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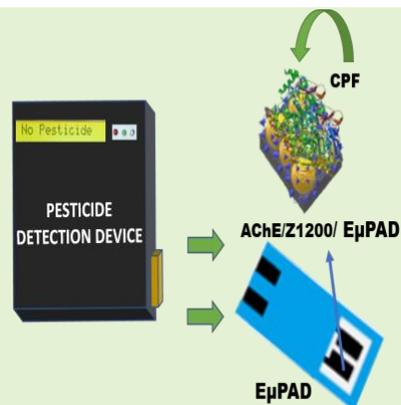
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Development of Field Deployable Sensor for Detection of Pesticide from Food Chain

Shalini Nagabooshanam, Shruti Sharma, Souradeep Roy, Ashish Mathur, Satheesh Krishnamurthy, Lalit M Bharadwaj

Abstract—The use of pesticides in agriculture field remains a serious issue related to public health. This necessitates the need to develop a low cost/portable, sensitive and selective bio-sensing platform for detection of pesticides in food chain. In this work a low-cost biosensing platform for ultrasensitive detection of chlorpyrifos (CPF) is developed. Electrochemical micro Paper Analytical Device (E μ PAD) is fabricated by conventional screen-printing technology. Metal Organic Framework (Z1200 MOF) having zinc metal and imidazole ligand is used as a transducing element which facilitates biocompatible matrix for Acetylcholinesterase (AChE) enzyme immobilization. The limit of detection for CPF is found to be 3 ng/L with sensitivity 0.521 k Ω /ng L⁻¹/mm². The sensor required 100 μ L of reagent and was tested with a linear concentration range of 10 ng/L to 1000 ng/L with response time of 5s. The sensor is further integrated with portable electronics based on Arduino microcontroller and Artificial Intelligence (AI) which provided economical, portable and user-friendly sensing platform. The stability of the sensor was 30 days. The developed sensor was also tested with real samples and the sensor response is in agreement with conventional technique.

Index Terms—Electrochemical, Impedance, Paper Microfluidics, Pesticides, Portable Device.



I. Introduction

ORGANOPHOSPHATE (OP) pesticides are widely used in agriculture to protect crops from pests and insects in order to increase production [1]. But the persistence and leaching of these toxic substances in food, air, soil and water bodies causes major health hazards such as respiratory problems, muscular dystrophy, depression, neurological disorders and even death in human beings [2]–[5]. Among the class of pesticides, OPs causes irreversible reaction with acetylcholinesterase (AChE) enzyme [6]. AChE enzyme helps in the regulation of acetylthiocholine (ATC) a neurotransmitter [7]. Chlorpyrifos (CPF) is most commonly

used in agriculture and this toxic substance tends to cause serious issues in ecosystem due to their long-term residual accumulation in environment.

The available conventional methods for detection of OPs include mass spectroscopy, gas chromatography, liquid chromatography, high-performance liquid chromatography and enzyme linked immunosorbent assay [8]–[10]. Even though these methods are sensitive and reliable, they require high maintenance and operating costs, analytical laboratory, skilled manpower, large sample volume and are time consuming [11]. Current need is to develop a sensing platform, which requires less sample volume, less response time and which is user-friendly, low cost, flexible and portable [12].

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For the past few decades electrochemical bio-sensors have drawn more attention because it offers sensitive detection platform with low detection limits and reduced sample volume and response time [13]–[17]. Among the various electrochemical detection techniques Electrochemical Impedance Spectroscopy (EIS) has been widely used for biochemical sensing applications. The advantage of EIS compared to other electrochemical detection technique is it offers high signal-to-noise-ratio, lesser assay time, rapid response time and low detection limits [18].

The Metal Organic Frameworks (MOFs) are new class of porous materials consisting of metal ions linked with organic bridging ligands which offer many advantages such as high surface area, tunable porosity and a varied choice of ligands which can be used to vary pore size [19], [20]. MOFs have

great potential in the fields of sensing, gas storage, drug delivery systems, and catalysis [21]–[24]. MOF provide a biocompatible matrix for biomolecule immobilization which helps in improving the sensitivity and selectivity [25].

