

Imprinted Polymer Inclusion Membrane Based Potentiometric Sensor for Determination and Quantification of Diethyl Chlorophosphate in Natural Waters

Varada Vishnuvardhan¹, Yakkala Kalyan¹, Krishnapillai Padmajakumari Prathish², Battala Gangadhar¹, Yadamari Tharakeswar¹, Talasila Prasada Rao², Gurijala Ramakrishna Naidu^{1*}

¹Department of Environmental Sciences, Sri Venkateswara University, Tirupati, India

²National Institute for Interdisciplinary Science & Technology, CSIR, Trivandrum, India

E-mail: naidugrk@yahoo.com

Received January 3, 2011; revised January 26, 2011; accepted May 6, 2011

Abstract

Biomimetic potentiometric sensor for the determination of diethyl chlorophosphate was developed using imprinted polymer inclusion membrane strategy. Semi-covalent imprinted and non-imprinted polymer particles were synthesized and found that non-imprinted polymer inclusion membrane was unstable in contrast to imprinted polymer inclusion membrane in determination and quantification of diethyl chlorophosphate. Imprinted polymer inclusion membrane based sensor found to be pH dependant with a 5 min equilibrium response time at pH = 10.5 and linearly responds to diethyl chlorophosphate in the concentration range of 1×10^{-9} to 1×10^{-4} and 1×10^{-4} to 1×10^{-2} mol·L⁻¹ with a detection limit of 1×10^{-9} mol·L⁻¹ (0.17 ppb). It was found that diethyl chlorophosphate response was selective against various selected interferents like pinacolyl methylphosphonate, dimethyl methyl phosphonate, methylphosphonic acid, Phorate and 2, 4-D. The developed sensor was found to be stable for 3 months and can be reusable more than 30 times without losing sensitivity. The developed sensor was successfully applied for the determination of diethyl chlorophosphate in natural waters.

Keywords: Sensor, Imprinted Polymer Inclusion Membrane, Potentiometry, Diethyl Chlorophosphate, Natural Waters

1. Introduction

Growing concerns with regard to determine the trace amounts of chemical warfare agents (CWAs) in environment, necessitate to the continuous development of simple analytical methods that can be employed as long term monitoring aids, used primarily as alarms. Nerve agents, in particular, are among the most lethal CWAs. The uses of nerve agents by terrorist organizations or even states are significant as they can be readily synthesized by simple chemical reactions and often have an extremely high toxicity. Highly toxic nerve agents such as G series agents-(GA) Tabun, GB (Sarin), GD (Soman), GF and V series agents-VE, VG, VM and VX are powerful inhibitors of acetyl cholinesterase, which is critical in nerve function [1]. The use of nerve agents in

1988 that killed thousands of Kurdish villagers and 1991 Gulf war further emphasized the threat of chemical warfare [2]. Two Sarin gas attacks in Matsumoto and Tokyo, Japan in 1994-1995 and events in the United States in 2001 have confirmed this horrible reality [3]. Due to the lethality of these agents, detection and monitoring of nerve agents are of prime importance in overall safety and security of humans, animals and plants. Therefore, it is necessary to develop detection systems that are portable, inexpensive, simple, rapid, selective and sensitive for analyzing environmental security threats.

The molecular imprinting technique [4] continues to be a fascinating field of analytical chemistry offering strategies for creating molecule-specific recognition matrices with recognition capabilities analogous to those of

biological receptors [5]. The shape, size and positions of the functional groups in the recognition sites generated are complementary to those of the original analyte. Thus, molecularly imprinted polymers (MIPs) rebind their original analytes in preference to related molecules. MIPs have considerable potential for applications in the areas of clinical analysis, medical diagnostics, environmental monitoring and drug delivery. Imprinted polymer materials possess several other virtues viz. physical and chemical stability, storage endurance and imprint memory which is essential for preparation of recognition membranes in a robust and reusable sensing device. Moreover, MIPs are usually cheaper and more accessible high affinity recognition materials in contrast to many biological entities [6].

