Gold nanocrystals modified electrochemical aptasensor for ultra-trace level detection of malathion in environmental samples

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Research Article

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Abstract

The abnormal concentrations of organophosphorus pesticides (OPs) in agricultural residues have raised serious concerns regarding food safety and environmental pollution. The trace level detection of these toxic OPs has therefore garnered great attention in the past few decades. Herein, gold nanocrystals modified electrochemical aptasensor has been reported for the detection of a widely used organophosphorus pesticide, malathion. By virtue of the high specificity of the aptamer towards malathion and enhanced surface area provided by the nanocrystals, the designed probe exhibits excellent selectivity and sensitivity for malathion. The conformational changes of aptamer due to its specific binding to malathion induces a large variation in the electrochemical responses of the redox moiety, methylene blue. The gold nanocrystals@aptamer probe exhibits excellent sensitivity in the linear range from 1 pm to 100 nm with 1 pM as the detection limit. Owing to its high reliability and robustness in the spiked samples, the developed nanoprobe paves the way for ultra-trace level detection of malathion in environmental samples.

1 Introduction

Organophosphorus pesticides (OPs) are the most widely employed pesticides and pose serious health concerns owing to their accumulation in the environment. They are the potent inhibitors of cholinesterase, an enzyme critical in the proper functioning of central and peripheral nervous system. The inhibition of the enzyme blocks the breakdown of the transmitter choline thereby preventing the nerve transmission and inflicts severe complications such as rhinorrhea, restlessness, miosis/mydriasis, convulsions, respiratory failure, pulmonary edema, flaccid paralysis and death in few cases. Thus, the sustained prevalence of pollution and health issues arising due to the over exploitation of OPs continue to develop rapid, accurate and specific methods for the onsite monitoring of OPs residues in real samples [1].

The detection of OPs has been carried out using various conventional techniques such as chromatography, mass spectrometry, chemiluminescence, fluorescence, piezoelectricity, surface plasmon resonance [2]. Although the techniques have been fairly sensitive, they are expensive, require cumbersome procedures, sophisticated instrumentation, trained personnel, long operation time thereby restricting their use in real time applications. In addition, various nanoparticles based colorimetric biosensors have also been designed which offers detection in pico-molar range however, the non-specific aggregation of NPs may provide erroneous results. Among various modes of detection, the outstanding properties of electrochemical mode of detection such as simplicity, rapidity, reliability, low cost, good detection limit, less sample requirement, fast response, high sensitivity, less bulky instrumentation, portability, ease of use serve as ideal mode of detection [3] [4]. Consequently, based on enzyme inhibition principle, numerous electrochemical biosensors have been developed for the detection of OPs. The non-inhibition methods based on hydrolysis of OPs by organophosphorus hydrolase (OPH) have also been successfully established [5]. However, the enzyme-based methods usually require large surface area for immobilization, prevention from enzyme leakage and maintenance of the enzymes’ activities and thus,
may add to the cost of the sensor. In view of these drawbacks, aptamer-based biosensors have come into play owing to their excellent sensitivity, good selectivity, and rapid detection. Aptamers are single-stranded DNA/RNA oligonucleotides, selected in vitro using SELEX, having a unique 3-D structure that enables its binding to their specific target molecule with high affinity [6] [7]. The potential of aptamers lie in its diverse range of applications, including diagnostics, targeted therapeutics, molecule imaging, gene delivery and drug delivery. Moreover, the flexibility of aptamers in chemical modifications and designing various kinds of probes makes them better alternatives to conventional antibodies which are usually immunogenic, thermally less stable compared to aptamers and require tedious procedures for their generation [8]. In particular, aptamers hold great promise in the detection of small molecules as they overcome the difficulties which normally arise during the generation of antibodies [2]. The selective analyte recognition ability of aptamers has been extensively applied against a wide range of target molecules which include DNA, RNA, proteins, pesticides, metal ions [9].

Keeping in view the harmful implications of OPs and the urgent need to develop rapid detection strategies, we have designed a facile, rapid and highly selective aptamer-based biosensor for OPs. The constructed electrochemical aptasensor is based on the conformational change of the aptamer induced by the specific target binding. The aptamer functionalized with a thiol group at one end was employed as the probe for electrochemical sensing of organophosphorus pesticide while methylene blue (MB) served as redox moiety. The presence of thiol group facilitated the anchoring of aptamer to the gold nanocrystals (AuNCs) modified electrode [10] [11]. The specific binding of the OP to the aptamer induced the conformational change of the aptamer and eventually resulted in the large variation in the voltammetric signal. Malathion was chosen as representative OPs owing to its acute toxicity and widespread use. To the best of our knowledge, this is the first electrochemical based detection of pesticides, organophosphorus pesticides in particular employing aptamer and methylene blue as detection probe. The methodology was facile and rapid thereby allowing highly sensitive and selective determination of OPs. Therefore, the manuscript is a preliminary step towards the exploitation of such interactions in pesticides detection.

