

## Measurement of Sensitivity and Oxygen of Blood by Amperometric Biosensor using GO<sub>x</sub> Enzyme for Detection of Cancer

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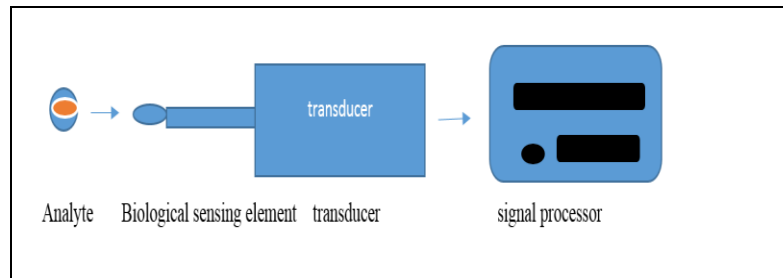
### Abstract

*The detection of cancer is a tough task with limited components. The methods adopted in detection of cancer are Biopsy, Biomarkers etc. Biopsy method involves invasive methods which takes longer time for detection of cancer. Biomarkers require AFM, X rays, MRI scan for recognition of biomolecules which exhibit malignant behavior. Latest techniques like amperometric biosensor which is electro chemical in nature with Nano technology can detect cancer at an earlier stage within a stipulated amount of time. The output of amperometric biosensor is current which is the measure of oxygen gas which is very low in cancer living cell. Cancer cells are also referred to as Hypoxia cells where they inhale less amount of oxygen and release less amount of carbon dioxide in exhalation. In this paper detection of cancer is done through amperometric biosensor with one bulk solution and results are compared with another bulk solution. The best suited bulk solution with very high sensitivity to cancer cells is chosen as the final bulk solution for confirmation of cancer cell. An electro chemical method called Direct Current Cyclic voltammetry is used for detection of cancer. GO<sub>x</sub> enzyme is used for diabetes detection, but in this paper, GO<sub>x</sub> is used for detection of cancer by calculating redox potentials. It is found that redox potential of cancer cell is very low in magnitude and output current falls due to low electron release in cancer cell. GO<sub>x</sub> is mobilized and immobilized on the surface of the biosensor and the corresponding current is calculated. The bulk solution is the key solution of a biosensor for is effective working. The bulk solution is composed of potassium ferro cyanide, titanium dioxide, potassium nitrate and sulphuric acid. The working of biosensor for detection of cancer is verified with different bulk solutions is verified and found that potassium ferro cyanide is best suited for detection of cancer with high sensitivity and oxygen level. This happened due to high electron binding affinity in cyanide compound, high molecular weight and volume..*

**Keywords:** Amperometric biosensor, Bulk solution DCCV, GO<sub>x</sub>, Cancer

### 1. Introduction

An amperometric biosensor is one which contains a ligand and a transducer. The ligand is a biological chemical used in detection of cancer. [1] A transducer is one which converts biological signal into electrical signal (current).



**Figure 1: Block diagram of amperometric biosensor**

The analyte is one on whom the analysis is made, is fed as input to the biosensor the biological sensing element senses the analyte blood sample and transforms into electric signal by the transducer [2]. The electrical signal is displayed through oxidation and reduction cycles of DCCV technique. DCCV refers to direct current cyclic voltammetry

## 2. Materials and Methods

**Chemicals used:** Potassium ferro cyanide, Sodium chloride, Sulphuric Acid, Titanium dioxide, Hydrochloric acid, Phosphate Buffer, Glucose oxidase, Ferro ferri

**Electrodes used:** Glassy carbon, Gold, Stainless steel, Calomel, Platinum, Silver –silver chloride.

**Equipment used:** Micro pipette, Polishing pad, Biosensor cell, Connector between biosensor and PC, Reflection Glass

**Soft wares used:** E/H electro com software, Origin 16 for plotting sensitivity and Oxygen levels, Excel Software.

## 3. Real time Experimentation

Sensitivity impression on the same are the key elements for cancer cells detection Biosensor characterization is validated in cyclic voltammetry in finding cancer stage in a blood sample. CV is used in finding peak levels of current and voltage through voltammograms. In this work the objective is fulfilled through CV in finding the oxygen levels in a given blood sample. CV results are obtained after providing the blood analyte only. Before providing the blood analyte, the experimental setup should be well prepared in terms of chemical materials and electrodes. The redox potentials are calculated from the CV result. These redox potentials provide the oxygen level of the cancer cell. Oxygen level, in its term, is the main indication of cancer stage. Cancer cells do not react to the redox potentials, so their sensitivity is low and this is second factor for cancer cells detection. With the help of an Origin software, the CV plot is displayed and further results analyzers are carried out. The effect of the cell oxygen level on its CV plot along with cell. The above mentioned information gives an indication for CV usefulness and importance. The amount of potassium ferro cyanide is calculated as Volume of water X density of the chemical which is available in data sheet of CSIR lab, Karraikuddi, TN, India.