Because of the high toxicity of CWAs, less toxic structural analogs that directly mimic or imitate the actual CWAs of simulant diethyl chlorophosphate (DCP) detection is very important task for verification studies since the corresponding simulant can be used to indicate the presence of the nerve agents produced or used. There have been many innovations for the detection of this species including colorimetric detection methods [7,8] surface acoustic wave devices [9,10], enzymatic assays [11], interferometry [12] and fluorescent sensors [13-15]. However, all are plagued by at least one limitation or other such as slow response, lack of selectivity, poor sensitivity, operational complexities or non-portability. Even though sensitivities are high, most of the fluorescent detectors performances have been demonstrated in non-aqueous media, which makes them unsuitable for real time analysis, which may require tedious extraction procedures. Potentiometric sensors, 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. The development of MIP based sensors with potentiometric transduction does not require the template or print molecule to be extracted from the membrane to create membrane potential and does not have to diffuse through the membrane, so that there is no size restriction on the template molecule, the main achilles heel of MIP's until recently [16-18]. Zhou, *et al.* [19] reported for the first time MIP based potentiometric sensor for methylphosphonic acid, an ultimate degradation product of nerve agents by coupling surface imprinting technique with a nanoscale transducer, indium tin oxide. The literature reveals that the prepared imprinted polymer membranes can be effectively used for the detection of nerve agents by fabricating them into potentiometric sensors [19-21]. These sensing devices are essentially based on use of polymer materials prepared via non-covalent strategy. However, due to non-persistent nature of nerve

agents, in non-covalent strategy, the decomposition products of nerve agents lead to a variety of binding sites depending on the nature of decomposition products. The previous studies [22] had succeeded in construction of semi-covalent strategy based in-situ monolithic polymer membrane based sensor for DCP via single pot synthesis. The extraction of all possible degradation products of the chemical warfare agents using molecularly imprinted polymers were developed by various research groups [20,23,24]. The semi-covalent strategy has been successfully demonstrated in the present study for the fabrication of imprinted polymer inclusion membrane (IPIM) based sensor for the detection and quantification of DCP, a simulant of soman present in spiked natural waters. As this technology does not require large instrumentation, it has feasibility for routine monitoring studies for the determination of chemical warfare agents or their simulants, where sampling is difficult and this method can be extensively applied for the determination of trace amounts of contaminants in environment, pharmaceutical and food processing industries and in biomedical applications

2. Experimental

2.1. Reagents and Electrochemical Equipment

Diethyl chlorophosphate (DCP), 4-vinyl aniline (VA), dimethyl methyl phosphonate (DMMP), pinacolyl methylphosphonate (PMP), methylphosphonic acid (MPA) were obtained from Aldrich (Milwaukee, WI, USA). Phorate, 2,4-D were obtained from SUPELCO, Pennsylvania, USA. 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), 2,2'-azobis isobutyronitrile, 2-nitrophenyl octyl ether (NPOE), di-n-octyl phthalate (DOP), bis-(2-ethylhexyl) sebacate (BEHS), tris-(2-ethylhexyl) phosphate (TEHP) and high molecular mass poly (vinyl chloride) (PVC) were purchased from Aldrich (Milwaukee, WI, USA). All other chemicals were of analytical grade reagents. Stock standard solution of ($0.1 \text{ mol}\cdot\text{L}^{-1}$) DCP was prepared by dissolving 1.725 g of DCP in 100 mL of deionized water. The solutions of 1.0×10^{-2} to $1.0 \times 10^{-11} \text{ mol}\cdot\text{L}^{-1}$ were prepared by aqueous dilution of a definite volume from the stock standard solution. Deionized water was used throughout the experiment. Potentiometric response characteristics were studied with an ELICO makes Ion analyzer, Model No. LI 126 (ELICO, Hyderabad, India).

2.2. Synthesis of Semi-Covalent Imprinted and Non-Imprinted Polymer Particles

Diethyl chlorophosphate imprinted particles were syn-

thesized as follows, 1 mmol of DCP, 1 mmol of VA to form covalent bond with DCP thus resulting in covalent interactions, 8 mmol HEMA, 32 mmol of EGDMA, 0.03 g of AIBN and 5 mL of methanol (porogen) were taken in a 50 mL round bottom flask. The mixture was purged with N_2 for 5 min. and the flask was sealed under inert atmosphere. Then it was kept for stirring in an oil bath maintained at a temperature of $60^\circ C$. After 2 h, the material obtained was ground and sieved and the particles with sizes between 50 - 105 m were collected. The resulting imprinted particles obtained were washed with methanol and then leached with $0.1 \text{ mol}\cdot\text{L}^{-1}$ HCl for 1 h. During leaching (hydrolysis) the template DCP is stripped off as the diethylphosphonic acid. So the cavity formed in the polymer matrix on leaching presumably corresponds to that of diethylphosphonic acid. During the rebinding process, the analyte DCP when added to the sample solution containing tris buffer will undergo hydrolysis to form diethylphosphonic acid [25], which is same as that of the leached template. Therefore, it can be construed that template rebinding takes place by non-covalent interactions. The covalent imprinting and subsequent rebinding via non-covalent interactions will be termed as semi-covalent strategy. The non-imprinted polymer particles were synthesized, washed and treated analogous to the imprinted polymer in the absence of template, *i.e.* DCP during synthesis.

