometallic gold (Au), silver (Ag) and bimetallic Au-Ag nano-systems with control over composition. Due to the occurrence of organic surface corona of antibiotic, these nano-systems can act like natural enzymes as reported by our group. Moreover, since isoniazid is first-line antibiotic agent to prevent and treat both the latent and active tuberculosis infections; these materials may be suitable for the same with enhanced efficacy. To validate our hypothesis, we have conducted *in-vitro* studies on these Au and Ag based nano-systems to scrutinize their potential peroxidase enzyme-like behaviour. Our investigations have revealed antibiotic corona and metal core reliant *in-vitro* enzyme-like behaviour.

Keywords: nano-systems, isoniazid antibiotic, surface corona, composition, enzyme-like

1 INTRODUCTION

Innovative approaches to engineer nanoparticles with desired physicochemical characteristics open new opportunities to utilise such materials for assorted applications [1]. In this context, various methods have been developed to prepare metal nanoparticles. However, most of the metal nanoparticles synthesis routes have primarily used chemicals for their synthesis and stability, which is major limiting factor for these materials in biological applications. Such chemical based synthesised nanoparticles may have potential unwanted toxic impact to biological entity. To overcome these issues, in the recent past, we and other research groups have developed synthesis methodologies to prepare Au and Ag nanoparticles by using amino acids and biological routes; and reconnoitred their potential in various applications [2-10].

Strategically, in the current research, we have further explored the competence of isoniazid antibiotic to

synthesize monometallic Au, Ag and bimetallic Au-Ag nano-systems with control over varying metal core composition and antibiotic surface corona as shown in Figure 1. Here, isoniazid works as reducing as well as stabilizing agent for these metal based nano-systems. Captivatingly, the presence of isoniazid corona on Au and Ag nano-systems may offer biological identity to these nanoparticles to perform variety of biological activities, which was confirmed by demonstrating *in-vitro* peroxidase-like behaviour of these nano-systems.

2 EXPERIMENTAL SECTION

2.1 Reagents and Materials

Tetrachloroauric acid (HAuCl₄), silver nitrate (AgNO₃), potassium hydroxide (KOH), isoniazid and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich and used as received. Deionized MilliQ water was employed to prepare various solutions and to synthesize metal based nano-systems.

2.2 Synthesis of isoniazid-coupled Au, Ag and Au-Ag nano-systems

To synthesize isoniazid-coupled Au, Ag and bimetallic Au-Ag nano-systems, 100 mL aqueous solutions with 1 mM KOH containing 0.5 mM isoniazid were allowed to boil. In the boiling solutions, [AuCl₄]⁻, Ag⁺ or both [AuCl₄]⁻ and Ag⁺ ions were added to obtain Au, Ag and three different compositions of Au-Ag bimetallic nano-systems, respectively. The total concentrations of metal ions were kept constant at 0.2 mM in all the reactions as summarised in Table 1.

2.3 Physicochemical characterization of isoniazid-coupled nano-systems

The homogeneous colloidal solutions of isoniazid synthesised Au, Ag and Au-Ag nano-systems were characterized by UV-Visible spectrophotometer (Shimadzu

3 RESULTS AND DISCUSSION

In the recent past, we have reported that amino acids can be employed as reducing agents for Au and Ag ions to create mono or bimetallic nanoparticles, respectively [2-9, 11]. Here, we further discovered the capability of isoniazid to synthesize Au, Ag and Au-Ag bimetallic of varying composition with antibiotic surface corona. Besides, the occurrence of antibiotic surface on metal nano- systems offer natural identity to these nanoparticles to perform enzymes-like behaviour, which was established by demonstrating *in-vitro* peroxidase enzyme-like behaviour.

