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PII: S0003-2670(15)00764-3

DOI: 10.1016/j.aca.2015.06.004

Reference: ACA 233945

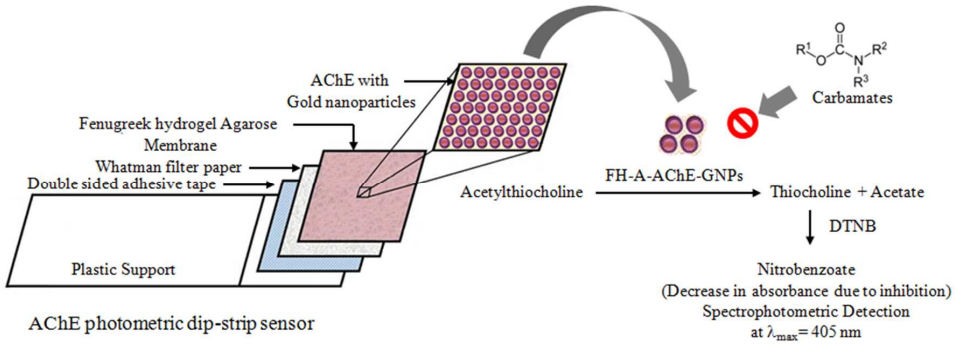
To appear in: *Analytica Chimica Acta*

Received Date: 12 September 2014

Revised Date: 18 May 2015

Accepted Date: 16 June 2015

Please cite this article as: R.M. Kestwal, D. Bagal-Kestwal, B.-H. Chiang, Fenugreek hydrogel-agarose composite entrapped gold nanoparticles for acetylcholinesterase based biosensor for carbamates detection., *Analytica Chimica Acta* (2015), doi: 10.1016/j.aca.2015.06.004.



Title: Fenugreek hydrogel-agarose composite entrapped gold nanoparticles for acetylcholinesterase based biosensor for carbamates detection.

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ABSTRACT

A biosensor was fabricated to detect pesticides in food samples. Acetylcholinesterase was immobilized in a novel fenugreek hydrogel- agarose matrix with gold nanoparticles. Transparent thin films with superior mechanical strength and stability were obtained with 2% fenugreek hydrogel and 2% agarose. Immobilization of acetylcholinesterase on the membrane resulted in high enzyme retention efficiency (92%) and a significantly prolonged shelf life of the enzyme (half-life, 55 days). Transmission electron microscopy revealed that, gold nanoparticles (10 to 20 nm in diameter) were uniformly dispersed in the fenugreek hydrogel-agarose-acetylcholinesterase membrane. This immobilized enzyme-gold nanoparticle dip-strip system detected various carbamates, including carbofuran, oxamyl, methomyl, and carbaryl, with limits of detection of 2, 21, 113, and 236 nM (S/N=3), respectively. Furthermore, the fabricated biosensor exhibited good testing capabilities when used to detect carbamates added to various fruit and vegetable samples.

Keywords: Acetylcholinesterase, Fenugreek hydrogel, Agarose, Gold nanoparticles, Pesticides, Carbamates.

Abbreviations: Agarose (A); Acetylcholinesterase (AChE); acetyl thiocholine chloride (ATC); 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB); fenugreek hydrogel (FH); gold nanoparticles (GNPs); Limit of detection (LOD); maximum residual limits (MRL); transmission electron microscopy (TEM)

1. INTRODUCTION

Pesticides are widely used in agriculture and depending on their aqueous solubility; they either remain in the soil or enter surface and ground waters. Compounds resulting from pesticide degradation can persist in animals, vegetables, and water sources and become more concentrated as they move up in the food chain [1]. Unfortunately, many of these compounds are highly toxic, with the majority being hazardous to both human health and the environment. Therefore, reliable and rapid measurement of pesticides in water and foods is of great importance [2].

Several analytical methods can be used to detect pesticides with high sensitivity, but they often involve tedious sample preparation steps, and they need to be conducted by highly skilled personnel, using expensive equipment [3-6]. Consequently, there is an impetus to develop biosensors for detecting pesticides, with an objective of simplifying or eliminating sample preparation, with less technical expertise and analytical equipment. Although several enzymes can be used to develop biosensors for pesticide detection, those based on acetylcholinesterase (AChE) are preferred when rapid toxicity screening with high specificity is required [7]. The main application of AChE biosensors is detection of pesticides, based on enzyme inhibition. To date, most studies have focused on detection of organophosphorus insecticides, whereas only a few involved carbamate insecticides, even though the latter are widely used due to their broad spectrum of activity [8].

Recently reported sensors for detecting carbamates include prussian blue (PB)-multi-walled carbon nanotubes (MWCNTs) modified screen-printed electrodes (SPEs) used to immobilize AChE for detecting carbamate insecticides [9]. This sensor could detect pirimicarb with a limit of detection (LOD) 0.532 ng L^{-1}. Another AChE biosensor developed using polyaniline and multi-walled carbon nanotubes core–shell modified glassy carbon electrode, detected carbamate

pesticides in fruits and vegetables, with LOD of 1.4 and 0.95 μM for carbaryl and methomyl, respectively [10]. In another study, Assis et al,. reported an AChE-based optical biosensor which detected two carbamates (carbaryl and carbofuran) at concentrations of 33.8 and 0.92 μM, respectively [11].

