Nanostructured biosensor to estimate the freshness of fish

Sontara Konwar Boruah[1], Bikash Agarwal[2], Sunandan Baruah[3] and Prabin Kumar Boruah[4]

[1] Department of Chemistry, Assam Don Bosco University,
Guwahati-781017, Assam, INDIA.
sontara.boruah@dbuniversity.ac.in

[2,3] Department of Electronics and Communication Engineering, Assam Don Bosco University,
Guwahati-781017, Assam, INDIA.
[2]*bikash.agarwal@dbuniversity.ac.in*
[3]*sunandan.baruah@dbuniversity.ac.in*

[4] Department of Chemistry, Gauhati University,
Guwahati-781014, Assam, INDIA.
Prabin.boruah@gmail.com

Abstract*: *Freshness of fish is of importance for consumers because of its connection with health and taste. A mixture of complex volatile biogenic amines collectively known as total volatile basic nitrogen (TVB-N) are released from the fish spoilage due to enzyme-catalyzed decomposition of biogenic amines by spoilage microorganisms. The release of TVB-N, due to microorganism growth in fish samples are considered as potential analytical indicator of fish spoilage. The developed sensor contains a mixture of pH sensitive anionic dye, cresol red (CR) and cationic surfactant cetyltrimethyl ammonium bromide (CTAB) above the critical micellar concentration. The mixture of CR-CTAB (1:1) was immobilized on the reduced graphene oxide (rGO) modified electrode surface. A change in static voltage was observed across the CR-CTAB/rGO biosensor when TVB-N are released from spoiled fish under room temperature. The developed biosensor can be used for the real time measurement of fish freshness.

Keywords: Biogenic amines, Nanostructured biosensor, dimethylamine, cresol red and reduced graphene oxide.

(Article history: Received 19 September 2017 and Accepted 27th April 2018)

1. INTRODUCTION

At present the freshness of fish quality judgment assessment is done on the basis of appearance, texture, smell and color by trained assessors. Generally after death, the number of specific spoilage organisms on skin, gill surface increases gradually and spreads within the various tissues. Biogenic amines are formed by bacterial enzymatic decarboxylation of free amino acids. The biogenic amines are biological active compounds with aliphatic, aromatic or heterocyclic organic bases [1-3]. Biogenic amines such as histamines, putrescine, cardaverine, agmatine, spermidine, trimethylamine, pyrrolidine, piperidine, nitrosamine, sperimine and ammonia etc. are produced in spoiled fish. Ingestion of toxic biogenic amines in spoiled fish and fish products results in food poisoning and cancer [4]. A mixture of volatile biogenic amines collectively known TVB-N are released from the spoilage of fish due to growth of enzyme-catalyzed decomposition by spoilage microorganisms. The release of TVB-N, due to microorganisms growth in fish samples are considered as potential analytical indicator of fish spoilage [5]. This work reports the development of a nanostructured biosensor to estimate the real time of freshness of fish. The developed sensor contains a mixture of pH sensitive anionic dye CR and a cationic surfactant CTAB above the critical micellar concentration and reduced graphene oxide. Due to large surface area, biocompatibility and high conductivity, graphene is used to immobilize CR and CTAB to promote the electron transfer between the electrode and CR-CTAB [6].

1. EXPERIMENTAL

2.1. Instruments and Reagents

UV-Visible spectroscopy was carried out on UV-1800 Shimadzu UV spectrophotometer. The FT-IR was recorded using Affinity-I Shimadzu spectrophotometer. The morphology of the graphene oxide and reduced graphene oxide were characterized by field emission scaning electron microscope (FESEM) model Carl Zeiss Sigma VP.

Graphite powder was purchased from Sigma Aldrich. Sulphuric acid (H_2SO_4 98%), Potassium permanganate ($KMnO_4$ 99%), Hydrogen peroxide (H_2O_2 30%) and Sodium nitrate ($NaNO_3$) were purchased from Merk. CTAB was purchased from HiMedia Laboratories Pvt.Ltd. Fresh Rohu (Labeo rohita) fish was collected from the local pond of Assam.

Graphene oxide (GO) was synthesized from graphite powder according to Hummer's method [7-9].

