Estimation of some Organophosphorus Pesticides Using Carbon Paste Electrode Coupled with Molecularly Imprinted Polymers

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Abstract:
The aim of this study was to develop a sensor based on a carbon paste electrodes (CPEs) modified with used MIP for determination of organophosphorus pesticides (OPPs). The modified electrode exhibited a significantly increased sensitivity and selectivity of (OPPs). The MIP was prepared by thermopolymerization method using N,N-diethylaminoethymethacrylate (NNDAA) as functional monomer, N,N-1,4-phenylenediacylamide (NNPDA) as cross-linker, the acetonitrile used as solvent and (Opps) as the template molecule. The three OPPs (diazinon, quinalphos and chlorpyrifos) were chosen as the templates, which have been selected as base analytes which used widely in agriculture sector. The extraction efficiency of the imprinted polymers has been evaluated by various parameters affecting to optimize the selective pre-concentration of OPPs from aqueous samples. The extraction efficiency of the MIPs-OPPs from environmental water samples was evaluated using carbon paste electrode and analytical parameters of the method, the slopes, linearity and detection limits of the liquid electrodes were ranged from 24.8 – 31.3 mV/decade , (10^{-1} - 10^{-7} ) mg L^{-1}and (2.2-8.2x10^{-6} ) mg L^{-1}, respectively with correlation coefficient (r) (0.9991-0.9998) and repeatability were established. The method was validated and successfully applied to determined OPPs compounds from environmental water samples.

Keywords: Molecularly imprinted polymers, organophosphorus pesticides, pesticides determination, N, N-diethylaminoethymethacrylate monomer.

Introduction:
The organophosphorus pesticides (OPPs) have often been used in the cultivation of agricultural and used for decades and the system continuous (1). These OPPs cause acute or chronic toxicity to human, environment and the biota thus tighten the need for active analytical procedures to observe prospect hazards. GC and HPLC were used for analyzed most OPPs (2). Generally, for analysis analyte must a pretreatment step in order to minimize the effect the matrix interference which used solid-phase extraction (SPE) for this purpose(3-5)

Molecularly imprinted polymers (MIPs) are useful for multi technique make up molecular gatherings of wanted properties and chemical structures (6). During the past era, MIPs have showed a good response as selective sensors and have been widely used for per concentration of samples in SPE processes (7-9), the research have proved that MIPs can be efficiently used in this field (10-11). MIPs technology has develop and increased applicability in analytical chemistry, which show a different and rapid methods for synthesis a polymer matrix for analyte with applications ranging for determination of pesticide using chemical sensing of complex chemical reactions such as ibuprofen (12) and warfarin sodium (13) and Mebeverine Hydrochloride (14) based on molecularly imprinted polymer method.

In this work, the selective use of CPEs technique for the analysis of organophosphorus pesticides is reported. Moreover, MIP- CPEs was developed using Organophosphorus pesticides OPPs as a template, and the specification of the electrodes were studied and the electrodes used for determination of the pesticides in water samples. The target analytes with structures similar to diazinon, quinalphos and chlorpyrifos were considered in this study are shown in Fig 1.

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Figure 1. Structures of target analytes
Materials and Methods:

All prepared solutions and measurements at room temperature using WTW were made (Germany), model pH meter 720. UV-Visible double beam spectrophotometer interfaced with computer via Shimadzu UV probe data system program (Version 1.10), 1.00 cm quartz cell(model (UV-1800 PC) SHIMADZ (Japan)). The temperature of polymerization reaction was controled by a water bath Memmert-854 (Schwabach, Germany). Potential values were measured with constant stirring of the test solution depends on indicator electrode used of carbon paste and reference electrode used was single junction saturated calomel electrode (SCE). The solutions used for interference study were obtained from Merck Company. Graphite fine powder extra pure, Quinalphos 100mg C_{12}H_{15}N_{2}O_{3}P_{5} (M.wt 298.30 g.mol^{-1}), Diazinon 250 mg C_{12}H_{16}N_{2}O_{3}P_{5} (M.wt 304.35 g.mol^{-1}), Chlorpyrifos 100mg C_{8}H_{11}Cl_{3}NO_{3}P_{5} (M.wt 350.59 g.mol^{-1}) tricresyl phosphate (TCP) (99.7%) and dibutyl butyl phosphonate (DDBP) (99.7%) were obtained from Aldrich Company.