Fenugreek (*Trigonella foenum-graecum*) is an annual herb in the family leguminosae. Fenugreek seeds contain mucilage (28%) and gums (23%) and are a rich source of polysaccharide galactomannan. Fenugreek gum is used as a thickener, stabilizer and emulsifier in many food products. Among 14 commercial gums tested in an oil/water emulsion model system, fenugreek gum exhibited the highest stabilizing properties [12, 13]. Similar to guar gum and locust bean gum, fenugreek gum is composed of galactose and mannose, which have high viscosity in aqueous solutions [14, 15]. It was also found that guar gum, with high galactose content, swells and dissolves readily in cold water, whereas locust bean gum (LBG) needs heating to dissolve. The solubility of tara gum is intermediate [16]. Typically, fenugreek gum (FG) has mannose to galactose (M/G) ratio of about 1, guar gum (GG) about 2, tara gum (TG) about 3, and the locust bean gum (LBG) M/G ratio is usually about 4. Many properties of galactomannans are related to the M/G ratio. The higher the M/G ratio, the lower the solubility. Thus, in comparison to these other gums, fenugreek gum has higher water solubility and swellability due to more galactose content [17]. Being a sugar-based hydrogel, fenugreek gum is an excellent matrix for drug-delivery systems and enzyme immobilization because of its high water content, homogeneity, stability, swellability and expected non-toxicity [18].

Although different studies have characterized numerous properties of fenugreek galactomannans, an enzyme immobilization application in biosensors has apparently not been reported. The objective of the present study was to fabricate novel nano-bioconjugates to develop a fast,

reliable and economical method for quantifying trace concentrations of carbamates. Fenugreek hydrogel (FH) and agarose (A) sol-gel were used to immobilize gold nanoparticles (GNPs) and AChE for fabrication of an optical biosensor.

2. MATERIALS AND METHODS

2.1. Materials and chemicals

Fenugreek (*Trigonella foenum-graecum*) seeds were obtained from a supermarket in Pune, Maharashtra, India. Acetylcholinesterase from electric eel (*Electrophorus electricus*), acetylthiocholine chloride (ATC), gold (III) chloride trihydrate, pesticides and other chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Instrumentation

Transmission electron microscopy was performed using a Hitachi H-7100 Electron Microscope (Hitachi, Tokyo, Japan). A Sunrise Basic ELISA reader (Tecan, Maennedorf, Switzerland) was used for analyzing 96-well plates. In addition, a Helios Alpha UV-Visible spectrophotometer (Thermo Spectronic, Cambridge, UK) and Branson 5510 Ultrasonic instrument (Branson Ultrasonic Corp., CT, USA) were also used.

2.3. Synthesis of gold nanoparticles

Gold nanoparticles were prepared as described by McFarland et al., [19]. A solution of $HAuCl_4$ (1.0 mM) was prepared in 20 mL deionized water (DIW). The solution was heated on a stir/hot plate, with continuous stirring. Once the solution began to boil, 2 mL of sodium citrate (38.8 mM) was added drop wise until the color changed from yellow to deep red indicating formation of gold nanoparticles (GNPs). This solution of GNPs (0.95 mM) was allowed to cool and was

stored at room temperature until further use. To confirm the formation of GNPs, absorption spectra of the GNPs solution as well as $HAuCl_4$ solution were taken and compared, as GNPs have a characteristic absorption peak around 520 nm, which is absent in case of $HAuCl_4$ solution. TEM was also performed to check the quality of GNPs synthesized.

2.4. Enzyme activity

Acetylcholinesterase (AChE) activity was determined based on the method of Ellman et al., with some modifications for a microplate reader [20]. Briefly, 90 µL of phosphate buffer (0.1 M, pH 8.0) was added to the microwell plate. Then 100 µL of 5, 5'-dithiobis-(2-nitrobenzoic acid) DTNB (0.6 mM in $NaHCO_3$ solution) and 100 µL of acetyl thiocholine chloride (ATC) substrate (5 mM) were also added to the microwell plate. The enzyme reaction was initiated by addition of appropriately diluted AChE enzyme (10 µL). After addition, the content of the plates was mixed gently and incubated at room temperature (25ºC). Conversion of DTNB to 5-thio-2-nitrobenzoic acid (TNB) was estimated by taking OD at 405 nm after 10 min as a measure of the hydrolysis of ATC into thiocholine. Formation of thiocholine was calculated by the extinction coefficient of TNB ($\varepsilon = 1.36 \times 10^4$ mL $mmol^{-1}$ cm^{-1}).

2.5. Immobilization of AChE in fenugreek hydrogel (FH) - agarose (A) membrane

For preparation of fenugreek hydrogel, fenugreek fine powder was obtained from dry fenugreek seeds using a grinder. The fine fenugreek powder thus obtained was weighed (2.0g) and added to 100 mL distilled water and was stirred for 30 min on a magnetic stirrer, followed by centrifugation at 6000 rpm (30 min, 25°C). The clear viscous supernatant containing gums and

pectins (fenugreek hydrogel, 2%, w/v) was stored at 4°C pending further use, while the insoluble precipitate was discarded.

Agarose solution (2%) was prepared in distilled water at 100°C with constant stirring until it gelatinized. For enzyme immobilization, fenugreek hydrogel (0.5 mL, 2%) and agarose (0.5 mL, 2%) were mixed together at 80°C and allowed to cool to 60°C for membrane casting. The blank (without enzyme) and enzyme membranes were prepared for testing. Enzyme (50 µL of AChE, 100 mU) was added to freshly prepared gel solutions and thoroughly mixed. In addition, 50 µL of GNP solution (0.9 mM) was added to the above mixture. This solution was vortex mixed for 5 sec to prevent aggregation and to ensure uniform distribution of enzyme and nanoparticles in the solution. Soda-lime glass coated with an inert plastic material was used as a support for membrane casting [21]. A simple drop-cast method was used for gel deposition. After overnight drying, membranes were carefully detached from the glass support. The resulting enzyme-nanoparticles-immobilized membranes were stored at 4°C under moisture-free conditions.