2 Experimental Section

2.1 Materials

Hydrogen tetrachloroaurate (III) trihydrate, 2-mercaptoethanol, 6-mercaptohexanol and aptamer sequence for malathion 5’ATCCGTCACACCTGCTCTTATACACAATTGTTTTTCTCTTAACTTCTTGACTGCTGGTGTTGGCTCCCGTAT-3’ with disulfide(S–S) modification at the 5’ end were acquired from Sigma Aldrich (India). Potassium ferrocyanide and potassium ferricyanide were purchased from Finar (India). All the reagents were of analytical grade and the experiments were performed in milli-Q water having a resistivity of 18.2 MΩ cm. The glassware was rinsed in aqua regia prior to use.
2.2 Methods

2.2.1 Electrochemical Measurements

All the electrochemical measurements were performed on conventional three-electrode system. Ag/AgCl and Pt wire were used as reference and auxiliary/counter electrodes respectively. Glassy carbon electrode (GCE) was employed for various modifications as a working electrode. The standard potassium ferrocyanide and ferricyanide was used as electrolyte for carrying out the cyclic voltammetric measurements while the square wave voltammograms were obtained using MB.

2.2.2 Preparation of working electrodes

The cleaning of the bare electrode (2 mm in diameter) was carried out by immersing the electrode in 30% HNO₃ solution followed by rinsing with ultrapure water. The electrode was then polished to get mirror like surface with 0.3 µm alumina slurry for 5 min. Next, the electrode was alternatively sonicated in ethanol and ultrapure water for 5 min in order to remove the residual alumina. The activation of electrode was carried out electrochemically with 20 successive cyclic voltammetry (CV) scans from −0.4 to 1.6 V in 0.5 M H₂SO₄ at 100 mV/s. Then, CV of 5 mM potassium ferrocyanide/ferricyanide in 0.1 N KCl was recorded.

2.2.3 Fabrication of AuNCs modified working electrodes

In order to modify the GCE with AuNPs, 10 CV cycles were employed from −0.1 V to 0.3 V in aqueous 25 mM HAuCl₄. Next, the electrode was thoroughly rinsed with ultrapure water to remove excess of salt and again, CV of [Fe(CN)₆]⁴⁻/³⁻ couple was measured.

2.2.4 Preparation of the electrochemical aptasensor

100 µL of 10 µΜ aptamer was incubated wit 2 % 2-ME for 1 hr at rt. Afterwards, the AuNPs modified GCE was immersed in the above prepared aptamer solution overnight and washed thoroughly with DI. In order to block the non-specific sites, the electrode was placed in 1 mM 6-mercaptohexanol solution for 1 hr followed by thorough washing with PBS and DI. Finally, the CV was recorded in 2.5 mM [Fe(CN)₆]⁴⁻/³⁻ solution having 0.1 N KCl. The modification of the working electrode was characterized using CV measurements.

2.2.5 Electrochemical detection of malathion

To test the presence of malathion, the developed electrochemical aptasensor was dipped into 20 mM methylene blue solution for 30 min so as to adsorb MB molecules. Then, the modified electrode was incubated in 1X PBS (pH 7.2) having different concentrations of malathion solution for 1 hr and then SWV was employed to obtain the peak current of MB. In order to test the selectivity, various non-target pesticides were used and treated in the same manner.
3 Results And Discussion

3.1 Modification and characterization of electrode with gold nanocrystals@aptamer

To begin with the electrochemical response of the working electrode was investigated using ferro-ferri redox couple. Initially, the CV of 1 X PBS was recorded without adding any electrolyte. The absence of any voltammogram clearly indicated the proper surface cleaning of the working electrode (Fig. 1). Though, with the addition of 5 mM $[\text{Fe(CN)}_6]^{4-/3-}$ in 0.1 N KCl, the characteristic CV of $[\text{Fe(CN)}_6]^{4-/3-}$ redox was obtained however, the shape of the peak was not appropriate. Consequently, the GCE was activated using sulphuric acid resulting in enhanced current response in the CV.

