6.1. Introduction

For the past one decade, there has been a great deal of interest in the synthesis of biocompatible gold nanoclusters (AuNCs) using BSA as the source of protein, which act as reductant and stabilizer [1-3]. The good biocompatibility and low toxicity make these NCs as an important entity for researchers and to make them in catalysis [4], bioimaging [5,6] and optical sensing [7-9] applications.

In recent years, intensive research interest has been put into the design of new materials modified electrodes for non-enzymatic determination of hydrogen peroxide (HP). Metallic nanoparticles [10], carbon nanotubes [11], carbon dots [12,13], graphene quantum dots [14] and metal-organic framework [15] modified electrodes have been used for the determination of HP. In addition, solid-state electrochemiluminescence sensor was also developed to determine HP with the aid of AuNCs-silica nanoparticles composites modified electrode [16]. Although extensive studies have been reported in the literature for the sensing of biomolecules using the colloidal solution of AuNCs only little effort has been made for their attachment on electrodes for the electrocatalytic applications. Chen and his co-workers attached AuNCs on Au electrode through thiol based linker or drop casting method and then used for oxygen reduction reaction [17,18]. Even though, they have exploited the AuNCs for the modification of electrode no attempt is made to characterize the attached AuNCs by SEM and other techniques and also not used for other applications. Hence, the objective of the present chapter is to utilize the photoluminescent FA-AuNCs and
BSA-AuNCs studied in Chapter 4 to attach on GC electrodes and exploit the resulting AuNCs modified electrode for the non-enzymatic determination of HP.

6.2. **Attachment of red and blue luminescent gold nanoclusters on GC electrodes for the sensitive determination of HP**

6.2.1. **Characterization of BSA and FA-AuNCs**

The characterization of red luminescent BSA-AuNCs and blue luminescent FA-AuNCs by UV-vis, spectrofluorimetry and HR-TEM was discussed in Chapter 4 in detail (Section 4.3.1 and 4.2.1). Since the synthesized AuNCs were highly stable an attempt is made to attach them on GC electrode for the non-enzymatic determination of HP. The BSA-AuNCs were directly attached on GC electrode similar to BSA-AuNPs attached on GC electrode as discussed in Chapter 2 (Section 2.12). On the other hand, FA-AuNCs were attached on GC electrode via 1,6-hexadiamine (HDA) linker similar to attachment of FA-AuNPs on GC electrode (Section 2.10).

6.2.2. **Characterization of BSA-AuNCs modified GC substrates by SEM, EDS and DRS**

The morphology of the BSA-AuNCs modified GC substrate was investigated by SEM. Fig.6.1 shows the SEM images recorded at different magnifications for BSA-AuNCs modified GC plate (Fig.6.1A-C). The SEM image shows that they were spherical in shape and completely covered on GC substrate. The average particle size was found to be 22 nm (Fig.6.1D). The SEM studies confirmed the successful modification of AuNCs on GC substrate.
It has been already mentioned in Chapter 4 (section 4.3.2) that the size of colloidal BSA-AuNCs was found to be 1.8 nm. When the same AuNCs were attached on GC substrate, their size was increased to 22 nm. This suggests that AuNCs after attached on GC plate become particles.

**Fig.6.1.** SEM images of BSA-AuNCs modified GC plate recorded at different magnifications (A-C) and (D) particle size distribution.
Further, EDS analysis was used to find the elements present on the surface with their chemical compositions. Fig. 6.2A shows the EDS obtained for AuNCs on GC substrate. The peaks obtained at 2.1 and 9.7 keV are the characteristic peaks of Au and 0.39 keV is the characteristic peak of N, respectively. The presence of both Au and N peaks confirmed the successful modification of AuNCs on GC substrate. Further, the modification of AuNCs on GC electrode was characterized by diffuse reflectance spectroscopy. Fig. 6.2B shows the diffuse reflectance spectrum (DRS) of AuNCs modified GC substrate. It shows a broad absorption band around 580 nm due to the SPR band of AuNPs.

**Fig.6.2.** (A) EDS analysis and (B) DRS of BSA-AuNCs modified GC substrate.
It is well known that AuNCs does not show any characteristic absorption band in the visible region. Therefore, the appearance of the broad band around 580 nm clearly confirmed that AuNCs after attached on GC plate were changed to AuNPs.