2.6. Entrapment efficiency of AChE

A square piece (5 x 5 mm) of AChE-immobilized membrane was rinsed with assay buffer to remove unbound/excess enzyme. Further, it was incubated with 300 µL AChE substrate (100 µL of phosphate buffer (pH 8.0, 0.1M), 100 µL of DTNB (0.6 mM in $NaHCO_3$ solution) and 100 µL of ATC substrate (5 mM)) at room temperature for 10 min. The reaction was stopped by removal of the membrane, and enzyme activity was estimated by recording OD at 405 nm (determining hydrolysis of ATC into thiocholine). Entrapment efficiency was calculated as percentage residual activity as compared to the soluble enzyme. Entrapment efficiency of membranes with and without GNPs was also calculated to check the influence of GNPs on immobilized AChE

activity. Michaelis Menten constant for both free and immobilized enzyme systems was calculated using a Lineweaver-Burk plot.

2.7. FH-A-AChE- GNPs dip-strips fabrication

To fabricate dip-strips, transparent polypropylene plastic sheets were cut in strips (5 x 50 mm). Whatman filter paper (5 x 5 mm) was fastened (with double-sided adhesive tape) to one end of the strip, to act as a test area. To make an enzyme-immobilized membrane, a stock solution was prepared containing fenugreek hydrogel agarose (1 mL), AChE (50 µL, 100 mU) and GNPs (50 µL, 0.95 mM). This stock solution was vortex mixed for 5 sec to prevent aggregation and to ensure uniform distribution of enzyme and nanoparticles in the solution. Finally 50 µL of the stock solution was drop-casted on the test area of the dip-strips. The FH-A-AChE-GNPs dip-strips were allowed to dry at room temperature overnight in a dust free environment. The next day, newly formed AChE immobilized dip-strips were stored in an airtight container at 4°C.

2.8. Microscopic characterization of FH-A-AChE-GNPs dip-strips

Size and morphology of synthesized GNPs in solution were investigated using transmission electron microscopy (TEM). Furthermore, TEM was also done to assess the presence and distribution of nanoparticles in the immobilized FH-A-AChE-GNPs membrane.

2.9. Response curve of FH-A-AChE-GNPs dip-strips

For response curve analysis, the enzyme-immobilized membrane was detached from the FH-A-AChE-GNP dip-strips. The detached transparent membrane was placed in a 96 well microwell plate with 100 µL of phosphate buffer (pH 8.0, 0.1M). Then, 5 µL of substrate DTNB (0.6 mM in $NaHCO_3$ solution) was added and absorbance was recorded every 30 s for 15 min to evaluate

enzyme responses to the substrate. For blank correction, a membrane without enzyme was used for each reading. Similar evaluations were done with soluble enzyme in lieu of immobilized enzyme, to compare enzyme responses to substrate before and after immobilization.

An extended analysis was performed for the FH-A-AChE-GNPs membrane by further addition of 5 µL of substrate 15 min after completion (repeated 3 times). Both the soluble and immobilized enzyme systems were subjected to the addition of substrate, and the resulting response curves were compared.

2.10. Effects of pH and temperature on the dip-strip sensor

The biosensor was tested at various temperatures and pH. The FH-A-AChE-GNPs dip-strip sensitivity for temperature was studied within the range of 10–100°C. In addition, pH-dependent studies of soluble and FH-A-AChE-GNPs dip-strips were also done. Acetate buffer (pH 3.0–5.0), phosphate buffer (pH 6.0–8.0) and glycine sodium hydroxide buffer (pH 9.0-10.0) were used in the assay system.

2.11. Pesticide detection: linear range and limit of detection (LOD)

Inhibition profiles of the FH-A-AChE-GNPs dip-strips were assessed with four pesticides: carbaryl (sparingly water soluble/organic solvent soluble), carbofuran (sparingly water soluble/organic solvent soluble), methomyl (water soluble) and oxamyl (water soluble). For each pesticide (inhibitor), calibration was plotted, first using a wide concentration range and subsequently using a narrow linear range (to determine the linear range of the system) and finally the LOD [21].

2.12. Fabrication reproducibility and storage stability of FH-A-AChE-GNPs dip-strips

The fabrication process of dip-strips was evaluated for suitability of the novel composite material used. Adhesion of the membrane to plastic support was critical. Ten enzyme assays were performed using dip-strips from different batches to assess fabrication reproducibility. Furthermore, storage stability of the dip-strips was investigated by determining the activities of the immobilized enzyme every 5th day when stored at 4°C under dry conditions, until the residual activity of the immobilized enzyme was <50% of initial activity. For comparison, the storage stability of soluble enzyme stored under similar conditions was also assessed. Interference in pesticide detection was checked in presence of different compounds including glucose (0.5 mM), sucrose (0.5 mM), ascorbic acid (0.5 mM), citric acid (0.5 mM) and metal ions including copper ions (10nM), ferrous ions (10nM) and zinc ions (10nM). Further interference in pesticide detection in presence of other pesticides (carbofuran, oxamyl, methomyl, and carbaryl) was also checked.

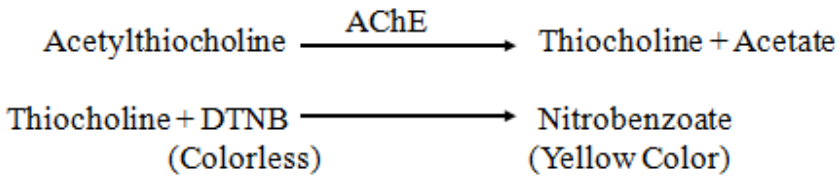
2.13. Application of FH-A-AChE-GNPs dip-strips: Real sample testing

Fabricated dip-strips were checked for practical reliability using samples of fruits and vegetables including, orange (*Citrus sinensis*), ber (*Ziziphus mauritiana)*, tomato *(Solanum lycopersicum)* and cabbage *(Brassica oleracea).* All the fruits and vegetables were purchased from a local market in Taipei. Each sample was rinsed, chopped into small pieces and juice (50% v/v) was prepared using a juicer after addition of phosphate buffer (pH 8.0). Each sample was spiked with a known amount of pesticide and vortexed to prepare a uniform solution. Thereafter, test samples containing pesticides were centrifuged at 10,000 rpm for 5 min, and the supernatant was transferred in 96 well plates (300 µL per well). The enzyme immobilized dip-strips were incubated with the sample in the test plate for 15 min with shaking (50 rpm). After incubation,

dip-strips were transferred to the substrate plate and incubated with 300 µL of substrate for 10 min with shaking (50 rpm). After incubation, dip-strips were removed and absorbance of the substrate (product) plate was recorded at 405 nm to estimate pesticide content.