In this present work, a low cost and portable paper-based sensing device was developed to detect CPF in food chain. Economical electrochemical micro paper analytical device ($E\mu$ PAD) was fabricated by simple screen-printing technology using a conductive carbon ink. MOFs provides better sensitivity and AChE enzyme provides selectivity in the detection of CPF. $E\mu$ PAD provides various advantages such as low electrode fabrication cost, less sample volume, disposability, less assay time and ease of fabrication. The developed $E\mu$ PAD is further integrated with portable electronics.

II. EXPERIMENTAL

A. Materials and Methods

Acetylcholinesterase (AChE), Acetylthiocholine chloride (ATCl), Chlorpyrifos and Z1200 MOF (Basolite Z1200) were purchased from Sigma Aldrich. Sodium Chloride (NaCl), Sodium dihydrogen orthophosphate dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), disodium hydrogen orthophosphate anhydrous (Na_2HPO_4) were purchased from Fischer Scientific. $E\mu$ PADs were fabricated in-house by screen printing technology using conductive carbon ink. The hydrophobic barrier having the microfluidic well capacity of 100 μL was made using self-adhesive polymeric sheets. The constructed $E\mu$ PAD is having two electrode configuration in which both are carbon electrodes. All experiments were performed using Milli-Q Deionized water having resistance 18.2 $\text{M}\Omega$.

B. Instrumentation

Electrochemical Impedance Spectroscopy (EIS) data were obtained using Precision Impedance Analyser, 6500B Series from Wayne Kerr. The EIS was conducted over a frequency range of 100 Hz to 1MHz at 10 mV amplitude. The impedance spectra were recorded using 0.1M phosphate buffer saline (PBS) solution optimized at pH 7.0 in the presence of 10 μM ATCl. Fourier transform infrared (FTIR) spectroscopic analysis was performed using Frontier Perkin Elmer Infra-red spectrometer. Scanning Electron Microscopy (SEM) was done using SEM QUANTA 200F.

C. Fabrication of AChE/Z1200/ $E\mu$ PAD Sensor Platform

The step wise fabrication of AChE/Z1200/ $E\mu$ PAD was shown in Fig.1. The screen printed $E\mu$ PAD was modified with Z1200 by drop casting 2 μL of Z1200 (1 mg/mL) solution dispersed in ethanol. Next, 2 μL (0.1 U) of AChE enzyme was immobilized on the Z1200/ $E\mu$ PAD by physisorption method and incubated at room temperature for 20 minutes. The fabricated sensor was stored at 4°C in the refrigerator for further utilization.

D. Preparation of Real Samples

First, 10 g of sample (Tomato) were crushed using mortar and pestle and 10 ml of PBS pH 7 solution was added. Secondly, the sample was centrifuged at 3000 rpm for 15 minutes and the supernatant was separated. These samples

were prepared by adding known amount of CPF to the supernatant solution [26].

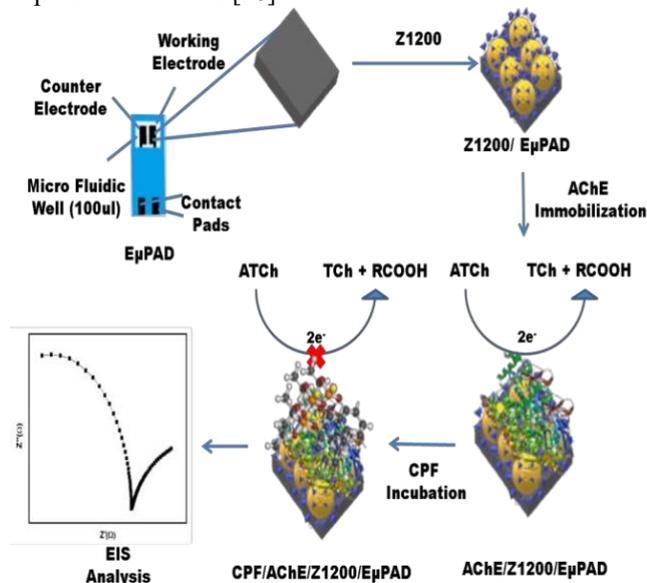


Fig. 1 Schematic the fabrication of AChE/Z1200/ $E\mu$ PAD by drop casting Z1200 MOF and AChE enzyme immobilization for the detection of CPF by EIS with sensing mechanism.

