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Research Article

Evaluation and Validation Studies of Cyclic Voltammetry Transducer-based Formaldehyde Biosensor in Vegetables Extract

MONIKA KUNDU[1]*, PRAMEELA KRISHNAN[1], ANANTA VASHISTH[1], SURENDER DUHAN[2]

[1]*Division of Agricultural Physics, ICAR-Indian Agricultural Research Institute, New Delhi-110012*
[2]*Deenbandhu Chhotu Ram University of Science and Technology, Murthal, Haryana*

ABSTRACT

In present study, cyclic voltammetry transducer-based formaldehyde biosensor has been tested and validated in vegetables extracts. Formaldehyde is a widespread pollutant in air, common adulterant in foods and water. It has severe health hazards due to its carcinogenicity. The study involved measuring the current responses of a range of electrodes, including those based on carbon nanotubes with immobilized formaldehyde dehydrogenase. The fabricated biosensor has been tested in real samples of vegetables extract (Spinach and Tomato) and the results have been compared and validation with HPLC method also. Real sample analysis yielded remarkable results, with the obtained Relative Standard Deviation (RSD) values falling below 1.67, highlighting the sensor's efficiency and reliability. Additionally, the sensor demonstrated an outstanding recovery rate of over 92%. The calculated detection limits (LOD) for formaldehyde in vegetables extract samples of spinach and tomato were determined to be 0.03 mg/L and 0.04 mg/L, respectively. Furthermore, the limits of quantification (LOQ) were found to be 0.06 mg/L and 0.08 mg/L for spinach and tomato samples, respectively. The LOD obtained using the HPLC technique was 0.03 mg/L for spinach and 0.05 mg/L for tomato samples. The limits of quantification (LOQ) were found to be 0.07 mg/L and 0.06 mg/L for spinach and tomato samples, respectively using HPLC method. These findings established the future application of biosensor in the detection of formaldehyde adulteration in vegetables extracts and other liquid food products within the agricultural and food supply chain.

Key words: Cyclic voltammetry, Formaldehyde, Limit of detection, Limit of quantification, Vegetables extraction, Adulteration

Introduction

In our rapidly evolving world, driven by the wheels of science and technology, new technologies for food processing and preservation emerge daily, promising convenience and simplifying our lives. However, in the midst of this rush toward smart and instant solutions, there is a concerning disregard for food quality and safety. This has led to a disturbing increase in cases of food toxicity and even reported deaths (Lebelo *et al.*, 2021). It is important to recognize that food has a direct impact on our health and, by extension, on the growth and development of nations. Therefore, it is essential that we redirect our focus to understand the root causes of these incidents. The modern market is inundated with ready-to-eat and ready-to-serve foods. Consumers are often willing to pay a premium for these products, with the expectation of receiving a thoroughly quality-tested product in return. Both raw and

