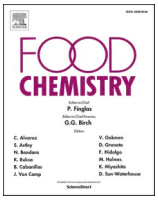
Food Chemistry 400 (2023) 134075

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Pesticide analytical screening system (PASS): A novel electrochemical system for multiplex screening of glyphosate and chlorpyrifos in high-fat and low-fat food matrices

Durgasha C. Poudyal [a,1], Vikram Narayanan Dhamu [a,1], Manish Samson [a], Sriram Muthukumar [b], Shalini Prasad [a,*]

[a] Department of Bioengineering, University of Texas at Dallas, Richardson, TX 75080, United States
[b] EnLiSense LLC, Allen, TX 75013, United States

ARTICLE INFO

Keywords:
Label-free
Electrochemical sensors
Electrochemical impedance spectroscopy (EIS)
Pesticide
Glyphosate
Pesticides in food
Cucumber
Protein powder

ABSTRACT

On-field detection of pesticide residue in complex-food matrices is a challenge when it comes to analytical detection involving multistep extraction and purification. In this study, we test the feasibility of an electrochemical portable device for detection of spiked pesticides Glyphosate (Glyp) and Chlorpyrifos (Chlp) in low-fat and high-fat food matrix. The immunoassay based two-plex sensing platform was fabricated using respective antibody glyphosate on one side and chlorpyrifos antibody on other side. The sensor response was tested using non-faradaic electrochemical impedance spectroscopy (EIS), which showed a linear response in Glyp/Chlp concentrations from 0.3 ng/mL to 243 ng/mL with limit of detection 1 ng/mL for low fat and 1 ng/mL to 243 ng/mL with LOD 1 ng/mL for high-fat matrix respectively. The laboratory-based benchtop data was then compared with portable device for feasibility of application as portable device. Such electrochemical portable sensing approach can be a future commercial field testing tool.

1. Introduction

Pesticides have been used extensively for decades to protect harvests and crops from pests such as insects. However, organophosphate pesticides have emerged as the most dominant form of pesticides in this sector - accounting for nearly 40 % of all pesticides produced (Rather et al., 2017; Wilkowska & Biziuk, 2011). Some examples of organophosphate pesticides such as malathion and chlorpyrifos are among the most widely used pesticides in both commercial and residential applications (Sturza et al., 2017). Annually in the United States alone, almost 80 million pounds of organophosphate-based pesticides are used and are applied to nearly 65 % of all pesticide-treated crops (Popp et al., 2013). Organophosphate pesticides is known to cause a toxic reaction in the human body, inhibiting the acetylcholinesterase (AChE) enzyme which is responsible for the neurotransmission of signals and could result in impaired respiratory tract and neuromuscular activity (Guyton et al., 2015; Kaushal et al., 2021; Mesnage et al., 2015). Continued inhibition of the AChE enzyme can cause permanent depolarization which can lead to seizures and eventual death (González-Alzaga et al., 2014). Due to the rampant use of organophosphate pesticides, contaminated groundwater and a variety of other vectors have exposed people to varying levels of these pesticides (González-Alzaga et al., 2014). Pesticide Safety Levels (PSLs) represent the concentration of organophosphate pesticide residue in daily consumption that would be required to reach the adjusted acute reference dose of the pesticide. Comparisons of these PSLs with regulatory levels indicate that only 1.9 % of over 4,320 PSLs were below regulatory standards (Jara & Winter, 2019). In most cases, PSL's were much higher than existing tolerance levels with PSLs being at least 100 times higher than the tolerance levels in 50.1 % of the comparisons (Jara & Winter, 2019). These comparisons show the alarming rate at which pesticide consumption is increasing and the deleterious effects that it can have on the health of a population. Due to the sharp increase in pesticide consumption and the growing levels of cross-contamination with people, there's an increased desire for detection systems that can identify trace amounts of these compounds (Rather et al., 2017). The gold standard laboratory method used for the analytical detection of pesticides is the GC–MS technique - Gas Chromatography – Mass Spectrometry (Guan et al., 2009). It has a reliable lower limit of detection for

* Corresponding author.
E-mail address: shalini.prasad@utdallas.edu (S. Prasad).
[1] Contributed equally.

https://doi.org/10.1016/j.foodchem.2022.134075
Received 1 April 2022; Received in revised form 4 August 2022; Accepted 28 August 2022
Available online 5 September 2022

organophosphate pesticides ranging from 0.01 ppm to 100 ppm. However, the major drawback of the analytical based testing method includes several pre-processing steps requiring expert and inability to use on-field rapid detection. A widely used testing approach for organophosphate pesticides in real food matrices is the CideLite broad-spectrum detection system. The CideLite detection system is an enzyme-based bioluminescent assay that can detect organophosphate metabolites within a 15-minute period. Despite its tailored sensitivity to organophosphates and its higher accuracy rate than AChE-based detection assays, the CideLite detection system isn't applicable for field-deployment and isn't a portable system requiring a Charm II Analyzer along with microplates and a full laboratory setup to detect bioactivity. There is an increased desire for a portable, field-applicable detection system due to the prevalence of pesticide usage and the increasing numbers of people who are potentially ingesting toxic chemicals. Extensive prior pre-processing of samples as well as requiring laboratory equipment is not only an expensive undertaking for farmers or regulatory agencies but generally has heightened error due to their long experimental procedures (Gilbert-López et al., 2009; González-Alzaga et al., 2014; Guan et al., 2009; Wilkowska & Biziuk, 2011). Farmers or normal consumers wishing to track pesticide levels generally don't have scientific expertise and the current options on the market require stringent pre-processing as well as technical knowledge regarding several experimental procedures. A portable, electroanalytical tool that is easy to use and can reliably detect organophosphate pesticides in an unprocessed food matrix allows responsible individuals or agencies to track pesticide levels in crops and make educated decisions regarding pertinent courses of action. Current electrochemical sensors use the AChE enzyme for the detection of organophosphates. Enzyme-based biosensors require immobilization of the enzyme on the support material and then use the immobilized system for the detection of the enzyme's substrate (Sturza et al., 2017). An AChE- based electrochemical sensor uses Nano composites of a complex of chitosan-titanium oxide-graphene for the organophosphate detection (Cui et al., 2018). This detection method had a range of detection of 0.036 μM to 22.6 μM with a detection limit of 29 nM within a time of 25 min. The chitosan-based electrochemical platform had a heightened biocompatibility and a larger surface area which aided in the sensing of the organophosphate. Another alternate biosensor for the detection of organophosphate was a design using 3D Nano flowers made up of graphene and copper oxide (Cui et al., 2018). The stability of the sensor was high with good selectivity and had a limit of detection of 0.31 ppm which indicated that it could reliably and effectively detect organophosphate pesticides. However, AChE-based biosensors have multiple shortcomings such as vulnerability to inhibition, lack of stability and a need of a substrate at the same quantification level as the pesticide itself (Liu et al., 2012). However, in this study portable immunoassay-based electrochemical sensor is used to detect organophosphate-based pesticides such as Glyphosate and Chlorpyrifos at concentrations of 1 ng/mL indicating that the sensor can detect even trace amounts of pesticide residue in food matrices. This is also the first electrochemical sensor that can actively detect pesticide in a real food sample that hasn't been processed with laboratory equipment. This high translational strength is a true asset of this electrochemical sensor in terms of its field-deployability and efficiency as a point-of-care analytical diagnostic. The two matrices being used as testing mediums for this electrochemical platform are a high-fat and low-fat matrix (protein powder and cucumber respectively). The inherent difficulty of dealing with a high-fat matrix is the complexity of the matrix itself - consisting of large triglycerides (Arduini et al., 2017; Gilbert-López et al., 2009). The isolation of the pesticide residue from the high-fat matrix involves time-consuming techniques such as gel-permeation chromatography and solid-phase extraction (Arduini et al., 2017). However, this electrochemical sensor can detect pesticide residues at low limits of detection in high-fat matrices without impairing the structural stability of the matrix. This portable electrochemical sensing platform (pesticide analytical screening system abbreviated as PASS in the manuscript title) with the ability to detect pesticide concentrations selectively and reliably in real field matrices without any prior pre-processing or laboratory equipment make it an ideal alternative to analytical methods and commercial market solutions.