III. RESULTS AND DISCUSSION

A. SEM and FTIR Analysis

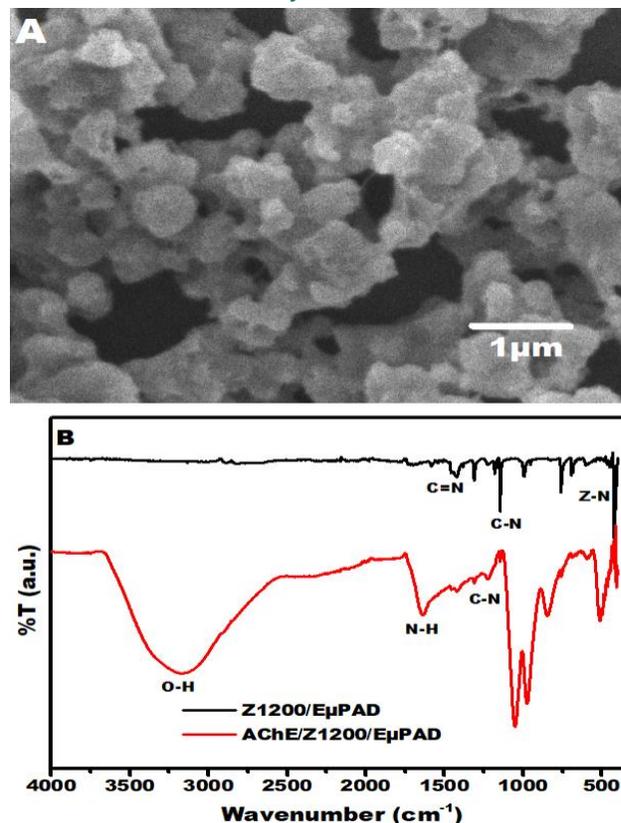


Fig. 2 A) Surface morphology characterization of as purchased Z1200 MOF by SEM, B) FTIR analysis of Z1200/E μ PAD and AChE/Z1200/E μ PAD.

Fig.2A shows the surface morphology analysis of the as purchased Z1200 MOF by SEM which shows uniform size distribution around $\sim 0.5 \mu\text{m}$ porous morphology. The rhombic dodecahedron structure of Basolite Z1200 MOF is well reported in the literature and the SEM analysis confirms the same [27]–[29]. Fig.2B shows FTIR spectroscopic analysis done at each modification steps. For the Z1200/E μ PAD, the peak at 1583 cm^{-1} was due to the C=N stretching vibration of imidazole, and the bands in the range of $1315\text{--}1500 \text{ cm}^{-1}$ were associated with the imidazole ring stretching [29]. The strong bands at 1132 and 1009 cm^{-1} were attributed to the C–N stretching of the imidazole units and the peaks from 400 to 450 cm^{-1} corresponds to Z–N in which confirms the binding of Zn^{2+} to the N present in the imidazole ligand [29]. In AChE/MOF/ μ E along with the Z1200 MOF bands, the additional band at 1630 cm^{-1} represents NH bending and scissoring mode of AChE enzyme and at 1200 cm^{-1} to 1450 cm^{-1} the bands were attributed to C–N stretching vibrations [30]. The peak around 3175 cm^{-1} is due to the presence of free hydroxyl stretching mode in AChE enzyme [31].

B. Electrochemical Characterization of Fabricated Electrode

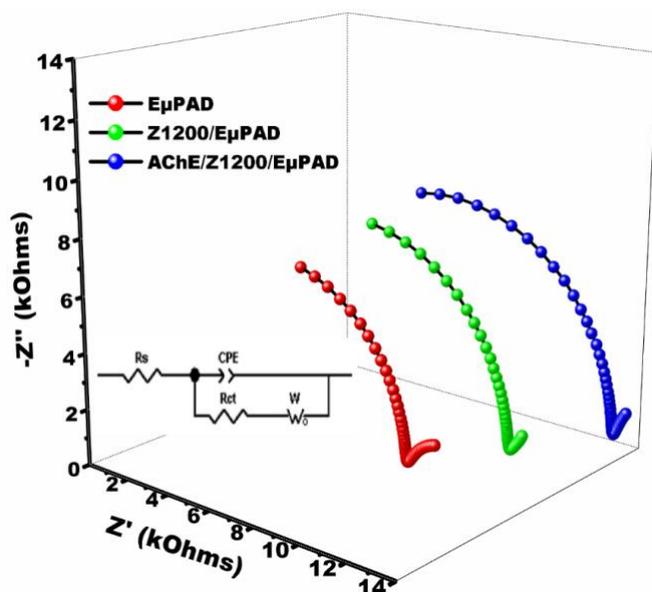


Fig. 3 Electrochemical characterization of E μ PAD, Z1200/E μ PAD and AChE/Z1200/E μ PAD in the presence of 0.1 M PBS pH 7.