**Step1:** Take ferro ferri solution with the help of micropipette and drop caste on the biosensor cell.

**Step 2:** If the output voltage is around 0.1V, the biosensor is ready for characterization.

**Step 3:** Next, the calculated amounts of Potassium ferro cyanide and Sodium chloride are taken into biosensor cell.

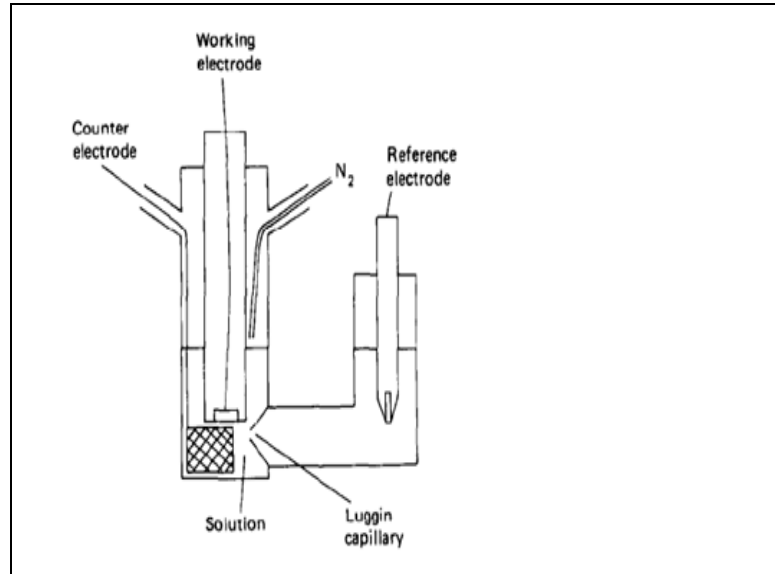
**Step 4:** The compound Potassium ferro cyanide and sodium chloride is called bulk solution is dumped into the biosensor cell with Glass substrate inside.

**Step 5:** The entire set up is connected to PC with E/h software with fixed scan rate of 5 mv/Sec and variable scan rate of 100 mv/sec to 900 mv/sec.

**Step 6:** After ferro ferri, the redox couple of biosensor is tested and observed in the PC and corresponding measurements are taken.

**Step7:** The Sensitivity (LIMIT OF DETECTION) is calculated as  $3 \times \text{Standard Deviation} \times \text{Slope of Voltage and currents in oxidation and reduction}$ .

**Step 8:** Oxygen level is calculated as  $\text{sensitivity} \times \text{solubility coefficient} \times \text{partial pressure}$ . [4]



**Figure 2: The Biosensor With 3 Terminals With Bulk Solution**



**Figure 3: The biosensor cell with Re Dox output in E/H Software. The output is in the form of duck shape**



**Figure 4: Interface of Biosensor with E/H Software PC**

#### **4. Cyclic Voltammetric Study of Ferro Cyanide/Ferricyanide and Titanium dioxide and Sulphuric Acid**

The bulk solution is composed of potassium ferro cyanide and Sodium chloride. Voltammogram refers to redox potentials of a chemical in the form of a bulk solution. Cyclic voltammetry gives redox behavior of chemical species within a wide potential range. The current starts flowing from working electrode which acts as anode towards counter electrode which acts cathode. The calculated potential is compared with the reference potential [9], high potential is applied to working electrode and is scanned in both forward and reverse scan. It continues even though the potential is now scanning in the negative direction resulting in a cathodic current which peaks. The ferro cyanide releases good number of electrons in forward scan and reverts back in reverse scan. The electron flow is high from anode to cathode. In forward scan if the counter electrode is calomel, the order of current is of micro amperes, if platinum is used the order of current is milli amperes. The current increases [10] in forward scan as well as in reverse scan due to high electrolytic property of the bulk solution. The resultant potential is given average of anodic peak potential and cathodic peak potential.

$$E_o = (E_{pa} + E_{pc}) / 2$$

The bulk solution is now changed from potassium ferro cyanide and NaCl to sulphuric acid and titanium dioxide. It is found that the new bulk solution decomposes [11] early due to open circuit between anode and cathode. The readings are confined to few, resulting in low anodic current and low cathodic current. The forward scan and reverse scan readings are taken in both fixed scan rate and variable scan rate. The current initially increased and decreased following voltammograms theory, but the electron release is very low due to open circuit of junction. As the electron release is very low under bare conditions, the sensitivity, oxygen level is very low which is not suitable for diagnosing cancer. The sensitivity and oxygen level are found to be decreased for different scan rates in fixed and variable domains.