2. Materials and methods

2.1. Chemicals and reagents

Glyphosate antibody, Chlorpyrifos antibody (Fitzgerald Antibodies, USA), Glyphosate and Chlorpyrifos antigen, Methanol, Dimethyl Sulfoxide (DMSO), Phosphate-buffered saline (pH 7.4) were procured from Sigma Aldrich, USA. Dithiobis succinimidyl propionate (DSP) and superblock was procured from Thermo Scientific, USA. All the chemicals in this study were of analytical grade and were used without further purification. All dilution solutions were prepared using de-ionized water.

2.2. Experimental instrumentation

Fourier transform infrared (FTIR) spectroscopy study was performed using the Nicolet iS50 FTIR.

spectrometer (at spectral scans of 256 at 4 cm^{-1} resolution at the wavelength ranging from 4000 to 600 cm^{-1}) to analyze the binding of glyphosate (Glyp-Ab) and chlorpyrifos antibody (Chlp-Ab) to the gold electrode sensing platform. The surface binding of the crosslinker and antibody (Glyphosate and Chlorpyrifos) to the gold sensing platform were also electrochemically confirmed using cyclic voltammetry. The surface image of the of the gold sensing platform before and after modification were captured using a HIROX microscope (series RH 2000).

All electrochemical measurements were performed using a laboratory based Gamry Reference 600 potentiostat (GAMRY Instruments) and portable device method using an EmstatPico modular system. The sensor platform is a two electrode system consisting of a portable printed circuit board platform having both gold surfaces as working and reference electrodes respectively. The laboratory designed gold sensor platform was a print on a PCB-FR4 substrate having ENIG gold finishing on copper-clad board as shown in the Fig. 1C (control) (Poudyal, Dhamu, Samson, et al., 2022). The data and graphs in work were plotted using GraphPad Prism, Origin Pro 2017 and the graphics of the reaction mechanism were depicted using Bio-render.

2.3. Fabrication/modification of the two-plex electrode sensing platform

Prior to the modification, the two-plex gold sensing electrode surface was washed three times with Isopropyl Alcohol (IPA) followed by washing with deionized water to remove the adsorbed surface impurities (Dhamu, Poudyal, Muthukumar, et al., 2021; Dhamu, Poudyal, Telang, et al., 2021; Poudyal, Dhamu, Samson, et al., 2022). On the dry two-plex electrode surface, 30 μL of 10 $mmolL^{-1}$ Dithiobis succinimidyl propionate (DSP) dissolved in Dimethyl Sulfoxide (DMSO) was suspended onto each side of the gold electrode surface. The chip was then allowed to incubate for a period of 90 min (all the modifications and experiments were performed inside the Faraday Cage) (Darder et al., 1999; Durocher et al., 2009). After this incubation period, electrochemical characterization methods such as Electrical Impedance Spectroscopy (EIS) and Chronoamperometry (CA) measurements were recorded. The electrode surface was then subsequently washed with Dimethyl Sulfoxide (DMSO) twice to eliminate the unbounded crosslinker remaining on the electrode surface followed by PBS wash for three times (Dhamu & Prasad, 2020). Onto the two-plex electrode surface bounded with crosslinker, 20 μL of 500 μg/mL Glyphosate antibody (Glyp-Ab) was suspended one side while the other side with 20 μL of Chlorpyrifos antibody (Chlp-Ab) of concentration 500 μg/mL. The antibodies suspended (Glyp-Ab and Chlp-Ab) two-plex electrode was

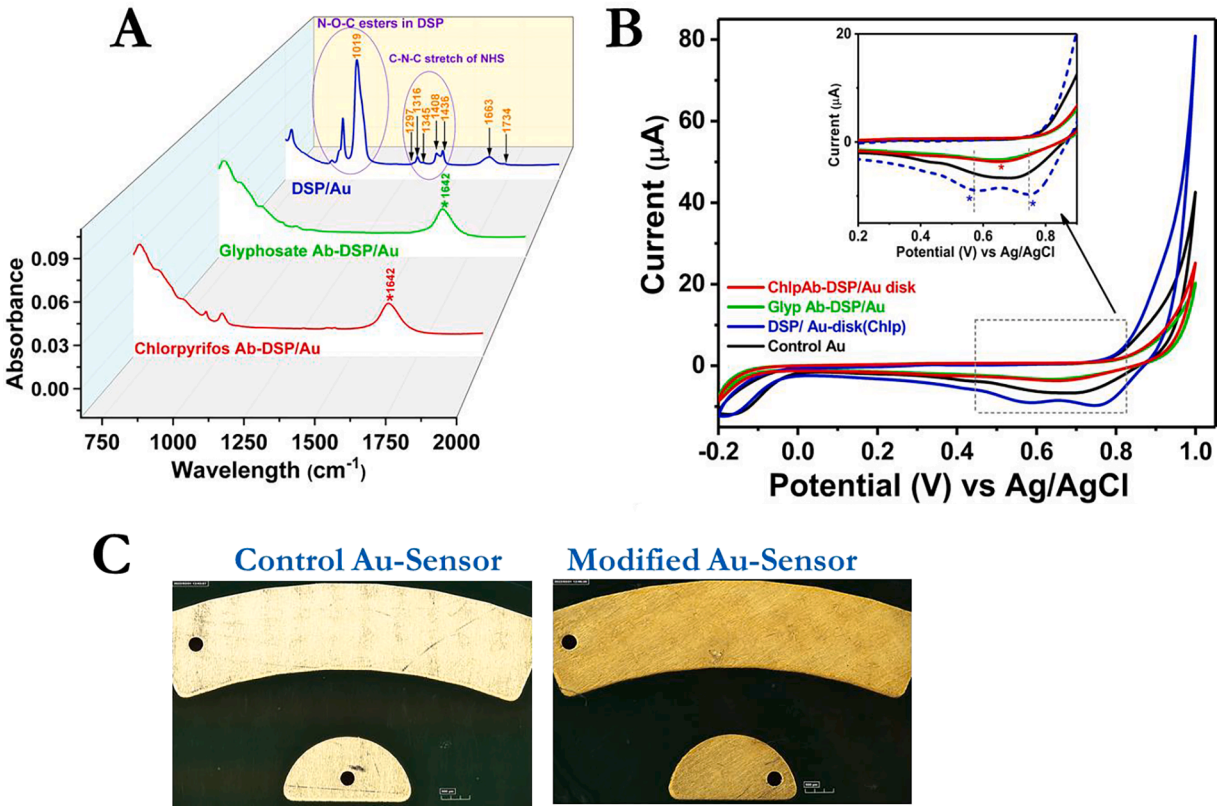


Fig. 1. (A) The FTIR spectra of the gold electrode after immobilization of the thiol-based crosslinker (blue spectrum represented as DSP/Au), Glyphosate-antibody immobilized on DSP/Au (green spectrum) and Chlorpyrifos-antibody bounded on the DSP-Au sensing substrate (red spectrum). (B) Electrochemical confirmation of each modification step of the Au-sensing substrate performed using three electrode system in PBS (pH 7.4), inset represents the cyclic voltammetry of the Au-control disk (represented by black line), DSP immobilized Au-disk (blue line), Glyphosate antibody and Chlorpyrifos antibody bounded DSP on Au-Disk surface represented in green and red line respectively. (C) Optical images (Hirox images) of the control Au-sensor substrate and antibody (Glyp-Ab or Chlp-Ab) modified Au-Sensor substrate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

allowed to incubate for 30 min. After completion of the incubation, the EIS and CA measurements were recorded to confirm the modification steps electrochemically. The chip surface was then washed with 60 μL of PBS (once) to remove any excess antibody and lastly 30 μL of Superblock (Sb) was dispensed on each side of the electrode and incubated for 10 min to reduce nonspecific binding by hydrolyzing the functional sites of DMSO (Darder et al., 1999). The electrodes surface was washed with PBS twice and stored at 4 ^{0}C for further use.