The impedance response at each electrode fabrication stage (Fig. 3), was recorded in the frequency range of 100 Hz to 1 MHz with sinusoidal amplitude of 10 mV . The Nyquist plot indicated a semi-circular feature at higher frequencies. The Randles equivalent circuit (Fig.3 inset) was used to model the electrode-electrolyte interface at each stage of surface modification. Meanwhile, the presence of straight line at 45° to Z' axis corresponded to analyte diffusion from electrolyte towards electrode surface, and is modelled by Warburg

element (W). The R_{ct} at bare E μ PAD was found to be $8.9 \text{ k}\Omega$, which was lower in comparison to that of Z1200/E μ PAD (R_{ct} $10.8 \text{ k}\Omega$) surface. A high R_{ct} at the latter was attributed to decayed electron transfer process because of Z1200 being less conductive. The organic ligand possesses nitrogen groups, as can be seen in FTIR spectrum of Z1200/E μ PAD, which were responsible for the slow electron transfer at this surface. After immobilizing AChE enzyme onto Z1200, the R_{ct} further increased to $12.9 \text{ k}\Omega$. This was attributed to the poor interfacial charge transfer due to insulating nature of the enzyme, which led to electrode surface passivation after its immobilization [32]. Therefore, the distinct R_{ct} recorded at each surface modification stage confirmed the successful fabrication of AChE/Z1200/E μ PAD electrodes.

C. Analytical Sensing Performance

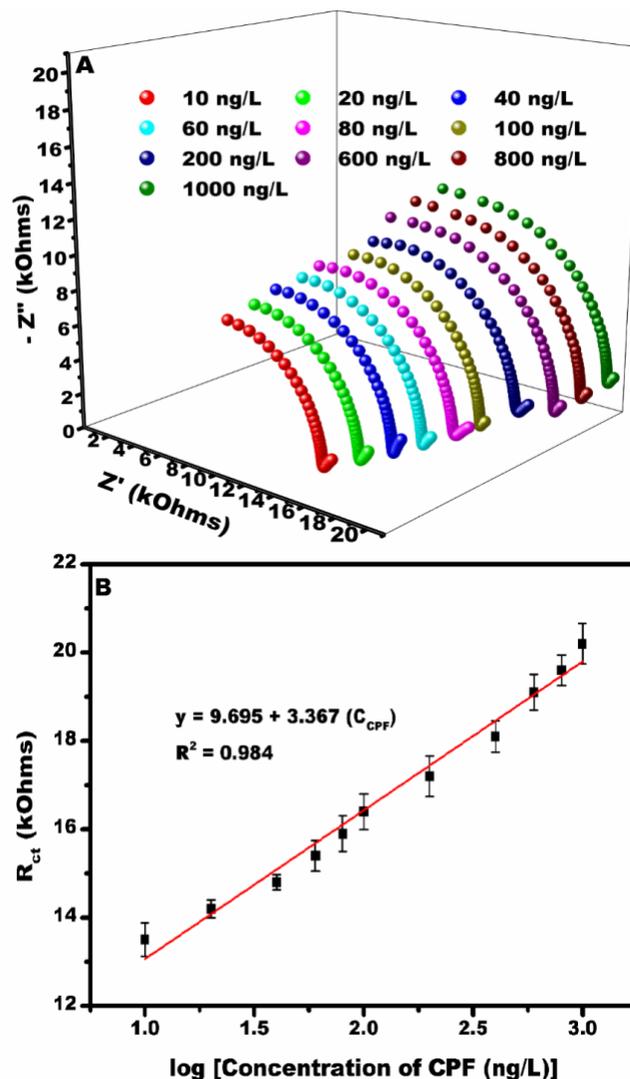


Fig. 4 A) Nyquist plot at different concentrations of CPF ranging from 10 ng/L to 1000 ng/L measure in 0.1 M PBS pH 7 containing $10 \mu\text{M}$ ATCl and B) Calibration plot at different concentrations of CPF (C_{CPF}) versus charge transfer resistance R_{ct} in range from 10 ng/L to 1000 ng/L (The experiments were repeated by preparing three replicas of electrodes and the average number is reported).