UV-visible analysis can be used to distinguish between alloy or core-shell type of bimetallic nanoparticles. Therefore, UV-visible absorption spectroscopic analysis were carried out to confirm alloy nature of Au-Ag nano-systems over core-shell bimetallic particles as depicted in Figure 2. Core-shell nanoparticles produce two different SPR bands, wherein individual band intensities will depend on the comparative percentage of each metal existing in the system. The similar condition will rise from a dispersion comprising separate Au and Ag nanoparticles in a single system. Contrariwise, only a single SPR character will occur for metal alloys between those of two independent metal nanoparticles [9, 12, 13]. Isoniazid antibiotic reduced Au_{100}^{Iso} and Ag_{100}^{Iso} nanoparticles showed their respective SPR bands at ca. 525 nm and 405 nm, respectively. Likewise, a single absorption spectrum was identified for $Au_{75}Ag_{25}^{Iso}$ (Au-rich alloy), $Au_{50}Ag_{50}^{Iso}$ (equimolar Au and Ag alloy), and $Au_{25}Ag_{75}^{Iso}$ (Ag-rich alloy) nano-systems. Isoniazid synthesized bimetallic alloys indicated their SPR features at ca. 499 nm, 457 nm and 430 nm for $Au_{75}Ag_{25}^{Iso}$, $Au_{50}Ag_{50}^{Iso}$ and $Au_{25}Ag_{75}^{Iso}$, respectively. The clear blue shift in Au SPR features with an increase in the Ag fraction, provides an evidence of a Au-Ag bimetallic alloy formation, which is in support to previous studies [5, 9]. However, is the case of $Au_{50}Ag_{50}^{Iso}$ and $Au_{25}Ag_{75}^{Iso}$ minor additional features were observed indicating slight amount of heterogeneous population of Au and Ag nanoparticles, in respective samples.

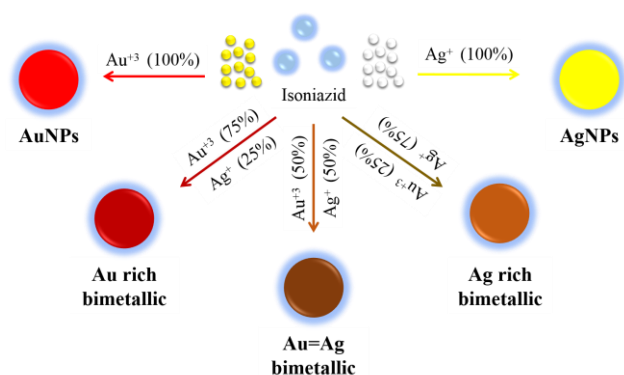


Figure 1: Schematic representation of development of isoniazid surface corona on metal nano systems with control over metal core.

Sample Name	HAuCl ₄ (mM)	AgNO ₃ (mM)
Au_{100}^{Iso}	0.20	0.00
$Au_{75}Ag_{25}^{Iso}$	0.15	0.05
$Au_{50}Ag_{50}^{Iso}$	0.10	0.10
$Au_{25}Ag_{75}^{Iso}$	0.05	0.15
Ag_{100}^{Iso}	0.01	0.20

Table 1: Relative concentration of HAuCl₄ and AgNO₃ used for the synthesis of Au, Ag and Au-Ag bimetallic nano-systems.

UV-1800) operated at a resolution of 2 nm. Zeta (ζ) Potential and dynamic light scattering (DLS) measurements on different solutions were performed at Malvern Instruments Aimil Ltd, Bangalore, India using Malvern Zetasizer Ver. 7.10 (Serial number MAL1045544)

2.3 Peroxidase enzyme-like activity

Metal core reliant peroxidase enzyme-like action of Au, Ag, and Au-Ag bimetallic nano-systems was observed using a chromogenic substrate TMB by catalysing its oxidation in the presence of H₂O₂.