*Corresponding author,
Email: mkkundu07@gmail.com

processed agricultural products are subject to a wide range of agricultural chemicals to extend their shelf life. While these chemicals are used within prescribed limits to protect consumers and ensure sustainability in agriculture, the extensive use of formalin as a preservative is a significant concern (Siddiquee and Ampon, 2016; Miah *et al.*, 2013). Formalin, commonly used as an antiseptic, disinfectant, and food preservative, poses a substantial threat to human health. It is highly toxic, strongly carcinogenic, and an elevated dose can lead to various health issues, such as diarrhea, vomiting, gastrointestinal ulcers, and eye, nose, and throat irritation (Nirmal Kumar *et al.*, 2021). Inhalation of formalin gas in excess can lead to asthma, pulmonary edema, and an increased risk of respiratory cancer. In some cases, it can even have adverse effects on reproductive health and cause dermatitis. Despite existing food legislation, adulteration remains largely uncontrolled. This is due, in part, to a lack of adequately trained personnel and proper laboratory facilities. Traditional quantitative analysis of formaldehyde in food samples involves techniques like HPLC, gas chromatography, and chemiluminescence, which are not only time-consuming but also require skilled personnel and expensive instrumentation (Wahed *et al.*, 2016). What's urgently needed is a rapid, sensitive, portable, and cost-effective technology for detecting formaldehyde adulteration in food. Researchers have explored various innovative methods, including electrical, optical, biochemical, and electrochemical approaches, to address quality and safety concerns in agriculture. However, chemical kits available in the market are often considered less reliable due to accuracy and specificity issues for detecting and quantifying specific adulterants (Waralun and Suwanaruang, 2019). Thus far, most studies have focused on conventional techniques, with a notable lack of rapid, sensitive, and cost-effective devices for quantifying formaldehyde in food. One promising approach involves the development of a formaldehyde sensor based on differential pulse voltammetry (DPV), as demonstrated for fish samples. This sensor employs the deposition of an ionic liquid, gold nanoparticles (AuNPs), and chitosan (CHIT) onto a glassy carbon electrode, achieving a low detection limit of 0.1 ppm (Hoque *et al.*, 2018). A recent study successfully quantified formalin in food extracts non-destructively using fabricated molecular imprinted polyacrylonitrile engraved graphite electrode (Nag *et al.*, 2022). The potential for commercial applications arises if this method can be transformed into a portable, cost-effective, and user-friendly device (Arfat Sharif *et al.*, 2018). Thus there is immediate need to devise novel methods and techniques that are highly specific, precise and rapid for the detection of formaldehyde contamination in agricultural produce.

In our study, we propose the evaluation and validation studies of a fabricated biosensor based on formaldehyde dehydrogenase-carboxyl-functionalized carbon nanotubes (FDH-CNT) for detection of formaldehyde in vegetables. Due to the unique characteristic properties of the organic materials i.e., carbon nanotubes, it is most preferred choice among researchers for biosensing applications (Mphuthi *et al.*, 2017). To support our research, we conducted comprehensive surface morphology and in-depth structural studies employing electron microscopy and diffraction techniques. Additionally, we examined the transmission and absorption characteristics of the materials used in our previously reported studies (Fig. 1). The pursuit of food safety and quality is of utmost importance, and our research endeavours to contribute to this critical mission by providing a reliable, cost-effective, and efficient method for detecting formaldehyde adulteration in food.

Materials and Methods

Cyclic voltammetry transducer-based biosensor was monitored using diûerent analyte solutions of

Fig. 1. Steps for the sample preparation for formaldehyde sensing

formaldehyde. The overall reaction of formaldehyde in different vegetable samples was widely studied. The sensor consists of carbon nanotubes as the nanomaterial substrate and this nanomaterial has been coated over the indium tin oxide coated glass substrate using electrophoretic deposition techniques. To evaluate the biosensing performance of the fabricated biosensor FDH/CNT/ITO different samples from vegetables, spinach (Spinacia oleracea) and tomato (Solanum lycopersicum), were obtained from the local market, New Delhi. Different groups of samples were randomly adulterated with formaldehyde using the spiked concentration method and others blank were used as control (Fig. 3).

Sample Preparation

To conduct the formaldehyde analysis in various vegetables extract, we carefully selected fresh and healthy vegetables from the local market. These vegetables were washed properly with distilled water and then peeled. We then prepared the extract using the pulp obtained, which was subsequently filtered through a muslin cloth. The analysis of formaldehyde was carried out using the standard addition method as described in previous studies (Fig. 1) (Kundu *et al*., 2019).

HPLC Measurements

For the comparative analysis of the results, we employed a C18 Luna column with a pore size of 120 Å (25cm × 4.6 mm inner diameter, 5µm particle size) from Spincotech Pvt. Ltd. Mumbai. This column was connected to a High-Performance Liquid Chromatography (HPLC) system (model LC-6AD) equipped with a photodiode array (PDA) detector (SPD-M20A) from Shimadzu Corporation, Japan. The instrument was set to operate at a wavelength of 254 nm. The total runtime for each analysis was fixed at 10 minutes. The mobile phase used for formaldehyde detection was a mixture of water and methanol in an optimized ratio (30:60, v/v) under isocratic conditions. The flow rate for the mobile phase was set at 1.0 mL per minute, and the injection volume was fixed at 30 µL for all the samples tested. The sampling speed was set at 3 µL/sec, and data recording was done using LC solution software. Calibration graphs were generated for the HPLC data, and correlation coefficients were determined for each type of sample using both techniques.