The experiment is repeated with stainless steel and potassium nitrate; the results were not fruitful.

##### **4.1 Selectivity**

This feature is most important in biosensor because this depends on the potential of bio receptor to detect the specific analyte in a mixture of sample and contaminants. For example; specific interaction of antibody with particular antigen. [12]

#### **4.2 Reproducibility**

It is just reproducibility of biosensor to program identical output after repetition of the experimental setup. [13]

#### **4.3 Stability**

It is the ability of biosensor to be non-susceptible in ambient conditions in and around the bio sensing system. Any disturbance can modulate the output signals of biosensor for its measurement which may lead to error and can affect the efficiency of the biosensor. [14]

#### **4.4 Sensitivity**

Sensitivity can be defined based on its limit of detection (LOD). In many medical application, biosensors have to detect analyte as small as in the range of concentration of ng/mL or fg/mL, to affirm the existence of analyte in the sample. For example, in Prostate cancer, the prostate specific antigen (PSA) concentration will have a value of nearly 4 ng/mL in blood.[20-25]

#### **4.5 Linearity**

It attributes to the accuracy of the measured response. It can be depicted mathematically as  $y = mc$ , where  $c$  is the concentration of the analyte,  $y$  is the output signal and  $m$  is the affectability of the biosensor. [15-19]

#### **4.6 Mobilization of Enzyme Stage Simulation**

The theoretical explanation of the mobilization stage is explained in detail [16] However, the objective here is to illustrate the working procedure within this stage in simulation and this to be applied with the real-time experiments .Within this stage, the bulk solution is potassium ferro cyanide ( $K_4FeCN_6$ ), Sodium chloride (NaCl), PANI solution, GOx and phosphate buffer (mobilized enzyme) is added, the electron flow is increased more between working electrode and counter electrode with respect to reference electrode and hence the lowest resistance of the bulk solution and the highest value in comparison with previous stage of reduction current as it is shown in figure 13. The oxidation current, in this case, will take more time for saturation.

#### **4.7 Immobilization Of Enzyme**

Within this stage, the bulk solution is potassium ferro cyanide ( $K_4FeCN_6$ ), Sodium chloride (NaCl), PANI solution, GOx, phosphate buffer (immobilized enzyme condition) [17] is added, the electron flow is decreased more between working electrode and counter electrode with respect to reference electrode and hence the resistance of the bulk solution will be increased in comparison with previous stage of reduction current as it is shown in figure 13. The oxidation current, in this case, will be reduced as shown in figure 14.

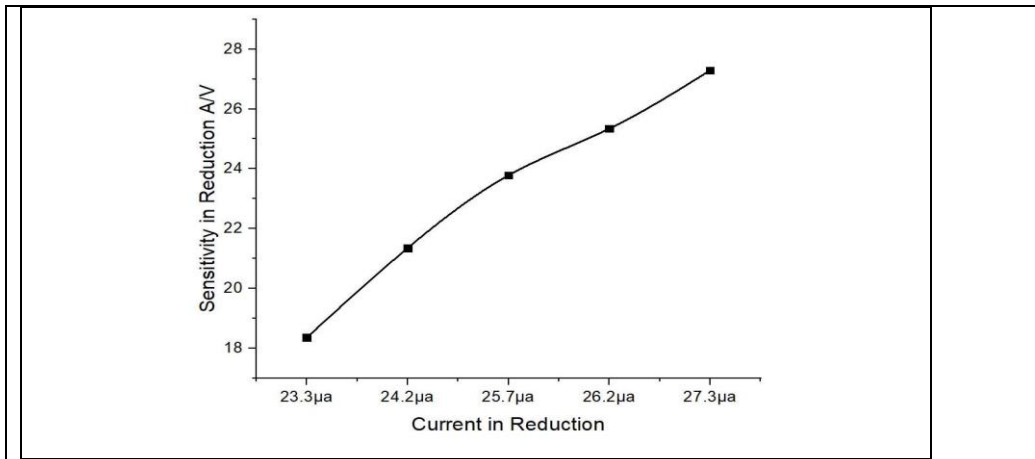
#### **4.8 Observation**

The glucose oxidase enzyme when reacts with cancer blood sample, the bioelectric potentials drop significantly, the redox potentials drop very drastically. Resulting in micro ampere current for cancerous blood sample.

