

## Full Paper

# Design and Development of Imprinted Polymer Inclusion Membrane-Based Field Monitoring Device for Trace Determination of Phorate (*O,O*-Diethyl *S*-Ethyl Thiomethyl Phosphorodithioate) in Natural Waters

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## Abstract

A biomimetic potentiometric field monitoring device was developed for the trace determination of phorate (*O,O*-diethyl *S*-ethyl thiomethyl phosphorodithioate) in natural waters. The sensing element was fabricated by the inclusion of phorate imprinted polymer materials in the polyvinyl chloride (PVC) matrix. The sensor surface can be reused without conditioning unlike most other conventional sensors. Operational parameters such as amount and nature of plasticizers sensing material, pH and response time were optimized. The response characteristics of the non-imprinted (NIPIM) and imprinted polymer inclusion membrane (IPIM) sensors for phorate were compared under optimum conditions. The IPIM sensor responds linearly to phorate in the concentration in the ranges  $1 \times 10^{-9}$  to  $1 \times 10^{-6}$  M and  $1 \times 10^{-6}$  to  $1 \times 10^{-5}$  M of different slopes with a detection limit of  $1 \times 10^{-9}$  M. The selectivity was tested with various common organophosphorous (OP) pesticides and herbicides. In addition to superior sensitivity and selectivity of IPIM over NIPIM-based sensor, IPIM-based phorate sensor was found to be stable for 3 months and can be used for more than 40 times without any loss in sensitivity. The applicability for analyzing ground, river and tap-water samples was successfully demonstrated via recovery studies.

**Keywords:** Imprinted polymer inclusion membrane sensor, Phorate, Potentiometry, Organophosphorous pesticides, Natural waters

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## 1. Introduction

Natural waters are contaminated with various pesticides and insecticides because of their widespread use in commercial and residential applications. Organophosphate (OP) neurotoxins comprise a unique class of contaminants, since they are powerful inhibitors of acetyl cholinesterase, which is critical in nerve function [1–5]. Phorate (*O,O*-diethyl *S*-ethyl thiomethyl phosphorodithioate, CAS No: 298-02-2) Figure 1, is a widely used, typical thioether containing organophosphate pesticide. The U.S. Environmental Protection Agency market estimates based on crop life America annual surveys for 2001 usage include 2–3 million pounds of active ingredient of phorate in the United States alone [6]. It is still an extremely difficult challenge to detect low concentrations of OPs accurately in environmental samples. Soil and water samples are very likely to contain OP pesticides due to heavy urban and rural use of these compounds.

Routine analysis of OPs in environmental and biological samples was carried out using analytical techniques such as

gas or liquid chromatography and mass spectrometry [7]. These techniques generally have high sensitivity but are complex and costly, require skilled technicians and large laboratory based instrumentation. Additionally, most of these techniques require sophisticated and time consuming extraction procedures such as liquid solid extraction (LSE) or solid phase microextraction (SPME) [8–10]. Biological methods such as immunoassay have also been reported [11]. Long analysis time and extensive sample handling with multiple washing steps limit the applications. More compact and low cost instruments coupled to smaller sensing probes are highly desirable for facilitating the task of on-site

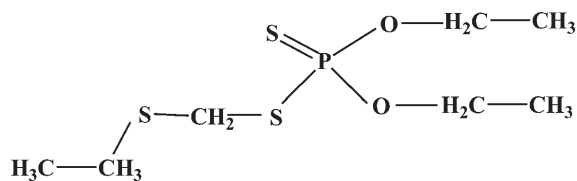


Fig. 1. Chemical structure of phorate.

monitoring of OP compounds. Eventhough enzyme based optical or electrochemical biosensors are available, they are prone to certain drawbacks [12–15]. Potentiometric sensors, being a subgroup of chemical sensors, are attractive for practical applications, as they are associated with small size, portability and low energy consumption and cost compared to other group of sensors.