### 6.2.3. Characterization of BSA-AuNCs modified GC substrate by XPS

Fig.6.3 shows the X-ray photoelectron spectra (XPS) of AuNCs modified GC substrate. XPS survey spectrum showed Au4f, N1s and C1s elements for AuNCs modified substrate (Fig.6.3A), which confirmed the successful modification of AuNCs on GC substrate. The nature of Au present on the AuNCs was further analyzed by deconvoluting the Au4f region by Gaussian functions after background correction. The Au4f spectrum depicts the characteristic Au4f_{5/2} and Au4f_{7/2} peaks, respectively at 86.8 and 83.1 eV with a spin-orbit coupling of 3.7 eV (Fig.6.3B). This indicates the presence of zero valency Au.

The N1s spectrum of AuNCs modified substrate was deconvoluted into two components at 398.4 and 399.4 eV and were attributed to sp^2 hybridized N atom linkage (≡N-) and free amino group (–NH₂) (Fig.6.3C). This result supported the direct attachment of AuNCs on substrate. Further, it also indicates that a few unreacted free amino groups present on their surface even after attached on GC substrate.
The C1s spectrum of the AuNCs modified GC plate was deconvoluted into three component peaks at 284.0, 286.3 and 287.9 eV (Fig. 6.3D) and were attributed to C=C, -C-N and C=O bonds on the AuNCs modified substrate. Further, the peak at 286.3 eV (C-N) was ascribed to carbon attached to the nitrogen groups, which further confirms the attachment of AuNCs on the GC substrate.

**Fig. 6.3.** XPS of GC/BSA-AuNCs substrate: (A) survey spectrum, deconvoluted spectra of (B) Au4f, (C) N1s and (D) C1s regions.
6.2.4. Characterization of GC/AuNCs electrode by cyclic voltammetry

The modification of BSA- and FA-AuNCs on GC electrode was further confirmed by cyclic voltammetry. Fig.6.4A shows the cyclic voltammograms (CVs) obtained for AuNCs modified GC electrode in 0.2 M PB solution at pH 7.2. The Au oxidation and reduction peaks were appeared at +0.93 and +0.48 V, respectively and it again confirms the successful modification of BSA-AuNCs on GC electrode. Similarly, FA-AuNCs modified GC electrode also showed the Au oxidation and reduction peaks at +0.95 and +0.50 V, respectively (Fig.6.4B). The oxidation and reduction peaks of gold oxide remain stable even after 1st and 5th cycles (a and a’). This suggests that the both BSA- and FA-AuNCs attached on GC electrode were highly stable.

Fig.6.4. CVs obtained for (A) GC/BSA-AuNCs and GC/FA-AuNCs electrodes in 0.2 M PB (pH 7.2) solution at a scan rate of 50 mV s⁻¹ (1st cycle (a) and 5th cycles (a’)).
6.2.5. Electrochemical impedance spectroscopy studies of AuNCs modified electrode

Fig.6.5 shows the Nyquist, Bode amplitude and Bode phase angle plots for bare GC, GC/BSA-AuNPs and GC/BSA-AuNCs electrodes in 1 mM K$_3$[Fe(CN)$_6$] containing 0.2 M PB solution (pH 7.2) at scanning frequencies from 0.01 to 100000 Hz. Here, impedance behavior of BSA-AuNPs modified GC electrode was also included for comparison. Fig.6.5A shows the Nyquist plots and are best fitted with Randles circuit model (Rs[C1-Rp]) (Fig.6.5A inset), where Rs refer to the solution resistance, C1 refers to the constant phase element and Rp refers to the polarization resistance. The charge transfer resistance (R$_{CT}$) values for bare GC, GC/AuNPs and GC/AuNCs electrodes were found to be 28.5, 5.68 and 3.91 kΩ respectively. The R$_{CT}$ value of AuNCs modified electrode was significantly lower than bare GC and AuNPs electrode, indicating high conductive nature of AuNCs modified electrode. Fig.6.5B exhibits the Bode amplitude plots for bare GC, GC/AuNPs and GC/AuNCs electrodes. The |Z| value for all the electrodes in the frequency range from 10$^4$ to 10$^6$ Hz is constant, suggesting that solution resistance is almost same. Fig.6.5C shows the Bode phase angle plot of bare GC, GC/AuNPs and GC/AuNCs electrodes. The bare GC, GC/AuNPs and GC/AuNCs modified electrodes show the phase angle of 77.85°, 63.35° and 57.65°, respectively. When compared to bare GC and GC/AuNPs electrodes, AuNCs modified electrode shows less phase angle value and it indicates that the electron transfer reaction was facile at this electrode. The Bode-phase plot of GC/AuNCs gives a single phase maximum, corresponding to
one time constant, i.e. one relaxation process seen on the Bode plot. Similarly, bare GC and GC/AuNPs electrodes also show same one time constant on Bode plot. Hence, the same equivalent circuit was used for all the electrodes in order to fit and analyze the EIS data.