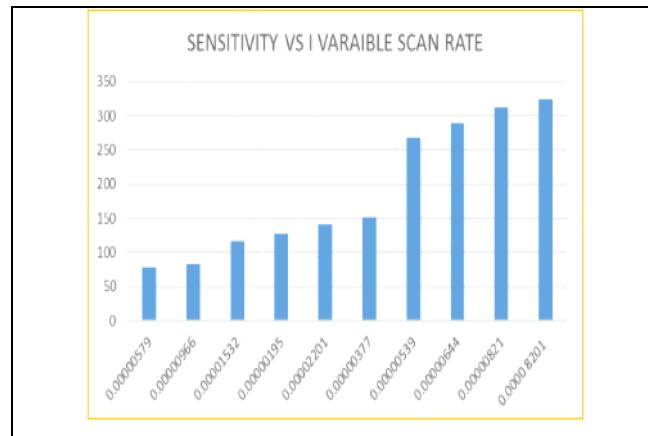
**Table 1: Illustration of cancer testing using GOX Enzyme**

Chemical Used For Testing Cancer	Current	Result
Gox+ normal blood	Milli amperes	Negative –no cancer
Gox+cancerous blood sample	Micro amperes	Positive- confirmed cancer in meta stage
Gox+cancerous blood sample	Pico amperes	Positive-confirmed cancer in advanced stage

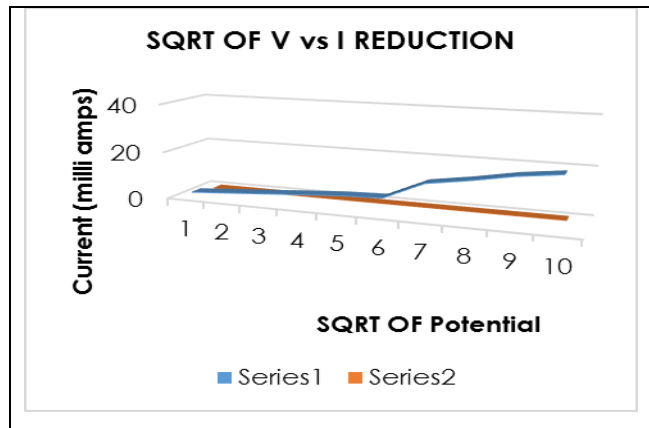
**6.Results and Discussion**



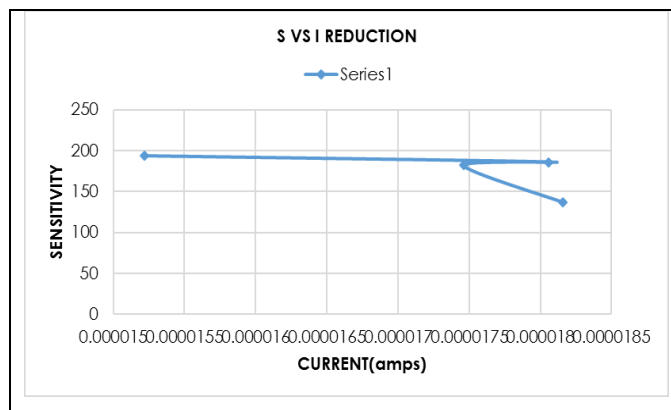
**Figure 5: The Increase In Sensitivity Vs Current In Reduction Of Poattasium Ferro Cynide Bulk Solution In Fixed Scan Rate**



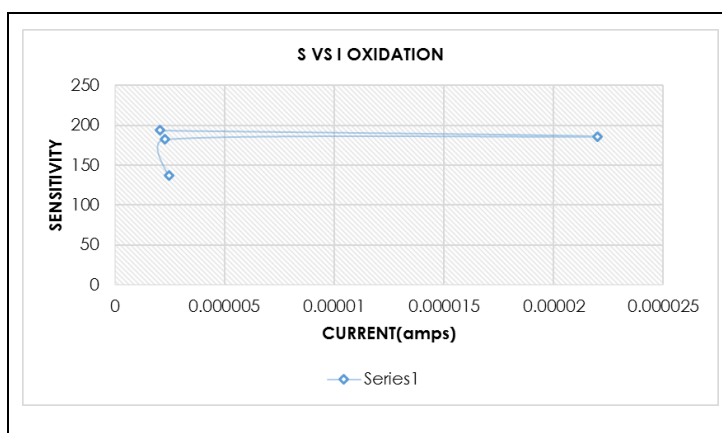
**Figure 6: The Increase In Sensitivity Vs Current In Variable Scan Rate Of Potassium Ferrocynade Bulk Solution. This Illustrates This Bulk Solution Is Fit For Cancer Detection.**