New frontiers in the field of molecular imprinted polymers (MIPs) have created synthetic materials that can mimic the function of biological receptors but with less stability constraints [16, 17]. These biomimetic receptors can readily and unequivocally distinguish the target OP pesticide from structurally and functionally similar class of compounds. Thus, molecularly imprinted thin films of sol-gel materials with specific binding sites of parathion have been developed by Marx et al. [18]. The films were cast on glassy substrate and glassy carbon electrodes to detect parathion in aqueous solutions by GC-FPD and cyclic voltammetry respectively. Li et al. [19] has developed an electrochemical sensor for the determination of parathion based on *p*-tert-butyl calix[6]arene-1, 4-crown-4 sol-gel film. Jenkin et al. [20] has reported MIP based fiber optic sensors for OP pesticides and insecticides. An overview of biomimetic sensors for toxic pesticides and inorganics based on various transducers was given by Rao et al. [21]. As of now, there are no literature reports on the utilization of imprinted polymer inclusion membrane (IPIM) based sensors for the trace determination of OP pesticides. This paper describes the fabrication of one such IPIM based biomimetic potentiometric sensor for routine monitoring of phorate down to  $5 \times 10^{-9}$  M levels in natural waters by inclusion of phorate imprinted material in polyvinyl chloride (PVC) matrix.

## 2. Experimental

### 2.1. Reagents

Phorate, Parathion, Atrazine, Dichlorovos, ethion, disulfoton, diazinon, 2,4-D, 2,4,5-T were obtained from SUPELCO, USA. Methacrylic acid (MAA), Ethylene glycol dimethacrylate (EGDMA), 2,2'-Azobisisobutyronitrile (AIBN), di-*n*-octylphthalate (DOP), 2-nitrophenyloctyl ether (NPOE), bis(2-ethylhexyl) sebacate (BEHS), tris(2-ethylhexyl) phosphate (TEHP) and high molecular mass poly(vinyl chloride) (PVC) were obtained from Aldrich (Milwaukee, WI, USA). All other reagents used were of analytical grade and deionized water was used throughout. Stock standard solution of phorate ( $10^{-4}$  M) was prepared in  $10^{-3}$  M phosphate-saline buffer ( $10^{-3}$  M  $\text{NaH}_2\text{PO}_4$  +  $10^{-3}$  M KCl). The solutions of  $1.0 \times 10^{-5}$  to  $1.0 \times 10^{-9}$  M of phorate were prepared by aqueous dilution of a definite volume from the stock standard solution by maintaining a overall concentration of  $10^{-4}$  M in  $\text{NaH}_2\text{PO}_4$  and  $10^{-4}$  M in KCl.

### 2.2. Equipment

Potentiometric response characteristics were studied with an ELICO make Ion analyzer, Model No.LI 126 (ELICO, Hyderabad, India).

### 2.3. Synthesis of Phorate Imprinted and Nonimprinted Polymer Materials

Phorate imprinted polymer materials were prepared by bulk polymerization method. 1 mmol of phorate and 8 mmol of MAA were taken in an RB flask and allowed for prearrangement for 5 min. It is then followed by the addition of 20 mmol of EGDMA and 2 mmol of AIBN and 5 mL of porogen (Toluene) was added. The mixture was then purged with  $\text{N}_2$  for 5 min and the flask was sealed under this inert atmosphere. It was then kept stirring in an oil bath at  $60^\circ\text{C}$  to start the polymerization process. After 24 h, the obtained polymer materials were crushed and sieved and the particles having in the range 50–105  $\mu\text{m}$  were collected. The polymer materials were washed with acetone to remove the unpolymerized reactants. The phorate template was removed by soxhlet extraction with glacial acetic acid for 1 h followed by toluene for 2 h. Then, the particles were suspended in acetone and allowed to settle for 4 h. The sedimented particles were discarded and those not sedimented were collected by centrifugation. This process was repeated two times. The resulting MIP particles were dried to constant weight under vacuum at  $60^\circ\text{C}$  and were used in the following experiments. Nonimprinted polymer (NIP) particles were prepared analogously without the addition of phorate during polymerization.

### 2.4. Membrane Preparation and Sensor Fabrication

The IPIM and corresponding nonimprinted (NIPIM) sensor was constructed by following the general procedure mentioned below. The phorate imprinted and non-imprinted polymer particles synthesized in Section 2.3 (90 mg) were included to 0.2 mL of NPOE (DOP, BEHS, TEHP) and 2.5 mL of THF containing 90 mg of PVC. The resulting solution was homogenized and poured into a Teflon mould of 17 mm diameter. Polymer membrane of ca. 0.45 mm thickness was obtained by the slow evaporation of the solvent. The membranes were glued to one end of a pyrex glass tube with Araldite. The tube was then filled with an internal filling solution of  $10^{-4}$  M phorate in 0.1 mM of each of  $\text{NaH}_2\text{PO}_4$  and KCl buffer. A schematic diagram of membrane formation and fabrication of biomimetic potentiometric sensor is given in Figure 2. The sensor was kept in air when not in use.

