Chapter 6

A portable detection kit using gold nanoparticles modified carbon paste electrode for screening uricemia patients

6.1. INTRODUCTION

Rural health care is a booming area in medical research. Considering its vast potential, engineers, scientists and medical practitioners work hand in hand to develop the medical technologies for rural health care centres. The main requirement is to implement the technology for developing various detection kits which can screen or detect various contagious diseases without the help of sophisticated lab environment. This research helps in the easy and accurate diagnosis of the health problems of the population living in slums and rural areas without having to go to multi specialty hospitals. These kinds of portable kits and devices using nanoparticles have already been developed for diabetes screening (Song et al., 2010), typhoid detection (Rajan et al., 2013) etc.

This work deals with the high end application of gold nanoparticles modified electrodes in the development of a portable detection kit for the primary screening of uricemia patients. The electrochemical sensors developed using nanoparticles for biomedical application have been proved to possess a very good sensitivity (Wang, 2005). The gold nanoparticles show good response to various chemicals like uric acid (Yogeswaran, 2007), ascorbic acid (Zhang and Jiang, 2005) etc. This property of gold nano particle enables it to act as a very good sensing material for biomedical applications.

Uric acid is the main breakdown product of purine metabolism in human body (Scheele et al., 1776). The uric acid is mainly excreted from the human body through human urine, human blood and sweat (Betrand, 2006). The level of uric acid varies according to the health condition. Therefore, the detection of uric acid level has become a major marker to find the presence of diseases such as urolithiasis (Pak, 2008), gout (Banach et al., 2005), nephritis (Dorland, 2012), Lesch-Nyhamn syndrome (Luo et al., 2006), alcoholism etc., in the human body. The normal level of
uric acid in human urine is 250mg to 750mg (i.e., 1.4 mM to 4.4 mM) per 24-hour sample (Nyhan, 2005). The abnormal concentration of uric acid leads to two different disorders, namely hyperuricemia and hypouricemia. Hyperuricemia occurs when the uric acid level in the urine rises above the normal. It leads to the common diseases such as urolithiasis, Lesch-Nyhan syndrome, gout (Martha, 1976). The decrease in the level of uric acid in body fluid leads to a condition called hypouricemia. Nephritis, myeloma, alcoholism, etc. are the main diseases caused due to this condition (Cruddin and Francis, 1905).

Determination of uric acid in serum plays an important role in laboratory medicine (Becker et al., 2006; Zhao et al., 2007). The common methods for serum uric acid assay include (a) the photometric method (Follin et al., 1912; Blauch et al., 1939), (b) liquid chromatography with detection by either UV absorbance or mass spectrometry (Lim et al., 1978; Ingerbersten et al., 1982; Sakuma et al., 1987), and (c) the uricase based methods (Moss, 1980; Sanders et al., 1980). The most commonly used biochemical analysis methods of uric acid in urine are 24hr urine analysis. The above said tests are either colorimetric or repetitive testing methods where the accuracy of the results is usually not satisfactory. These tests require a sophisticated environment and a lab facility. Hence, it cannot be used for rural health programs or mobile health centers.

Therefore, this detection kit has been developed as a portable one which does not require any sophisticated lab atmosphere for uric acid detection. The fact that concentration and disease condition will be instantly displayed renders the kit a real-time screening device.

6.2. MATERIALS AND METHODS

The electrochemical sensing system has a three electrode assembly, chemically modified gold nanoparticles modified carbon paste composite electrode as working electrode (Au-CPE) (Fig 1.), Ag/AgCl as reference electrode (CH instruments, CH111) and Platinum (Pt) electrode (CH instruments) as the counter electrode.
6.2.1 PREPARATION OF WORKING ELECTRODE

6.2.1.1 PREPARATION OF AU NANOPARTICLES

Gold nanoparticles (AuNPs) used in the present work were prepared using *Coleus forskohlii root extract* as reported in our earlier work (Saraschandra et al., 2014). The extract of *forskollii roots* were prepared and different concentration of this extract was mixed with the boiling solution of 10mL of $1 \times 10^{-3}$ M of HAuCl$_4$ and stirred at 80°C for 15 min which gives 4mg of suspension of AuNPs in ruby red colour (Saraschandra et al., 2014).