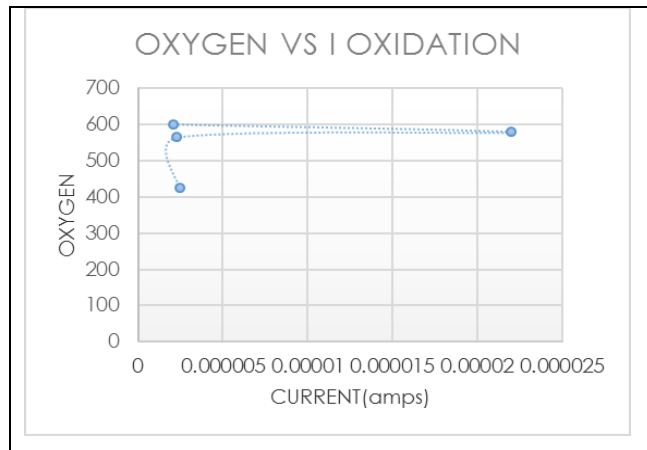
**Figure 7: The Variation Of Current Vs Square Root Of Potential In 3D Plot Of Poattassium Ferro Cynade Bulk Solution For Normal Cells Following Michealeas Menon Equation**



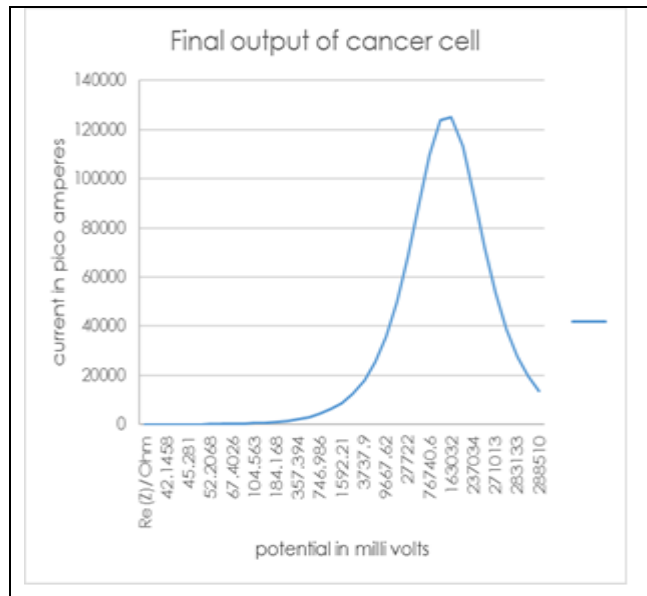
**Figure 8: Decrease In Sensitivity Vs Current In Tio2+H2So4 Bulk Solution In Reduction For Normal Cells.This Illustrates This Bulk Solution Is Not Appropriate For Cancer Detection.**



**Figure 9: Decrease In Sensitivity Vs Current In Tio2+H2So4 Bulk Solution In Oxidation For Normal Cells**

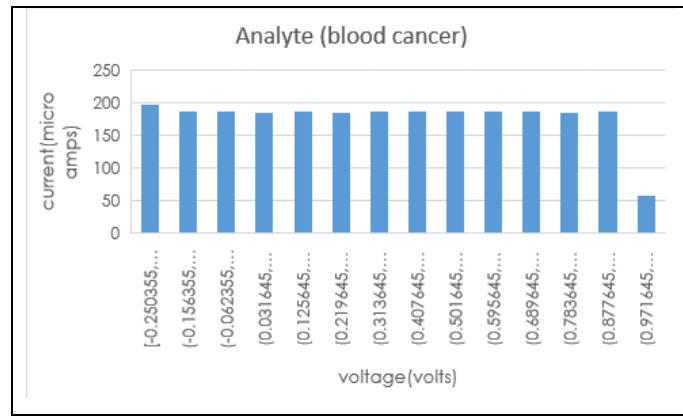


**Figure 10: Decrease In Oxygen In Ppm Vs Current In Amperes In  $TiO_2+H_2SO_4$  Bulk Solution In Oxidation. This Illustrates This Bulk Solution Is Not Fit For Cancer Cell Detection.**