### 2.5. EMF Measurement

The sensor was conditioned in 25 mL of 0.1 M Tris buffer with pH 10 for 30 min. The response of the sensor was

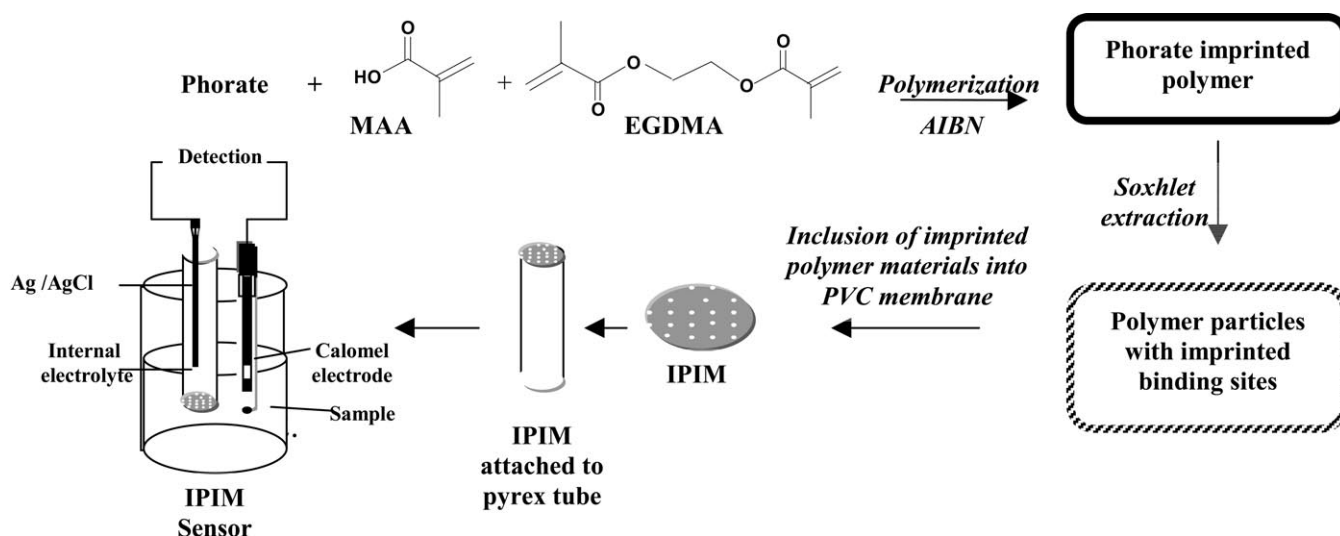


Fig. 2. A schematic representation of imprinted polymer inclusion membrane-based field monitoring device for phorate.

examined by measuring the electromotive force (EMF) of the following electrochemical cell.

Ag, AgCl  $1.0 \times 10^{-4}$  M phorate in 0.1 mM phosphate saline | PVC membrane | sample || KCl (saturated) | HgCl<sub>2</sub>, Hg.

The potential response of the sample solutions containing different concentrations [ $1 \times 10^{-10}$  M to  $1 \times 10^{-5}$  M] of phorate in 50 mL of 0.1 M Tris buffer (pH 10) were measured. The EMF was plotted as a function of phorate concentration.

## 2.6. Analysis of River/Ground/Tap Waters

The pHs of ground, river or tap water samples (ca. 45 mL) were adjusted to pH 10 after the addition of 5 mL of 1 M Tris buffer using HCl or NaOH. The samples were analyzed via standard addition method using the above fabricated IPIM-based phorate potentiometric sensor by following the analytical procedure mentioned above.

## 3. Results and Discussion

### 3.1. Effect of Membrane Composition

The sensor performance depends a lot on the optimal design of the membrane. Literature reports on conventional potentiometric sensors for inorganics shows that the response behavior of the sensor depends on the various features such as nature of plasticizer, nature and amount of sensing material used [22–25]. The various parameters of phorate sensor membrane have been optimized under similar lines.