It is well documented in literature about the surface enhancement of the electrodes by NPs thereby facilitating the process of electron transfer. Owing to the excellent properties, easy synthesis, high stability, facile functionalization of AuNPs; they were selected for modification of the working electrode. The modification of GCE with gold nanocrystals resulted in significant current enhancement which validated the successful deposition of the nanocrystals on the electrode. Following the successful attachment of the crystals on the electrode surface, the electrode was subjected to coating with aptamer specific for the target analyte. Initially, the S-S linkage of the thiolated aptamer was reduced with 2-mercaptoethanol (2-ME). Following this, the aptamer was attached to the gold crystals modified electrodes by virtue of strong Au-S binding. The successful attachment of the malathion specific aptamer onto gold nanocrystals was manifested from the decrease in the current response as the negatively charged self-assembled layer of aptamer repels the $[\text{Fe(CN)}_6]^{4-/3-}$ anions thereby hindering the electron transfer process and therefore, resulted in an overall decrease in the signal (Fig. 2).

3.2 Design of the nanoprobe

The schematic of the working principle for the electrochemical detection is illustrated in Scheme 1. In this methodology, aptamer served as the bio-recognition element for malathion and MB as the electrochemical tag. There are several reports in literature that confirms the binding of MB to single strands of DNA via electrostatic interactions between DNA phosphate backbone and MB. Moreover, MB also possesses specific binding to guanine bases and therefore, can interact strongly with DNA. The interactions between MB and DNA were thus exploited to devise a simple and highly sensitive platform for the electrochemical detection of malathion. At the start, the AuNPs were deposited on the surface of GCE for providing high surface area and further functionalized with thiolated aptamer, due to strong Au-thiol chemistry. To reduce the non-specific adsorption of interferents, 6-MCH was used for blocking. The designed probe follows the principle that in the absence of any target analyte, MB gets bound to the aptamer thereby giving its characteristic signal in SWV. However, when target molecule i.e. malathion exists in the sample, the aptamer owing to its strong binding with malathion no longer remains bound to MB which then dissociate itself from the aptamer resulting in a decrease in the peak current of MB. The
presence of malathion results in large variation in the MB SWV signal and thus, the applicability of the AuNCs@aptamer probe was tested for highly sensitive and selective detection of malathion.

### 3.3 Probe sensitivity for malathion detection

Linearity is a critical parameter for any sensor. Therefore, the feasibility of the probe was thoroughly investigated. After the successful functionalization of GCE with AuNCs@aptamer, the viability of the proposed aptasensor was checked by incubating the modified electrode, MB with malathion and subsequently the corresponding SWV curves of MB were recorded. Figure 3 clearly reveals that a significant peak was observed for MB in the absence of malathion while the peak current decreased with 1 nM malathion. The results clearly indicated that the current is due to MB bound in the single strands of DNA and owing to the specific interaction of aptamer with malathion, the conformational changes in the aptamer resulted in an overall decrease in the peak current of MB. Thus, the results showed that the changes in the peak current can be easily applied for the detection of target of interest.

The analytical ability of the proposed electrochemical aptasensor was then evaluated by investigating the SWV of MB in the presence of different malathion concentrations. Figure 4 shows the relationship of current with varying malathion concentrations. The results showed that the peak current decreased linearly with increasing malathion concentrations. The developed aptasensor exhibited high sensitivity in the linear range from 1 pM to 100 nM with $R^2 = 0.9945$. A very low limit of detection i.e. 1 pM as compared to other electrochemical methodologies for malathion was achieved with the designed aptasensor (Table 1). The results analysis clearly indicated that the developed methodology offers high sensitivity and can applied for the onsite screening of pesticides residues in environmental samples.

<table>
<thead>
<tr>
<th>Linear Range</th>
<th>LOD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001 nM-100 nM</td>
<td>1 pM</td>
<td>Present method</td>
</tr>
<tr>
<td>9.99 to 99.01 nM</td>
<td>4.08 nM</td>
<td>[12]</td>
</tr>
<tr>
<td>$2.0 \times 10^{-7}$ M to $14.0 \times 10^{-7}$ M</td>
<td>0.2 µM</td>
<td>[13]</td>
</tr>
<tr>
<td>-</td>
<td>1 nM</td>
<td>[14]</td>
</tr>
<tr>
<td>0.01 to 0.5 µg/mL</td>
<td>1 ng/mL</td>
<td>[15]</td>
</tr>
<tr>
<td>0.1–70 nmol L$^{-1}$</td>
<td>0.1 nmol L$^{-1}$</td>
<td>[16]</td>
</tr>
<tr>
<td>0.1 to 20 ng mL$^{-1}$</td>
<td>0.1 nM</td>
<td>[17]</td>
</tr>
</tbody>
</table>

### 3.4 Specificity towards malathion
In order to examine the cross reactivity of non-target pesticides such as atrazine, chlorosulfuron, 2,4 D, diuron commonly used in agricultural fields and ions (Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\)), careful study was carried out using the devised nanoprobe. As illustrated in Fig. 5, even the 100-fold concentrations of non-target analytes caused insignificant decrease in the current response of the probe and only malathion induced the decrease in the current signal signifying the high specificity towards malathion detection.