Fig.4 shows the analytical sensing performance of the fabricated AChE/ Z1200/ E μ PAD towards the detection of CPF. The impedance spectra at different concentrations of chlorpyrifos (C_{CPF}) was obtained within the frequency range of 100 Hz – 1 MHz, and is shown in Fig.4A. It can be observed that R_{ct} increases as CPF concentration was elevated from 10 ng/L to 1000 ng/L. Randle's equivalent circuit (same as mentioned in fig.3 inset) was constructed to measure R_{ct} value by curve fitting using ZView software. It has been reported that CPF act as an inhibitor of AChE enzyme by blocking the latter's active sites, thereby hindering the interfacial electron transfer [33], [34]. The increase in R_{ct} with increase in CPF concentrations can be attributed to the fact that the active sites of AChE was blocked by CPF molecules which hindered the oxidation of electroactive ATCl substrate. This leads to the formation of an insulating layer of CPF over AChE coating, and ultimately impedes the flow of electrons from electrolyte to electrode surface [34]. The AChE/Z1200/E μ PAD based sensor was calibrated at 1 kHz and exhibited linear nature within CPF concentration of 10 ng/L to 1000 ng/L as shown in Fig. 4B. A direct proportionality was established between C_{CPF} and R_{ct} with the latter being highest at 20.5 k Ω for 1000 ng/L, and lowest at 13.8 k Ω for 10 ng/L. The regression line equation of calibration plot was calculated to be R_{ct} (k Ω) = 3.367 log C_{CPF} (ng/L) + 9.695 and $R^2 = 0.984$. The Limit of Detection (LoD) and sensitivity was calculated to be 3 ng/L and 0.521 k Ω /ng L $^{-1}$ /mm 2 respectively [35].

D. Stability and Interference Analysis

Identical electrodes were fabricated to study the stability of the developed sensor. The electrodes were stored in refrigerator at 4 $^{\circ}$ C and were tested at 5-day interval in 0.1 M PBS pH 7 containing 20 ng/L of CPF as shown in Fig.5A. It was found that the sensor response was stable for 30 days. In order to test the sensor with field samples the possible interferants were also taken in account as shown in Fig.5B. The possible interferants were chosen on the basis of their frequency of occurrence in real samples. The most probable interferants as per the available literature were found to be heavy metals (Pb, As, Cr, Cd etc). Interference study was carried out by testing the AChE/ Z1200/ E μ PAD electrode in the presence of 20 ng/L concentration of heavy metals such as chromium (Cr), arsenic (As), cadmium (Cd) and lead (Pb) in 0.1 M PBS pH 7. It was observed that there was minimal change in the R_{ct} depicting negligible interference by heavy metals during the detection of CPF by AChE/ Z1200/ E μ PAD. However, the detailed study need to be carried out to affirm the selectivity of the sensor as the enzyme activity can be inhibited by other possible inhibitors.

