CHAPTER 4

NON–ENZYMATIC SENSING OF SALIVARY THIOCYANATE IONS USING Ag@Cu BIMETALLIC NRDs MODIFIED ELECTRODES

4.1 INTRODUCTION

Thiocyanate is present in organism metabolites, pesticide residues and industrial waste waters.\textsuperscript{109-111} It is a normal constituent in human saliva, urine and serum which are introduced through vegetables, including cabbage and cauliflower and as a drug for thyroid treatment and arterial hypertension.\textsuperscript{112-117} The concentration of SCN\textsuperscript{–} depends upon the nature of food habits which is between 0.5 and 2 mM with an average level of 1 mM.\textsuperscript{118} Besides, cigarette smoking habit is a significant resource of increasing SCN\textsuperscript{–} in the human body. HCN is a potentially toxic substance generating in the cigarette smoke by the combustion of nitrogen containing compounds in tobacco, which inhibits the activity of the enzyme cytochrome oxidase.\textsuperscript{119,120} During the practice of cigarette smoking, dissolved HCN gas in saliva is metabolized to SCN\textsuperscript{–} through sulfuration by the enzyme mitochondrial rhodase with thiosulphate.\textsuperscript{121-124} It is schematically shown in Scheme 4.1 A. The concentration of SCN\textsuperscript{–} stood increased up to 6 mM in human saliva due to the heavy practice of cigarette smoking. Thus, the increased concentration of SCN\textsuperscript{–} did affect by the following pathways like interfering in the secretion of iodine by the thyroid glands, increasing the risk of cancer, exerting adverse cardiovascular hazards and affecting the protein dialysis in the human body.\textsuperscript{125-128} These factors validate the importance of an exact and direct determination of the trace amount of SCN\textsuperscript{–} in water, food or in biological
samples. Thereby, the detection of SCN$^-$ in human saliva has been considered as a biomarker for the habit of cigarette smoking.

**Scheme 4.1** A: Biochemical detoxification of HCN followed by the generation of thiocyanate (SCN$^-$) in human saliva. B: Electrooxidation of the generated SCN$^-$ at the Ag@Cu bimetallic NRDs electrode.

Different methods have been developed for the sensing of SCN$^-$ such as chromatography, ion selective electrodes, electrophoresis, spectrofluorometry, spectrophotometry, polarography, amperometry, FTIR spectroscopy and colorimetry. On the other hand, most of these methods were laborious and time consuming. Organometallic compounds and metal porphyrins were used as an electrochemical sensors for SCN$^-$. An ion selective potentiometric sensor using crown ether was developed for the selective sensing of SCN$^-$ with the
detection limit of 0.03 μg ml\(^{-1}\). Kinetic spectrophotometry technique was used for the SCN\(^{-}\) sensing with the detection limit 0.016 μg ml\(^{-1}\). Compared to all these methods in the study of SCN\(^{-}\), metallic NPs based electrochemical sensors show better sensitivity, selectivity and linear range. Nano–silver coated multi–walled carbon nanotubes modified GCE was developed and used for the detection of SCN\(^{-}\). An amperometric sensor with silver NPs modified electrode was prepared for the determination of SCN\(^{-}\). Sulfadimidine metal complexes modified electrodes and ErBa\(_2\)Cu\(_3\)O\(_7\) modified electrodes were constructed for the sensing of SCN\(^{-}\). Mostly, in electrochemical sensors, SCN\(^{-}\) determinations were studied based on the complex formation of SCN\(^{-}\) with metallic NPs where the oxidation potential of metal NPs shifted with respect to the complex formation. On the other hand, the direct determination of SCN\(^{-}\) without any complex formation is very limited. Gold NPs decorated multi–walled carbon nanotubes modified carbon paste electrode was used for the detection of SCN\(^{-}\). In the present investigation, the electrooxidation of SCN\(^{-}\) will be discussed without any complex formation using Ag@Cu bimetallic NRDs modified GCEs. It is schematically represented in Scheme 4.1 B.

