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Fenugreek hydrogel–agarose composite entrapped gold nanoparticles for acetylcholinesterase based biosensor for carbamates detection

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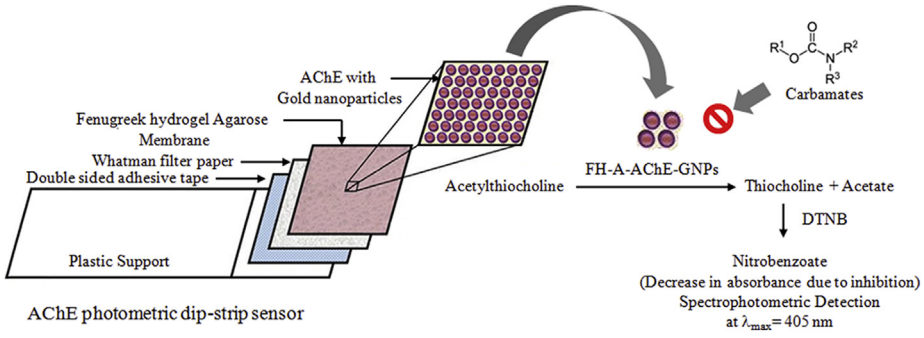
HIGHLIGHTS

- 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.

GRAPHICAL ABSTRACT



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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 (10–20 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.

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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].

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

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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 (*T. foenum-graecum*) seeds were obtained from a supermarket in Pune, Maharashtra, India. Acetylcholinesterase from electric eel (*Electrophorus electricus*), acetyl thiocholine 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 (25 °C). 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 (2.0 g) 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.

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