2.4. Preparation of low-fat (cucumber) and high-fat matrix (protein shake)

To demonstrate the low-fat test sample matrix for this study, fresh organic cucumber sample was purchased from WHOLE FOODS® market. The cucumber solution was prepared by first thoroughly washing the exterior of the cucumber, this was done to remove any adsorbed surface-level impurities. The cucumber was then sliced into smaller pieces for easier processing (whole cucumber with skin). It was then added to the blender (using Oster® One-Touch Blender with 800-Watt Motor and auto-Programs) where it was processed using the salad/chop function for 5 min. Upon reaching a semi-liquid, semi-solid state, the cucumber solution was then processed using the Pulse feature for an additional 5 min. The 100 mL of well blended cucumber slurry was then diluted with 100 mL amount of PBS (1:1 ratio of cucumber to PBS) to create a 50 % concentration of Cucumber - PBS solution and Pulsed (feature in blender) for another 1 min to homogenize the solution matrix. After the processing steps in the blender, it was transferred to a plastic container and allowed to rest overnight in the refrigerator (4 ^{0}C). This resting time (20 h) allowed the mixture to form two layers in the suspension and create a purely liquid layer naturally that could be used for further concentrations. Upon extracting the supernatant from the cucumber suspension, the solution was added to several vials, and they were subsequently spiked with Glyphosate and Chlorpyrifos antigen in the dynamic concentration range of 0.3 ng/mL, 1 ng/mL, 3 ng/mL, 9 ng/mL, 27 ng/mL, 81 ng/mL and 243 ng/mL respectively.

Similarly, to demonstrate the high-fat test sample matrix for this study, we choose an organic protein powder, a plant-based Protein powder from Orgain Organic Protein ^{TM}. The 100 % protein solution was prepared by first weighing 10 g of protein powder and 100 mL of PBS (7.4) solution. The mixture was blended (using Oster® One-Touch Blender in the mix shake feature for 5 min. The solution matrix was then diluted to make 50 % protein-PBS solution (the 50 %-50 % Protein-PBS concentration was optimized after running several experiments). The 50 % protein mix was then allowed to stand overnight (20 h) in the refrigerator (4 °C). After 24 h, the supernatant solution was slowly withdrawn and used as the stock high fat matrix. The matrix solution was then subsequently spiked with Glyphosate antigen and Chlorpyrifos antigens separately in the dynamic concentration range of 0.3 ng/mL, 1 ng/mL, 3 ng/mL,9 ng/mL, 27 ng/mL, 81 ng/mL and 243 ng/mL respectively.

3. Results and discussion

3.1. Characterization of the modified two-plex electrode surface

The affinity-based detection is the sensing phenomenon employed for this study. Therefore the binding/chemisorption of the thiol-based crosslinker to the control Au-substrate and subsequent immobilization

of the antibody's Glyphosate-Ab/ Chlorpyrifos to the crosslinker DSP (Dithiobis succinimidyl propionate) was confirmed using the FTIR spectroscopy. A glass surface was deposited with thin Au-film (by electron vapor deposition) modified with the antibody's similar to the Au-sensor platform (discussed in the section 2.3) was used for this study. As shown in Fig. 1A, blue spectrum represents the binding of the thiol-crosslinker to the Au-substrate as depicted by the N—O—C esters peak at 1019 cm^{-1}, CO stretching of NHS at 1734 cm^{-1} and C—N—C stretch of the NHS esters bonds (marked at 1436 cm^{-1},1408 cm^{-1}, 1345 cm^{-1}, 1316 cm^{-1} and 1297 cm^{-1}) (Poudyal, Dhamu, Samson, et al., 2022). On immobilization of the antibody's (both Glyp-Ab and Chlp-Ab represented by green spectrum and red spectrum respectively) to the crosslinker-Au surface, the characteristics NHS esters peaks were observed masked, while the enhanced aminolysis peaks at 1642 cm^{-1} were observed for both the antibodies (Darder et al., 1999; Durocher et al., 2009).

The characteristics binding phenomenon were also validated by electrochemical method as shown in Fig. 1B. The cyclic voltammetry measurement was studied on three electrode system using commercial 2 mm Au-disk as working, Ag/AgCl (Sat. KCl) and Pt wire as reference and auxiliary electrodes respectively. The CV were recorded in PBS (pH 7.4) with scan rate of 50 mV/s in the potential window −0.2 V to 1 V (vs Ag/AgCl) at each step of electrode modification (mimicking modification steps discussed in section 2.3). As shown in Fig. 1B, after crosslinker immobilization the Au-Disk surface showed reduction peaks at ~ 0.585 V and ~ 0.747 V showing capacitive behavior probably due to the S-Au bonding on the Au-surface. On the other hand, subsequent loading of the antibody to the thiol modified gold surface, the two peaks get masked which can be attributed to the binding of the antibody to the crosslinker surface. The characteristic binding feature of the electrode were also recorded using impedance at each stage of fabrication represented in the Fig. S2 (supporting information). Fig. 1C represents the optical images (HIROX microscope series RH 2000) obtained for the designed two-plex sensor platform, before modification and after modification, a uniform thin-film of bounded modification layer is observed on the antibodies-DSP modified Au-sensor substrate even after the washing the surface with buffer (PBS pH 7.4).

3.2. *Electrochemical detection of pesticides in high-fat and low-fat on modified two-plex sensor platform*

The detection of pesticides residue has always been a challenge due to the complexity in the food-matrix. The traditional analytical method involves multistep sample processing with large sample size or quantity requirement, with time-consuming tedious and expensive process. Most recent development commonly known as as QuEChERS method (quick, easy cheap, effective, rugged and safe) has popular as it reduced the multistep process and function over a range of pesticide with small sample quantity as compared to the older method of analysis (Wilkowska & Biziuk, 2011). This method is a based on the micro-extraction using the solvent acetonitrile and purification using the dispersive solid phase extraction. On the other hand, when it comes to the detection of the residue levels in the food or related commodities, the assessment involves the residue level not exceeding Maximum residue limit (MRL) as per the regular-derived safety standard limit (Vicini et al., 2021). Therefore, for the regular assessment in terms of consumer levels or in-situ on-field study or testing of the safe threshold limit, a portable electrochemical sensor device would be more convenient, quick, and cheap assessing tool. However, with the varying food matrix properties, the complexity of the detection may vary. Therefore, in this study, we chose two food matrices, low-fat with cucumber as one of the representative sample and organic protein powder (dietary supplement