2.2. Fabrication of two electrodes configuration on printed circuit board

A two electrode configuration was fabricated on a copper board. Firstly, the electrodes were washed with acetone and isopropyl alcohol solvent in an ultrasonic bath. Secondly, aqueous 25µL graphene oxide (GO) was coated on the two electrodes such that the two electrodes and middle gap space between the electrodes are fully coated with GO. Then the GO coated electrode was dried at 40ºC in an oven for 10 minutes. Thirdly, 10µL sodium borohydride (50mM) was added on the dry GO surface layer to reduce the GO. Then the rGO (Figure 1a) coated electrodes were dried in air for overnight. Thus, Cu-electrode coated with rGO was obtained. Fourthly, a mixture of 20µL CR-CTAB (1:1) solution was immobilized on Cu/rGO electrode and subsequently dried in air oven at 40ºC for 10 minutes.

2.3. Experimental setup

A polypropylene container 4.5cm internal diameter and 6.5cm height with cover was used for the analysis. The experimental set-up is shown in the Figure 1.

A piece of fresh fish (0.5g) was taken inside the air tight polypropylene container and the fabricated electrodes were installed on the inner side of the container cover fixed with adhesive tape. The six sensor electrodes were affixed to the cover of the polypropylene container was sealed with fast cure epoxy to create a permanent gas tight seal, to prevent leakage of volatile amine gases. Using connecting wires the observed static voltage was then acquired in the personal computer system by using the virtual instrument application called Lab VIEW software and using a data acquisition (DAQ) card (NI USB-6009 of National Instruments, Singapore) for continuous monitoring of the freshness of fish. The experiment was carried out with multiple sensors to observe the response. The Lab VIEW software used to control DAQ card which connect the devices to the computer and convert the signal from analog to digital. The acquired signal was then processed and analyzed to get information about the freshness of fish with respect to time. The block diagram of the hardware and software system used in the Lab VIEW to acquire the electrical signal from the sensors and different virtual instrumental blocks available in the signal processing section of Lab VIEW is shown in the Figure 1b. The proper operation and connections for this card can most easily be checked using

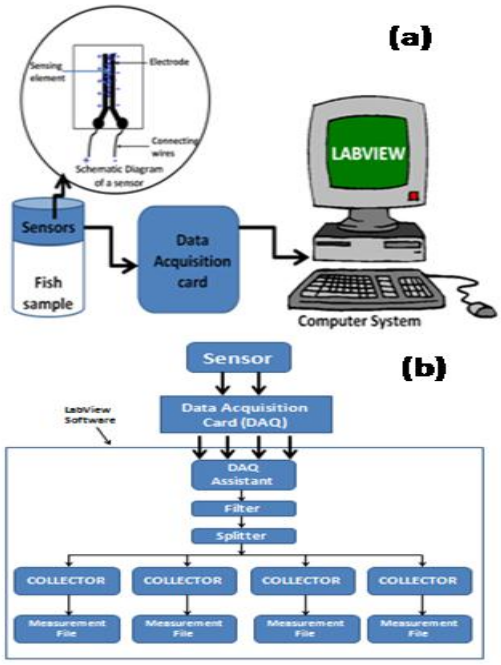
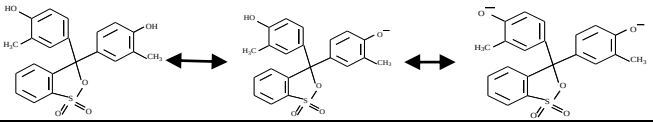


Figure 1: Block diagram of the fish biosensor and (1b) Block diagram of the hardware and software.

the National Instruments Measurement and Automation Explorer (MAX). At the outset, the output of the sensor system was connected to analog input channels of the DAQ. As shown in Figure 1b the acquired signals are read by the Lab VIEW software using a Virtual Instrument called DAQ Assistant. The combined data is then filtered using a LPF of cut off frequency 1 Hz to remove the ac level (any noise signal) inserted due to acquisition. A splitter is used to separate the data from different sensors as individual value to record their output. "Collectors" are used to collect the input signals and returns the most recent data, up to the specified maximum number of samples per channel. In this block the number of samples used is 1 sample per minute. Then the samples are given to another file called "Measurement File" which writes the acquired data from a sensor to an excel file for further analyze the data.

A typical sensor solution contained a pH sensitive anionic dye CR (2% w/w), cationic CTAB. The mixture of CR and CTAB (1:1) was sonicated for 30 minutes until dissolution was complete. The molecular structure of acidic, neutral and base form CR is shown in the Figure 2.