Preparation of molecularly imprinted polymers with bulk methods

Imprinted polymer preparation has been described in detail in our previous study (15). According to the mentioned procedure, 2mmol of template (diazinon, quinalphos and chlorpyrifos) and 4 mmol of (NNDAA) were dissolved in 6 mL of porogenic solvent acetonitrile in a glass polymerization test tube. After oscillating for 30 min, (NNPDA, 25 mmol) was added as cross-linker and benzoyl peroxide (BPO, 15 mg) as initiator, nitrogen gas was passed through the test tube for 10 min. The glass tube was sealed under vacuum and placed in water bath at 65°C for 12 h. The bulk polymers obtained was crushed, ground and sieved through 75 µm sieve. The polymer particles obtained were washed with 10% (acetic acid methanol 1:5) solution until diazinon, quinalphos and chlorpyrifos template were difficult to detect by UV-Visible spectrophotometry. After that, the particles extracted with methanol to remove residual acetic acid and washed with methanol. After washing processes the particles were collected and dried at 60 °C in an oven under vacuum for 5h. The same treatment carried out with non-imprinted polymers which contained every component except the template molecule.

The steps of molecularly imprinted polymer synthesis are illustration of the concept and typical generation of MIPs; they produce polymer materials that are formed in the presence of a template molecule that can subsequently be removed and yet leave an imprinted cavity in the polymer model that will recognize and permit for an environmentally sensitive reversible rebinding of a specific template molecule or structurally related compounds. The morphology of MIP and NIP membranes for diazinon before and after washing is showed by images of electron microscope in Figure 2. A pore on the surface (Fig. 2a) about 50 μm may indicate the binding sides to the polymer. Figure 2b shows that clear holes about 10 μm in size have been obtained and which were removed by soxhletextraction.

Figure 2. SEM photograph of the surface of diazinon MIP, a) after washing b) before washing

The Electrode Preparation

Holder (14 cm length) made from Teflon, has a hole at one end (8 mm) diameter and (4.5 mm deep) was used for packing the carbon paste, was used as the electrode body. A stainless steel rod used as conductor the electrical to the center of the holder. The rod freely moves up and down to press the paste down when a regeneration of the surface of the electrode is needed. Preparation of membrane requires several steps, these include mixing 0.23 g PVC, 0.6 g of the plasticizer and 0.04 g of the MIP i.e. diazinon (electrode I), quinalphos (electrode II) and chlorpyrifos (electrode III). After
homogenization, 6-7 mL of THF was added and stirred.

The preparation of carbon paste was prepared by mixing 20 mg of the above mixture with graphite powder (250 mg) and different types of plasticizers: tricresyl phosphate (TCP) and dibutyl butyl phosphonate (DDBP). To obtain a homogenous paste a careful mixing with mortar and must be done and sieving 75Mm. Afterwards trituration via pressing bushy with the pestle to pack into the hole of the electrode bodies smoothly and polishing by a wet filter paper.

**Potential Measurements**

At temperature 20°C with continuous magnetic stirring of the test solution (50 mL), all electrode response was measured. The electrode performance was studied by measuring the potential of standard solutions of the pesticides using a concentration ranged from $10^{-1}$ to $10^{-7}$ mg.L$^{-1}$ prepared by serial dilution. The response time, slope, detection limit and operative life were calculated from the calibration curve. Depending on the IUPAC recommendations data, the electrochemical performance of the three suggested sensors were determined. Two methods applied to measured selectivity coefficients, separate solution and matched potential methods using 0.01 mg L$^{-1}$ each of diazinon, quinalphos and chlorpyrifos and the interfering ions. pH of solutions was measured and adjusted the pH with 0.1 M of hydrochloride acid and or hydroxide sodium.