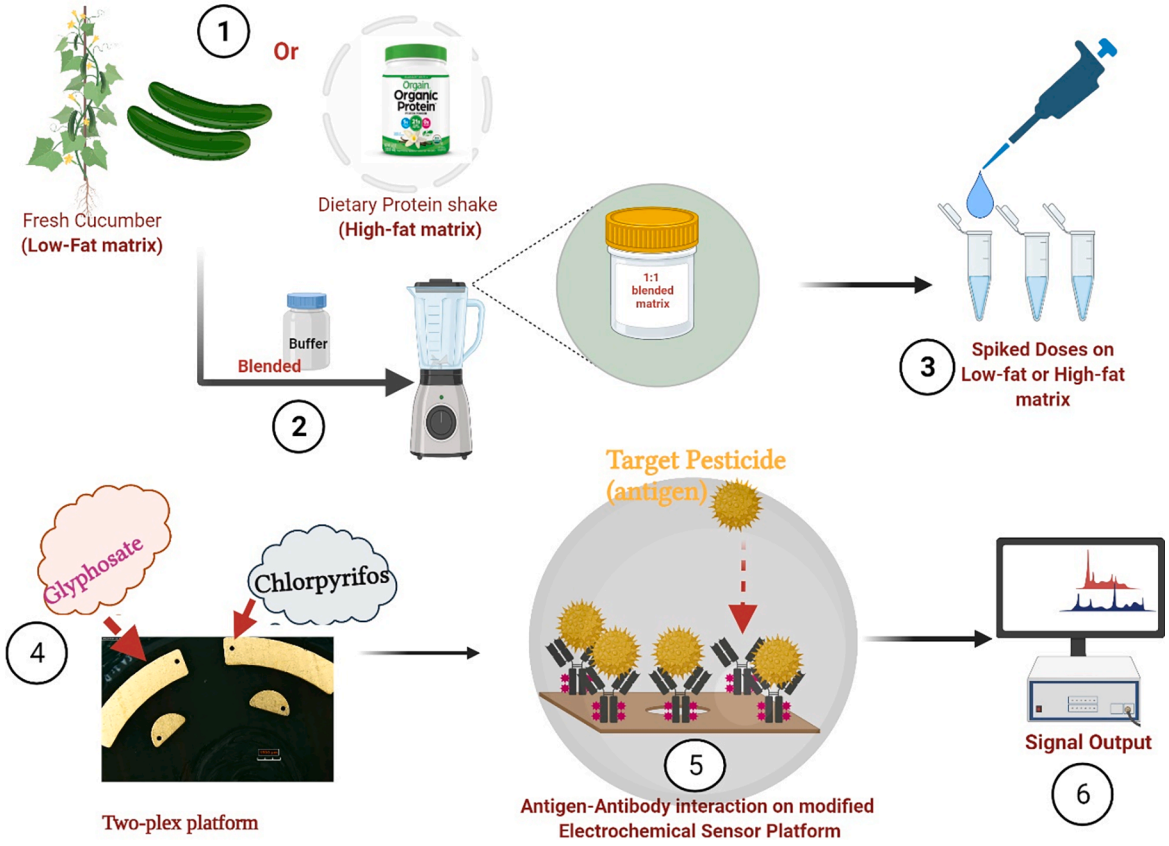


Fig. 2. Schematic of the sample matrix preparation of high-fat/low-fat matrix and electrochemical detection of Glyphosate and Chlorpyrifos on the modified two-plex sensor platform.

consumed commonly as protein shake) as the high-fat food matrix. A schematic representation of the matrix process and electrochemical detection is shown in Fig. 2. The immuno-assay or antibody-based modification was chosen for the two-plex sensor platform to simultaneously detect two common pesticides, Glyphosate and Chlorpyrifos. The electrochemical measurement due to the non-faradaic event occurring at the electrode surface was used to gauge the signal using electrochemical impedance spectroscopy (EIS) at a small applied 10 mV AC bias (Poudyal, Dhamu, Paul, et al., 2022). The double layer charge modulation at the electrode surface due to the binding of the target antigen to the specific antibody (Glyp-Ab on side and Chlp-Ab on the other side) is observed as a signal change with the added antigen concentration.

4. Electrochemical signal response of low fat and high matrix on the modified two-plex sensor platform.

The performance of the modified two-plex sensor for the simultaneous detection of the Glyphosate (Glyp) and Chlorpyrifos (Chlp) antigens were confirmed electrochemically using non-faradaic Electrochemical impedance spectroscopy (EIS). The EIS is very sensitive electrochemical tool which can gauge the interaction on the antibody modified electrode sensor platform as function of binding of the antigen (Bahadir & Sezgintürk, 2016). All measurements in this study were first recorded using the standard benchtop laboratory potentiostat (a Gamry Series 600 Potentiostat) followed by a portable Emstat Pico Development Module which serves as an inquiry into the feasibility of the sensing platform as a point-of-care, portable, field device. The two-plex sensor was employed for low-fat matrix initially to detect the Glyp and Chlp simultaneously and then similarly the study was extended to high-fat matrix for the both the pesticides (side by side simultaneously).

The performance characterization of the modified sensor was done by measuring the EIS and creating their respective calibrated dose response (CDR) plot. For the CDR measurements, the respective spiked doses containing Glyphosate antigen (Glyp) and Chlorpyrifos antigen (Chlp) were separately prepared by serially dilution (3 factor) of the antigen concentrations starting from zero dose (ZD) or control matrix, 0.3 ng/mL, 1 ng/mL, 3 ng/mL, 9 ng/mL, 27 ng/mL, 81 ng/mL and 243 ng/mL in the cucumber matrix (as discussed in section 2.4).

On each side of the two-plex sensor platform, 90 μL of the control matrix (ZD) was suspended into the modified electrode surfaces and the solution was allowed to incubate for 30 min. Fig. S1 (supporting) represents the open circuit potential (OCP) study performed on the high-fat matrix. The initial shift (drift) in the potential was observed from 0 to 1580 *sec*, which may be attributed to the adsorption of ingredients such as minerals, additives or vitamins present in protein. While after 1580 s, the system moves towards stability with the decay or drift of 3.3 μV/s. Therefore, the initial 30 mins incubation time was maintained to allow the system/matrix to stabilize on the sensor platform. However, on the subsequent dosing the response was not affected with the lower incubation period and hence 12 min incubation period was maintained.

After the incubation period, EIS and CA measurements were recorded to determine the signal baseline for further comparison. The ZD (control matrix solution) was then removed slowly using a pipette. Subsequently, the lowest concentration dose of 0.3 ng/mL of Glyphosate (Glyp) or Chlorpyrifos (Chlp) was then dispensed onto the electrode surface modified with their respective antibody and allowed to incubate for 12 min for the sufficient binding between antigen and the antibody