Results and Discussion

The calculated detection limits (LOD) for formaldehyde in vegetables extract samples of spinach and tomato were determined to be 0.03 mg/L and 0.04 mg/L, respectively. Furthermore, the limits of quantification (LOQ) were found to be 0.06 mg/L and 0.08 mg/L for spinach and tomato samples, respectively (Fig. 3 and Fig. 4). The slight disparities in LOD and LOQ values between different vegetables samples might be attributed to matrix effects or interferences, as reported by researchers (Waralun,

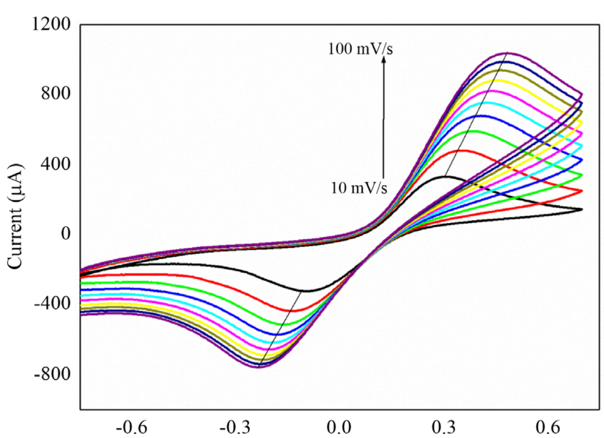

Fig. 2. Scan rate optimization studies from 10 mV/s to 100 mV/s for the formaldehyde sensor

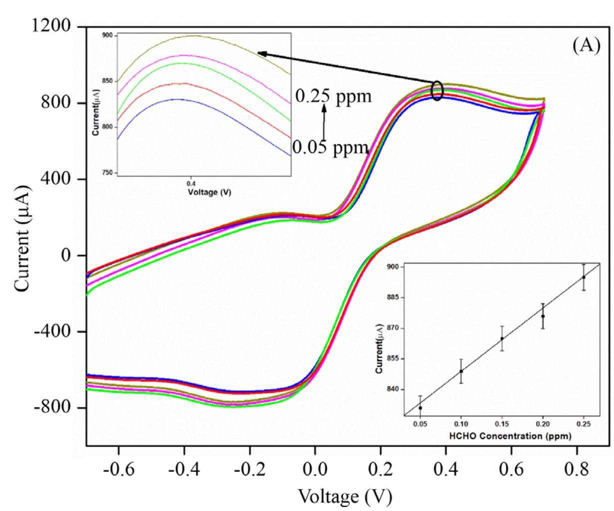

Fig. 3. Cyclic voltammetry transducer-based biosensor response for varying formaldehyde concentrations (0.05 ppm-0.25 ppm) in spinach samples

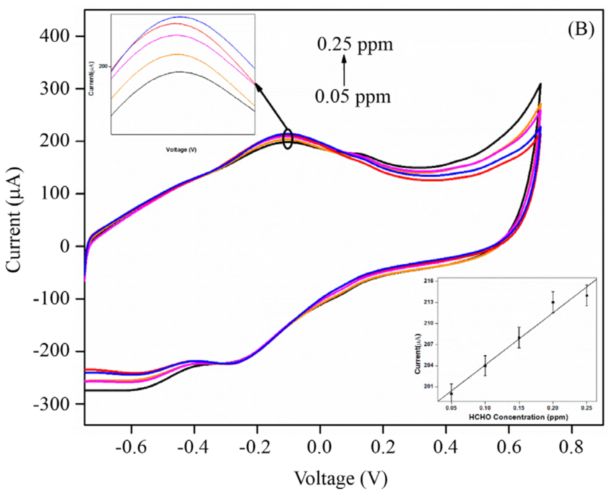

Fig. 4. Cyclic voltammetry transducer-based biosensor response for varying formaldehyde concentrations (0.05 ppm-0.25 ppm) in tomato samples