6.2.1.2 PREPARATION OF AUNPS MODIFIED CARBON PASTE COMPOSITE ELECTRODES

The Au-CPE was prepared by mixing graphite powder (Sigma Aldrich, 99% purity) and paraffin wax (Sigma Aldrich, 99% purity). The mixture was heated to 40°C till it became a paste. The prepared AuNPs suspension was taken at different levels of AuNPs i.e., 2 mg, 2.5 mg, 3 mg, 3.4 mg, 4 mg and was mixed with the molten wax separately to prepare a paste. This paste was filled in different glass tubes, and copper wires were inserted to take out the electrical connection and to prepare Au-CPE electrode with circular cross section of area 0.196 cm$^2$ as shown in Fig 6.1.

Fig. 6.1. Gold nanoparticles modified carbon paste composite electrode
6.2.2 PREPARATION OF URIC ACID SOLUTIONS

Uric acid is sparingly soluble in water (60 mg/dm$^3$ is the maximum solubility) (Shmaefsky et al., 2004). Hence, 0.3 mM solution of uric was prepared by dissolving appropriate amount of uric acid in distilled water. This uric acid was used only to check the response of the composite electrode (however, for calibration and sensing, the range of uric acid and pH selected in the artificial urine were similar to the biological urine samples).

6.2.3 PREPARATION OF ARTIFICIAL URINE

Artificial urine or synthetic urine depicts the characteristics of natural human urine. Artificial urine was prepared according to the biological procedure by adding 0.39 M urea (Sigma Aldrich), 0.171 M sodium chloride (MERCK), 0.0804 M potassium chloride (MERCK) and 0.036 M sodium phosphate (Loba Chemie) into double distilled water to make a solution. The pH of this solution was adjusted to 5 and later 0.022 M creatinine and 0.399 M albumin were added (S.D Fine Chemicals) and the solution was made up to 500 ml. The artificial urine was made to exhibit uricemia condition by adding uric acid in concentration that was different from the prepared solution to make samples from 1-3 mM of uric acid. These solutions were used to calibrate the sensor device before checking the real time samples.

6.2.4 COLLECTION OF REAL SAMPLES

The 24hrs urine samples were collected from a clinical laboratory. The clinical uric acid reading was initially monitored using the set standard procedure, and from the obtained readings, five normal samples and five diseased samples were selected for studying the performance of the gadget.

6.2.5 ELECTRONIC KIT FABRICATION

After checking the response of the Au-CPE, development of the entire gadget for real time detection was taken up. The motherboard of the gadget included a pre-amplifier circuit, a rectifier, analog to digital converter, a microcontroller and an LCD display. The programme was coded and burnt in the microcontroller in such a way that when
the electrode checked the sample, the display showed the concentration of the uric acid as well as the disease condition.

6.3 RESULTS AND DISCUSSION

The sensitivity and selectivity of the Au-CPE was checked by taking the sensor response using cyclic voltammetry (CHI6003D Instrument) technique.

6.3.1 CALIBRATION OF AU-CPE ELECTRODE

Au-CPE electrodes were prepared by incorporating different amounts of AuNPs in the carbon paste. The main aim of this was to arrive at minimum amount of AuNPs required for detectable response in the voltammogram. This was arrived at by running the voltammograms using 0.03 mM uric acid in PBS. Based on voltammetric runs with AUCPE electrodes containing 2 mg, 2.5 mg, 3 mg, 3.4 mg, 4 mg AuNPs, were carried out. Based on these trials, the minimum amount of AuNPs in the composite electrode was arrived as 4 mg, which was used in all the trials conducted with Au-CPE. The voltammetric response of 0.03 mM uric acid with working electrode (Au-CPE) containing different amount of AuNPs is shown in the Fig 6.2 given below.

![Voltammetric response of different electrodes for uric acid concentration](image)

Fig 6.2: The voltammetric response of different electrodes for the concentration of 0.03 mM uric acid
6.3.2 AU-CPE (4mg) RESPONSE TO URIC ACID

The sensitivity of the working electrode for different concentrations of uric acid was checked in the voltage window of -1 V to 1 V for better resolution. The voltage scan rate was fixed at 0.1 V/s for all the voltammetric runs.

All the Au-CPEs fabricated were checked for response with the prepared uric acid solutions to arrive at minimum amount of AuNPs required in the Au-CPE for detectable response. It was observed that the composite electrode containing 4 mg AuNPs showed good response for different concentrations of uric acid, as shown in Fig 6.3. Two peaks were observed in the response, where the peak at negative voltage window was the response of carbon in the electrode which was confirmed by running cyclic voltammetric runs for blank carbon paste electrode (without AuNPs) and the Au-CPE electrode with buffer saline solution as the electrolyte (Fig 6.4).