Now–a–days, either alloy or core–shell bimetallic NPs were commonly used in sensing applications. In the present work, we have directly grown Ag@Cu bimetallic NRDs on the GC electrode surface by simple and an efficient seed mediated growth method. The synergistic effect would be generated due to an interaction between the neighbouring metal atoms such as Ag and Cu in the bimetallic NRDs which enables high catalytic activity for the direct determination of SCN\(^{-}\) in a sub micromolar concentration with better reproducibility and stability. Importantly, the performance of the sensor subjected to SCN\(^{-}\) oxidation was studied in real sample such as human saliva samples collected from cigarette smokers and
non-smokers. In addition, the interference of several cations and anions was studied in the SCN\(^-\) determination.

### 4.2 RESULTS AND DISCUSSION

#### 4.2.1 Surface characterization

SEM analysis and EDX measurements were carried out to study the surface morphology and elemental composition at Ag@Cu bimetallic NRDs decorated electrode. Figure 4.1 shows the SEM images of the synthesized Ag@Cu bimetallic NRDs at different modified electrodes. It indicates the presence of linear NRDs and few triangular shaped NRDs with the average size distribution (diameter) of 42 ± 2 nm. Triangular NRDs are the transient structures formed in between the growth of nanospheres into linear NRDs.\(^{74}\)

![SEM images of Ag@Cu bimetallic NRDs at different modified electrodes.](image)

Both linear and triangular NRDs exist on the electrode surface were specifically studied and confirmed the existence of Ag and Cu bimetallic structures by EDX measurement (Figure 4.2). Thus, SEM and EDX
measurements satisfactorily elucidated the presence of Ag@Cu bimetallic NRDs on the electrode surface.

![Figure 4.2 EDX pattern of Ag@Cu bimetallic NRDs modified electrode.](image)

4.2.2 Electrooxidation of SCN$^-$ at Ag@Cu bimetallic NRDs modified electrode

CV was used to study the electrochemical response of SCN$^-$ at the modified GC electrode. Figure 4.3 shows the comparative CVs of bare GCE (a), Ag$_{\text{seeds}}$ modified GCE (b), and Ag@Cu bimetallic NRDs modified GCE in the presence (c) and the absence (d) of 1 mM SCN$^-$ in PB. Ag@Cu bimetallic NRDs modified electrode shows an intense anodic peak (c) due to the electrooxidation of SCN$^-$ at 0.91 V, whereas no observable anodic peak was noticed in the absence of SCN$^-$ (d) or in the presence of SCN$^-$ at Ag$_{\text{seeds}}$ modified GC (b) electrode.
In the absence of any complex formation, the oxidative peak potential for SCN$^-$ would be appeared around 0.9 V.$^{147,154}$ It is coinciding with the present investigation where the electrooxidation of SCN$^-$ is achieved without any complex formation. Compared to bare GCE, Ag@Cu bimetallic NRDs modified electrode shows a two-fold enhancement in the anodic peak current towards SCN$^-$ electrooxidation with a sharp decrease in the overpotential of 400 mV. Either the smaller size distribution or high inter-distance between Ag$_{seeds}$ would be the limiting factor which prevents the oxidation process at Ag$_{seeds}$ modified electrode. In seed mediated growth approach where Ag$_{seeds}$ have a capacity to grow as bimetallic NRDs in the presence of Cu which resulted in a synergistic effect in increasing its active surface area and

Figure 4.3 CV responses of bare GCE (a), GCE/Ni/Ag$_{seeds}$ (b) and GCE/Ni/Ag@Cu bimetallic NRDs in the presence (c) and absence (d) of 1 mM KSCN in 0.1 M PB (pH 6) at a scan rate of 50 mV/s.
sensitivity. This in turn executes the enhancement in the electrooxidation of SCN\textsuperscript{−}. Equation (4.1) shows the oxidative reaction of SCN\textsuperscript{−} in aqueous medium.

\[
\text{SCN}^- + 4\text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + \text{CN}^- + 8\text{H}^+ + 6\text{e}^-
\]  \hspace{1cm} (4.1)