2.3. Casting of Semi-Covalent Imprinted and Non-Imprinted Polymer Inclusion Membranes

The polymer inclusion membranes were cast by the following procedure mentioned below. DCP imprinted polymer particles (90 mg) synthesized via semi-covalent strategy were dispersed in 0.2 mL of NPOE and were mixed with tetrahydrofuran (THF) (2.5 mL) solution of PVC (90 mg). The solutions were homogenized and then poured into a Teflon mould having an internal diameter of 21 mm. The THF on evaporation at room temperature results in the formation of imprinted polymer inclusion membranes of thickness ~ 0.45 mm. In a similar manner non-imprinted polymer inclusion membranes were also casted.

2.4. Sensor Fabrication and EMF Measurement

The imprinted and non-imprinted membranes cast via inclusion by semi-covalent strategy were glued to one end of a pyrex glass tube with Araldite. The tube was then filled with an internal filling solution of $10^{-3} \text{ mol}\cdot\text{L}^{-1}$ DCP. A schematic diagram of membrane formation and fabrication of biomimetic potentiometric sensor

is given in (Figure 1). The sensor was kept in air when not in use.

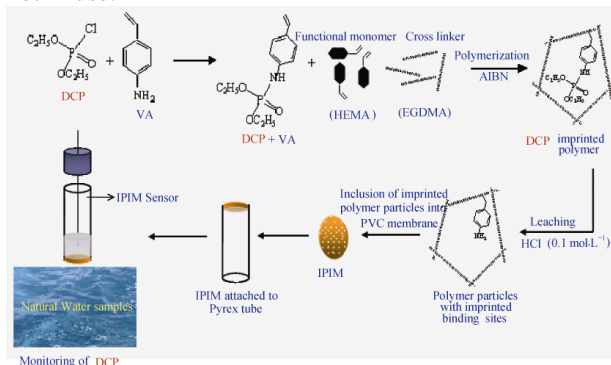


Figure 1. A Schematic representation of membrane formation and fabrication of IPIM based sensor for DCP.

Table 1. Effect of plasticizers on potential response of IPIM based sensor for each decade.

DCP ($\text{mol}\cdot\text{L}^{-1}$)	Potential response (mV/decade)			
	NPOE	TEHP	DOP	BEHS
1×10^{-7} to 1×10^{-6}	3.0	-	-	-
1×10^{-6} to 1×10^{-5}	3.0	-	1.0	-
1×10^{-5} to 1×10^{-4}	6.0	2.0	1.5	-
1×10^{-4} to 1×10^{-3}	18.0	12.0	3.0	10.0
1×10^{-3} to 1×10^{-2}	40.0	21.0	21.0	24.0

Sensors were conditioned in $10^{-5} \text{ mol}\cdot\text{L}^{-1}$ DCP solution $+0.1 \text{ mol}\cdot\text{L}^{-1}$ tris buffer (adjusted to pH 10.5) for 24 h and then stirred in tris buffer for 1/2 h to remove bound DCP ions after which the membranes would generate stable potentials. The test solution whose pH was maintained at 10.5 after the addition of 5 mL of $1 \text{ mol}\cdot\text{L}^{-1}$ tris buffer was taken and response of the sensor was examined by measuring the electromotive force (EMF) of the following electrochemical cell. $\text{Ag-AgCl} | 10^{-3} \text{ mol}\cdot\text{L}^{-1} \text{ DCP} | \text{DCP membrane} || \text{test solution} | \text{Hg-HgCl}_2\text{-KCl}$ (saturated). The potential responses of the sample solutions containing different concentrations (1.0×10^{-11} to $1.0 \times 10^{-2} \text{ mol}\cdot\text{L}^{-1}$) of DCP in 50 mL of $0.1 \text{ mol}\cdot\text{L}^{-1}$ Tris buffer (pH 10.5) was measured. The EMF was plotted as a function of DCP concentration.

2.5. Analysis of Natural Water Samples

The river water or ground water samples (~ 45 mL) were adjusted to pH = 10.5 after the addition of 5 mL of $1 \text{ mol}\cdot\text{L}^{-1}$ tris buffer using HCl or NaOH. The samples were analyzed using the above fabricated IPIM based potentiometric sensor by following the analytical procedure mentioned in section 2.4.