![Cyclic voltammetric response of Au-CPE with varying concentration of uric acid](image)

Fig 6.3 Cyclic voltammetric response of Au-CPE with varying concentration of uric acid
The intensity of the peak which appeared in the voltage range at 0.6V in Fig 6.3 increased as the uric acid concentration was increased. This confirmed the sensitivity of the AuNPs towards uric acid detection. The primary response of the Au-CPE guaranteed the detection of uric acid and confirmed the minimum amount of AuNPs required in the composite electrode for detection of uric acid.

The calibration of working electrode was checked by plotting the response curve between the various concentrations of the uric acid and the peak current produced by the Au-CPE electrode, as shown in Fig 6.5.
Fig. 6.4. Response of (a) blank CPE (b) Au-CPE with PBS
6.3.3 AU-CPE RESPONSE TO ARTIFICIAL URINE

The selective response of the modified electrode is very important in its selectivity since, in natural urine, apart from uric acid, many other chemicals as well as proteins are present. It is, therefore, necessary to ensure that the gadget should selectively show response to uric acid in human urine. In order to check the selectivity, the response of optimized Au-CPE (containing 4mg of AuNPs) was checked using different concentrations of artificial urine including simulated uricemia samples. The voltammetric response of optimized Au-CPE for different artificial urine samples is shown in Fig 6.6.

Similar to the linear response in sensitivity, it is necessary to calibrate the selectivity response of the electrode. Thereby, the linear response of working electrode with artificial urine samples was carried out, and is shown in Fig 6.7. The peak current also showed linear increase with increasing concentration of uric acid in the artificial urine and hence it was used as a parameter for analysing the uric acid levels in real samples.

From the voltammograms, it was inferred that the Au-CPE showed selective response to uric acid in the voltage range of 0.5 to 0.6 V with the artificial urine samples and
that there was no interference observed in this voltage range from other constituents or from the proteins in the urine. This confirmed that the Au-CPE could be employed for accurate sensing of uric acid in the samples of real urine.

Fig 6.6 The voltammetric response of Au-CPE for artificial urine samples
6.3.4 DETECTION KIT FOR URICEMIA

6.3.4.1 FABRICATION OF COMPONENTS FOR THE KIT

The three electrode system used in the above voltammetric experiments was incorporated in the kit which simultaneously detected the uric acid in the sample and displayed its concentration as well as the diseased condition. The circuit mainly contained three different sections, namely sensor part, processing circuit and display. The blueprint of the circuit diagram is shown in Fig 6.8.

The sensor section contains working (Au-CPE), reference (Ag/AgCl) and counter (Pt wire) electrodes. The output from this section was the voltage amplitude produced when it was used to detect different concentrations of uric acid. These voltage amplitude values were used as inputs to design the rest of the circuit.

Fig. 6.7 Calibration graph for the response of Au-CPE for artificial urine samples
The processing circuit contained a non-inverting operational amplifier, a filter circuit and a microcontroller. The voltage signal was amplified through an amplifier with a gain of 10. The ripples and distortions in the voltage were removed using a filter circuit. The output of the filter circuit is given to the PIC 16F886 microcontroller. The microcontroller was programmed to take the voltage from the working electrode to one of its input ports, and to calibrate it using the following equation,

\[
\text{Concentration} = \left( \text{Voltage} \times 1.4 \right) + 1 \quad \text{---------- (6.1)}
\]

After calibration, it was designed to check the concentration of uric acid from the sample and to display it on the LCD screen showing the uricemia condition, wherever seen.

The circuit of the detection kit contains different sections like amplifier circuit; filter circuit, analog to digital converter, microcontroller and LED display. The circuit components have been fabricated into a PCB board as shown in Fig 6.9.
6.3.4.2 MICROCONTROLLER CODING

#include<htc.h>

#include<stdio.h>

#include "adc.h"

#include "lcd.h"

__CONFIG(0x20F4);

__CONFIG(0x3FFF);

#define _XTAL_FREQ 4e6

void main()

{

    ANSEL=0x01;