The effective surface area was calculated by using the Randles-Sevcik equation (5.1). It was found to be $57 \times 10^{-3}$, $61 \times 10^{-3}$ and $81 \times 10^{-3}$ cm$^2$ for bare GC, GC/AuNPs and GC/AuNCs electrodes, respectively. The obtained higher effective surface area of AuNCs modified electrode was attributed to the large surface area provided by AuNCs. Further, the heterogeneous electron-transfer rate constant ($k_{et}$) for the redox probe Fe(CN)$_6^{3-/4-}$ at the modified electrode can be calculated using equation

$$K_{et} = \frac{RT}{n^2F^2} AR_{CT} C^0$$  

\hspace{10cm} (6.1)
The calculated $k_{et}$ values are $1.4 \times 10^{-4}$, $6.7 \times 10^{-4}$ and $7.4 \times 10^{-4}$ cm s$^{-1}$ for bare GC, GC/AuNPs and GC/AuNCs electrodes, respectively. The obtained higher $k_{et}$ value at GC/AuNCs electrode indicates that the electron transfer reaction was faster at this electrode than bare GC and GC/AuNPs electrodes.

6.2.6. Electrochemical reduction of HP at BSA-AuNCs and FA-AuNCs modified electrodes

Fig. 6.6 shows the CVs obtained for 0.5 mM HP at bare GC, GC/BSA-AuNCs and GC/HDA/FA-AuNCs modified electrodes in 0.2 M PB solution (pH 7.2) at a scan rate of 50 mV s$^{-1}$. The bare GC electrode does not show any response in the absence of HP (curve a).

![Graph](image)

**Fig. 6.6.** CVs obtained for 0.5 mM HP at (b) bare GC, (c) BSA-AuNCs and (d) FA-AuNCs modified GC electrodes in 0.2 M PB solution (pH 7.2) at a scan rate of 50 mV s$^{-1}$. (a) Bare GC electrode in the absence of HP.
An ill-defined shoulder wave was observed around -1.2 V for HP reduction at bare GC electrode (curve b). However, both BSA-AuNCs and FA-AuNCs modified GC electrodes showed a sharp reduction peak for HP at -0.72 V with much enhanced reduction current (curves c and d). The obtained higher reduction current was attributed to the higher effective surface area of the AuNCs modified electrodes. The reduction peak of HP was highly stable at both the AuNCs modified electrodes. Among the two AuNCs modified electrodes, FA-AuNCs modified electrode showed a slightly higher reduction current for HP. However, modification of these AuNCs on GC electrode requires a linker whereas BSA-AuNCs can be directly attached on GC electrode. Therefore, sensitive and selective determination of HP was carried out BSA-AuNCs modified electrode alone.