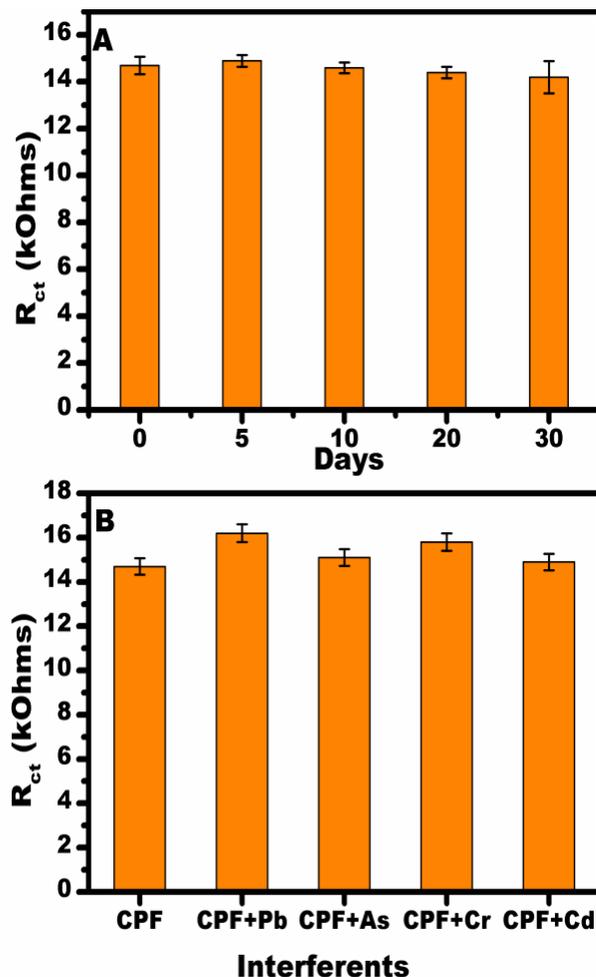


Fig. 5 A) Stability study of AChE/Z1200/E μ PAD at 5-day interval for 30 days measured in 0.1 M PBS pH 7 containing 20 ng/L of CPF. B) Interference Analysis of AChE/Z1200/E μ PAD measured in 0.1 M PBS pH 7 containing 10 μ M ATCl at 20 ng/L concentration of CPF and heavy metals (Pb, As, Cr, Cd) mixture with CPF.

E. Integration with Portable Electronics

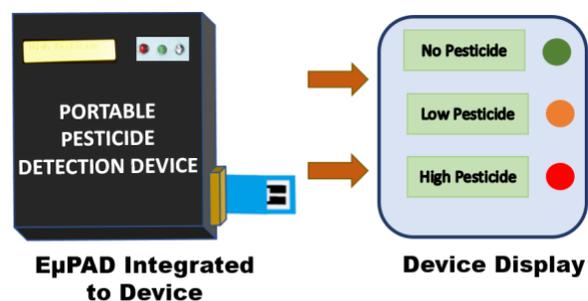


Fig. 6 Schematic of AChE/Z1200/E μ PAD integration with portable device.

After obtaining the calibration plot from conventional EIS system an attempt was made to fabricate miniature/hand-held screening device which can be field deployable. Apart from

other mentioned advantages this set up requires low operational power and minimal scientific skill set for operation. The device was fabricated based on our earlier reported protocol [36]. The Fig.6 shows the schematic of AChE/Z1200/E μ PAD integration with portable device and actual image of the developed device. According to the Maximum Residual Limit (MRL) value of CPF (50 μ g/L) the device is calibrated to display the output. If the CPF concentration is below 1 μ g/L the device displays low pesticide with orange LED, if it is above 50 μ g/L the device displays high pesticide with red LED and in the absence of pesticide the device displays no pesticide with green LED.

F. Real Sample Analysis

The sensor was tested with real samples using both techniques it was found that the developed device produces an output with ~96% accuracy as compared to EIS technique as shown in Table 1.

Table I. Comparative analyses between EIS and developed portable device at different concentrations of CPF spiked in tomato juice.

Spiked Concentration (ng/L)	Found Concentration by EIS (ng/L)	Found Concentration by Portable Device (ng/L)	% Correlation
1000	995.4 \pm 3.4	980.3 \pm 3.9	98.4
100	94.1 \pm 2.8	89.6 \pm 3.1	95.2
10	9.8 \pm 2.1	9.3 \pm 2.6	94.8

IV. CONCLUSION

Economical, portable, user friendly sensing platform is developed for screening of pesticide contamination in food samples. The developed sensor module is based on paper therefore eliminating plastic contamination as well as reducing the cost thus providing economic and environmental friendly solution. The fabricated sensor provided LoD of 3 ng/L with sensitivity 0.521 k Ω /ng L⁻¹/mm². The sensor required 100 μ L of reagent and was tested with a linear concentration range of 10 ng/L to 1000 ng/L with response time of 5s. The data obtained from the developed device correlates well with the conventional sensing methods. The sensor can also be modified for screening other OPs in food chain. The device is also capable of sending data wirelessly to portable device. More work needs to be done to extend possible integration to Artificial Intelligence (AI) module.

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