Acid form (Orange color) Neutral form (Yellow color) Base form (Red color)

Figure 2: Molecular structure of Cresol red (o-Cresol sulfonephthalein). pH 7.2-8.8.

2. RESULTS AND DISCUSSIONS

3.1. Sensor characterization using UV-Visible spectroscopy

UV-Visible spectra of CR, CTAB in distilled water and in the presence of CTAB are shown in the Figure 3. The absorption spectrum of CR in distilled water shows absorption band at 434nm to n-π* transitions. CR in the presence of CTAB above CMC, it is observed that absorption band at 434nm decreases with the appearance of a new band at 584nm. It clearly suggests that the new band at 584nm arises due to the electrostatic and hydrophobic interactions between the dye CR and monomer of the surfactant forming CR-CTAB ion pairs. These interactions can be explained by considering that the negatively charged sulfonate groups $^{-}SO_3^{-}$ of CR prefer to cluster with the positively charged cationic head groups $-N^{+}(CH_3)_3$ of CTAB [10,11].

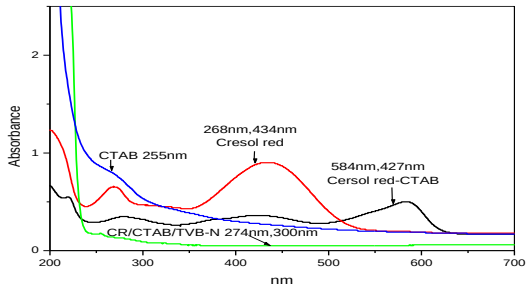


Figure 3: UV-Vis spectra of Cresol red, CTAB, CR-CTAB, CR-CTAB-TVB-N in Water.

3.2. Sensor response to air as reference blank

Figure 4 shows typical sensor responses to air as blank reference. The sensor output static voltage (mV) was

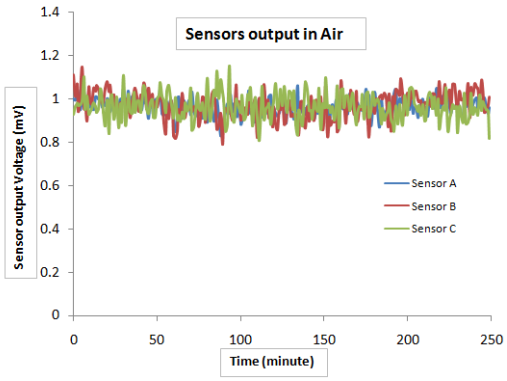


Figure 4: Typical Sensor A, Sensor B and Sensor C responses to air as a reference blank at room temperature.

recorded upto 25 minutes. Figure 4 shows that three sensors (Sensor A, Sensor B and sensor C) output static voltage remains constant throughout the experimental period.

3.3. Sensor response to tetra ethylamine gas

Figure 5 shows typical sensor output static voltage responses to tetra ethylamine gas. To investigate the sensor responses to the basic tetra ethylamine gas, 10µl tetra ethylamine was placed inside the polypropylene container. The sensors output static voltage (mV) were recorded upto 80 minutes. Figure 5 shows that, the static output static voltage signal of sensor A, sensor B and sensor C were found to depend on the concentration of the tetra ethylamine gas. As the volatile basic tetra ethylamine gas increases, the static output voltage signal increases upto 60 minutes then after that output static voltage remains almost unchanged. This experimental evidence indicates the CR dye protonation-deprotonation process requires the presence of an effective proton transport medium to ensure efficient shuttling of protons from acidic dye (proton donors) to basic tetra ethylamine (proton acceptor).

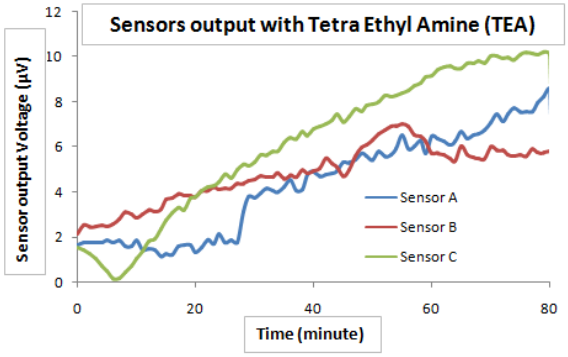


Figure 5: Typical Sensor A, Sensor B and Sensor C responses to tetraethyl amine gas asreference at room temperature.