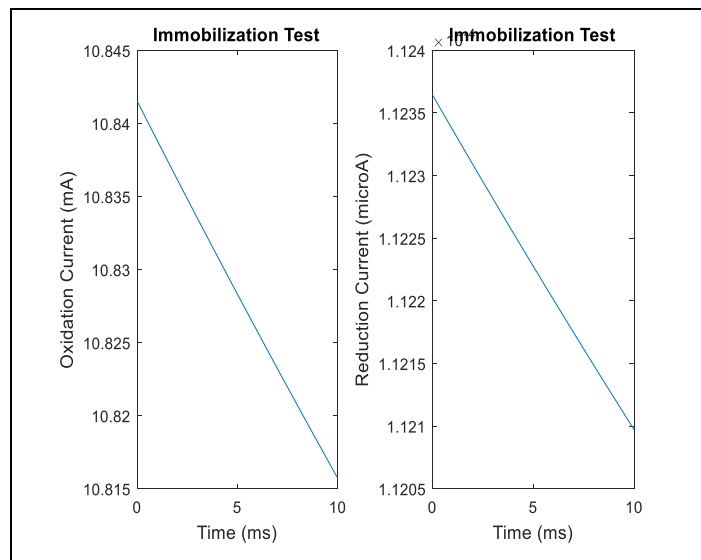


**Figure 11: Final REDOX Output Of Amperometric Biosensor In Detection Of Cancer Cell With Potassium Ferro Cyanide And Sodium Chloride Bulk Solution. This Is An Accurate Result Which Confirms Cancer In Metastasis Stage**

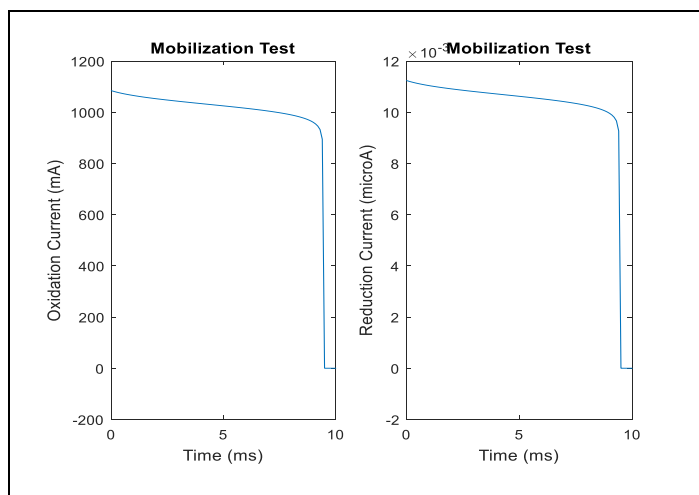




**Figure 12: The Final Output Of Amperometric Biosensor After Glucose Oxidase Encounters Cancer Blood Sample In DCCV Technique With Potassium Ferro Cynade Bulk Solution[6]**



**Figure13: Immobilizaion of glucose oxidase enzyme at the working electrode resulting inlow oxidation current for cancer cells**



**Figure 14: The Gox Enzyme Is Mobilized At Working Electrode Resulting In High Oxidation Current For Normal Cells**

**Table 2: Comparison Chart: This Illustrates The Final Selection Of Bulk Solution To Be Used In A Biosensor For Cancerous Cell Detection.**

<b>BULK SOLUTION</b>	<b>SENSITIVITY</b>	<b>OXYGEN LEVEL</b>	<b>RESULT (Detection of cancer cells)</b>
Potassium ferro cyanide +Sodium chloride	Increased	Increased	Best Preferred
Titanium dioxide +Sulphuric acid	Decreased	Decreased	Least preferred
Stainless steel and potassium nitrate	Decreased	Decreased	Not an option

## 5. Conclusion

In this paper we compared 3 different bulk solutions for detection of cancer cells. It is found that potassium ferro cyanide and sodium chloride bulk solution is best suited for detection of cancer cells when compared with other bulk solutions which are composed of sulphuric acid and titanium dioxide and stainless steel. This is because of high electron binding affinity and resistances to cancer cell encounter. This supports T cells in faster recognition of cancer cells. We conclude potassium ferrocyanide and sodium chloride is best suited for analyzing cancer blood samples.

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