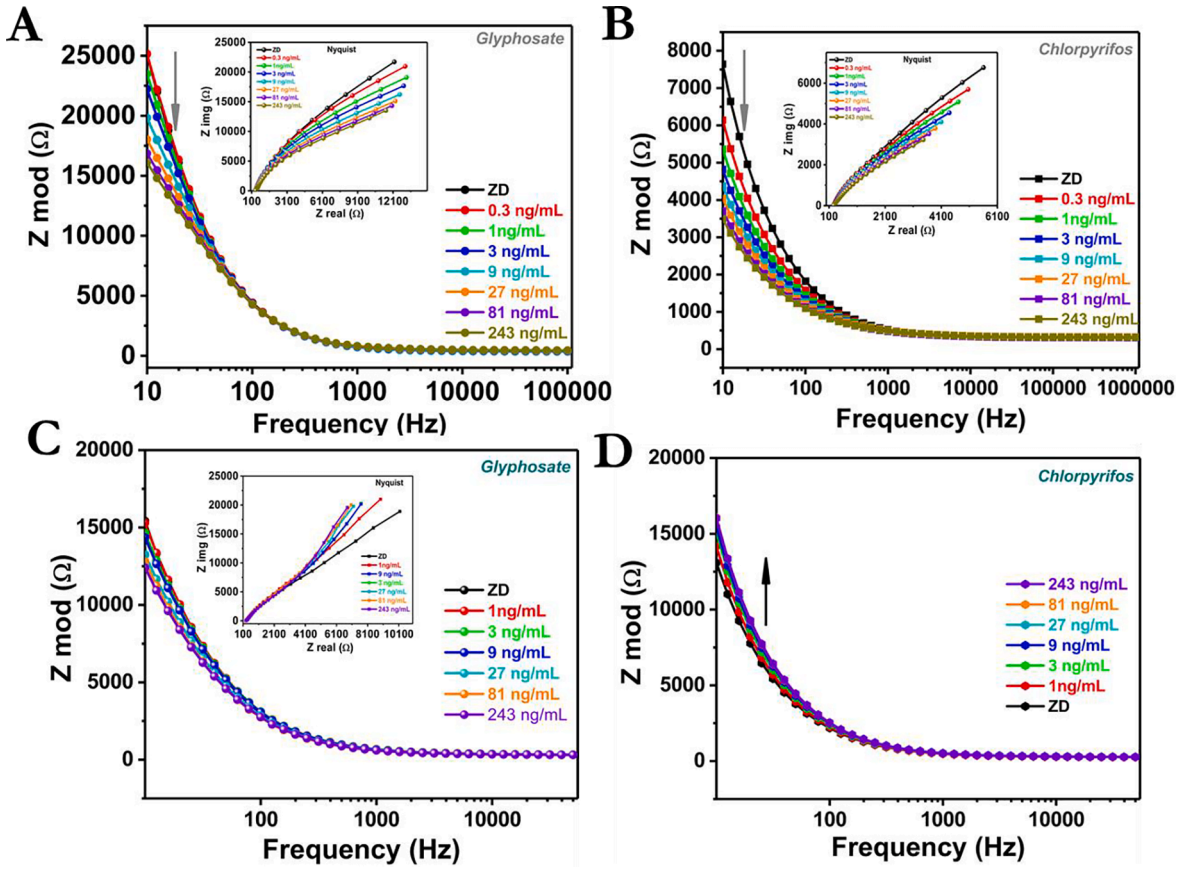


Fig. 3. (A,B) Represents the characteristics Bode response plot for the CDR study for the Glyp and Chlp antigens in two-plex platform respectively for the spiked dose concentrations ZD, 0.1 ng/mL to 243 ng/mL in the low fat matrix (cucumber). (C,D) represents the characteristics response plots obtained for high fat matrix for the spiked Glyp and Chlp antigens respectively. Insets plots are their characteristics Nyquist response (CDR) plot for the spiked doses on the modified two-plex sensors platform.

modified on the electrode surface. EIS measurements were recorded after the incubation process was completed. The procedure was repeated for remaining spiked doses of the antigens 1 ng/mL, 3 ng/mL, 9 ng/mL, 27 ng/mL, 81 ng/mL and 243 ng/mL respectively for both side of the two-plex sensor platform. Fig. 3 represent the raw impedance data obtained from the EIS measurements (at frequency range 10 Hz-10^6 Hz) and can be plotted as Bode plot (Z_{mod} (Ω) plotted against the frequency (Hz) and Nyquist plot (Z_{real} vs Z_{imag}) for the spiked concentration doses 0.3 ng/mL to 243 ng/mL.

Fig. 3 A, dose dependent raw Bode-plot obtained from EIS measurements, for the spiked Glyphosate (Glyp) in low fat matrix, Z_{mod} (Ω) plotted against the frequency (Hz), shows the decrease in the Z_{mod} value for the control (baseline) as 25000 Ω to 15500 Ω with the increasing concentrations of the spiked Glyphosate doses. The linear decrease in the Z_{mod} as function of the added antigen may be attributed to the increasing binding of the added antigen concentrations and consequently resulting in the modulation of the double layer and signal variation at the large frequency range (Daniels & Pourmand, 2007; Tanak et al., 2019). Highest signal to ratio was obtained at 100 Hz, which was chosen frequency to analyze/extract and generate the calibrated dose response plot. Additionally, the inset Nyquist data plot (plot of Z_{img} vs Z_{real}) for the same measurement was observed to show decrease in the imaginary impedance with the increasing concentration of the antigens bending towards more real value depicting the binding of the antigens. This observation can be attributed to the non-faradaic process involving no redox material (ferri/ferrocyanide), the imaginary part of the impedance is known to be inversely proportional to the electrical double layer capacitance.

In case of other pesticide Chlp (Fig. 3B), similar observation was noted were the plot of Z_{mod} vs Frequency (Hz), shows decrease in the Z_{mod} value (7500 Ω for baseline to the 3500 Ω for the highest dose) and the Nyquist tends toward more to the real value depicted the binding interaction between the antibody modified electrodes and antigen for the concentration ranging from 0.3 ng/mL to 243 ng/mL, showing slightly saturation towards the higher concentrations region.

Our study was extended for the two-plex pesticide substrate for High-fat matrix (protein shake). The solutions concentration for CDR study were similarly prepared as discussed for the low-fat matrix. The bode plot as shown in Fig. 3C shows the linear response from 1 ng/mL to 243 ng/mL spiked concentration doses of Glyp-antigen with the Z_{mod} (Ω) value decreasing from 16000 Ω (for baseline) to 12,500 (Ω) for the highest concentration and Nyquist show similar features as low-fat matrix. However, for the chlorpyrifos in the high-fat protein matrix as shown in Fig. 3D, the Z_{mod} (Ω) response is seen increasing linearly toward higher value with the increasing concentrations of the added chlorpyrifos doses 12,500 (Ω) for control baseline to higher doses 16,500 (Ω). The observed feature of the Z_{mod} (Ω) value response signal can be correlated to the matrix solution charge influence by the various added mineral during the processing. During the incubation or zero doses the charge or the nutrient would tend to adsorb on the modified electrode surface, therefore the signal were observed only from 1 ng/mL of the antigens. The antigen Chlorpyrifos being highly non-polar in nature, the response signal due to the antigens and the interaction to the electrode surface or the charge modulation on the double layer may be influenced by the nutrients charge of the matrix as the first layer adsorbed which is followed by binding of the added antigen concentration, giving the response as the increase Z_{mod} value. Highest signal to ratio was obtained at 100 Hz, which was chosen frequency to analyze/extract and generate the calibrated dose response plot for High-fat matrix as well.