4.2.3 pH optimization

The PB was used as the supporting electrolyte for the electrooxidation of SCN\textsuperscript{−}. The pH of PB tends to influence SCN\textsuperscript{−} electrooxidation. Figure 4.4 shows the electrooxidative current response towards 1 mM SCN\textsuperscript{−} between pH 2 and 9. It infers that there is a linear increase in the SCN\textsuperscript{−} electrooxidation current from pH 4 to 6. Afterwards, a steady decrease was observed for increasing pH from 6 to 9. Hydroxide ions might interfere and generate as oxide layers on the electrode surface which reduces the current response at more basic condition. In acidic condition SCN\textsuperscript{−} protonation occur, which reduced the current response.\textsuperscript{148} Thus the maximum oxidative current response for SCN\textsuperscript{−} occurs at pH 6. Hence, the supporting electrolyte was optimized with pH 6 for the electrooxidation of SCN\textsuperscript{−}. On the other hand, there was no change in the oxidative peak potential toward SCN\textsuperscript{−} electrooxidation with respect to pH variation.
Figure 4.4 The oxidative peak current response obtained at 0.91 V toward the electrooxidation of 1 mM KSCN at different pH between 2 and 9 in 0.1 M PB at Ag@Cu bimetallic NRDs modified GCE.

4.2.4 Effect of varying concentration

CV was used to study the effect of [SCN$^-\$]. Figure 4.5 shows the CV responses for [SCN$^-\$] between 1 mM and 9 mM in PB at Ag@Cu bimetallic NRDs electrode which infers that the oxidation current stood linearly with increased concentration of SCN$^-\$. Figure 4.5(inset) shows a linear relationship with respect to the [SCN$^-\$] vs. current response with an equation, $I_{pa} = 76.4 \ [\text{SCN}^-] \ (\text{mM}) + 10.66 \ (R^2 = 0.9859)$. CV is an efficient tool to study between the low and high concentration of the analyte with utmost accuracy. Three different concentrations of SCN$^-\$ (1 μM, 0.01 μM and 0.001 μM) were investigated using freshly prepared Ag@Cu bimetallic NRDs electrode for studying its sensitivity such as detection limit.
Figure 4.5 CVs of $[\text{SCN}^-]$ at GCE/Nf/Ag@Cu bimetallic NRDs modified electrodes in 0.1 M PB (pH 6.0) $[\text{SCN}^-] = 1$ mM (a), 3 mM (b), 5 mM (c), 7 mM (d) and 9 mM (e)]. Scan rate: 50 mV/s. The inset figure shows the plot of $[\text{SCN}^-]$ vs anodic peak current.

Figure 4.6 shows the CV response in the absence (a) and presence (b) of 10 nM SCN$^-$ in PB at Ag@Cu bimetallic NRDs electrode. A significant response for the SCN$^-$ determination was achieved with a low detection limit of 10 nM with S/N=3. In order to validate this result, CA analysis was carried out with the detection limit of 10 nM SCN$^-$ at Ag@Cu bimetallic NRDs electrode. It seems that the voltammetric results were justified by CA analysis.
Figure 4.6 CV responses at the modified electrode in the absence (a) and presence of 10 nM SCN⁻ (b) in 0.1 M PB (pH 6.0).

4.2.5 Effect of scan rate

The influence of scan rate toward the electrooxidation of SCN⁻ at Ag@Cu bimetallic NRDs modified electrodes was investigated. Figure 4.7 shows the CV response of Ag@Cu bimetallic NRDs modified GCE in the presence of 1 mM SCN⁻ in PB (pH 6) at different scan rates from 0.01 to 0.1 V/s. Figure 4.7(inset) shows the plot of the anodic peak currents vs. square root of scan rate which infers that the oxidation current increases with respect to the square root of scan rate with a linear regression equation, $I_{pa} = 176.56 (V s^{-1})^{1/2} + 1.283$ ($R^2 = 0.9849$). It indicates that the electrooxidation of SCN⁻ at Ag@Cu bimetallic NRDs modified electrode follow a diffusion—controlled process. Further, the total number of electrons transferred in the
electrooxidation of SCN$^-$ was estimated using the equation for irreversible systems. Hence, the number of electrons transferred is calculated as 5.4 using the diffusion coefficient as $1.38 \times 10^{-5}$ cm$^2$ s$^{-1}$ for SCN$^-$. 

**Figure 4.7** CVs recorded at GCE/Nf/Ag@Cu bimetallic NRDs in the presence of 1 mM KSCN in 0.1 M PB (pH 6) at 0.01 (a), 0.03 (b), 0.05 (c), 0.07 (d), and 0.09 (e) V s$^{-1}$. The inset figure shows the plot of the anodic peak currents vs. square root of scan rate.