6.2.7. Effect of scan rate

The CVs obtained for 0.5 mM HP at GC/BSA-AuNCs electrode in 0.2 M PB solution (pH 7.2) at scan rate ranging from 10-120 mVs\(^{-1}\) are shown in Fig.6.7A. It shows that the reduction peak current of HP increases while increasing scan rate from 10 to 120 mVs\(^{-1}\). A good linearity was observed between the plot of cathodic peak current against square root of scan rate with a correlation coefficient of 0.9970 (inset), suggesting that the reduction of HP is due to diffusion controlled process at GC/BSA-AuNCs electrode.
6.2.8. Determination of HP by differential pulse voltammetry (DPV)

Fig. 6.7B shows the differential pulse voltammograms (DPVs) obtained for the reduction of HP in the presence of 5 to 100 µM at GC/AuNCs electrode in 0.2 M PB solution (pH 7.2). It shows a reduction peak at -0.65 V for 5 µM HP. The reduction peak current of HP increases while increasing its concentration from 10 to 100 µM HP. The plot of peak current against concentration of HP linearly increases with a correlation coefficient of 0.9951 (inset). These results indicate that the GC/AuNCs electrode is highly suitable for the determination of HP.

6.2.9. Amperometric determination of HP at GC/AuNCs electrode

Fig. 6.8A shows the amperometric i-t curve for the reduction of HP at GC/AuNCs electrode in a homogeneously stirred 0.2 M PB solution (pH 7.2) by applying a constant potential of -1.0 V. Addition of 0.5 µM HP in each step with
a time interval of 50 s, the current response increases and the steady state current response was obtained within 3 s. The dependence of response current on the concentration of HP was linear from $0.5 \times 10^{-6}$ M to $4.0 \times 10^{-6}$ M with a correlation coefficient of 0.9958. The amperometric measurements were also carried out for a wide range concentration of HP. **Fig.6.8B** depicts the amperometric i-t curve for HP at GC/BSA-AuNCs electrode in a homogeneously stirred PB solution (pH 7.2). The modified electrode showed the initial current response due to 0.05 µM HP and further addition of 0.1 µM HP into the same solution with an interval of 50 s, the current response was increased and a steady state current response was attained within 3 s.

**Fig.6.8.** Amperometric i-t curve for the determination of HP at GCE/BSA-AuNCs in 0.2 M PB (pH 7.2) solution at 50 s interval. (A) Each addition increases the concentration of 500 nM of HP and (B) each addition increases the concentration of (a) 50, (b) 100, (c) 250, (d) 500, (e) 750, (f) 1000 nM, (g) 2.0, (h) 5.0, (i) 7.5 and (j) 10 µM HP. Insets: Plots for current versus concentration of HP (A and B).
The current response was increased for further addition of 0.25, 0.5, 0.75, 1.0, 2.0, 5.0, 7.5 and 10 µM HP to the same solution with a time interval of 50 s. The amperometric current was increased linearly with increasing HP concentration from 5.0×10⁻⁸ M to 1.0×10⁻⁵ M with a correlation coefficient of 0.9926 and the LOD was found to be 1.7×10⁻¹⁰ M (S/N=3). The sensitivity of GC/BSA-AuNCs electrode was found to be 50.3 μA μM⁻¹.

<table>
<thead>
<tr>
<th>Electrode system</th>
<th>pH</th>
<th>Linear range</th>
<th>LOD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCE/Au/PtNPs</td>
<td>7.2</td>
<td>100×10⁻⁹-50×10⁻⁶ M</td>
<td>60×10⁻⁹ M</td>
<td>10</td>
</tr>
<tr>
<td>GCE/MOF/NPs</td>
<td>7.2</td>
<td>1.0×10⁻⁶-5.0×10⁻³ M</td>
<td>67×10⁻⁹ M</td>
<td>15</td>
</tr>
<tr>
<td>CNTs/MnO₂</td>
<td>7.8</td>
<td>1.2×10⁻⁶-1.8×10⁻³ M</td>
<td>8×10⁻⁷ M</td>
<td>19</td>
</tr>
<tr>
<td>GCE/CQD/Na/Cu₂O</td>
<td>7.4</td>
<td>5.0×10⁻⁶-5.3×10⁻³ M</td>
<td>2.8×10⁻⁶ M</td>
<td>20</td>
</tr>
<tr>
<td>GCE/CDs/Co-Fe</td>
<td>7.0</td>
<td>0.1×10⁻⁶-23.1×10⁻⁶ M</td>
<td>4.0×10⁻⁸ M</td>
<td>21</td>
</tr>
<tr>
<td>GCE/GQD/NPs</td>
<td>7.4</td>
<td>0.25×10⁻⁶-1.0×10⁻³ M</td>
<td>12.0×10⁻⁸ M</td>
<td>22</td>
</tr>
<tr>
<td>GCE/AuNCs/SiNPs</td>
<td>LiClO₄ medium</td>
<td>6.5×10⁻⁶-32.6×10⁻⁶ M</td>
<td>-</td>
<td>23</td>
</tr>
<tr>
<td>GCE/BSA-AuNCs</td>
<td>7.2</td>
<td>5.0×10⁻⁸-1.0×10⁻⁵ M</td>
<td>1.7×10⁻¹⁰ M</td>
<td>This work</td>
</tr>
</tbody>
</table>