**Results and Discussion:**

**Characteristics of the CPEs Composition**

The potentiometric sensors behavior with CPEs depending on the composition of the electrode material used and the conditions of the contact solution. It is known that the response of an electrode depends on two important parts the first is the nature and amount of an MIP and second was the nature and the amount of the plasticizer. The influence of the plasticizer type and concentration on the characteristics of the studied diazinon, quinalphos and chlorpyrifos electrodes were investigated using two plasticizers have different polarities, including (TCP) and (DDBP) and MIP (w/w) ratios were studied to determine the influence of the amount of plasticizer in the electrode. From Calibration curve of OPPs electrodes were obtained, slop, detection limit, linearity and another properties are in show Fig. (3).

The obtained results are presented in Table 1. These results show that the potentiometric responses of the electrodes modified with conventional plasticizers were satisfactory, where as those of the proposed electrode towards diazinon, quinalphos and chlorpyrifos electrodes were greatly improved in the presence of MIP as both an ionophore and a plasticizer. These electrodes have successfully applied for subsequent studies.

![Fig 3. Calibration curve of OPPs electrodes: (a) Electrode (I); (b) Electrode (II). (c) Electrode (III).](image-url)
Table 1. Characteristic of OPPs-MIP selective electrode by using (NDAA) as a functional monomers at different plasticizers

| Elect. No. | MIP Content (mg) | Plasticizer (0.8 ml) | Graphite powder (mg) | Conc. range mg L⁻¹ | Slope mV/decade | Detection limit mg L⁻¹ | Correlation coefficient mg L⁻¹ |
|------------|------------------|----------------------|----------------------|---------------------|-----------------|------------------------|-------------------------------|
| Diazinon (I) | 20               | TCP                  | 250                  | 10⁻¹ - 10⁻⁷         | 26.7            | 4.6x10⁻⁶               | 0.9998                        |
| Diazinon (II) | 40               | DDBP                 | 250                  | 10⁻¹ - 10⁻⁷         | 28.4            | 8.2x10⁻⁶               | 0.9995                        |
| Quinalphos (III) | 20               | TCP                  | 250                  | 10⁻¹ - 10⁻⁷         | 24.8            | 7.2x10⁻⁵               | 0.9991                        |
| Quinalphos (IV) | 40               | DDBP                 | 250                  | 10⁻¹ - 10⁻⁷         | 25.3            | 6.2x10⁻⁶               | 0.9998                        |
| Chlorpyrifos (V) | 20               | TCP                  | 250                  | 10⁻¹ - 10⁻⁷         | 29.6            | 2.2x10⁻⁶               | 0.9997                        |
| Chlorpyrifos (VI) | 40              | DDBP                 | 250                  | 10⁻¹ - 10⁻⁷         | 31.3            | 3.2x10⁻⁶               | 0.9993                        |

The effect of pH

The effect of pH on the electrodes potentials of (diazinon, quinalphos and chlorpyrifos) was investigated by measuring the e.m.f. of the cell of diazinon, quinalphos and chlorpyrifos solutions at two different concentrations 1 × 10⁻² and 1 × 10⁻⁵ mg.L⁻¹, at pH ranged was from 2–8. The pH was adjusted in each time by adding appropriate amounts of hydrochloric acid and/or hydroxide sodium solution. However variation in potential below pH= 2 and above pH=8 was observed. This variation is due to the interference of hydrogen ion in the charge transfer process at lower pH and formation of hydroxyl complex of OPPs at higher pH. The effect of pH on the electrode potentials for OPPs electrodes are shown in Fig. 4(a), 4(b), and 4(c).

Figure 4. Effect of pH on the potentiometric response of OPPs electrodes: (a) Electrode (I); (b) Electrode (II); (c) Electrode (III).
Selectivity measurement

Selectivity is one of the main characteristics of specific ion electrodes to verify the possibility of trustworthy measurement in target sample. It has been examined via two methods, firstly Separate Solution Method (SSM), and secondly Matched Potential Method (MPM) depending on a method given by IUPAC. The first method (SSM) is determination of the selectivity coefficient as given by Ion Selective Electrodes. SSM method has some limitations in accordance with interfering ions with a non-Nernstian behavior due to difference in the charges of ions. Thus a second method termed as Matched Potential Method (MPM) has been recommended in particular as the primary or interfering ion not satisfies according to Nernst response, or when the concerned ions did not equal in charge. Selectivity coefficient was calculated using the following equation:

\[ \log K^{\text{pot}} = \left[ \frac{(E_a - E_\Delta)}{(2.303RT/zF)} \right] + (1 - z_A/z_B) \log a_A \]