4.1. *Spike and recovery study for the low-fat and high-fat matrix using laboratory benchtop method*

Fig. 4 demonstrate the calibrated dose response (CDR) (N = 18 replicates), spike and recovery (N = 3) study for the Low-fat and High-fat matrix obtained on the modified sensor platform. Fig. 4 A represents the sensor response as a function of the added varied concentrations of Glyp, plotted in the form of percentage change in the Z_{mod} value from the baseline. The higher signal to ratio was observed at 100 Hz in the impedance measurements, which was the chosen single frequency used to extract data to generate the CDR in Low-fat matrix. The percentage

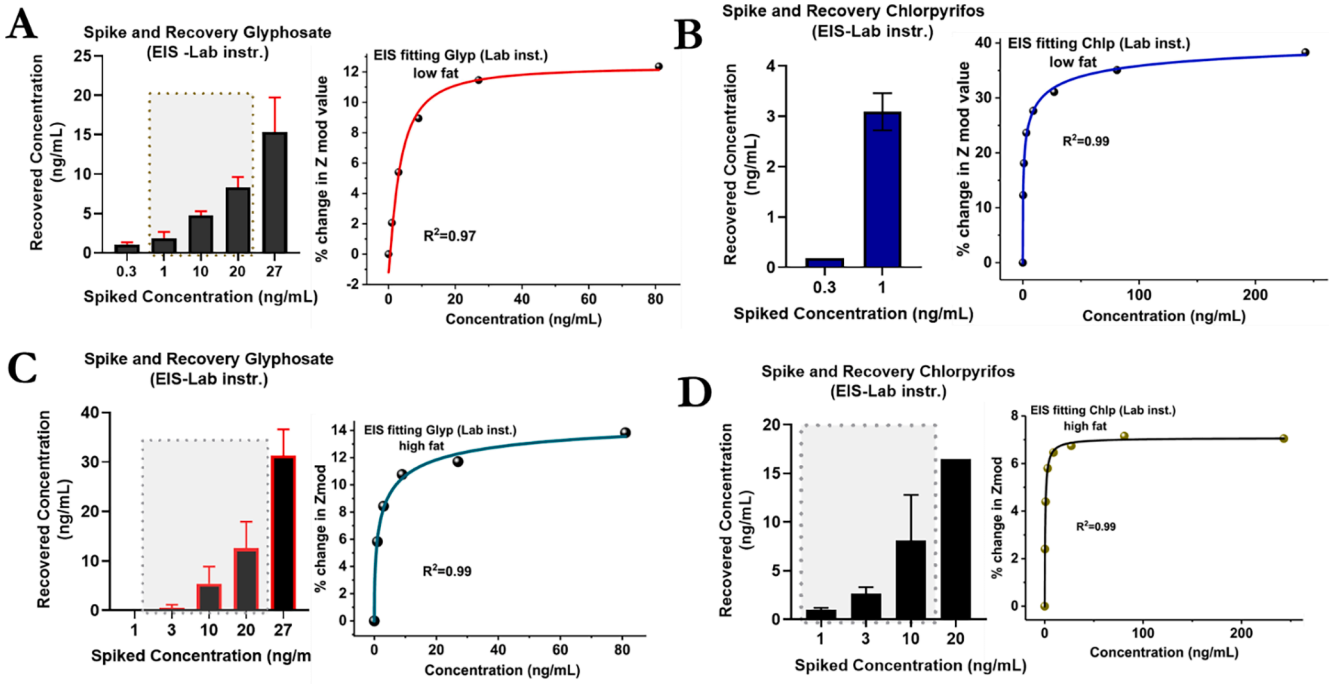


Fig. 4. The Electrochemical performance of the two-plex sensor platform in laboratory benchtop method. (A,B) Represents the calibrated dose response plot obtained for the spiked concentration doses of Glyphosate and Chlorpyrifos from 0.3 ng/mL to 243 ng/mL in Low-fat (Cucumber) matrix. Similarly (C,D) are their CDR response in High-fat matrix. The respective right-side plots are their spiked and recovery performance.

change in Z_{mod} value for spiked Glyp in Low-fat matrix (cucumber matrix) from 0.3 ng/mL to 243 ng/mL was found to be 2 % to 12.5 % with the limit of detection 0.3 ng/mL. The CDR plot was fitted using the non-linear regression model with the $R^2 = 0.97$, the equation was used to calculate the recovered concentration. Fig. S3 (supporting information) shows representative CDR plot with specific signal threshold (SST) which was calculated to distinguish signal from noise (3*SD of blank + mean of Blank/ZD). The left figure represents the five randomly spiked concentrations concentration of Glyp (0.3 ng/mL, 1 ng/mL, 3 ng/mL, 9 ng/mL and 27 ng/mL) and this range covers the lowest threshold range of the Glyp in the food matrix.

Similarly for the High-fat matrix (as shown in Fig. 4 C), the percentage change in Z_{mod} value for spiked Glyp from 0.3 ng/mL to 243 ng/mL show similar change from be 4.8 % to 14 % with the limit of detection 3 ng/mL. The spiked doses concentration were found successfully recovered at Low-fat matrix and were within the ±10 % to 15 % error. In Chlorpyrifos antigen as shown in Fig. 4 B, in Low-fat matrix, the percentage change in Z_{mod} value (N = 18 replicates) ranges from 12 % to 38 % and with the limit of detection 0.3 ng/mL with the coefficient of determination $R^2 = 0.99$ and their respective recovery study were performed. Whereas in case of Chlp in High-fat matrix (as shown in Fig. 4D), the percentage change of 2.2 % to 7 % was observed ($R^2 = 0.99$, for N = 18 replicates), with the LOD of 1 ng/mL and reproducible recovered concentration from 1 ng/mL to 2 ng/mL (error ± 20 %).

4.2. Spike and recovery and correlation study between the benchtop and portable device

After successful performance study of the two-plex sensor platform to detect pesticides (Glyp and Chlp) in both High-fat and Low-fat matrix, a feasibility test for the possible translation to portable device were performed. All the electrochemical performance were tested using the portable device system similar as the test performed for laboratory instrument (discussed in section 3.3). As shown in Fig. 5 (A&C), the calibrated dose response for the Glyphosate in Low-fat matrix (Cucumber) showed the percentage change from 1.8 % to 7% with the LOD 1 ng/mL (coefficient of determination $R^2 = 0.99$). Whereas the Chlp in Low-Fat matrix (Fig. 5B) showed the percentage change from 1 % to 12.5 %. For the study of recovery performance for the randomly picked concentration doses within the CDR range, the device platform in low-fat matrix showed sensitive response in low dose 1 ng/mL and high concentration doses (10 ng/mL and 20 ng/mL).

Although the high fat matrix is complex matrix (Guan et al., 2009) containing several minerals and ions, the presence of the ions layer showed stable and linear recovery response performance for both the antigen (Glyp and Chlp). Fig. 5 (C,D) demonstrate the calibrated response plot of Glyp (percentage change of 2 % to 11 % from baseline, with coefficient of determination $R^2 = 0.99$) and Chlp (percentage change of 4.2 % to 13.8 % from baseline, $R^2 = 0.98$) both showing limit of detection as low as 1 ng/mL. Interestingly in this high fat matrix, despite of the presence of large ions and protein sources, adsorption which may affect the double layer surface charge of the modified electrode, the recovery response were very sensitive at all doses (error within ± 10). This may be attributed to the formation of charge diffuse layer, generating a better signal even in the presence of the interferent. Additionally, for reference, the tabular form of spike and recovery data for both the low and high-fat matrices of each target pesticides has been added in the newly added supplementary section as Figs. S4 and S5 respectively.