#### 3.1.1. Influence of Plasticizer

The response mechanism of the sensor strongly depends on the mobility of electroactive species, thereby reducing the resistance. Incorporation of suitable plasticizer influences the working concentration range of potentiometric sensor by enhancing the mobility of target analytes. Moreover, it ensures flexibility to the membrane. As it is shown that the plasticizers improves the electrochemical properties of potentiometric sensors including dysprosium ion imprinted polymer based sensor [22–24] the effect of different plasticizers on the performance of phorate sensor was investigated. Figure 3 shows the potential output of phorate IPIM sensor with different plasticizers NPOE, BEHS, TEHP and DOP. From the Figure, it is evident that membranes with NPOE and BEHS alone offers better response characteristics in the concentration range  $1 \times 10^{-9}$  to  $1 \times 10^{-5}$  M compared to TEHP and DOP sensors. Also, the magnitude of potential difference for these two membranes is higher in each decade. Considering the stability of EMF output and consequent better precision of NPOE-based sensor over BEHS, NPOE is preferred for fabricating phorate IPIM sensor. This observation is coherent with earlier [26, 27] reports on which can be attributed to the higher dielectric constant of the plasticizer, i.e., plasticizer NPOE having high dielectric constant of 24.0 giving better characteristics than DOP ( $\epsilon = 5.0$ ), BEHS ( $\epsilon = 4.0$ ) and TEHP ( $\epsilon = 4.8$ ). It is pertinent to mention here that the phorate membranes were found to be brittle in the absence of plasticizer and the sensor performance could not be studied.

#### 3.1.2. Effect of PVC to MIP Particles Ratio

Since the phorate IPIM sensor performance depends heavily on the affinity sites available for template binding,

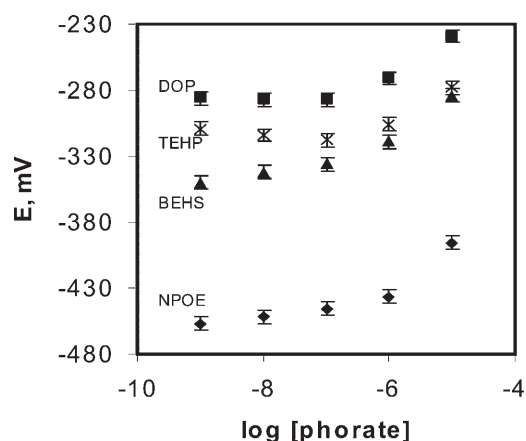


Fig. 3. Potential output of phorate IPIM sensor fabricated using various plasticizers.

optimum ratio of PVC to MIP is essential. Table 1 shows the performance of the membrane sensor while varying the amount of MIP particles in the PVC matrix. From the table, it is clear that the membranes with 1:1 and 2:1 ratios are equally good or rather increasing the amount of MIP particles beyond a particular limit does not enhance its response behavior in contradiction to our expectation. This may be due to the inhomogeneous nature of the 2:1 membrane thereby restricting the interaction of template with affinity sites. In the case of membranes with 1:0.5 ratio, the total number of binding sites available for the rebinding of phorate is relatively low, for the membrane to respond effectively. Hence, the 1:1 membrane was selected for further studies.

### 3.2. Effect of pH

The pH of the test solution plays a crucial role in deriving optimum IPIM sensor performance. The influence of pH on the phorate IPIM sensor response has been studied by varying it from 8 to 11 in steps of 0.5 after addition with 5 mL of 1.0 M tris(hydroxymethyl) aminomethane (Tris) buffer. The results indicate that the optimum pH for constant and maximum response behavior over the entire concentration range of phorate ( $1 \times 10^{-9}$  to  $1 \times 10^{-5}$  M) is 9.5 to 10.5. Hence, the pH of the test solution was adjusted to ca. 10 after the addition of 5 mL of 1.0 M Tris buffer. Tris buffer plays a vital role in the stabilization of these phosphorus thioesters in the alkaline medium rather than hydrolyzing it in contrast to other alkaline buffers working in this pH range. This is

due to the weaker nucleophilicity and higher basicity of Tris buffer, which helps us in dealing with these pesticides.