3. RESULTS AND DISCUSSION

For pesticide detection, a nanomaterial-enzyme based optical biosensor was fabricated and characterized using a fenugreek hydrogel - agarose composite entrapped AChE with gold nanoparticles. The reaction scheme for the enzyme is as follows:



3.1. Characterization of immobilized AChE

For acetylcholinesterase immobilization, a total of 9 nano-biocomposites were screened with fenugreek hydrogel (1%, 2%, and 3%) and agarose (1%, 2% and 3%) and their combinations thereof. The combination with fenugreek hydrogel (FH) (2%) and agarose (A) (2%) was the most efficient (Data not included). It yielded a transparent thin film with superior mechanical strength and stability. Enzyme immobilization efficiency for AChE with and without GNPs were 85.16% and 92.28%, respectively (Table S1, Supplementary Material). These immobilization efficiencies were better than the 70% and 66% reported in two of the recent studies regarding AChE biosensors fabrication, [23, 24]. The K_m of the free and immobilized AChE immobilized with GNPs were 0.348 and 0.243 mM, respectively. The change in K_m was probably due to the hydrophilic nature of the bio-composite.

Preferred Position For Figure 1.

3.2. FH-A-AChE-GNPs dip-strip fabrication and TEM analysis

For pesticide detection FH-A-AChE-GNPs dip-strips were fabricated with dimension of 5 x 50 mm. In the test zone (5 x 5 mm) 100 mU of AChE was immobilized along with GNPs in a FH-A matrix (Figure 1A). The GNPs synthesis was confirmed by performing absorption spectra of GNPs solution, where a characteristic absorption peak was observed around 520 nm, which was absent in case of $HAuCl_4$ solution (Figure S1, Supplementary Material). While, TEM analysis of the free gold nanoparticles in solution showed that the particle size of the GNPs were in the range of 10-20 nm (Figure 1B). Furthermore, the size and shape of immobilized GNPs in FH-A-AChE-GNPs membrane were similar to those of the free GNPs in solution. Therefore, we inferred that GNPs were not affected by high temperatures during immobilization. The GNPs were uniformly dispersed in the membrane, either individually or in small clusters, thus providing maximum electrochemical and real surface area for enzyme immobilization and electron transfer during the enzymatic reaction [25]. In addition, GNPs in biosensors are also known to provide a biocompatible microenvironment for the immobilized biomolecules, which may contribute to their stability and bioactivity [26].

Preferred Position For Figure 1.

3.3. Response characteristics of the FH-A-AChE-GNPs membrane

To determine the optimal time scale for the progress curve of the FH-A-AChE-GNPs membrane, a drop test was done as shown in Figure 2A. Measurements were done as a function of time for the enzymatic reaction, with steady state absorbance achieved after 10 min of exposure of the biosensor to substrate. Based on the process curve, 10 min of incubation time was selected for subsequent studies. A typical response curve of the biosensor after successive addition of 5 mM ATC is shown (Figure 2B). There was a clear change in optical density, due to a rapid response

Preferred Position For Figure 2.

of the sensor following addition of ATC, which apparently was able to rapidly diffuse through the porous structure of the sol–gel matrix.

3.4. Effect of pH and temperature on immobilized AChE

For pesticide detection, FH-A-AChE-GNPs dip-strips were fabricated and the response of dip-strips was tested at various temperatures and pH (Figure 3A). Although the optimum pH of the enzyme (pH 8.0) was not altered after immobilization, enzyme activities of the immobilized AChE markedly improved at pH 6.0, 7.0 and 9.0. Similarly, it was previously reported that the optimal pH for AChE did not change, but that the pH curves became wider in cases of calcium-alginate and alginate/κ-carrageenan-immobilized AChE [27]. In another study, the curves also became wider and even the AChE optimum pH changed from 7.0 to 8.0 [28]. A broader pH curve after immobilization could be due to various interactions between the enzyme and the polymeric matrix (e.g. ionic interactions, hydrogen bonding, etc.). A similar response pattern was observed in case of FH-A-AChE without GNPs indicating that GNP incorporation doesn't alter the pH optimum of the immobilized enzyme. Even for temperature study, the GNPs played a neutral role without disturbing the optimum temperature profile (data not shown). Enzyme sensitivity for temperature was studied within the range of 10–100 °C (Figure 3B). There was increased thermal stability above 40 °C for the immobilized enzyme compared to the soluble form. Previously it has been reported that the optimum temperature of immobilized AChE increased from 38 to 40 °C, with significantly increased thermal stability at 50 and 60 °C [29]. The high thermal stability could enable the membrane to be used at a higher temperature, and can increase the enzyme activity by increasing the diffusion rate of substrates and products across the membrane [30].

Preferred Position For Figure 3.