**Table 6.1.** Comparison of various modified electrodes for the determination of HP with GC/BSA-AuNCs modified electrode
The wide range of determination and LOD obtained for HP at GC/AuNCs electrode was compared with the reported papers [12-18] (Table 6.1). It can be seen from Table 6.1 that the present modified electrode showed the lowest LOD with wide concentration range determination of HP compared to the reported papers. Besides, the present modification of electrode is simple and reproducible.

6.2.10. Effect of interferences

The determination of HP in the presence of common and physiological interferences such as uric acid, glucose, ascorbic acid, Na$^+$, Zn$^{2+}$, Ni$^{2+}$, Cl$^-$, NO$_3^-$ and SO$_4^{2-}$ was studied at GC/AuNCs electrode by amperometry. Fig.6.9A shows the amperometric i-t curve obtained for HP at GC/AuNCs electrode in the presence of above interferences in 0.2 M PB solution (pH 7.2). The increased initial current response was due to the addition of 0.5 µM HP (a). While adding 0.5 mM each uric acid, glucose and ascorbic acid (b-d) to the same solution the current response was not increased. However, the current response was increased similar to the early step after the addition of 0.5 µM HP to the same solution. Further addition of 0.5 mM each Na$^+$, Zn$^{2+}$ and Ni$^{2+}$ (e-g) to the same solution caused no change in current response. Similarly, the addition of 0.5 mM each Cl$^-$, NO$_3^-$ and SO$_4^{2-}$ (h-j) did not change the current response. These results indicated that the determination of 0.5 µM HP is possible even in the presence of 1000-fold excess of common and physiological interferences.
6.2.11. Real sample analysis

The practical application of GC/BSA-AuNCs electrode was utilized by determining the concentration of HP in human blood serum samples (Fig.6.9B). The human blood serum sample was diluted to 50 times using 0.2 M PB solution (pH 7.2). The DPV of human blood serum sample does not show any reduction peak in the potential window of HP studied (curve a). However, after the addition of 10 µM HP in human blood serum, the reduction peak was appeared at -0.52 V due to the reduction of HP (curve b). Further increasing the concentration of HP to 20 µM, again the current response was increased (curve c). The corresponding results are given in Table 6.2. The obtained good recovery results suggest that the GC/BSA-AuNCs electrode can be utilized for the determination of HP in human blood serum sample.

![Graph A](image1.png)

![Graph B](image2.png)

**Fig.6.9.** (A) Amperometric determination of HP in the presence of interferents. (a) 0.5 µM HP, 0.5 mM each (b-d) UA, glucose, AA and (e-g) Na⁺, Zn²⁺ and Ni²⁺ and (h-j) Cl⁻, NO₃⁻ and SO₄²⁻. (B) DPVs obtained for (a) human blood serum and after the addition of (b) 10 and (c) 20 µM HP to human blood serum sample at GC/BSA-AuNCs electrode in 0.2 M PB (pH 7.2) solution.
The stability of the GC/AuNCs modified electrode was examined by recording DPVs for every 15 min interval in 0.2 M PB solution containing 20 µM HP. It was found that the reduction current of HP remains almost identical with a relative standard deviation of 1.8% (±0.07%) for 5 times repetitive measurements indicating that the modified electrode has a good stability. Further, three different GC electrodes were modified with AuNCs and their response towards the reduction of HP was tested by 5 repeated measurements. The reduction peak current of HP for the three independent modified electrodes showed a relative standard deviation of 2.5% (±0.09%), confirming that the results are reproducible.