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Table 2. Result of selectivity coefficients using separate solution method and Matched Potential Method (MPM) for some interfering cations.

| Interfering Species | Electrode (I) | Log K | Electrode (II) | Log K | Electrode (III) | Log K |
|---------------------|--------------|-------|----------------|-------|----------------|-------|
|                     | SSM          | MPM   | SSM            | MPM   | SSM            | MPM   |
| Fe²⁺                | -1.52        | -1.98 | -2.17          | -1.87 | -2.21          | -1.92 |
| Al³⁺                | -3.52        | -3.67 | -2.95          | -2.87 | -2.81          | -1.99 |
| Co²⁺                | -6.45        | -5.98 | -6.28          | -6.17 | -5.95          | -6.23 |
| Ni²⁺                | -2.78        | -2.39 | -2.53          | -2.92 | -3.17          | -2.79 |
| Na⁺                 | -5.67        | -5.11 | -5.92          | -5.81 | -5.40          | -5.11 |
| K⁺                  | -2.93        | -2.76 | -2.52          | -2.83 | -3.11          | -2.98 |

| Interfering Species | Electrode (IV) | Log K | Electrode (V) | Log K | Electrode (VI) | Log K |
|---------------------|--------------|-------|----------------|-------|----------------|-------|
|                     | SSM          | MPM   | SSM            | MPM   | SSM            | MPM   |
| Fe²⁺                | -3.64        | -2.92 | -4.87          | -3.72 | -1.97          | -2.82 |
| Al³⁺                | -6.37        | -5.12 | -3.62          | -3.47 | -1.24          | -1.57 |
| Co²⁺                | -4.93        | -3.99 | -3.51          | -5.87 | -2.81          | -2.26 |
| Ni²⁺                | -1.62        | -2.81 | -6.72          | -6.21 | -2.86          | -2.61 |
| Na⁺                 | -6.23        | -6.89 | -4.92          | -5.12 | -4.82          | -5.17 |
| K⁺                  | -1.69        | -1.82 | -3.41          | -3.82 | -5.71          | -5.92 |

Analytical Applications

Diazinon, quinalphos and chlorpyrifos were determined in samples by using two potentiometric techniques direct method and standard addition method (SAM) carried as in Table 3. The proposed method was applied to determine (diazinon, quinalphos and chlorpyrifos) in underground water samples. The underground water samples were obtained by taking samples of groundwater from multiple areas of Iraq (Anbar, Najaf, Samawa). The support electrolytes were prepared by addition of 0.1 mg L⁻¹ of Na₂SO₄ to fresh underground water. To assess the proposed method, synthetic water samples were prepared by adding appropriate amounts of standard diazinon, quinalphos and chlorpyrifos solution to the underground water. The electro-analytical curves were in the range from 1.0 × 10⁻⁵ to 1.0 × 10⁻¹ mg L⁻¹ of diazinon, quinalphos and chlorpyrifos solutions. Figure 5 shows a linear increase in the measured response with the added concentration of organophosphorus pesticides solutions added into underground sample solution. These results indicate that the proposed methodology is suitable for the determination of diazinon, quinalphos and chlorpyrifos solutions in the underground water. Statistical calculations for the assay results showed suitable precision of the proposed method (Table 3). Moreover, the obtained values of RSD% for six replicates pure the suitability of the application of the developed electrodes to ward complex sample with good repeatability.
Figure 5. Calibration curves of underground water spiked in the range from 1.0x10^{-5} to 1.0x10^{-1} mg.L^{-1}

Table 3. Recoveries (%) and precision (RSD) of OPPs in water samples spiked with 0.001 mg.L^{-1}.