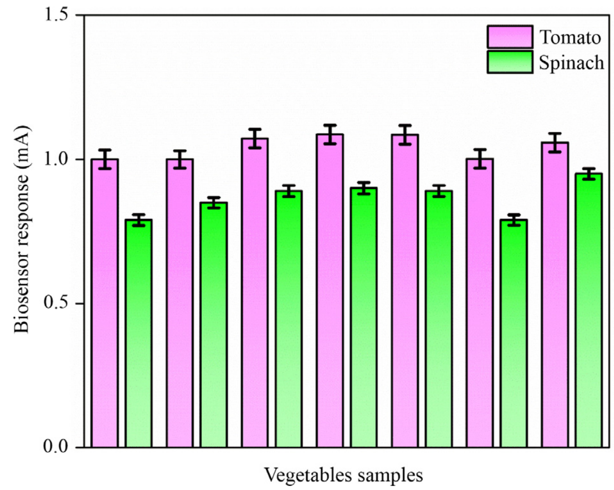

Fig. 5. Biosensor response of vegetable sample for testing the presence of formaldehyde

Chainonnok and Suwanaruang, 2019). Notably, the developed biosensor also exhibited excellent recovery rates, exceeding 92%, for both types of samples. It's worth noting that even in recently reported biosensor studies, validation with standard techniques using real samples has often been lacking (Gunawan and Sudarmaji, 2017; Sun *et al.*, 2019). In this research, we sought to bridge this gap by conducting a comprehensive comparative study with HPLC, thus establishing the reliability of the developed biosensor for formaldehyde detection. HPLC experiments were carried out to analyze the formaldehyde content in samples of both types of vegetables. The resulting chromatographic data were then compared with biosensing measurements. In the HPLC chromatographs, the retention times for formaldehyde were found to be approximately 6.1 and 6.6 minutes, for spinach and tomato samples respectively. It was observed that the peak areas exhibited linear variations with formaldehyde concentrations. The LOD obtained using the HPLC technique was 0.03 mg/L for spinach and 0.05 mg/L for tomato samples. The limits of quantification (LOQ) were found to be 0.07 mg/L and 0.06 mg/L for spinach and tomato samples, respectively using HPLC method. The acceptable comparative values obtained for LOD and LOQ using both biosensor based on cyclic voltammetry transducer and HPLC, indicated that the developed biosensor, along with in-situ applications, holds significant promise in the commercial sector due to its high specificity. In a t-test, the calculated t-value did not exceed the theoretical t-value at the 95% confidence level. This implies a strong agreement in the results between the standard technique (HPLC) and the developed biosensor, confirming the biosensor's suitability for in-situ detection applications (Fig. 5) (Table 1).

Table 1. Recovery rates of formaldehyde in vegetables samples

Sample	HCHO added (ppm)	Measured value (ppm)		Recovery (%)		RSD (%)		Error (%)	
		Biosensor	HPLC	Biosensor	HPLC	Biosensor	HPLC	Biosensor	HPLC
Spinach extract	0.10	0.096	0.094	96	97.9	0.24	0.48	6	8.0
	0.15	0.149	0.153	99.3	102	1.29	1.47	1.1	5.0
	0.20	0.189	0.199	94.5	103	1.67	1.78	0.8	4.0
Tomato extract	0.10	0.100	0.103	100	101	0.32	0.87	1.2	2.0
	0.15	0.149	0.145	99.4	97.2	1.34	1.45	1.5	1.9
	0.20	0.189	0.190	94.5	100.5	1.44	1.69	2.2	3.8