### Table 6.2. Determination of HP in human blood serum samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added (µM)</th>
<th>Found (µM)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>10</td>
<td>10.12</td>
<td>101.2</td>
<td>0.536</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.0</td>
<td>100.0</td>
<td>0.258</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.91</td>
<td>99.7</td>
<td>0.852</td>
</tr>
</tbody>
</table>

*aThree replicate measurements*

6.2.12. Stability and reproducibility of the GC/AuNCs electrode

The stability of the GC/AuNCs modified electrode was examined by recording DPVs for every 15 min interval in 0.2 M PB solution containing 20 µM HP. It was found that the reduction current of HP remains almost identical with a relative standard deviation of 1.8% (±0.07%) for 5 times repetitive measurements indicating that the modified electrode has a good stability. Further, three different GC electrodes were modified with AuNCs and their response towards the reduction of HP was tested by 5 repeated measurements. The reduction peak current of HP for the three independent modified electrodes showed a relative standard deviation of 2.5% (±0.09%), confirming that the results are reproducible.

6.3. Electrochemical reduction of nitrite ion at FA-AuNCs and BSA-AuNCs modified electrodes

Since determination of nitrite ion using BSA-AuNPs modified electrode has been successfully carried out in the previous chapter, similar nitrite ion
sensing studies are also carried out BSA-AuNCs and FA-AuNCs. Fig.6.10 shows the LSVs obtained for GC/HDA/FA-AuNCs electrode in the absence and presence of 0.5 mM nitrite ion. The GC/HDA/FA-AuNCs electrode in 0.2 M PB solution shows a small oxidation peak due to gold oxide at 0.97 V (curve a) in the absence of HP.

![Graph](image)

**Fig.6.10.** LSVs obtained for (a) absence and (b) presence of 0.5 mM NaNO₂ at GC/HDA/FA-AuNCs electrodes in 0.2 M PB solution (pH 7.2) at a scan rate of 50 mV s⁻¹.

On the other hand, it shows an oxidation current at 1.0 V for the oxidation of nitrite ion with 11 µA current (curve b) as well as it shows good stability against nitrite ion oxidation.

**Fig.6.11** shows LSVs obtained for GC/BSA-AuNCs electrode in the absence and presence of 0.5 mM nitrite ion. The GC/BSA-AuNCs electrode in 0.2 M PB solution shows the small oxidation peak due to gold oxide at 1.03 V.
(curve a) in the absence of HP. On the other hand, it shows an increased oxidation current at 1.1 V for the oxidation of nitrite ion with 10 µA current (curve b) as well as it also shows good stability against nitrite ion oxidation.

Fig.6.11. LSVs obtained for (a) absence and (b) presence of 0.5 mM NaNO₂ at GC/BSA-AuNCs electrodes in 0.2 M PB solution (pH 7.2) at a scan rate of 50 mV s⁻¹.

From the above results, it is concluded that FA-AuNCs modified electrode showed higher electrocatalytic activity than BSA-AuNCs modified electrode but it was less than that of BSA-AuNPs modified electrode.
6.4. Conclusions

The present study demonstrated the successful modification of AuNCs on GC electrode and their electrocatalytic activity towards the reduction of HP. The SEM and DRS studies revealed that the luminescent AuNCs became AuNPs after attached on GC substrate. The CV studies indicated that the attached AuNCs were highly stable on GC electrode. Further, the electrochemical impedance studies showed that the conductivity of BSA-AuNCs modified electrode is higher than BSA-AuNPs modified electrode. Further, the BSA-AuNCs modified electrode showed higher electrocatalytic activity towards HP by not only shifting its reduction potential towards less positive potential but also enhanced its reduction current in contrast to bare GC electrode. The higher electrocatalytic activity was ascribed to the large surface area provided by AuNCs. The amperometric current linearly increased when HP concentration was increased from 5.0×10^{-8} M to 1.0×10^{-5} M and the LOD was found to be 1.7×10^{-10} M (S/N=3). Further, the modified electrode showed excellent selectivity towards HP even in the presence of 1000-fold common interferences. The practical application of the present AuNCs modified electrode demonstrated by determining HP in human blood serum samples. This is the first report in which AuNCs have been directly attached on GC electrode and utilized for the sensitive and selective determination of HP.
6.5. References