3. Results and Discussions

The optimal design of the membrane enables the per-

formance of the sensor. As it is well known that the electrochemical properties of conventional potentiometric sensors depends on various features of the membrane such as nature of plasticizer, nature and amount of sensing material used as described elsewhere [26-28]. In view of this, the suitability of the membrane casted by dispersing the imprinted polymer particles in plasticizer and embedded in PVC were examined for selective recognition of DCP.

3.1. 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. In accordance with that the effect of different plasticizers on the performance of IPIM based sensor was investigated. **Table 1** shows the potential output of IPIM based sensor with different plasticizers NPOE, TEHP, DOP and BEHS. From the table, it is evident that membrane with NPOE alone offer better potential responses in the entire concentration range of DCP compared to TEHP, DOP and BEHS based sensors. Also, the magnitude of potential difference, stability of EMF output and better precision for NPOE based sensor is higher in each decade. The plasticizer NPOE having a high dielectric constant of 24.0 has given better response characteristics than that of DOP ($\epsilon = 5.0$), BEHS ($\epsilon = 4.0$) and TEHP ($\epsilon = 4.8$). This is in agreement with earlier reports [20,21]. It was also observed that the imprinted membranes casted without plasticizer were not suitable for use as recognition membranes as they are brittle.

3.2. Effect of MIP Particles to PVC Ratio

The ratio of MIP particles to PVC was found to play a key role in the sensor performance since the weight of MIP particles determine the number of binding sites available for selective rebinding of DCP. The effect of changing the ratio of imprinted polymer particles (prepared by semi-covalent strategy) to PVC was varied in the ratio 0.5:1, 1:1 and 2:1. The results indicate that 1:1 ratio gave the best results in terms of linear calibration range, limit of detection, response time and magnitude of potential change for each decade change of concentration of DCP (**Table 2**). In the case of membranes with 0.5:1 ratio, the total number of binding sites available for rebinding of DCP is relatively lower for the membrane to respond effectively. On the other hand, during the preparation of membranes with 2:1 ratio, the MIP particles were dispersed non-uniformly resulting in poor perfor-

mance. Since the potential response is not improved beyond particular limit on increasing the amount of MIP

Table 2. Effect of weight ratio of MIP particles to PVC on response of IPIM based sensor.

Weight of MIP particles (g)	Weight of PVC (g)	Weight ratio	Working concentration range (mol·L ⁻¹)
0.045	0.09	0.5:1	4.5×10^{-5} to 1×10^{-2}
0.09	0.09	1:1	1×10^{-9} to 1×10^{-4} and 1×10^{-4} to 1×10^{-2}
0.18	0.09	2:1	3.3×10^{-7} to 1×10^{-4} and 1×10^{-4} to 1×10^{-2}

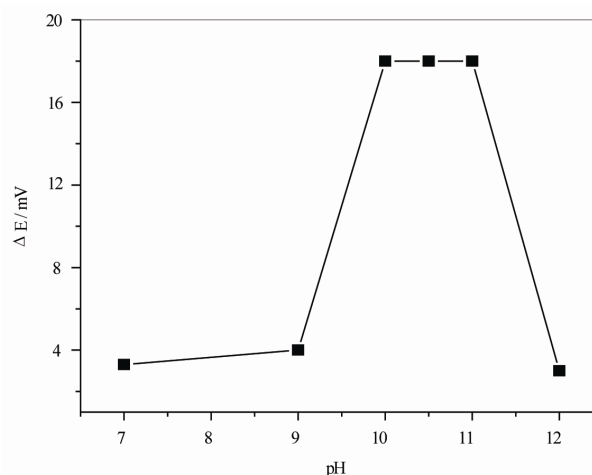


Figure 2. Effect of pH on response of the IPIM based sensor.

particles, further studies were restricted with membranes having 1:1 ratio.

3.3. Effect of pH of Test Solution

The effect of pH of test solution on the performance of IPIM based sensor response for decade change of DCP concentration *i.e.* 10^{-5} to 10^{-4} mol·L⁻¹ was studied by varying pH = 7 to pH = 12 after addition of 5 mL of 1.0 mol·L⁻¹ tris buffer (**Figure 2**). The results indicate that the optimum pH for constant and maximum potential response over the entire concentration range of DCP (1.0×10^{-9} to 1.0×10^{-2} mol·L⁻¹) was found to be 10.0 to 11.0. Hence, the pH of the test solution was adjusted to ~10.5 after the addition of 5 mL of 1.0 mol·L⁻¹ tris buffer.