However, it was observed that the results for spinach and tomato samples were significantly different ($p<0.05$), possibly due to the presence of different interferents in samples tested. Notably, the calculated LOD values are substantially lower than the minimal risk levels (MRLs) established by various international agencies for food adulteration. The Environmental Protection Agency (EPA) has set a maximum daily dosage of 0.2 ìg/g of body weight per day for formaldehyde (Ioannidou and Gilsenan, 2021; Nirmal Kumar *et al.*, 2021; Reza *et al.*, 2023). Formaldehyde, as a non-intentional impurity, is not permissible in foods beyond 5 mg/kg (Gelbke *et al.*, 2019; Reza *et al.*, 2023). These values even fall below the WHO permissible limit of 0.9 mg/L for formaldehyde detection in drinking water (Gelbke *et al.*, 2019; Lugwisha, Mahugija and Mwankuna, 2016).

Conclusions

In conclusion, we present an easy-to-make, low-cost, simple-to- use, and reliable formaldehyde detector that can be readily used by consumers, distillers, and law-enforcing authorities for easy formaldehyde contamination screening. This modular design could also be applied for electrochemical education and other uses. Aûordable detectors are particularly attractive for widely distributed use, especially in low-income economies where food safety is a concern. In future, research studies should further be conducted to lower system costs, improve the detector2 s sensitivity and reliability, reduce the size of the detector using microûuid chips and modiûed electrodes, and perform larger-scale tests in actual-use environments. We have successfully developed an innovative biosensor for the assessment of postharvest quality and safety, specifically tailored for the detection of formaldehyde. The incorporation of CNT has proven to significantly enhance the electrochemical responses, resulting in a biosensor with remarkable sensitivity, a low detection limit (in compliance with the prescribed Maximum Residue Limit set by the EPA for human consumption), an extensive linear detection range, excellent precision, and operational stability. This biosensor is designed to determine the presence of formaldehyde in vegetables extract. Furthermore, this biosensor demonstrates minimal interference from potential interferents such as acetaldehyde, methanol, ethanol, glucose, acetone, and formic acid. The results obtained by the biosensor for formaldehyde have shown strong correlations, as indicated by high R^2 values (0.95) when compared to results obtained using the HPLC technique at a significance level of $p<0.05$. Additionally, it has exhibited high sample recovery rates.

References

Aini, B.N., Siddiquee, S. and Ampon, K. 2016. Development of formaldehyde biosensor for determination of formalin in fish samples; malabar red snapper (lutjanus malabaricus) and longtail tuna (Thunnus tonggol). *Biosensors* **6**: 32. https://doi.org/10.3390/bios6030032

Arfat Sharif, M., Kumer, A., Bosir Ahmed, M. and Paul, S. 2018. Chitosan is a new target of chemical replacement to formalin in food preservative. *International Journal of Chemical Studies* **6**: 757-760.

Gelbke, H.P., Buist, H., Eisert, R., Leibold, E. and Sherman, J.H. 2019. Derivation of safe exposure levels for potential migration of formaldehyde into food. *Food and Chemical Toxicology* **132**: 110598. https://doi.org/10.1016/j.fct.2019.110598

Gunawan, B. and Sudarmaji, A. 2017. The use of polymer based gas sensor for detecting formalin in food using artificial neural network. *Telkomnika (Telecommunication Computing Electronics and Control)* **15**: 1641-1650. https://doi.org/10.12928/TELKOMNIKA.v15i4.6164

Hoque, Md.S., Jacxsens, L., Rahman, Md. B., Nowsad, A.A.K.M., Azad, S.M.O., De Meulenaer, B., Lachat, C. and Rahman, M. 2018. Evaluation of artificially contaminated fish with formaldehyde under laboratory conditions and exposure assessment in freshwater fish in Southern Bangladesh. *Chemosphere* **195**: 702-712. https://doi.org/10.1016/j.chemosphere.2017.12.111

