E-BOOK OF EXTENDED ABSTRACT

THE 14TH INTERNATIONAL INVENTION, INNOVATION & DESIGN COMPETITION 2025

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COLOUR IN A CLICK: ON-THE-SPOT CARBARYL DETECTION WITH ACHE@ZIF-8

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ABSTRACT

Our project introduces a biosensor platform where acetylcholinesterase (AChE) is encapsulated within zeolitic imidazolate framework-8 (ZIF-8) to enhance enzyme stability. The AChE@ZIF-8 is used in a gold nanoparticles (AuNPs) colorimetric assay to detect carbaryl rapidly with the naked eye. Upon exposure to carbaryl, the inhibition of AChE activity modulates the aggregation state of AuNPs, resulting in a visible color change. This method offers a fast, portable, and easy method for on-site detection of carbaryl residues in environmental and food samples.

Keywords: acetylcholinesterase, acetylcholine, AuNPs, encapsulation, ZIF-8, carbaryl

1. INTRODUCTION

Carbaryl is important in pest control and disease prevention, effectively ensuring and increasing agricultural, forestry, and animal husbandry production (Leong et al., 2020). The high toxicity of carbaryl arises from its ability to persistently block acetylcholinesterase (AChE) function in the central and peripheral nervous systems, inhibiting neurotransmitter transmission and eventually leading to death. The extensive and often indiscriminate use of pesticides in agricultural products has amplified the demand for quick residue analysis methods. Conventional approaches like gas chromatography, high-performance liquid chromatography (HPLC), or coupled gas chromatography mass spectrometry (GC–MS) are currently used as standard methods to detect pesticide residues in the agricultural sector. Although these techniques have low limits of detection and excellent selectivity, they either need costly chemicals, lengthy sample pre-treatment, or expert operators and sophisticated apparatus, limiting their use. These methods have been standardized; none of them are appropriate for on-site screening and rapid detection of pesticides (Weerathunge et al., 2019). To address the drawbacks of present techniques and fulfil future detection demands, alternative solutions are extremely desired. Acetylcholinesterase (AChE) is used to replace the traditional methods and act as a biomarker in detecting carbaryl due to its sensitivity. However, they are very fragile and unstable in harsh conditions. To overcome all the limitations, encapsulated acetylcholinesterase within ZIF-8 was used to enhance their stability and storage life term. Development of the rapid, portable, and on-site detection of carbaryl using AChE@ZIF-8.

2. METHODOLOGY

2.1 Synthesis of AChE@ZIF-8

The synthesis method of AChE@ZIF-8 was used in a copyrighted work (LY2024W09077). The samples were prepared in a water solution at room temperature using the previous procedures with slight modifications (Aziz et al., 2022). In brief, AChE (0.08 mg/mL, 750 µL) and $Zn(NO_3)_2.6H_2O$ solution (0.31 M, 250 µL) were mixed with a 2-methylimidazole solution (HmIm; 2.5 M, 2.5 mL), then aged for

30 minutes at room temperature. After mixing, a milky solution was formed instantly. The product was collected by centrifugation at 10,000 rpm for 15 minutes, then washed three times in deionised water and redissolved in 5 mL of deionized water. The pure ZIF-8 compound was synthesised using the same method but without the addition of the enzyme AChE to the HmIm solution. The obtained products were dried using freeze-drying before being characterised.

2.2 Detection of carbaryl using AChE@ZIF-8

The neurotoxin of carbaryl insecticide was tested as an inhibitor of AChE@ZIF-8 composite activity using conventional biosafety and chemical safety protocols and suitable personal protective equipment (PPE). With the slight modification methodology (Shah et al., 2021), 150 µL of Tris-HCl buffer (100 mM, pH 7.6), 25 µL of AChE (0.08 mg/mL), and various dilutions of the carbaryl (20 – 160 nM) were combined and incubated at 37 C for 10 minutes. Next, 20 µL of ACh (4 mmol/L) was added to each sample, and the reagents were incubated for another 20 minutes, followed by 60 µL of AuNPs and 60 µL of the above solution. The inhibitory activity of AChE@ZIF-8 was determined by various concentrations of carbaryl pesticide (20 nm -160 nm).