3.5. Detection of pesticides using FH-A-AChE-GNPs dip-strips

Pesticides detection capabilities of the biosensor were investigated by measuring the inhibitory effects on AChE activity by various concentrations of the pesticides including carbaryl, carbofuran, methomyl and oxamyl. Calibration plots of inhibition versus pesticide concentration were constructed to obtain the working dynamic range and LOD values for the respective pesticides (Table 1). Wide concentration inhibition profiles and linear relationships between inhibition percentage and pesticide concentration were obtained (Figure S2, Supplementary Material). Linear ranges for carbofuran, oxamyl, methomyl, and carbaryl were 2 to 10, 10 to 100, 100 to 500, and 200 to 1000 nM, respectively. For the fabricated dip-strip, the LOD for carbofuran was 2 nM (S/N=3), which seemed much lower than a previous report of 17 nM for optical biosensors [31].

Similarly, the LOD for carbaryl was 236 nM (S/N=3), which was lower than 536 nM reported for optical biosensors in the past [32]. Moreover, the LOD for methomyl was 113 nM (S/N=3), which is even better than a recently reported electrochemical biosensor which had an LOD of 950 nM for methomyl [10]. And the LOD for oxamyl was 21 nM (S/N=3). Most importantly, our dip-strip system was capable of detecting pesticide concentrations below international maximum residual limits (MRL) values [33] (WHO Pesticides MRLs, 2008; Table 1).

Preferred Position For Table 1.

3.6. Fabrication reproducibility and storage stability

The fabrication process of FH-A-AChE-GNPs dip-strips was evaluated for reproducibility (Figure 4A). Data from 10 recently fabricated dip-strips from various batches was compared for enzyme activity without any inhibitor. Responses of fabricated systems to their substrate ATC were essentially the same. Therefore, we inferred that the simple and easy physical entrapment method devoid of any chemical treatment was suitable for AChE.

Storage stability of the dip-strips was determined every 5^{th} day when stored at 4 °C under dry conditions (Figure 4B). For soluble enzyme, the half-life (loss of 50% of activity) was <10 days, whereas after immobilization, the half-life increased to 55 days. Therefore, the dip-strips had much better storage stability than soluble enzyme. Furthermore, 96% activity retention after 20 days was apparently better than 83% activity retention after 20 days and 89.1% activity retention in 15 days as reported previously [34, 35]. Similarly, there was 94% activity retention after 30 days, compared to a previous report of 88% activity retention after the same interval [36].

The interference studies results showed no noticeable interference in presence of different compounds at 0.5 mM concentration as well as metal ions at 10 nM concentration. However, one disadvantage of the reported biosensor is that, the pesticides at equivalent concentration severely interfere with detection of each other (carbofuran, oxamyl, methomyl, and carbaryl), similar to those reported in earlier reports [37, 38].

Preferred Position For Figure 4.

3.7. Application of FH-A-AChE-GNPs dip-strips: Real sample testing

The FH-A-AChE-GNPs dip-strips fabricated in this study were checked for practical reliability. For fruit and vegetable juices spiked with pesticides and tested using dip-strips, recovery ranged from 93 to 105% (Table 2). Therefore, the interference effect of the juice component as well as the matrix was negligible after dilution with phosphate buffer (pH 8.0) and the dip-strips

accurately estimated pesticide concentrations. Like other pesticide biosensors, we also used carbamates as target for fabricating our sensor system in this study. However, our sensor can have wider applications because other organophosphate pesticides, heavy metal ions and some organic compounds, etc. can also have inhibition effect on AChE biosensors [8, 9].

4. CONCLUSION

An optical FH-A-AChE-GNPs dip-strip based biosensor was fabricated for detection of various pesticides. A novel bio-composite matrix (including fenugreek hydrogel and agarose) was used to immobilize the enzyme and nanoparticles. Enzyme loading in the matrix was optimized and the biosensor was characterized for several parameters, including assessment of nanoparticles (with TEM), enzyme kinetics, and the effects of pH and temperature. The fabricated FH-A-AChE-GNPs dip-strips were also checked for fabrication reproducibility and storage stability. The enzyme stability was markedly improved due to immobilization. The LOD of the current biosensor for some pesticides seemed superior to other recently reported optical biosensors and were much lower than international MRL values. Real sample testing demonstrated the practical applicability of the fabricated optical biosensor.

ACKNOWLEDGMENT

We acknowledge financial assistance from the National Science Council, Taiwan (NSC 100-2221-E-002-032-MY2).

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LIST OF CAPTIONS FOR FIGURES AND TABLES

Figure 1A. Schematic representation of AChE photometric dip-strip sensor.

Figure 1B. Photomicrograph **(**transmission electron microscopy) of free and immobilized GNPs in FH-A-AChE-GNPs membrane.

Figure 2. Response characteristics of FH-A-AChE-GNPs membrane. (A) Response time during drop test and (B) Progress curve for sequential substrate addition.

Figure 3: Effect of immobilization on optimum parameters of AChE; (A) Optimum pH and (B) Optimum temperature.

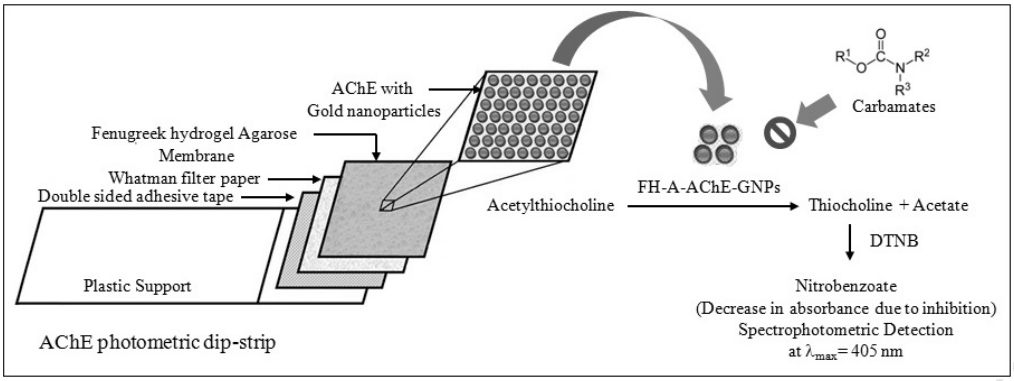
Figure 4: Fabrication reproducibility (A) and storage stability (B) of FH-G-AChE-GNPs dip-strips.