3.4. Sensitivity of the IPIM Based Sensor

The potential responses of the imprinted membrane and non-imprinted membrane based sensors fabricated under

the optimal conditions arrived above was studied and the results obtained are shown in (Figure 3). It can be noticed from the figure that the plot obtained for the IPIM based sensor gave a linear calibration curve in the con

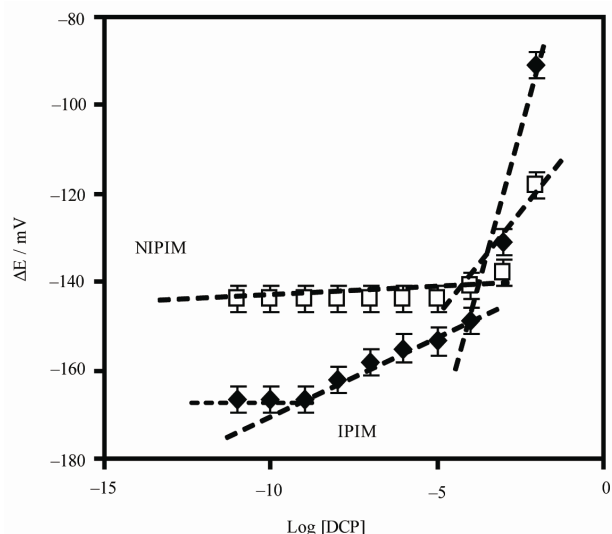


Figure 3. Potential response of the IPIM and NIPIM based sensors with respect to DCP concentration.

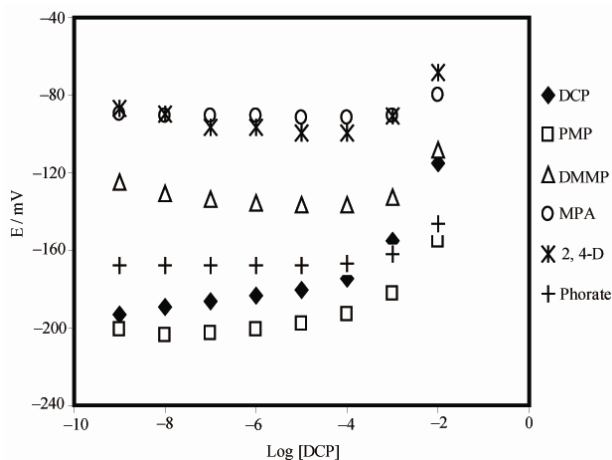


Figure 4. Potentiometric response of the IPIM based sensor to DCP and other selected interferents for each decade change of concentration from 1×10^{-9} to 1×10^{-2} mol L^{-1} .

centration range 1×10^{-9} to 1×10^{-4} and 1×10^{-4} to 1×10^{-2} mol L^{-1} of DCP. On the other hand, the non-imprinted polymer inclusion membrane (NIPIM) based sensor gave linear response for DCP in the concentration range 1×10^{-4} to 1×10^{-2} mol L^{-1} . It was observed that the absolute potentials obtained from NIPIM based sensor were unstable which is due to nonspecific binding of analyte in contrast to the specific site selective binding in the case of IPIM based sensor. In addition, the LOD for IPIM and NIPIM based sensors calculated based on

IUPAC recommendation [29] were found to be 1×10^{-9} mol L^{-1} and 1×10^{-4} mol L^{-1} , respectively. Whereas the higher ΔE value for IPIM based sensor over NIPIM based sensor in the entire concentration range is attributed to significant imprinting effect. The equilibrium

Table 3. Comparison of experimental selectivity coefficients of DCP against various selected interferents using NIPIM and IPIM based sensors.

Interferents	$K_{A,B}^{pot}$	
	NIPIM	IPIM
PMP	2.0×10^{-2}	3.3×10^{-3}
DMMP	1.0×10^{-1}	3.3×10^{-2}
MPA	1.0×10^{-1}	3.6×10^{-2}
Phorate	1.0×10^{-1}	2.5×10^{-2}
2,4-D	4.0×10^{-2}	5.0×10^{-2}

$K_{A,B}^{pot}$ = Potentiometric selectivity coefficient; A = DCP; B = Interferent.

response time was found to be 5 min. for the IPIM based sensors employing particles prepared by semi-covalent strategy.