### 3.3. Dynamic Response Time

Dynamic response time is another factor that measures the sensing ability of a sensor. The response time was recorded by varying the phorate concentration in the test solution from  $1 \times 10^{-9}$  to  $1 \times 10^{-5}$ . It was observed that 95% of the phorate IPIM sensor response was realized within 2 min and reaches equilibrium response in a time of about 5 min. To evaluate the reversibility of phorate sensor, a similar procedure in opposite direction was studied. The measurements performed in the sequence of high to low concentration (from  $1 \times 10^{-5}$  to  $1 \times 10^{-9}$  M) indicate that the IPIM sensor was reversible analogous to most of the conventional potentiometric sensors.

### 3.4. Sensitivity and Detection Limit

The response performance of the non-imprinted (NIPIM) and phorate imprinted (IPIM) sensors were compared under the optimum conditions (Fig. 4). As seen from the above Figure, the plot of  $E$  vs.  $\log$  [phorate] for an IPIM sensors gave linear calibration plot with 2 different slopes gave linear calibration plot with 2 different slopes in the range  $10^{-9}$  to  $10^{-6}$  and  $10^{-6}$  to  $10^{-5}$  M. It was observed that the absolute potentials obtained from non-imprinted polymer materials (ref. inset) were unstable (i.e., varying in a range of  $\pm 7$  mV) which is due to nonspecific binding of analyte in contrast to the specific site selective binding in the case of imprinted polymer materials. The stable potential of phorate imprinted membrane is attributed to the template (phorate) specific binding sites. The imprinting effect was further exemplified by the higher potential response of imprinted membrane in each decade compared to non-imprinted one (see inset) and the lower detection limit of  $1 \times 10^{-9}$  M for imprinted polymer membrane which is calculated based on IUPAC recommendation [28]. On the other hand, the NIPIM do not respond to phorate below  $1 \times 10^{-8}$  M and moreover the values were unstable even at higher concentrations of phorate.

### 3.5. Sensor Selectivity

Selectivity is undoubtedly an important issue in the implementation of imprinted polymer inclusion membrane based

Table 1. Effect of weight ratio of MIP particles to PVC on phorate IPIM sensor response.

Weight of MIP particles (mg)	Weight of PVC (mg)	Weight ratio	Working concentration range (M)
45	90	0.5: 1	$5 \times 10^{-7} - 10^{-5}$
90	90	1: 1	$10^{-9} - 10^{-6}$ & $10^{-6} - 10^{-5}$
180	90	2: 1	$10^{-9} - 10^{-6}$ & $10^{-6} - 10^{-5}$

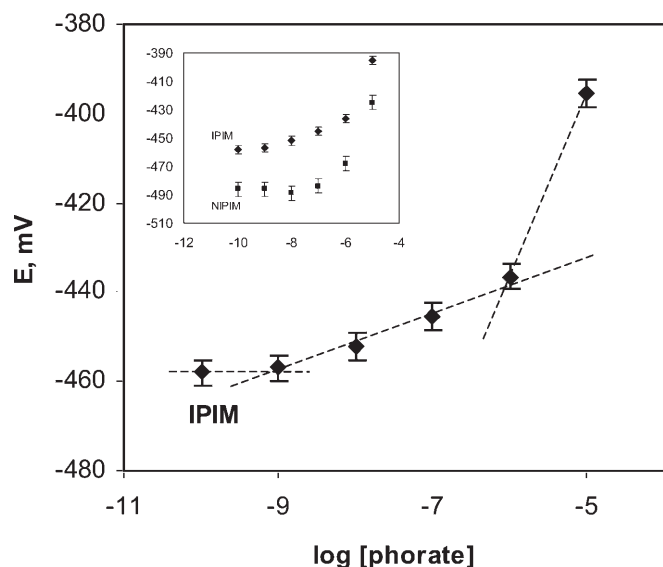


Fig. 4. Potential response of NIPIM and phorate IPIM sensors.

sensors. In environmental applications, the concentration of the target analytes can be quite low and reasonably high selectivity is essential for an effective treatment. Hence, the selectivity of the developed sensor with various common organophosphorous pesticides and herbicides was tested. The change in potentiometric response resulting from the exposure to coexisting species individually with IPIM and NIPIM-based sensors were recorded on changing the phorate concentration from  $1 \times 10^{-7}$  M to  $1 \times 10^{-6}$  M and the results obtained are shown in Figure 5. The species which are more likely to coexist with phorate do not respond with IPIM sensor except diazinon and disulfoton. This can be attributed to the fact that diazinon and disulfoton belong to the same thioether class. Even these species upto  $10^{-8}$  M

have no deleterious effect on the potential response of phorate. The IPIM sensor gave better response to phorate than NIPIM sensor, which substantiates the imprinting effect.