5. Cross-reactivity study

The selective performance of the fabricated sensor platform is very crucial in real time applications. When it comes to the real-life analysis, whether it is a fresh food matrix or the processed protein shake like matrix, variety of nutrients are expected to add complexity to the matrix and the electrode due to binding of inherent nutrient and minerals. Additionally, presence of the structural similar pesticides in similar and higher concentration will mislead the data. Therefore, the modified sensor platform was tested for the cross-reactivity by measuring the response of the cross-reactive antigen spiked in the concentration's doses from 1 ng/mL to 243 ng/mL in presence of each other in Low-fat and High -fat matrix as shown in Fig. 6 (A-D). The response plot of the cross-reactive study in Low-fat matrix is represented in Fig. 6 (A,B)

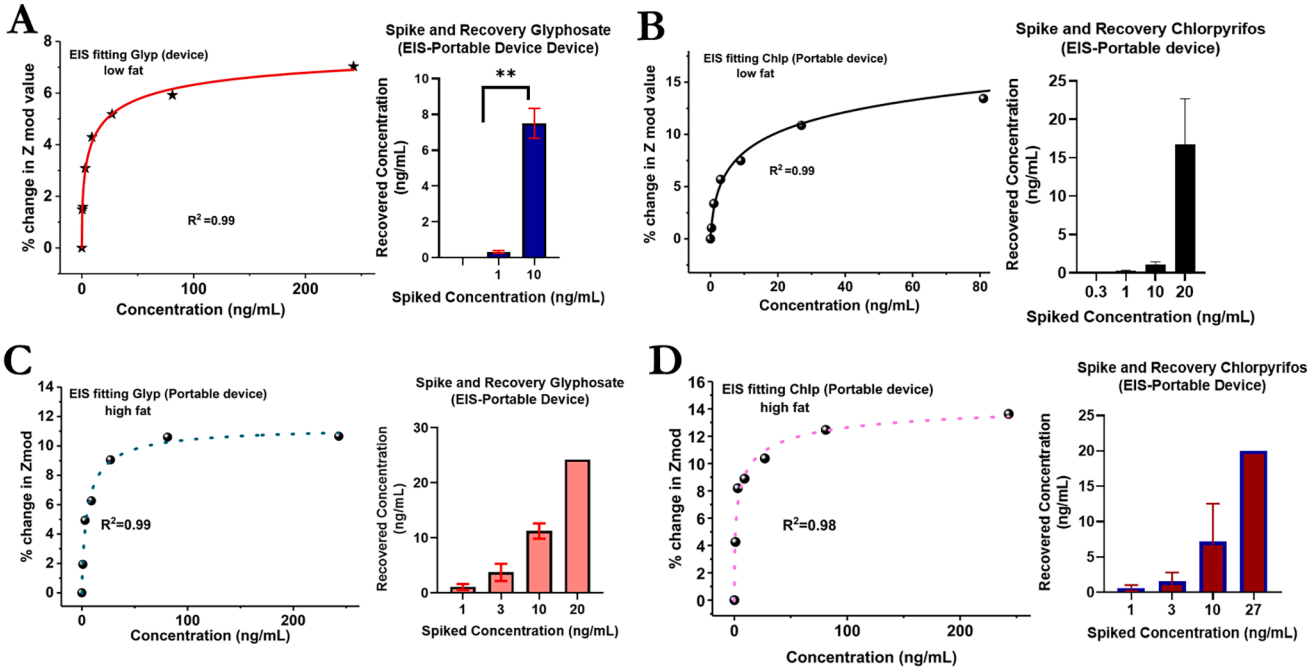


Fig. 5. The Electrochemical performance of the two-plex sensor using portable sensing device (A,B) Represents the calibrated dose response plot obtained for the spiked concentration doses of Glyphosate and Chlorpyrifos from 0.3 ng/mL to 243 ng/mL in Low-fat (Cucumber) matrix. Similarly (C,D) are their CDR response in High-fat matrix. The respective right-side plots are their spiked and recovery performance.

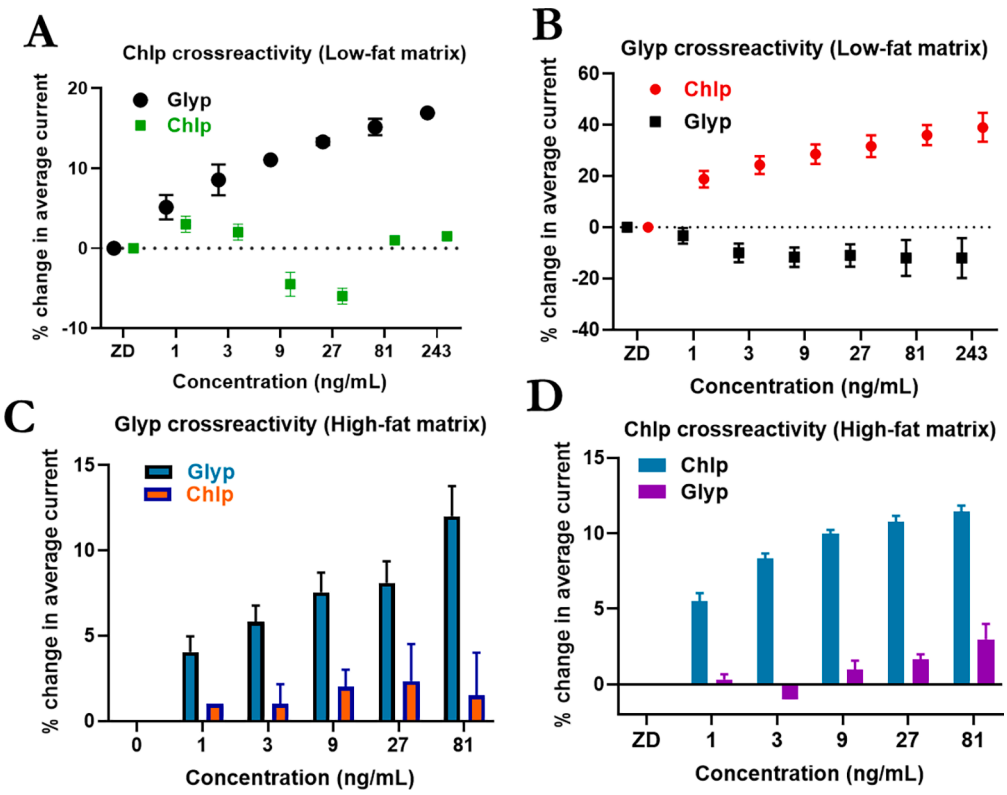


Fig. 6. The percentage change in the response profile observed for the spiked doses concentration of Glyp and comparison to the Glyphosate detection platform response for the Chlp antigen. (A, B) represented the respective percentage change to the opposite antibody in Low-fat matrix, (C,D) Cross-reactivity study of the Glyp vs Chlp in High-fat matrix.

represents, where the response due to chlp doses on the Glyp-Ab sensor platform showed 3 % change in total impedance only upto 1 ng/mL of spiked concentration. In case of the Chlp-Ab modified sensor platform, the response performance of Glyp showed the impedance showed completed opposite profile, confirming high selective performance with the respect to cross-reactive antigen. In case of High -fat matrix, as shown in Fig. 6 (A,B) spiked doses Chlp antigen doses on the Glyp-Ab modified sensor platform showed the positive percentage variation to 2.3 % and Glyp antigen on Chlp platform to be 3 %, the overall positive response is 3 times lower to the respective antigen. The positive response in case of the High-fat matrix can be clearly seen and expected due to influence of the charged ions or nutrient contribution on the subsequent dosing. Despite of the interference charge concentration, the diffusion of the antigen makes the system response more specific to larger concentrations as shown in Fig. 5B spike and recovery study of High fat matrix.

6. Conclusions

We successfully demonstrated two-plex modified electrochemical EIS based sensing tool for simultaneous detection of two pesticides in a single matrix either low-fat (cucumber) or high-fat. The immuno-assay/antibodies-based sensor modification helps to selectivity detect the target antigens in the low-fat (cucumber solution) clean matrix as well the high fat matrix with the pool of inherent nutrients in the solution suspension. The modified two-plex sensor performed well both in the laboratory-based testing as well as portable device for low, medium, and high dose range of pesticides between the two devices. The two-plex platform was tested for the cross-reactivity of the glyphosate vs chlorpyrifos. This is a successful first-time demonstration of the two-matrix on a portable electrochemical sensor platform. This open-up more possibility for exploring of such commercial testing device for on-field applications.