3.5. Selectivity of the IPIM Based Sensor

Selectivity refers to the extent of suitability of the developed IPIM based sensors to determine particular analyte in mixtures or matrices without interferences from other components. In environmental applications, the concentrations of the analytes are quite low and thus, high selectivity is essential for an effective monitoring. Hence, the selectivity of the developed IPIM based sensor with various common simulants (PMP and DMMP) and degradation product (MPA) of CWAs, pesticides like phorate and 2,4-D which may co-exist in real samples were tested. The response profiles of DCP and selected coexisting interferents obtained with IPIM based sensor fabricated with particles prepared by semi-covalent strategy are shown in Figure 4. The higher selectivity noticed in the case of IPIM based sensor can be attributed to the more rigid polymeric structure leading to more stabilized cavities. Similar imprinting effect can also be visualized from Table 3 and compares the selectivity coefficients of DCP over selected interferents obtained by IPIM based sensor with corresponding NIPIM based sensor by employing IUPAC method [30] as described elsewhere.

3.6. Stability and Reusability

Another important criteria for any sensing device in addition to sensitivity and selectivity is stability and reusability. The developed IPIM based sensors prepared by employing semi-covalent strategy were found to be stable with deviations less than 0.5 mV for 1×10^{-4} mol L^{-1}

DCP for 3 months and can be reused for more than 30 times without losing sensing ability.

3.7. Analytical Application to Natural Water Samples

It was successfully applied to natural water samples, as it

Table 4. Analysis of natural water samples.

Sample	Concentration of selected interferences in mixture spiked to natural waters (mol·L ⁻¹) ^a	DCP added (× 10 ⁻⁸ mol·L ⁻¹)	DCP found ^b (× 10 ⁻⁸ mol·L ⁻¹)	Recovery (%)
Ground water	-	1.0	1.01 ± 0.09	101
	10 ⁻⁸	1.0	0.99 ± 0.11	99
	10 ⁻⁷	1.0	0.98 ± 0.16	98
River water	-	1.0	1.00 ± 0.03	100
	10 ⁻⁸	1.0	0.98 ± 0.14	98
	10 ⁻⁷	1.0	1.01 ± 0.09	101

^aMixture of PMP, DMMP, MPA, Phorate and 2,4-D; ^bAverage of three determinations.

is clear from the selectivity studies that several interferences co-exist in real samples do not have any deleterious effect on IPIM based sensor performance. Ground and river water samples were analyzed by spiking known amounts of DCP and varying concentrations of interferent mixtures. The results thus obtained are shown in **Table 4**. The recovery obtained in the range 98% - 101% shows that the developed IPIM based sensor can reliably be used for monitoring the natural waters, which, if found, can alert the authorities for appropriate control measures.

4. Conclusions

Semi-covalent imprinted and non-imprinted polymer particles were synthesized and found that non-imprinted polymer inclusion membrane (NIPIM) was unstable in contrast to imprinted polymer inclusion membrane (IPIM) in determination and quantification of DCP. In addition, the sensor performance of the IPIM based sensor is remarkable with a detection limit of 1×10^{-9} mol·L⁻¹ (0.17 ppb) compared to corresponding NIPIM based sensor. The interferences co-exist in real samples do not have any deleterious effect on IPIM based sensor performance. The recovery studies of DCP from ground and river waters indicated the possibility of using the sensor investigated in the present work for nature water samples. The developed technology found to be an effective analytical tool as it requires simple sample preparation procedures and does not require large instrumentation as it is a miniature device that responds to a particular analyte in a selective way and can be effectively used for the determination of pollutants in the environmental

and biological samples [20,23]. Further studies are in progress to integrate other transducers with MIP materials for a chosen template.