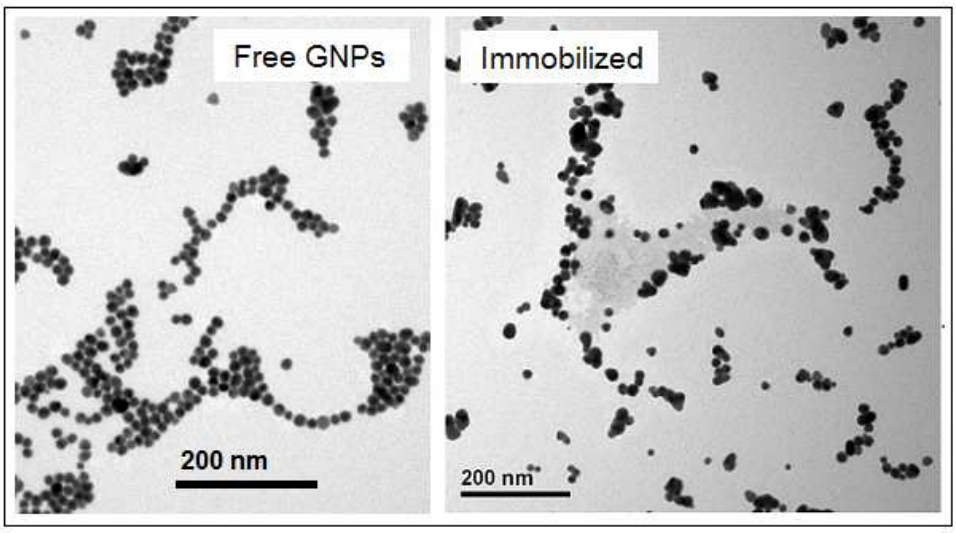
Table 1. Limit of detection of FG-A-AChE-GNPs dip-strip system for various pesticides.

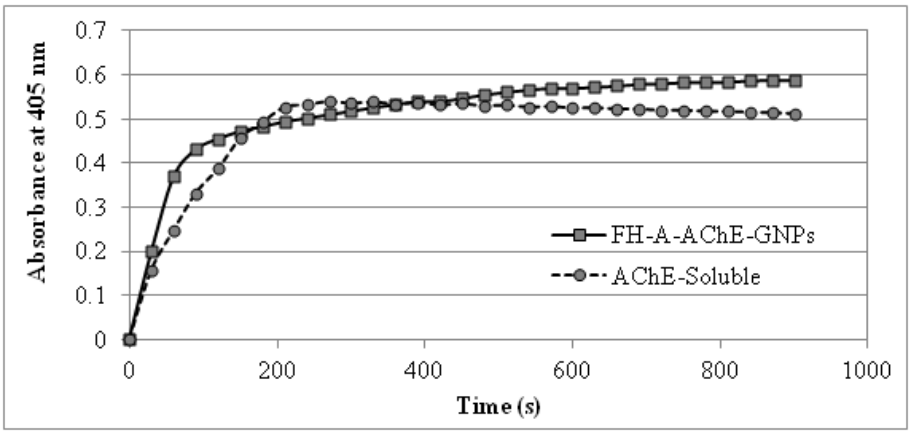
Table 2. Detection of pesticides in spiked juices using fabricated FH-A-AChE-GNPs dip-strips.

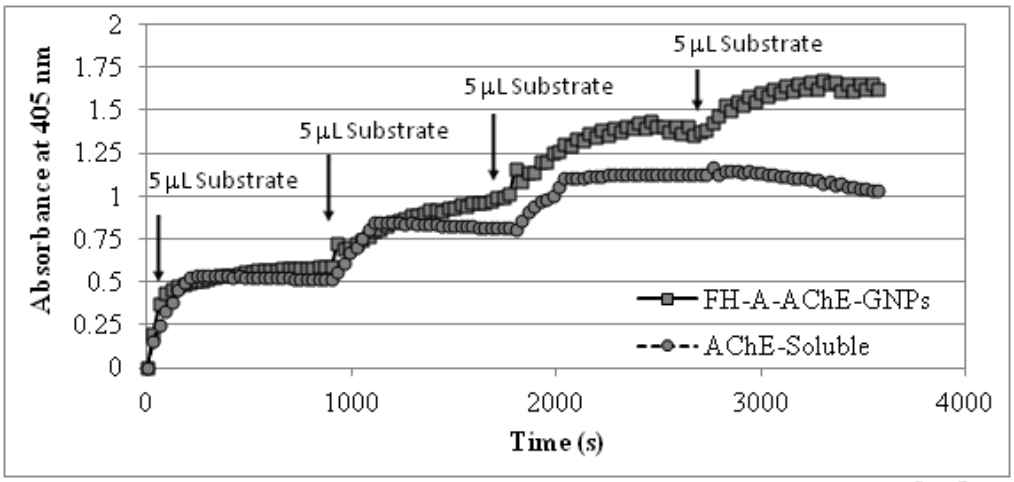
Pesticide	LOD for FG-A-AChE-GNPs Dip-strips system (nM)	WHO MRL (nM)
Carbofuran	2	452
Oxamyl	21	460
Methomyl	113	616
Carbaryl	236	2,485