3.4. Sensor response to TVB-N from spoiled fish sample

Figure 6 shows typical sensor responses to TVB-N levels in fish sample at room temperature. The sensors output static voltage (mV) were recorded upto 80 minutes. Figure 6 shows the static output signal of sensor A, sensor B and sensor C were found to depend on the concentration of TVB-N gas. As the volatile TVB-N gas increases, the static output voltage reaches a plateau at 60 minutes, there after the output static voltage remains almost unchanged.

Figure 7 shows the comparison of sensor responses to tetra ethylamine gas and TVB-N level in fish sample at room temperature. Figure 7 shows sensor output voltage was maximum at 60 minutes for both tetra ethylamine gas and TVB-N. This comparison results suggest that the fabricated fish biosensor electrode Cu/CR-CTAB/rGO response was conforming with the result of tetra ethylamine gas. After exposing the sensor electrodes to the fish sample, the TVB-N residues were extracted from the sensor electrode surface with distilled water. The UV-Visible spectrum of TVB-N in distilled water was recorded with absorption bands at 274nm and 300nm (Figure 3).

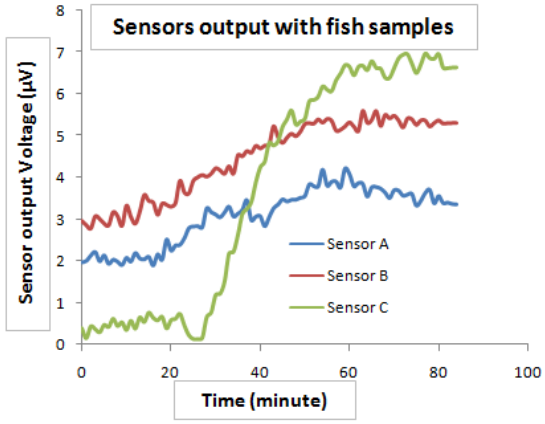


Figure 6: Typical Sensor A, Sensor B and Sensor C responses to TVB-N level in fish sample at room temperature.

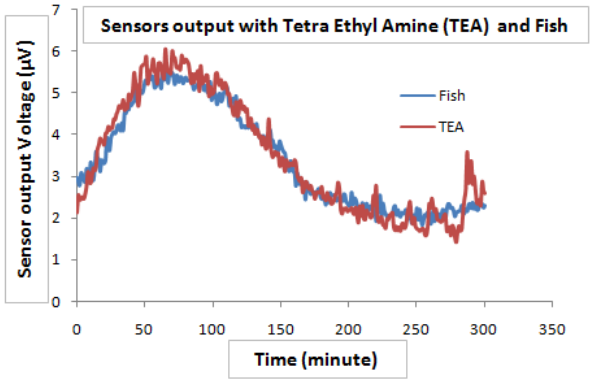


Figure 7: Comparison of Sensors response to tetraethyl amine gas and TVB-N level in fish sample at room temperature.

3. CONCLUSIONS

In this work, a very simple, fast responding biosensor prototype using a mixture of CR-CTAB (1:1), immobilized on the rGO modified Cu electrode for real time measurement of fish freshness. Experimental results showed the feasibility of using this biosensor for food industries, markets, domestic applications for the real time measurement of fish freshness. So the Cu/CR-CTAB/rGO biosensor may be a promising candidate for the fabrication of simple nanostructured biosensor for the detection of TVB-N from spoilage fish in future.

ACKNOWLEDGEMENT

The authors acknowledge Assam Don Bosco University for providing synthesis facilities and Department of Chemistry, Gauhati University, Guwahati-781014, Assam, India for providing instrumental facilities for analysis. The authors also acknowledge Institute of Advanced Study in Science and Technology(IASST), Guwahati-781035, Assam, India for providing access to FESEM.

REFERENCES

[1] Alexis Pacquit, King Tong Lau, Henry McLaughlin, June Frisby, Bird Quilty, Dermon Diamond, "Development of volatile amine sensor for the monitoring of fish spoilage". *Talanta* , **2006**, *69*, 515-520.

[2] Ismail Al Bulushi, Susan Poole, Hilton C. Deeth and Gary A. Dykes. "Biogenic amine in fish : Roles in intoxication, Spoilage and Nitrosamine Formation – A review", *Critical Reviews in Food Science and Nutrition*, **2009**, *49*, 4, 369-377. DOI:10.1080/10408390802067514.