| samples          | Potentiometric methods | Concentration Prepared/ mg.L^{-1} | Concentration Found/ mg.L^{-1} | %Rec.   | %RE | %RSD | F- experimental | F- theoretical |
|------------------|------------------------|-----------------------------------|--------------------------------|---------|-----|------|-----------------|----------------|
| underground water Anbar | Direct method          | 1.0x10^{-3}                      | 1.052 x 10^{-7}               | 105.24  | 5.24 | 2.73 | 10.2            | 19.3           |
|                   | SAM                    | 1.0448 x 10^{-3}                 | 104.48                        | 4.48    | 1.65 | 13.4 |                 |                |
|                   | Direct method          | 1.0x10^{-4}                      | 94.95 x 10^{-7}               | 94.95   | -5.05| 3.21 | 15.3            | 19.3           |
|                   | SAM                    | 1.0472 x 10^{-4}                 | 104.72                        | 4.72    | 2.24 | 17.4 |                 |                |
| underground water Najaf | Direct method          | 1.0x10^{-3}                      | 1.056 x 10^{-3}               | 105.60  | 5.60 | 3.38 | 13.2            | 19.3           |
|                   | SAM                    | 1.0458 x 10^{-3}                 | 104.58                        | 4.58    | 2.92 | 10.5 |                 |                |
| underground water Samawa | Direct method          | 1.0x10^{-4}                      | 1.0579 x 10^{-4}              | 105.79  | 5.79 | 2.20 | 12.6            | 19.3           |
|                   | SAM                    | 0.9756 x 10^{-4}                 | 97.36                         | -2.44   | 1.42 | 14.7 |                 |                |
|                   | Direct method          | 1.0x10^{-3}                      | 0.9638 x 10^{-3}              | 96.38   | -3.62| 2.82 | 11.4            | 19.3           |
|                   | SAM                    | 1.0496 x 10^{-3}                 | 104.58                        | 4.58    | 2.65 | 10.9 |                 |                |
|                   | Direct method          | 1.0x10^{-4}                      | 1.052 x 10^{-4}               | 105.24  | 5.24 | 1.48 | 16.5            | 19.3           |
|                   | SAM                    | 1.0186 x 10^{-4}                 | 101.86                        | 1.86    | 1.25 | 11.2 |                 |                |

Conclusion:
In this work, Organophosphorus pesticides (quinalphos, diazinon and chlorpyrifos) imprinted polymers were electrode prepared by bulk polymerization for membrane preparation. Carbon paste electrodes were used for potential measurement which gave acceptable electrode specification and used for pesticides determination in underground water samples.

Conflicts of Interest: None.

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تقدير مبيدات الفسفورية العضوية باستخدام قطب اللصق الكاربون المزدوج مع الطبعة البوليمرية الجزيئية

الخلاصة:

الهدف من هذه الدراسة هو تطوير محصصات محضرة من مواد بوليمرية لتقدير المبيدات الفوسفورية العضوية باستخدام قطب اللصق الكاربون. أظهرت الأقطاب المحضرة زيادة كبيرة في الحساسية والانتقائية للمبيدات الفوسفورية. تم تحضير الطبعة الجزيئية البوليمرية بواسطة طريقة البلمرة الحرارية مستخدما N,N-ثنائي اثيل امينو اثيل ميثا اكريليت (NNPDAA) كمجموعة وظيفة و 1,4-ثنائي فنيلين (NNPDA) كقوى كشابل باستخدام أسيتو نيترال كمذيب والمبيد كقالب جزيئي. استخدم في البحث ثلاثة مبيدات (ديازينون، كينوفوس و كلوربيريفوس) كمواد تشبك باستخدام إسترو تيرنر كمنيب والمبيد كقاب جزيئي. استخدم في البحث ثلاثة مبيدات (ديازينون، كينوفوس و كلوربيريفوس) كقوى كشابل، نظرا لاستخدامها المهمة في مجال الزراعة. وقد تقييم كفاءة الطبعة الجزيئية البوليمرية المحضرة من خلال تطبيقها على العينات المائية باستخدام تقنية إقطاب لصق الكاربون وتم تعيين عوامل عديدة منها الميل والتي تراوحت بين (24.8-31.3) مم. و (0.9991-0.9998) نسبيا. وطبقت الطريقة المترحة بنجاح لتعيين المبيدات الفوسفورية العضوية في عينات المياه البيئية.

الكلمات المفتاحية: بوليميرات مطبوع جزيئي، مبيدات فوسفات عضوية، تحديد المبيدات من داي اثيل امينونا ميني اكريليت مونمر