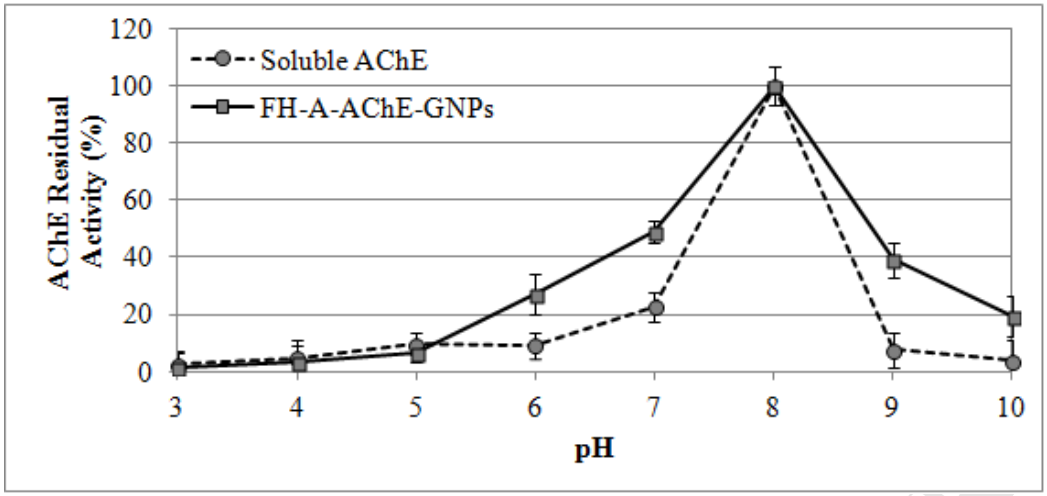
Fruit/ Vegetable Used	Pesticide	Concentration		Recovery (%)	Error (%)
		Added (nM)	Recovered (nM)		
Orange	Carbaryl	500	489.4 ±18.4	97.9	-2.1
(Citrus sinensis)	Carbofuran	500	518.1 ±25.5	103.6	3.6
	Methomyl	500	526.3 ±26.3	105.3	5.3
	Oxamyl	500	512.5 ±44.2	102.5	2.5
Tomato	Carbaryl	500	491.5 ±37.3	98.3	-1.7
(Solanum	Carbofuran	500	514.7 ±45.2	102.9	2.9
lycopersicum)	Methomyl	500	517.6 ±27.4	103.5	3.5
	Oxamyl	500	515.5 ±44.1	103.1	3.1
Ber	Carbaryl	500	504.5 ±49.2	100.9	0.9
(Ziziphus mauritiana)	Carbofuran	500	468.0 ±54.4	93.6	-6.4
	Methomyl	500	489.6 ±25.5	97.9	-2.1
	Oxamyl	500	481.0 ±56.3	96.2	-3.8
Cabbage	Carbaryl	500	484.3 ±23.2	96.9	-3.1
(Brassica oleracea)	Carbofuran	500	488.5 ±65.6	97.7	-2.3
	Methomyl	500	509.2 ±46.3	101.8	1.8
	Oxamyl	500	483.5 ±24.4	96.7	-3.3