5. Reference

- [1] S. Chauhan, R. D. Cruz, S. Faruqi, K. K. Singh, S. Varma, M. Singh and V. Karthik, "Chemical Warfare Agent," *Environmental Toxicology and Pharmacology*, Vol. 26, No. 2, 2008, pp. 113-122. [doi:10.1016/j.etap.2008.03.003](https://doi.org/10.1016/j.etap.2008.03.003)
- [2] A. B. Kanu, P. E. Haigh and H. H. Hill, "Surface Detection of Chemical Warfare Agents Simulants and Degradation Products," *Analytica Chimica Acta*, Vol. 553, No. 1-2, 2005, pp. 148-159. [doi:10.1016/j.aca.2005.08.012](https://doi.org/10.1016/j.aca.2005.08.012)
- [3] Y. Seto, M. Kanamori-Kataoka, K. Tsuge, I. Ohsawa, K. Matsushita, H. Sekiguchi, T. Itoi, K. Iura, Y. Sano and S. Yamashiro, "Sensing Technology for Chemical Warfare Agents and its Evaluation using Authentic Agents," *Sensors and Actuators B*, Vol. 108, No. 1-2, 2005, pp. 193-197. [doi:10.1016/j.snb.2004.12.084](https://doi.org/10.1016/j.snb.2004.12.084)
- [4] G. Wulff, "Molecular Imprinting in Cross-Linked Materials with the Aid of Molecular Templates-A Way towards Artificial Antibodies," *Angewandte Chemie International Edition in English*, Vol. 34, No. 17, 1995, pp. 1812-1832. [doi:10.1002/anie.199518121](https://doi.org/10.1002/anie.199518121)
- [5] G. Vlatkis, L. I. Andersson, R. Muller and K. Mosbach, "Drug Assay using Antibody Mimics Made by Molecular Imprinting," *Nature*, Vol. 361, 1993, pp. 645-647. [doi:10.1038/361645a0](https://doi.org/10.1038/361645a0)
- [6] N. Lavignac, C. J. Allender and K. R. Brain, "Current Status of Molecularly Imprinted Polymers as Alternatives to Antibodies in Sorbent Assays," *Analytica Chimica Acta*, Vol. 510, No. 2, 2004, pp. 139-145. [doi:10.1016/j.aca.2003.12.066](https://doi.org/10.1016/j.aca.2003.12.066)
- [7] H. O. Michel, E. C. Gorder and J. Epstein, "Detection and Estimation of Isopropyl Methylphosphonofluoridate and O-Ethyl S-Diisopropylaminoethylmethylphosphonothioate in Sea Water in Parts-per-Trillion Level," *Environmental Science and Technology*, Vol. 7, No. 11, 1973, pp. 1045-1049. [doi:10.1021/es60083a010](https://doi.org/10.1021/es60083a010)
- [8] V. Pavlov, Y. Xiao and I. Willner, "Inhibition of the Acetylcholine Esterase Stimulated Growth of Au Nanoparticles: Nanotechnology-Based Sensing of Nerve gases," *Nano Letters*, Vol. 5, No. 4, 2005, pp. 649-653. [doi:10.1021/nl050054c](https://doi.org/10.1021/nl050054c)
- [9] Y. Yang, H. F. Ji and T. Thundat, "Nerve agents detection using a Cu₂/l-cysteine bilayer-coated microcantilever," *Journal of American Chemical Society*, Vol. 125, No. 5, 2003, pp. 1124-1125. [doi:10.1021/ja028181n](https://doi.org/10.1021/ja028181n)
- [10] C. Hartmann-Thompson, J. Hu, S. N. Kaganove, S. E. Keinath, D. L. Keeley and P. R. Dvornic, "Hydrogen-Bond Acidic Hyperbranched Polymers for Surface Acoustic Wave (SAW) Sensors," *Chemistry of Materials*, Vol. 16, No. 25, 2004, pp. 5357-5364. [doi:10.1021/cm040346z](https://doi.org/10.1021/cm040346z)
- [11] K. E. LeJeune, J. R. Wild and A. J. Russell, "Nerve