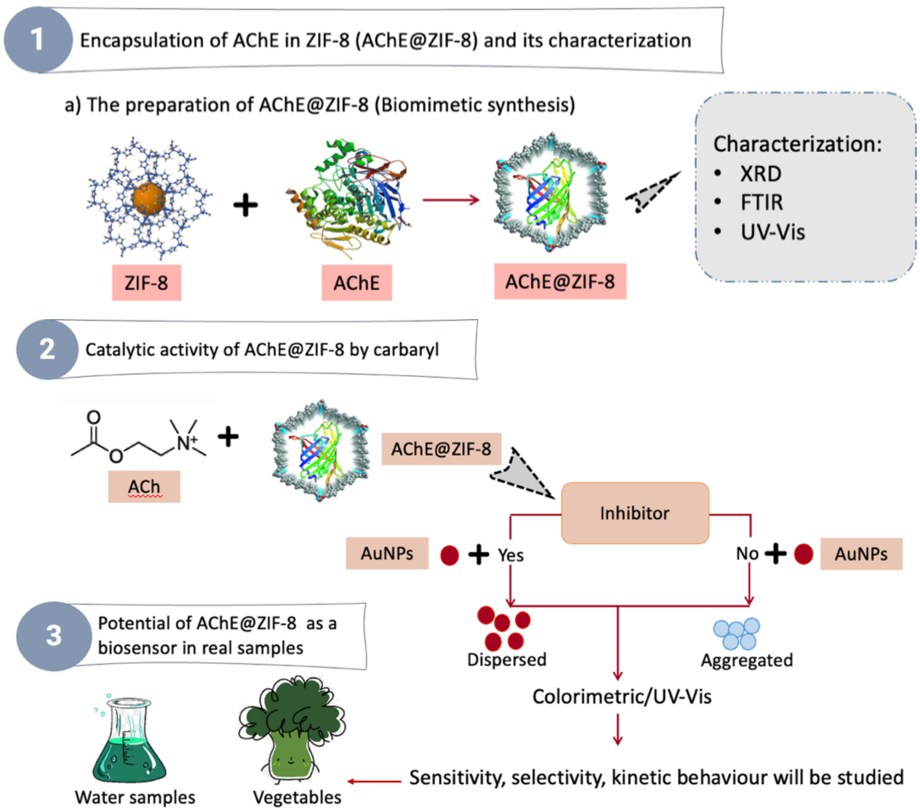

Figure 1 Flow chart of the overall research approach of the study.

3. FINDINGS

Figure 2 (a) PXRD and (b) FTIR spectra of ZIF-8 and AChE@ZIF-8 confirm the successful synthesis of the biocomposite material, maintaining the structural integrity of ZIF-8 after enzyme encapsulation. Figure 2 (c) shows the UV–Vis absorbance. The spectrum highlights the optical sensitivity of AuNPs in response to free AChE and AChE@ZIF-8. The blue curve represents the baseline AuNPs, with a sharp peak around 520 nm, indicating well-dispersed nanoparticles. Upon addition of AChE (orange) and AChE@ZIF-8 (dark red), a red shift and broadening of the surface plasmon resonance peak are observed, especially beyond 600 nm. This shift is attributed to the enzymatic hydrolysis of acetylcholine, producing choline, which induces the aggregation of AuNPs.

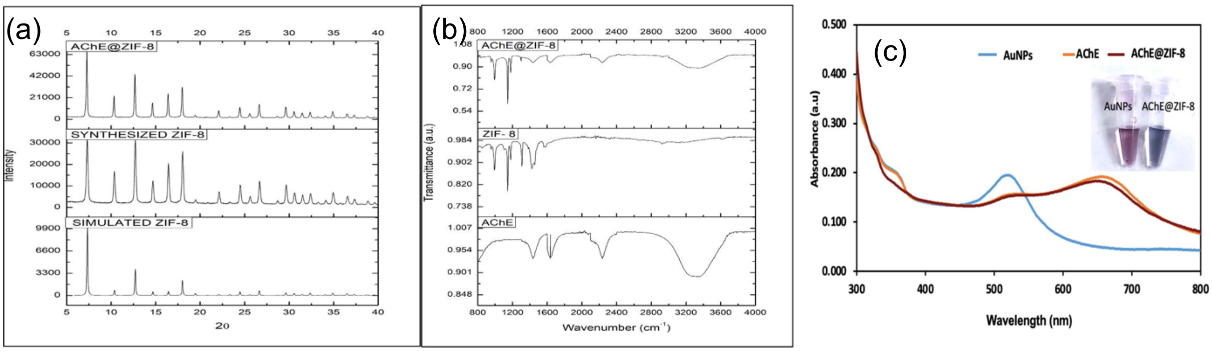

Figure 2 (a) PXRD analysis of simulated ZIF-8, AChE@ZIF-8 and ZIF-8, (b) FTIR analysis of AChE@ZIF-8, ZIF-8 and AChE and (c) Absorption spectra of AuNPs, AChE and AChE@ZIF-8.

4. CONCLUSION

In conclusion, a simple and green approach for producing a high-performance organised enzyme system for catalysis has been described. AChE was synthesised concurrently with ZIF-8 through a biomimetic mineralization process. Based on the technique, an enzyme inhibition approach was used to produce a quick colorimetric sensor for carbaryl measurement. The colorimetric sensor demonstrated outstanding stability, selectivity, and sensitivity (detection limit of 35 nM) due to the combination of ZIF-8's protective action and enzyme cascade catalysis. The test, with excellent sensitivity, accuracy, long-term storage, green synthesis and fits the detection criteria for on-site and robust visual monitoring of carbamate. The biosensor employs an AuNPs probe for high-specificity visual monitoring of carbamate. Due to the visual monitoring, this approach is more adjustable than other analytical procedures that rely on sophisticated gear. This colorimetric detection device may be used to monitor carbamate in a variety of environments and food processing applications.

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