The interference studies of various cations and anions were carried out in the presence of 5 μM SCN$^-$. Three order or four order higher concentrations of different cations such as Li$^+$, Na$^+$, K$^+$, Mg$^{2+}$ and Ca$^{2+}$ or anions such as PO$_4^{3-}$, SO$_4^{2-}$, NO$_3^-$, Cl$^-$ and CO$_3^{2-}$ were added and analyzed. In general, most of these cations and anions do exist as electrolytes in real
human saliva samples. Figure 4.8 shows the amperometric response of 10 mM concentrations of different cations and anions in the presence of 5 μM SCN⁻.

![Graph showing amperometric response](image)

**Figure 4.8** CA responses of GCE/Nf/Ag@Cu bimetallic NRDs for 10 mM different interfering cations and anions in the presence of 5 μM SCN⁻. 1 μM and 10 μM SCN⁻ were spiked before and after the addition of interfering ions.

In order to differentiate the CA signals between the inferences ions and SCN⁻ determination, 1 μM and 10 μM SCN⁻ were spiked after 40 s and 280 s, respectively. Noticeable amperometric current responses were recorded by spiking 1 μM or 10 μM SCN⁻ whereas no appreciable increase was observed with respect to the addition of different cations and anions. Thus, it clearly shows that the aforementioned ions didn’t interfere in the electrooxidation and in the accurate determination of SCN⁻ at the Ag@Cu
bimetallic NRDs modified electrode. Table 4.1 compared the Ag@Cu bimetallic NRDs based sensor toward SCN⁻ assay with the existing methods.

**Table 4.1** Comparative studies between Ag@Cu bimetallic NRDs modified electrode based sensor and other existing methods toward the linear range and detection limit in the determination of SCN⁻.

<table>
<thead>
<tr>
<th>Method</th>
<th>Linear range</th>
<th>Limit of detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag@Cu bimetallic NRDs based sensor</td>
<td>1 μM– 0.1 M</td>
<td>10×10⁻⁹ M</td>
<td></td>
</tr>
<tr>
<td>Potentiometric sensor</td>
<td>1 μM– 0.11 M</td>
<td>0.833 μM</td>
<td>[159]</td>
</tr>
<tr>
<td>Potentiometry</td>
<td>0.63 μM – 0.01 M</td>
<td>0.316 μM</td>
<td>[160]</td>
</tr>
<tr>
<td>Potentiometry</td>
<td>1 μM – 0.1 M</td>
<td>0.03 μg ml⁻¹</td>
<td>[145]</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>0.34 μM to 14 μM</td>
<td>0.085 μM</td>
<td>[163]</td>
</tr>
<tr>
<td>Ion selective electrodes</td>
<td>1 μM to 0.1 M</td>
<td>0.57 μM</td>
<td>[162]</td>
</tr>
<tr>
<td>Electrochemistry</td>
<td>1 μM – 1 mM</td>
<td>0.1 μM</td>
<td>[143]</td>
</tr>
<tr>
<td>Amperometry</td>
<td>0.5 μM– 0.4 mM</td>
<td>0.04 μM</td>
<td>[141]</td>
</tr>
<tr>
<td>Chromatography</td>
<td>8.6 μM to 0.17 mM</td>
<td>0.04 mg L⁻¹</td>
<td>[161]</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>25 μM to 0.5 mM</td>
<td>0.7 μM</td>
<td>[158]</td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>1.0 to 195 μM</td>
<td>0.5 μM</td>
<td>[129]</td>
</tr>
</tbody>
</table>

After 20 successive cycles, Ag@Cu bimetallic NRDs modified electrode shows a very good response toward SCN⁻ electrooxidation with
relative standard deviation (RSD) of 2.2 %. Further, the modified GCE was stored and studied after 3 weeks at room temperature and exhibits similar current response toward SCN⁻ electrooxidation. This indicates that the modified electrode possesses good reproducibility and stability. Thus, the newly developed sensor shows a better detection limit with a wide linear range and satisfactorily applied for studying real biological samples like human saliva samples of smokers and non-smokers.