- Agents Degraded by Enzymatic Foams," *Nature*, Vol. 395, 1998, pp. 27-28. [doi:10.1038/25634](https://doi.org/10.1038/25634)
- [12] H. Sohn, S. Letant, M. J. Sailor and W. C. Trogler, "Detection of Fluorophosphate Chemical Warfare Agents by Catalytic Hydrolysis with a Porous Silicon Interferometer," *Journal of American Chemical Society*, Vol. 122, No. 22, 2000, pp. 5399-5400. [doi:10.1021/ja0006200](https://doi.org/10.1021/ja0006200)
- [13] S. W. Zhang and T. M. Swager, "Fluorescent Detection of Chemical Warfare Agents: Functional Group Specific Ratiometric Chemosensors," *Journal of American Chemical Society*, Vol. 125, No. 12, 2003, pp. 3420-3421. [doi:10.1021/ja029265z](https://doi.org/10.1021/ja029265z)
- [14] S. B. Nagale, T. Sternfeld and D. R. Walt, "Microbead Chemical Switches: An Approach to Detection of Reactive Organophosphate Chemical Warfare Agent Vapours," *Journal of American Chemical Society*, Vol. 128, No. 15, 2006, pp. 5041-5048. [doi:10.1021/ja057057b](https://doi.org/10.1021/ja057057b)
- [15] T. J. Dale and R. Rebek, "Fluorescent Sensors for Organophosphorus Nerve Agent Mimics," *Journal of American Chemical Society*, Vol. 128, No. 14, 2006, pp. 4500-4501. [doi:10.1021/ja057449j](https://doi.org/10.1021/ja057449j)
- [16] T. Prasada Rao, K. Prasad, R. Kala and J. M. Gladis, "Biomimetic Sensors for Toxic Pesticides and Inorganics Based on Optoelectronic/Electrochemical Transducers—An Overview," *Critical Reviews in Analytical Chemistry*, Vol. 37, No. 3, 2007, pp. 191-210.
- [17] M. C. Blaco-hopez, M. J. Lobo-castanon, A. J. Miranda-ordieres and P. Tunon-Blanco, "Electrochemical Sensors Based on Molecularly Imprinted Polymers," *Trends in Analytical Chemistry*, Vol. 23, No. 1, 2004, pp. 36-48. [doi:10.1016/S0165-9936\(04\)00102-5](https://doi.org/10.1016/S0165-9936(04)00102-5)
- [18] S. A. Piletsky and A. P. F. Turner, "Electrochemical Sensors Based on Molecularly Imprinted Polymers," *Electroanalysis*, Vol. 14, No. 5, 2002, pp. 317-323. [doi:10.1002/1521-4109\(200203\)14:5<317::AID-ELAN317>3.0.CO;2-5](https://doi.org/10.1002/1521-4109(200203)14:5<317::AID-ELAN317>3.0.CO;2-5)
- [19] Y. Zhou, B. Yu, E. Shiu and K. Levon, "Potentiometric Sensing of Chemical Warfare Agents: Surface Imprinted Polymer Integrated with an Indium Tin Oxide Electrode," *Analytical Chemistry*, Vol. 76, No. 10, 2004, pp. 2689-2693. [doi:10.1021/ac035072y](https://doi.org/10.1021/ac035072y)
- [20] K. P. Prathish, K. Prasad, T. Prasada Rao and M. V. S. Suryanarayana, "Molecularly Imprinted Polymer-Based Potentiometric Sensor for Degradation Product of Chemical Warfare Agents Part.I. Methylphosphonic Acid," *Talanta*, Vol. 71, No. 5, 2007, pp. 1976-1980. [doi:10.1016/j.talanta.2006.09.002](https://doi.org/10.1016/j.talanta.2006.09.002)
- [21] V. Vishnuvardhan, K. P. Prathish, G. R. K. Naidu and T. Prasada Rao, "Fabrication and Topographical Analysis of Non-Covalently Imprinted Polymer Inclusion Membranes for the Selective Sensing of Pincolyl Methylphosphonate—A Simulant of Soman," *Electrochimica Acta*, Vol. 52, No. 24, 2007, pp. 6922-6928. [doi:10.1016/j.electacta.2007.05.005](https://doi.org/10.1016/j.electacta.2007.05.005)
- [22] K. P. Prathish, V. Vishnuvardhan and T. Prasada Rao, "Rational Design of in Situ Monolithic Imprinted Polymer Membranes for the Potentiometric Sensing of Diethyl Chlorophosphate—A Chemical Warfare Agent Stimulant," *Electroanalysis*, Vol. 21, No. 9, 2009, pp. 1048-1056. [doi:10.1002/elan.200804515](https://doi.org/10.1002/elan.200804515)
- [23] Z.-H. Meng and Q. Liu, "Determination of Degradation Products of Nerve Agents in Human Serum by Solid Phase Extraction using Molecularly Imprinted Polymer," *Analytica Chimica Acta*, Vol. 435, No. 1, 2001, pp. 121-127. [doi:10.1016/S0003-2670\(01\)00858-3](https://doi.org/10.1016/S0003-2670(01)00858-3)
- [24] Sophie Le Moullec, Arlette Begos, Valerie Pichan and Bruno Bellier, "Selective Extraction of Organophosphorus Nerve Agent Degradation Products by Molecularly Imprinted Solid-Phase Extraction," *Journal of Chromatography A*, Vol. 1108, No. 1, 2006, pp. 7-13. [doi:10.1016/j.chroma.2005.12.105](https://doi.org/10.1016/j.chroma.2005.12.105)
- [25] G. A. Sega, B. A. Tomkins and W. H. Griest, "Analysis of Methylphosphonic Acid, Ethyl Methylphosphonic Acid and Isopropyl Methylphosphonic Acid at Low Microgram per Litre Levels in Ground Water," *Journal of Chromatography A*, Vol. 790, No. 1-2, 1997, pp. 143-152. [doi:10.1016/S0021-9673\(97\)00747-4](https://doi.org/10.1016/S0021-9673(97)00747-4)
- [26] G. J. Moody, J. M. Slater and J. D. R. Thomas, "Poly (vinyl chloride) Matrix Membrane Uranyl Ion-Selective Electrodes Based on Organophosphorus sensors," *Analyst*, Vol. 113, No. 5, 1988, pp. 699-703. [doi:10.1039/an9881300699](https://doi.org/10.1039/an9881300699)