### 3.6. Stability and Reusability

The important criteria required for any sensing device in addition to sensitivity and selectivity is stability and reusability. The above fabricated IPIM based phorate sensor was found to be stable (deviation less than  $\pm 1.0$  mV for  $5 \times 10^{-9}$  M for phorate) for 3 months and can be reused for more than 40 times without any loss in sensing ability. Furthermore, the sensing membrane does not require any conditioning, i.e., no need for soaking in conditioning solutions after fabrication.

### 3.7. Analytical Applications

Since the interferences did not give any false positive signals, ground water samples were analyzed by spiking with known amounts of phorate. The results obtained are shown in Table 2 from which it is clear that the IPIM based phorate sensor can reliably be used for monitoring phorate contaminated natural waters containing host of other pesticides and herbicides.

## 4. Conclusions

This report describes a simple and elegant way of designing imprinted polymer inclusion membrane (IPIM) sensor for phorate based on molecular imprinting technology. The

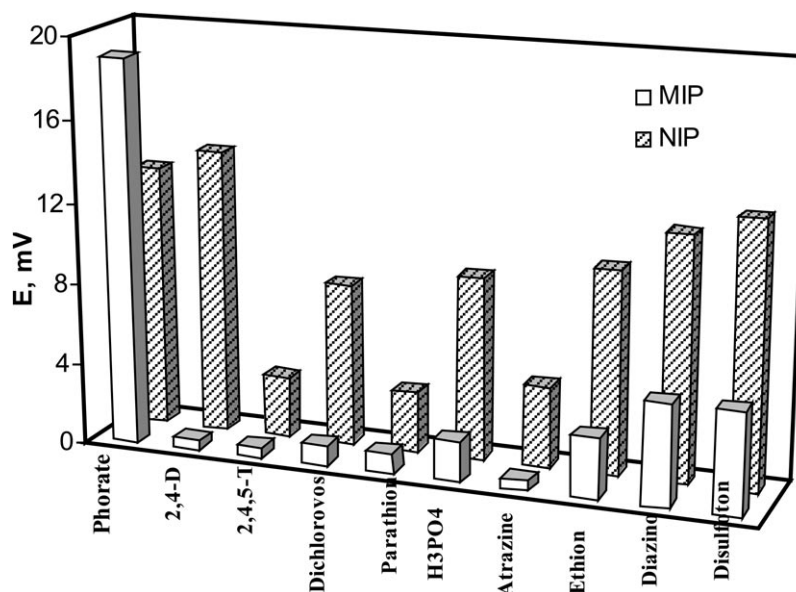


Fig. 5. Potentiometric response of phorate IPIM and NIPIM sensor against other pesticides in the concentration range  $1 \times 10^{-7}$  to  $1 \times 10^{-6}$  M.

Table 2. Analysis of ground water samples, collected from Trivandrum, India.

S.No.	Concentration of each pesticide in mixture added to ground water [a] (M)	Phorate added ( $\times 10^{-9}$ M)	Phorate found [b] ( $\times 10^{-9}$ M)	Recovery (%)
1.	–	2.0	$2.01 \pm 0.02$	100.5
2.	$10^{-9}$	2.0	$2.04 \pm 0.02$	102.0
3.	$10^{-8}$	2.0	$2.06 \pm 0.03$	103.0

[a] Mixture of parathion, dichorovos, atrazine, 2,4-D and 2,4,5-T.

[b] Average of triplicate measurements.

selectivity of the IPIM is remarkable compared to corresponding non-imprinted polymer inclusion membrane (NI-PIM) based potentiometric sensor. Again, the phorate sensor developed in this paper does not require any conditioning unlike most other potentiometric sensors. The stability, reusability, portability and absence of memory effect enables the present IPIM based phorate sensor device to find ready use in field monitoring studies and also thrives us to develop such site selective sensors for other organic pollutants as well.

## 5. Acknowledgements

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