In dark experimental conditions, equal volume (200 μ l) of Au and Ag based metal nano-systems were incubated with TMB and H₂O₂ for 600 sec to evaluate effect of nanoparticles metal composition on their peroxidase-like performance. It must be noted that prior to this study, all the nano-samples were normalized for 100 μ g/L concentration for Au in case of bimetallic system, whereas Ag fraction varied sequentially. Nevertheless, both monometallic Au and Ag were also used at 100 μ g/L concentration. The blue color developed as the reaction progressed over the time and it was monitored kinetically using an UV-visible spectrophotometer. The conversion of TMB substrate was measured at 650 nm and all the reactions were executed at room temperature. In the akin experimental settings, pristine isoniazid and precursor metal ions were also employed to assess their peroxidase-like activities.

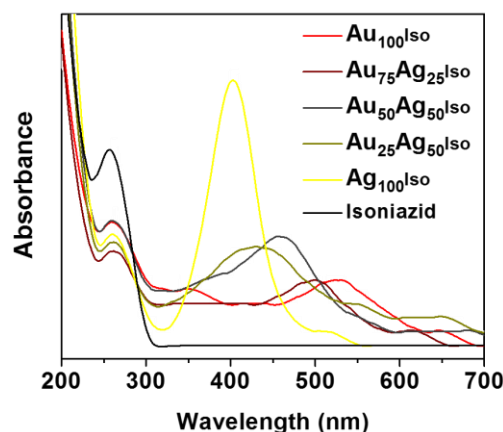


Figure 2: UV-visible absorbance spectra of pristine Au, pristine Ag and bimetallic Au-Ag nano-systems.

Sample name	Average size (nm)	ζ potential (milliVolts)
Au ₁₀₀ ^{Iso}	27.83	-38.47
Au ₇₅ Ag ₂₅ ^{Iso}	29.12	-36.10
Au ₅₀ Ag ₅₀ ^{Iso}	36.57	-37.93
Au ₂₅ Ag ₇₅ ^{Iso}	54.29	-28.70
Ag ₁₀₀ ^{Iso}	47.35	-36.70

Table 2: Average size and zeta potential valuse (surface charge) of Au, Ag and Au-Ag bimetallic nano-systems measured by DLS and Zeta potential analyser.

Average diameter of these nano-systems has been illustrated in Table 2 along with zeta (ζ) potential values. The characterization using DLS showed that all the isoniazid-coupled systems are falling in nano scale range. Wherein, Au and Au rich systems are below 30 nm in size while both Ag and Ag rich bimetallic alloys revealed average particle diameter around 50 nm. Interestingly, with the increment in the Ag fraction in alloys the average size of the particles increased and it was maximum for Au₂₅Ag₇₅^{Iso}.

The ζ potential values reflects surface charge of metal nanoparticles. The negative ζ potential values as illustrated in Table 2 were expected since these nanoparticles were stabilized with isoniazid, thus making them negatively charged at physiological pH. Remarkably, all the nano-systems possess high negative surface charge and it is -38.47, -36.10, -37.93, -28.70 and -36.70 for Au₁₀₀^{Iso}, Au₇₅Ag₂₅^{Iso}, Au₅₀Ag₅₀^{Iso} and Au₂₅Ag₇₅^{Iso} and Ag₁₀₀^{Iso}, respectively. Moreover, a high negative ζ potential value designate that these nano-systems have good stability, which is essential to avoid aggregation of these nano-systems. It is imperative to state that the solutions of isoniazid-coupled nano-systems were found highly stable under standard laboratory storage conditions at room temperature over twelve months.