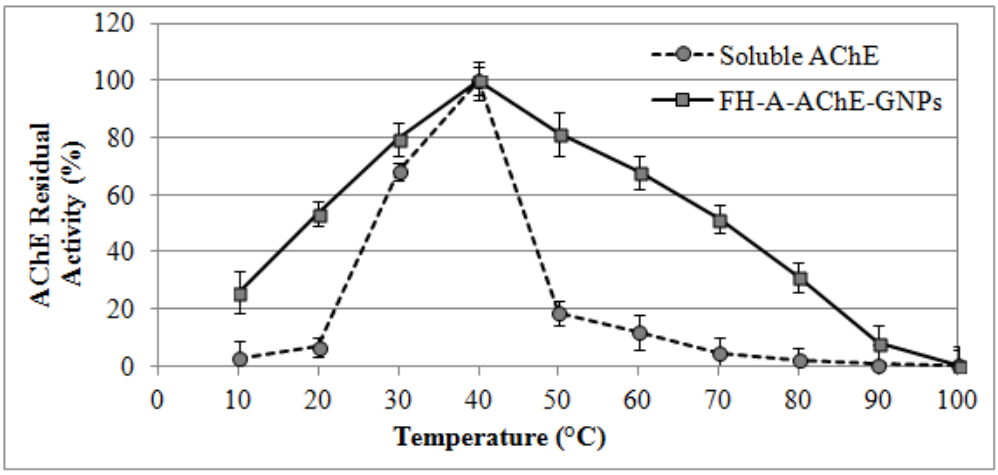


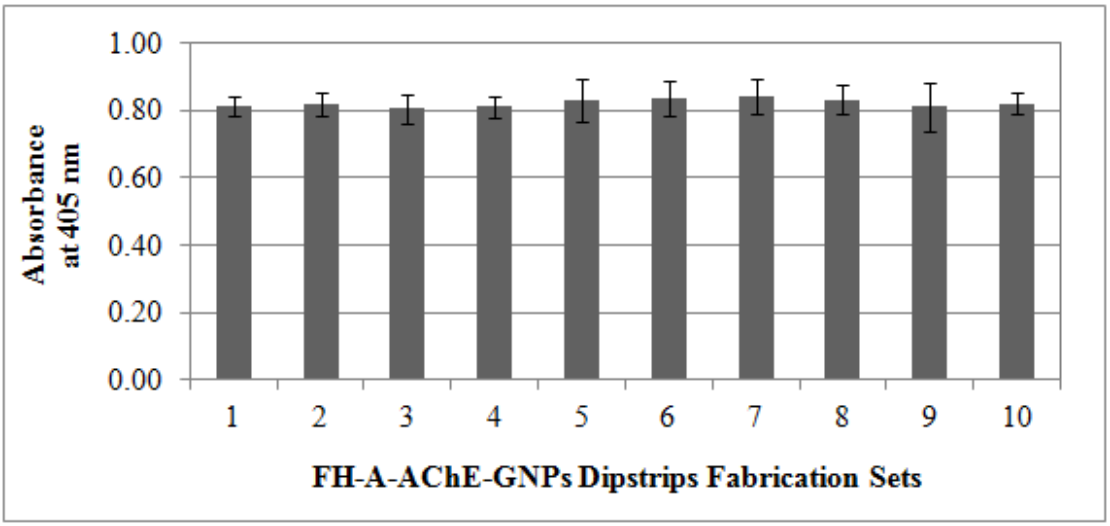


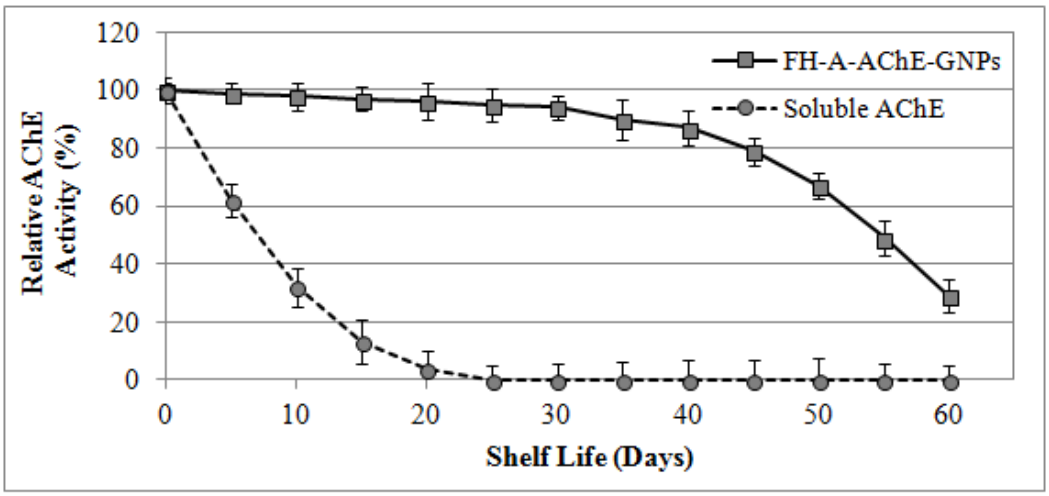












RESEARCH HIGHLIGHTS

Title: Fenugreek hydrogel-agarose composite entrapped gold nanoparticles for acetylcholinesterase based biosensor for carbamates detection.

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RESEARCH HIGHLIGHTS

- Acetylcholinesterase (AChE) dip-strip biosensor fabricated to detect carbamates.
- AChE entrapped in fenugreek hydrogel-agarose matrix with gold nanoparticles (GNPs).
- High enzyme retention efficiency (92%) and shelf life (half-life, 55 days).
- Detection limits of carbofuran, oxamyl and methomyl: 2, 21 and 113 nM.
- The biosensor had good testing capabilities to detect carbamates in food samples.

SUPPLEMENTARY MATERIAL

Title: Fenugreek hydrogel-agarose composite entrapped gold nanoparticles for acetylcholinesterase based biosensor for carbamates detection.

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Table S1: Influence of GNPs on Immobilization efficiency of AChE.

Immobilization System	AChE Units Immobilized (mU)	AChE Units after Immobilization (mU)	Immobilization Efficiency (%)
FH-A-AChE	5.000	4.258	85.16
FH-A-AChE-GNPs	5.000	4.614	92.28

Figure S1: Absorbance spectra of gold chloride ($HAuCl_4$) and GNPs.

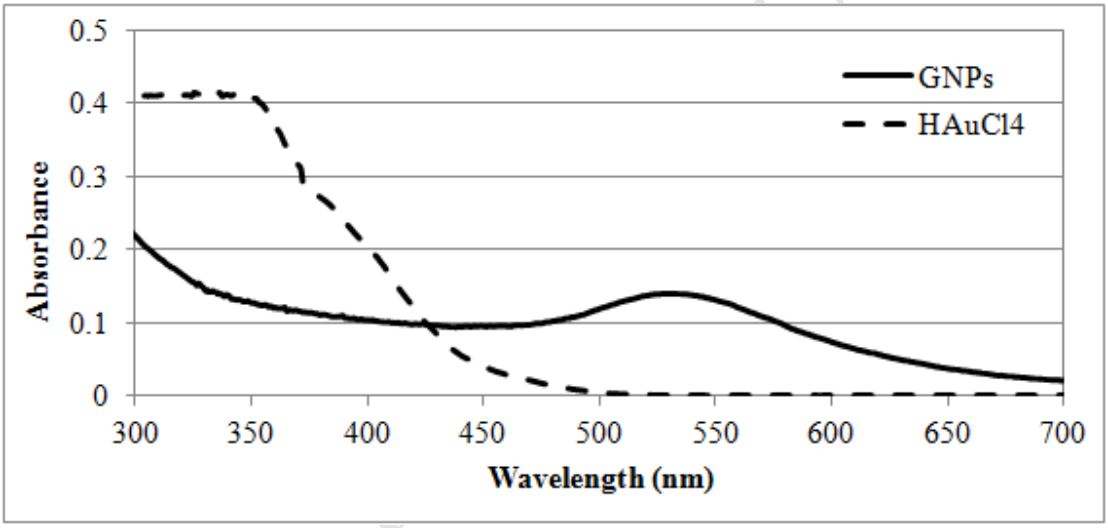

Figure S2: Linear inhibition concentration range of different pesticides for FH-A-AChE-GNPs dipstrips system.