[3] Boram Lee, Rosario Scopelliti and Kay Severin. "A molecular probe for the optical detection of biogenic amines", *Chem. Commun.*, **2011**, *47*, 9639-9641.

[4] Joanna Stadnik, Zbigniew J. Dolatowski. "Biogenic amines in meet and fermented meat products", *Acta Sci. Pol., Technol. Aliment*. **2010**, *9*, 3, 251-263.

[5] Hirofumi Nishikawa, Tatsuya Tabata, Seiichi Kitani. "Simple Detection Method of Biogenic Amines in Decomposed Fish by Intramolecular Excimer Fluorescence", *Food and Nutrition Sciences*, **2012**, *3*, 1020-1026. http://dx.doi.org/10-4236/fns-2012.37135.

[6] Xiao Yang, Bo Feng, Xiulan He, Fangping Li, Yonglan Ding and Junjie Fei. " Carbon nanomaterial based electrochemical sensors for biogenic amines", *Microchim Acta*, **2013**, *180*, 935-956. DOI:10.1007/s00604-013-1015-6.

[7] F.Y. Ban, S.R. Majid, N.M. Huang, H.N. Lim. "Graphene Oxide and Its Electrochemical Performance," International Journal of Electrochemical Science, **2012**, *7*, 4345-4351.

[8] A. Satti, P. Larpent, Y. Gun'ko. "Improvement of Mechanical Properties of Graphene Oxide/Poly(allylamine) composites by chemical Crosslinking", *Carbon*. DOI:10.1016/j.carbon.2010.05.030.

[9] Sontara K. Boruah, Prabin Kr. Boruah, Pradyut Sarma, Bipul Bezbaruah, Chitrani Medhi, Okhil Kr. Medhi. "Exfoliation of Graphene oxide nanosheets from pencil lead and in situ preparation of gold nanoparticles on graphene oxide nanosheets", *International Journal of Nanoscience*, **2013**, *Vol.12*, No.5, 1350034 (7 pages). DOI:10.1142/S0219581X13500348.

[10] Anwar Ali, Sahar Uzair, Nisar Ahmad Malik, Maroof Ali. "Study of interaction between cationic surfactant and cresol red dye by electrical conductivity and spectroscopy methods", *Journal of Molecular Liquids*, **2014**, *196*, 395-403.

[11] Issa M. El Nahhal, Shehata M. Zourab, Fawzi S. Kodeh, Florence Babonneau, Wafaa Hegazy. "Sol-gel encapsulation of cresol red in presence of surfactants", *J. Sol-Gel Sci Technol*, **2012**, *62*, 117-125. DOI: 10.1007/s10971-012-2693-y.

Author's Profile

Dr. Sontara Konwar Boruah is presently working as an assistant professor(Senior) in the department of Chemistry, Assam Don Bosco University, India. She completed her M.Sc in Chemistry and Ph. D in Chemistry from Gauhati University, Assam, India. Her area of research interest are bioinorganic Chemistry, Nano materials and biosensors.

Mr. Bikash Agarwal is currently serving as an Senior Assistant Professor in Electronics and Communication Engineering Department of Assam Don Bosco University, India. He completed his Bachelor of Engineering from Rajasthan University, Jaipur, India and his Master of Technology from Assam Don Bosco University, Assam, India. Currently he is also pursuing his Doctoral from Assam Don Bosco University. His research interest is in the development of nanomaterials for different applications including sensors and water purification.

Prof. Sunandan Baruah is currently serving as the Director of the Center of Excellence in Nanotechnology of Assam Don Bosco University, India. Prior to this he held teaching and research positions at Angstrom Laboratories, Uppsala University, Sweden and the Asian Institute of Technology, Thailand. He completed his Bachelor of Engineering from Assam Engineering College, Guwahati and his Master of Engineering and Doctor of Engineering from the Asian Institute of Technology, Thailand. His research interest is in the development of nanomaterials for different applications including sensors, photo-catalysis and solar cells.

Dr. Prabin Kumar Boruah is presently working as a Scientific Officer in the department of Chemistry, Gauhati University, Guwahati, India. He completed his M.Sc in Chemistry and Ph. D in Chemistry from Gauhati University, Assam, India. His area of research interest are bioinorganic Chemistry, Nano materials and biosensor devices