After in-depth physicochemical characterization, all these materials were subjected to *in-vitro* peroxidase-like activity at room temperature. Figure 3 (Panel A and B) illustrates metal core reliant kinetics of peroxidase-like activity of pristine Au and Ag nano-systems at comparable metal concentrations. From the Panel A, it is clearly evident that Au nano-system have significantly higher catalysing activity of TMB substrate signifying its higher peroxidase enzyme-like activity. It is further interesting to notice that although both the metal ions did not possess this capacity but in the form of nanoparticulate system in conjugation with isoniazid antibiotic these materials exhibited enzyme-like activity. Isoniazid itself revealed inherent peroxidase-like, which has been imparted on metal surface during the synthesis process and therefore both Au and Ag nano-systems displayed this activity. Panel A in Figure 3, shows

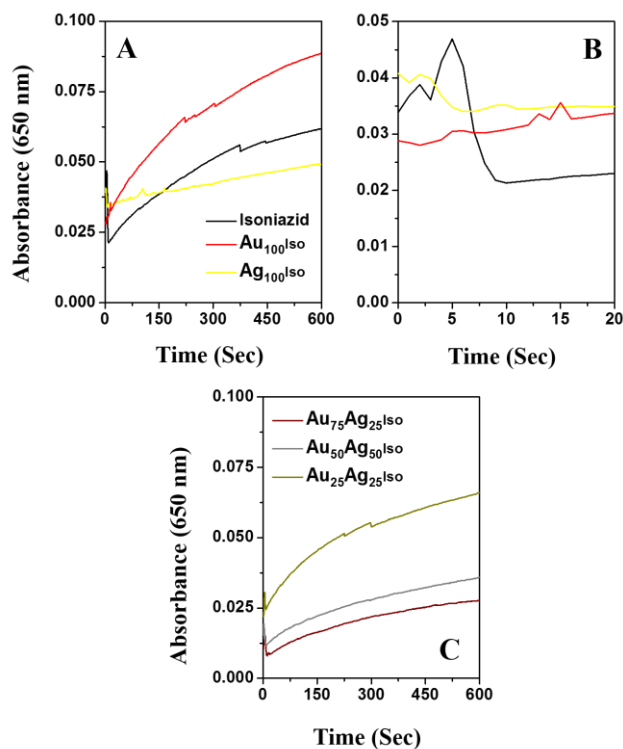


Figure 3: Peroxidase-like activities of pristine isoniazid, Au and Ag nano-systems are shown in Panel A and B; whereas, Panel C shows peroxidase-like activity of Au-Ag bimetallic nano-systems.

initial enzyme-like action of Au and Ag nano-systems. This is stimulating to notice that at the commencement to the reaction Ag nano-system has more activity compare to Au nano-system. The initial results are in support to our earlier reports [5].

Furthermore, to investigate the role of Ag in the overall activity of peroxidase-like behaviour isoniazid capped Au-Ag alloys were subjected for study under similar experimental conditions as displayed in Figure 3 (Panel C). Assessment of equivalent stoichiometric bimetallic alloys with different fraction of Ag, with respect to constant Au (as discussed in materials and method section) revealed diverse peroxidase-like activities confirming the role of Ag fraction in catalysing TMB. Here we observed that with the increasing fraction of Ag, the peroxidase enzyme-like behaviour also improved. The control experiments on metal ions revealed that they do not have any peroxidase-like activity as they could not catalyse oxidation of TMB to produce blue coloured reaction product confirming that this enzyme-like behaviour was intrinsic in nature for studied nano-systems which was introduced during their synthesis from free isoniazid and it was reliant on metal core along with surface corona.

4 CONCLUSIONS

In the present research work we demonstrate development of antibiotic surface corona on metal nanoparticles by employing isoniazid as reducing and stabilizing agent. The presence of isoniazid corona on Au and Ag based nano-systems can provide biological identity to these particles, which was confirmed by showing inherent peroxidase enzyme-like action of these materials. This behaviour was found to be dependent on antibiotic surface and metal core of the nano-system. Outcomes of this study have potential to open up new prospects toward careful design of antibiotic-functionalized metallic nano-systems, which will have significant potential to be used as new class of materials for variety of biological applications. Furthermore, it is believed that such materials may be suitable to recognise mycobacteria, particularly *Mycobacterium tuberculosis* due to the presence of isoniazid in surface corona, which need further investigations.

5 ACKNOWLEDGEMENTS

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