### 3.5 Malathion testing in real samples

The potential application of the designed probe was assessed with real samples. For this, the detection of malathion was carried out in spiked soil and lake water samples. Initially, the soil sample was collected from fields and filtered using 0.4 m filters followed by spiking with malathion. Similarly, lake water was pretreated, spiked and analyzed. Afterwards, the spiked samples were analyzed using same procedure as already described. The results are presented in Table 2 which clearly shows that the amount of malathion obtained using the developed aptasensor was in perfect correlation with the spiked amount thus, validating the potential of the methodology in real samples. Figure 6 shows the response of the aptasensor towards spiked and non-spiked samples. As evident, the unspiked sample did not show any significant decrease in the current and thus, change in current i.e. \(\Delta I\) was low suggesting that the MB is bound to the aptamer strands. However, the spiked samples showed a considerable decrease in the peak current and \(\Delta I\) was very high indicating the dissociation of MB from aptamer strands due to specific binding of aptamer towards malathion. Thus, it can be clearly stated that the developed aptasensor can be easily applied for the rapid monitoring of pesticides residues in real samples.

#### Table 2

<table>
<thead>
<tr>
<th>Source</th>
<th>Spiked Amount (nM)</th>
<th>Found Amount (nM)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake water</td>
<td>1</td>
<td>0.98</td>
<td>98</td>
<td>2.36</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.65</td>
<td>96</td>
<td>3.89</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>95</td>
<td>95</td>
<td>3.45</td>
</tr>
<tr>
<td>Soil sample</td>
<td>1</td>
<td>0.89</td>
<td>89</td>
<td>4.87</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8.97</td>
<td>90</td>
<td>5.67</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>92.69</td>
<td>93</td>
<td>5.98</td>
</tr>
</tbody>
</table>

### 4 Conclusions

To sum up, we have devised a label free, highly sensitive, specific and rapid electrochemical aptasensor for the detection of an extremely toxic organophosphorus pesticide, malathion. The detection was based on the change in SWV signals of MB arising due to target induced conformational changes in the aptamer. The LOD of the aptasensor was found to be 1 pM which is considerably lower than the available
electrochemical techniques. The detection was facile and allowed the rapid determination of OP in few minutes. The speed of SWV combined with the unique properties of AuNCs resulted in a highly sensitive and rapid platform for the detection of malathion. Moreover, the employment of aptamer as bio-recognition element provided high specificity to the aptasensor. Furthermore, the detection results in spiked samples clearly validated the applicability of the developed aptasensor in real environmental samples.

**Declarations**

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**Author contributions**

Rajni Bala: investigation, formal analysis, writing—original draft. Alisha Lalhall: data curation, writing—reviewing and editing. Rohit K. Sharma: resources, supervision. Nishima Wangoo: resources, supervision, funding acquisition.

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**Availability of data and materials**

The data and materials will be provided on request.

**Competing interests**

The authors declare no competing interests.

**Conflict of Interest**

The authors declare no competing interests.

**Research Involving Humans and Animals Statement**

This article does not contain a description of the studies performed by the authors involving people or using animals as objects.

**References**


**Scheme 1**

Scheme 1 is available in Supplementary Files section.

**Figures**
Figure 1

Cyclic voltammograms of (a) 1 X PBS, (b) $[\text{Fe(CN)}_6]^{4-/3-}$ redox couple without activation using sulphuric acid and (c) $[\text{Fe(CN)}_6]^{4-/3-}$ redox couple after activation using sulphuric acid
Figure 2

Cyclic voltammograms of $[\text{Fe(CN)}_6]^{4-/3-}$ redox couple with bare GCE, AuNPs modified GCE and malathion specific aptamer modified AuNPs-GCE
Figure 3

SWV curve of MB (a) in the absence of malathion and (b) in the presence of malathion

Figure 4

A

B

$R^2 = 0.9945$
(A) SWV curves of MB with varying malathion concentrations. (B) Calibration plot for malathion. Each point represents an average of three individual measurements and error bars indicate the standard deviation.

Figure 5

Electrochemical response of the aptasensor towards different interferents: 1 µM of other interferents and 1 nM malathion

Figure 6
Detection of malathion in (A) lake water and (B) soil sample. U represents the unspiked sample whereas S shows the sample spiked with 1 nM malathion. Experiments were carried out in triplicates and error bars show the standard deviation.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.png