CRediT authorship contribution statement

Durgasha C. Poudyal: Conceptualization, Methodology, Writing – original draft. **Vikram Narayanan Dhamu:** Conceptualization, Methodology, Writing – original draft. **Manish Samson:** Writing – review & editing. **Sriram Muthukumar:** Conceptualization, Methodology, Writing – original draft. **Shalini Prasad:** Conceptualization, Methodology, Writing – original draft, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Shalini Prasad and Sriram Muthukumar have a significant interest in Enlisense LLC, a company that may have a commercial interest in the results of this research and technology. The potential individual conflict of interest has been reviewed and managed by The University of Texas at Dallas, and played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report, or in the decision to submit the report for publication.

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134075.

References

Arduini, F., Forchielli, M., Scognamiglio, V., Kozitsina, A. N., & Moscone, D. (2017). Organophosphorous pesticide detection in olive oil by using a miniaturized, easy-to-use, and cost-effective biosensor combined with quechers for sample clean-up. *Sensors (Switzerland), 17*(1). https://doi.org/10.3390/s17010034

Bahadir, E. B., & Sezgintürk, M. K. (2016). A review on impedimetric biosensors. *Artificial Cells, Nanomedicine and Biotechnology, 44*(1), 248–262. https://doi.org/10.3109/21691401.2014.942456

Cui, H. F., Wu, W. W., Li, M. M., Song, X., Lv, Y., & Zhang, T. T. (2018). A highly stable acetylcholinesterase biosensor based on chitosan-TiO2-graphene nanocomposites for detection of organophosphate pesticides. *Biosensors and Bioelectronics*, *99*(May 2017), 223–229. doi: 10.1016/j.bios.2017.07.068.

Daniels, J. S., & Pourmand, N. (2007). Label-free impedance biosensors: Opportunities and challenges. *Electroanalysis, 19*(12), 1239–1257. https://doi.org/10.1002/elan.200603855

Darder, M., Takada, K., Pariente, F., & Abruña, H. D. (1999). Dithiobissuccinimidyl propionate as an anchor for assembling peroxidases at electrodes surfaces and its application in a H2O2 biosensor. *Analytical Chemistry, 71*(24), 5530–5537. https://doi.org/10.1021/ac990759x

Dhamu, V. N., Poudyal, D. C., Muthukumar, S., & Prasad, S. (2021). A highly sensitive electrochemical sensor system to detect and distinguish between glyphosate and glufosinate. *Journal of The Electrochemical Society, 168*(5). https://doi.org/10.1149/1945-7111/ac00f7

Dhamu, V. N., Poudyal, D. C., Telang, C. M., Paul, A., Muthukumar, S., & Prasad, S. (2021). Electrochemically mediated multi-modal detection strategy-driven sensor platform to detect and quantify pesticides. *Electrochemical Science Advances*, *June*, 1–14. doi: 10.1002/elsa.202100128.

Dhamu, V. N., & Prasad, S. (2020). ElectrochemSENSE: A platform towards field deployable direct on-produce glyphosate detection. *Biosensors and Bioelectronics, 170* (May). https://doi.org/10.1016/j.bios.2020.112609

Durocher, S., Rezaee, A., Hamm, C., Rangan, C., Mittler, S., & Mutus, B. (2009). Disulfide-linked, gold nanoparticle based reagent for detecting small molecular weight thiols. *Journal of the American Chemical Society, 131*(7), 2475–2477. https://doi.org/10.1021/ja808548x

Gilbert-López, B., García-Reyes, J. F., & Molina-Díaz, A. (2009). Sample treatment and determination of pesticide residues in fatty vegetable matrices: A review. *Talanta, 79* (2), 109–128. https://doi.org/10.1016/j.talanta.2009.04.022

González-Alzaga, B., Lacasaña, M., Aguilar-Garduño, C., Rodríguez-Barranco, M., Ballester, F., Rebagliato, M., & Hernández, A. F. (2014). A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. *Toxicology Letters, 230*(2), 104–121. https://doi.org/10.1016/j.toxlet.2013.11.019

Guan, H., Brewer, W. E., & Morgan, S. L. (2009). New approach to multiresidue pesticide determination in foods with high fat content using disposable pipette extraction (DPX) and gas chromatography-mass spectrometry (GC-MS). *Journal of Agricultural and Food Chemistry, 57*(22), 10531–10538. https://doi.org/10.1021/jf902373u

Guyton, K. Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., … Zeise, L. (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *The Lancet Oncology, 16*(5), 490–491. https://doi.org/10.1016/S1470-2045(15)70134-8

Jara, E. A., & Winter, C. K. (2019). Safety levels for organophosphate pesticide residues on fruits, vegetables, and nuts. *International Journal of Food Contamination, 6*(1), 1–8. https://doi.org/10.1186/s40550-019-0076-7

Kaushal, J., Khatri, M., & Arya, S. K. (2021). A treatise on Organophosphate pesticide pollution: Current strategies and advancements in their environmental degradation and elimination. *Ecotoxicology and Environmental Safety, 207*. https://doi.org/10.1016/j.ecoenv.2020.111483

Liu, D., Chen, W., Wei, J., Li, X., Wang, Z., & Jiang, X. (2012). A highly sensitive, dual-readout assay based on gold nanoparticles for organophosphorus and carbamate pesticides. *Analytical Chemistry, 84*(9), 4185–4191. https://doi.org/10.1021/ac300545p

Mesnage, R., Defarge, N., Spiroux de Vendômois, J., & Séralini, G. E. (2015). Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. *Food and Chemical Toxicology, 84*, 133–153. https://doi.org/10.1016/j.fct.2015.08.012

Popp, J., Pető, K., & Nagy, J. (2013). Pesticide productivity and food security. A review. *Agronomy for Sustainable Development, 33*(1), 243–255. https://doi.org/10.1007/s13593-012-0105-x

Poudyal, D. C., Dhamu, V. N., Paul, A., Samson, M., Muthukumar, S., & Prasad, S. (2022a). A novel single step method to rapidly screen for metal contaminants in beverages, a case study with aluminum. *Environmental Technology & Innovation, 28*. https://doi.org/10.1016/j.eti.2022.102691

Poudyal, D. C., Dhamu, V. N., Samson, M., Muthukumar, S., & Prasad, S. (2022b). Portable pesticide electrochem-sensor: A label-free detection of glyphosate in human urine. *Langmuir, 38*(5), 1781–1790. https://doi.org/10.1021/acs.langmuir.1c02877

Rather, I. A., Koh, W. Y., Paek, W. K., & Lim, J. (2017). The sources of chemical contaminants in food and their health implications. *Frontiers in Pharmacology, 8* (NOV). https://doi.org/10.3389/fphar.2017.00830

Sturza, J., Silver, M. K., Xu, L., Li, M., Mai, X., Xia, Y., Lozoff, B., Meeker, J., & Arbor, A. (2017). *Chinese Infants, 478–485*. https://doi.org/10.1016/j.envint.2016.04.035.Prenatal

Tanak, A. S., Jagannath, B., Tamrakar, Y., Muthukumar, S., & Prasad, S. (2019). Non-faradaic electrochemical impedimetric profiling of procalcitonin and C-reactive protein as a dual marker biosensor for early sepsis detection. *Analytica Chimica Acta: X, 3*. https://doi.org/10.1016/j.acax.2019.100029

Vicini, J. L., Jensen, P. K., Young, B. M., & Swarthout, J. T. (2021). Residues of glyphosate in food and dietary exposure. *Comprehensive Reviews in Food Science and Food Safety, 20*(5), 5226–5257. https://doi.org/10.1111/1541-4337.12822

Wilkowska, A., & Biziuk, M. (2011). Determination of pesticide residues in food matrices using the QuEChERS methodology. *Food Chemistry, 125*(3), 803–812. https://doi.org/10.1016/j.foodchem.2010.09.094