4.2.6 Real sample analyses

To test the efficiency of the developed sensor in real time application, SCN⁻ electrooxidation was attempted in human saliva containing dissolved cigarette smoke. The saliva samples of cigarette smokers and non-smokers were analysed. Briefly, 100 μl of saliva samples of smokers who were practicing 2 cigarettes/day, 5 cigarettes/day, 1 packet (10 nos.) of cigarettes/day and non-smoker saliva samples were diluted with 10 ml of PB and tested. Figure 4.8 shows the CV response of saliva samples of smokers and non-smokers. It convincingly recognized the presence of SCN⁻ in saliva samples without any participation of inferring species. Thus the observed results were directly related to the practice of cigarette smoking. It was correlated and compared with the previous studies carried out in gas chromatography and spectroscopic methods for SCN⁻ assay. The variation of SCN⁻ concentration with respect to the use of 1−5 and 11−20 cigarettes per day was studied and reported earlier.¹⁵⁵ It suggested that 0.5 mM SCN⁻ would exist in the non-smokers saliva where as a steady increase in the SCN⁻ concentration as 0.8 mM or 2 mM in the smokers saliva of 1−5 or 6−20 cigarettes per day, respectively. In the present investigations, the SCN⁻ oxidative current response for the saliva sample of the person using 5 and 10 cigarettes per day was found to be 27 μA and 33 μA, respectively. In real sample analysis, such as in human saliva samples, there is a
decrease in the SCN\textsuperscript{−} oxidative peak current, which is due to matrix effect of the co–exciting components such as proteins, enzymes etc.\textsuperscript{156}

Figure 4.9 CV responses of real human saliva samples of cigarette smoker and non-smokers in 0.1 PB (pH 6) at GCE/Nf/Ag@Cu bimetallic NRDs. CVs indicate the non–smoker (a), 2 cigarettes/day (b), 5 cigarettes/day (c) and 10 cigarettes/day (d), respectively

The estimated concentration of SCN\textsuperscript{−} level exists in the saliva of a person using 10 cigarettes per day is 1.5 mM. This was correlated with the standard existing gas chromatographic, spectroscopic and capillary electrophoresis methods where the concentrations of SCN\textsuperscript{−} in saliva of smokers were estimated as 1.65 ± 0.841 mM.\textsuperscript{155,157,158} It means that the optimum level of SCN\textsuperscript{−} in human saliva increased by the additive practice of cigarette smoking. Besides, for comparison, the saliva samples of persons
using beedi (a thin Indian cigarette filled with tobacco) and chewing tobacco were also tested in order to identify the presence of SCN\(^-\). Figure 4.9 shows the CV response of the saliva samples of a person using 15 beedi per day (a) and a person chewing tobacco (3 packets/day) (b).

![Graph showing CV responses](image)

**Figure 4.10** CV responses of real human saliva samples of a person using 15 beedi/day (a) and a person chewing tobacco (3 packets/day) in 0.1 PB (pH 6) at Ag@Cu bimetallic NRDs modified electrode.

It confirmed the presence of SCN\(^-\) in the saliva samples of a person using beedi whereas no observable voltammetric peak was recorded in the saliva samples of person chewing tobacco and the results were similar to the non smoker’s voltammetric results. Thus, the practices of cigarettes and beedi were resulted in the excess SCN\(^-\) accumulation in human saliva. Accordingly, the
mechanism of HCN detoxification in the cigarette smoke would pave the way for the generation of excess SCN$^-$ in human saliva. Hence, the present method could be considered as the better tool in SCN$^-$ sensing which will act as a good marker to distinguish between the saliva samples of cigarette smokers and non-smokers in short span of time.

4.3 CONCLUSIONS

In the present work, an efficient voltammetric sensor for the determination of SCN$^-$ using Ag@Cu bimetallic NRDs modified GCE was developed. The Ag@Cu bimetallic NRDs were satisfactorily prepared by simple, economic and accelerated seed mediated growth method. The as synthesized bimetallic NRDs were characterized by SEM and EDX measurements. Importantly, the presence of Cu on the NRDs surface increases the surface roughness and electrochemically active surface area. Thus the enhanced SCN$^-$ electrooxidation in PB (pH 6) was successfully achieved with high sensitivity, selectivity and low detection limit. Finally, the modified electrodes were satisfactorily tested in the sensing of SCN$^-$ in human saliva samples. The cigarette smokers and non-smokers saliva samples were tested and easily differentiated in short duration of time using the newly developed transducer. Thus, the developed bimetallic NRDs could be used as an efficient sensor for studying the trace amount of SCN$^-$ in real sample analysis.