}
TRWASA=0x01;
ANSELH=0x00;
PORTB=0x00;
TRWASB=0x00;

init_adc();
lcd_init();
lcd_clear();
lcd_goto_1(0);
lcd_puts("Uric Acid Sensor");
__delay_ms(4000);
lcd_clear();

char a[20];
unsigned long var;
int q,r;
float f;

while(1)
{
    var=0;

    for(char i=0;i<50;i++)
    {
        var=var+read_adc(0);
        var=(var/50);
    }
}
f = var*(5.0/1023);
if (f <= 0.1)
    f = 0;
else
    f = f - 0.1;

f = (f * 1.4) + 1;
q = (int)f;

r = (f - q) * 100;
sprintf(a, "Conc. = %.d%.d mM ", q, r);
lcd_goto_1(0);
lcd_puts(a);
lcd_goto_2(0);

if (f > 4.5)
    lcd_puts("Hyperuricemia ");
else if (f < 1.5)
    lcd_puts("Hypouricemia");
else
    lcd_puts("Normal");

__delay_ms(1000);
6.3.4.3 DETECTION KIT DISPLAY FOR KNOWN CONCENTRATION OF SAMPLES

The display was basically coded in three different stages. When the system was ON it displayed “URIC ACID SENSOR” for 3 seconds. After 5 seconds of contact of the sample with the electrode system, it displayed the uric acid concentration in the first line and the disease condition in the second line, wherever the parameters were present.

When the concentration of uric acid in the sample was less than 1.5 mM, the disease condition was displayed as ‘HYPOURICEMIA’, as shown in Fig 6.10. If the concentration of uric acid was between 1.5 mM and 4.5 mM, the display showed the condition as ‘NORMAL’ and if the concentration was above the normal range, the gadget displayed ‘HYPERURICEMIA’.
Fig 6.10 Display of uric acid levels in (a) Hypouricemia (b) Normal (c) Hyperuricemia conditions
6.3.4.4 KIT RESPONSE FOR REAL SAMPLES

A total of 10 samples were screened using the prepared gadget. The uric acid value was first measured clinically for all the samples to set a standard to compare the device. The value of uric acid for each sample was measured using the gadget and its clinical readings were compared. This is noted in Table 6.1.

Table 6.1: The uric acid readings produced from various samples using clinical methods and the gadget produced

<table>
<thead>
<tr>
<th>Sample No:</th>
<th>Clinical Test (mM)</th>
<th>Gadget Response (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
<td>1</td>
<td>1.53</td>
<td>1.55</td>
</tr>
<tr>
<td>2</td>
<td>2.25</td>
<td>2.25</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>2.5</td>
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<tr>
<td>4</td>
<td>4.21</td>
<td>4.23</td>
</tr>
<tr>
<td>5</td>
<td>5.37</td>
<td>5.35</td>
</tr>
<tr>
<td>6</td>
<td>6.11</td>
<td>6.11</td>
</tr>
<tr>
<td>7</td>
<td>4.43</td>
<td>4.46</td>
</tr>
<tr>
<td>8</td>
<td>0.56</td>
<td>0.6</td>
</tr>
<tr>
<td>9</td>
<td>1.23</td>
<td>1.4</td>
</tr>
<tr>
<td>10</td>
<td>2.31</td>
<td>2.3</td>
</tr>
</tbody>
</table>

**Correlation significant, α= 0.05.
The values obtained in different trials for both the cases showed almost similar value and the correlation significance (α) was calculated to be 0.05 which proved that there was a high correlation between both the cases. The correlation plots are shown in Fig 6.11.

![Correlation Plot](image)

Fig 6.11 The correlation plot for the response of the gadget as well as the clinical reading produced for uric acid concentrations in different real samples. [Inset: The comparison of the concentration reading produced for each sample in both the cases]

The readings produced for various samples from the already available clinical tests and the gadget response were found to be highly correlated to form a linear graph. In the inset of Fig 6.11, the bar graph compares the value produced for each sample in both the cases.

6.4. CONCLUSION

The ‘uric acid detecting kit’ was developed for screening uricemia patients. This employed a high end application of nanoparticles. This gadget’s core part is an electrochemical sensor electrode which was developed using chemically modified AuNPs modified carbon paste composite electrode (Au-CPE). The sensitivity and
selectivity of the Au-CPE were checked by conducting repeated experiments. The developed gadget showed a good level of accuracy with a detecting range of 0.01 to 7.5 mM in detecting uric acid count in human urine and in determining the diseased condition. Since detection kit does not require any sophisticated environment, it can be used in rural medical centres as well as in mobile health units to screen patients with uricemia conditions. This kit is portable, and the operating personnel do not need any additional technical skills to use it for quick and effective analysis. The device can be fabricated into a proper device form and can be used in clinical laboratories in future on trial basis before it is been available in market for purchase.