Ioannidou, S., Cascio, C. and Gilsenan, M.B. 2021. European Food Safety Authority open access tools to estimate dietary exposure to food chemicals. *Environment International* **149**: 106357. doi: 10.1016/j.envint.2020.106357

Kundu, M., Prasad, S., Krishnan, P. and Gajjala, S. 2019. A Novel Electrochemical Biosensor Based on Hematite (α-Fe_2O_3) Flowerlike Nanostructures for Sensitive Determination of Formaldehyde Adulteration in Fruit Juices. *Food and Bioprocess Technology* **12**: 1659–1671. https://doi.org/10.1007/s11947-019-02318-7

Lebelo, K., Malebo, N., Mochane, M.J. and Masinde, M. 2021. Chemical contamination pathways and the food safety implications along the various stages of food production: A review. *International Journal of Environmental Research and Public Health* **18**:5795. doi: 10.3390/ijerph18115795

Lugwisha, E.H.J., Mahugija, J.A.M. and Mwankuna, C. 2016. Levels of formaldehyde and acetaldehyde in selected bottled drinking water sold in urban areas in Tanzania. *Tanzania Journal of Science* **42**(1): 1-14.

Miah, Md. F., Tania, T.K., Begum, N.N. and Khan, Z.K. 2013. Effects of Formalin Contaminated Food on Reproductive Cycle and Lifespan of Drosophila Melanogaster. *Advances in Zoology and Botany* **1**: 65-70. https://doi.org/10.13189/azb.2013.010304

Mphuthi, N.G., Adekunle, A.S., Fayemi, O.E., Olasunkanmi, L.O. and Ebenso, E.E. 2017. Phthalocyanine Doped Metal Oxide Nanoparticles on Multiwalled Carbon Nanotubes Platform for the detection of dopamine. *Scientific Reports* **7**: 43181. https://doi.org/10.1038/srep43181

Nag, S., Pradhan, S., Das, D., Tudu, B., Bandyopadhyay, R. and Banerjee Roy, R. 2022. Fabrication of a Molecular Imprinted Polyacrylonitrile Engraved Graphite Electrode for Detection of Formalin in Food Extracts. *IEEE Sensors Journal* **22**: 42-49. https://doi.org/10.1109/JSEN.2021.3128520

Nirmal Kumar, V., Pillay, V.V., Ramakrishnan, U.K., Arathy, S.L. and Bhaskaran, R. 2021. Estimation of formaldehyde contamination in selected sea fish species sold in ernakulam district of Kerala state. *Journal of Punjab Academy of Forensic Medicine and Toxicology* **21**: 83-90. https://doi.org/10.5958/0974-083X.2021.00014.5

Reza, M.S. Al, Sudipto Das, S., Rahman, M.A., Rebeka, S., Lamyea Afrose, B., Mowsumi, K., Abedin, M.Z. and Luthfunnesa, B. 2023. The Impact of Formalin on Postharvest Quality, Shelf Life, and Nutritive Properties of Carrot, Papaya, Plum, Apple Plum, and Guava. *Current Research in Nutrition and Food Science* **11**: 320-338. https://doi.org/10.12944/CRNFSJ.11.1.24

Sun, X., Zhang, H., Hao, S., Zhai, J. and Dong, S. 2019. A Self-Powered Biosensor with a Flake Electrochromic Display for Electrochemical and Colorimetric Formaldehyde Detection. *ACS Sensors* **4**: 2631-2637. https://doi.org/10.1021/acssensors.9b00917

Wahed, P., Razzaq, M.A., Dharmapuri, S. and Corrales, M. 2016. Determination of formaldehyde in food and feed by an in-house validated HPLC method. *Food Chemistry* **202**: 476-483. https://doi.org/10.1016/j.foodchem.2016.01.136

Waralun, S., Chainonnok, S. and Suwanaruang, T. 2019. Iodometric Analysis of Formalin in Different Foods. *Research Journal of Applied Sciences* **14**: 235-238. https://doi.org/10.36478/rjasci.2019.235.238

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