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MOLECULARLY IMPRINTED POLYMER-COATED QUARTZ CRYSTAL MICROBALANCE FOR DETECTION OF PARATHION

(Polimer Molekul Tercetak Saduran Kristal Kuarza Penimbang Mikro bagi Penentuan Parathion)

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Abstract

Organophosphorus compounds (OPs) as important components of insecticides, pesticides, and chemical threat agents (CTA), have become more and more serious threats to the environment. Real-time monitoring of the threats is an urgent demand for environmental safety. The molecular imprinting polymer (MIP) technique is an attractive method for the generation of polymer-based molecular "memory" for a present target or group of target molecules. In this work, we integrate a novel MIP with quartz crystal microbalance (QCM) to build a simple sensor to selectively monitor OP in water samples in real-time. The MIP system was built up by polymerization of methacryalic acid (MAA) with divinylbenzene (DVB), initiated with 2,2'-azobis(2-methylpropionitrile) (AIBN) in presence of target. The polymer was characterized with FT-IR. Using parathion as a representative template, the MIP was coated on QCM by casting a semi-polymerized solution. The sensor could detect parathion in wide range from 290 ppb to 29 ppm. This sensor is stable, easy to prepare which can be reused and has the potential to be applied other analytes, which are threats to the environment.

Keywords: organophosphorus compounds, molecular imprinting polymer, quartz crystal microbalance, parathion

Abstrak

Sebatian organofosforus (OPs) adalah kompenen penting bagi racun serangga, racun mahkluk perosak dan agen kimia berbahaya yang telah menjadi ancaman kepada alam sekitar. Pemantauan terhadap ancaman ini telah meningkat berdasarkan kepada keselamatan alam sekitar. Teknik polimer molekul tercetak (MIP) adalah kaedah yang menarik molekul berasaskan polimer yang mempunyai sasaran terhadap molekul tertentu. Dalam kajian ini, MIP tulen bersama penimbang mikro kristal kuarza (QCM) telah disepadu untuk menghasilkan sensor mudah bagi pemantauan terpilih kehadiran OP di dalam sampel air bagi siutuasi sebenar. Sistem MIP telah dibangunkan berdasarkan pempolimeran asid metakrialik (MAA) dan divinilbenzena (DVD), dimulakan dengan kehadiran 2,2'-azobis(2-metilpropionitril)(AIBN) sebagai sasaran. Polimer telah dicirikan menggunakan FT-IR. Menggunakan parathion sebagai templat, MIP disadur ke atas QCM melalui teknik saduran larutan separa polimer. Sensor berupaya mengesan parathio pada julat yang besar dari 290 ppb hingga 29 ppm. Sensor ini adalah stabil, mudah disediakan, dimana boleh diguna semula dan berpotensi untuk diaplikasi terhadap analit lain yang mengancam alam sekitar.

Kata kunci: sebatian organofosforus, polimer molekul tercetak, penimbang mikro kristal kuarza, parathion

Introduction

Pesticides including insecticides are widely used in agriculture activities to provide more food productivity. As important components of pesticide and chemical threat agent (CTA), many organophosphorus compounds (OPs) are generally neurotoxic to all animal and human as cholinesterase inhibitors.[1-3] As shown in Figure 1, a typical organophosporus compound has a phosphorus atom with P=S or P=O bond and phosphate groups. Over-use of OPs has become more and more serious threats to the environment and human health. For example, natural water can be easily contaminated by OPs because it is widely spread and highly fluid, which would result in severe environmental and human health concerns. The residual OPs in food are another traditional problem, which is highly related to human health. To evaluate and control the contamination of OPs in environment, food or other places, many efforts were made on the development of different analytic methods in the last decades, which include chemosensor [4], capillary electrophoresis [5], immunoassay[6] and chromatography-mass spectrometry. Among these methods, the most popular methods are GC and HPLC or in tandem with mass spectrometer [7]. These methods normally are expensive, complex and require skilled personnel to operate. In addition, for trace analysis of complex samples, pre-treatments including separation and enrichment steps are generally required [8].Therefore, sensitive, fast, reliable and easy-handling methods are of great demand.

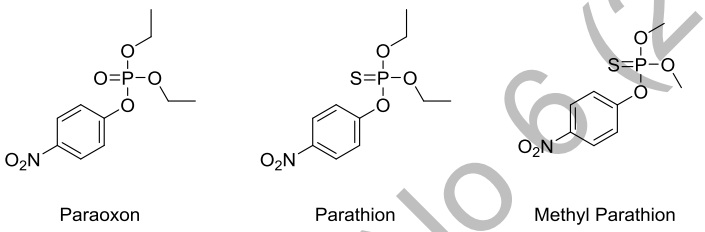

Figure 1. Structures of paraoxon, parathion and methyl parathion

Molecularly imprinted polymer (MIP) has grown fast in the last decades. MIP technique becomes an attractive method to construct of polymer-based molecular recognition elements for a preset target or a group of target molecules. The so-called artificial "enzyme" or "antibody", MIP has many applications, such as separation,[9] sensor [10] and immunoassay [11]. For example, MIPs have been used in selectively separation and enrichment of OPs from complex samples [12, 13]. MIPs have also been reported to make a sensor for Ops detection using electrochemical method by coating the MIPs on electrode [14]. This technique has many advantages including easy preparation, cost-effect, high stability, high affinity and high selectivity [15, 16].

Based on the piezoelectric properties of quartz crystals, the quartz crystal microbalance (QCM) is suitable for trace analysis, as a very sensitive pressure- and mass-sensing device. However, QCM itself has no selectivity. Therefore, different functional materials or chemicals were immobilized on the crystal surface to offer the desired selectivity. According to the well-known Sauerbrey equation, the frequency change of QCM, Δf, is proportional to the mass of the absorbed target of interest. Therefore, QCM-based sensor has been applied to many research fields, such as vapour analysis and biological analysis [17-19]. For example, an antibody against *A. hydrophila*, which is a bacteria of pathogen, was immobilized on QCM and the mass of target bacteria attached was monitored [20].

Parathion, also known as Folidol, is an organothiophosphate pesticide, which is widely used in agriculture and has high toxicity [21]. In this study, by combination of the sensitivity of QCM and the selectivity of MIP, we have developed a novel on-line biosensor for detection of parathion in water sample.

Materials and Methods

Instrument and device

QCM-D experiments were performed on a Q-Sense E4 QCM-D instrument (Q-Sense AB, Västra Frölunda, Sweden) with an AT-cut gold-coated quartz crystal (Model: AC5AP14, Diameter:14.0 mm, Jiaxing JingKong Electonic Co. Ltd, China), which has a response frequency of 5 MHz. All data were recorded at room temperature (25 ^{o}C). Sauerbrey's equation has been established for the AT-cut gold-coated quartz crystal:

$$\Delta f = -2 f_0^2 . (\rho_q \mu_q)^{-1/2} \Delta m / A \quad (1)$$

Δf is frequency shift (Hz) due to the mass change on the crystal, f_0 is the resonant frequency (Hz) of the original crystal, ρ_q is the density of quartz crystal (2.648 g. cm^{-3}), μ_q is the shear modulus (2.947×10^{11} $g \cdot cm^{-1} \cdot s^{-2}$) and A is the active electrode area (cm^2).

Chemicals
Parathion, methacryalic acid (MAA), divinylbenzene (DVB), 2,2'-azobis(2-methylpropionitrile) (AIBN), sulphuric acid (98%), Hydrogen peroxide (30%, w/w) and ethanol (AR grade) are purchased from commercial vendors. Ultra-pure water was processed by Q-POD® ultrapure water dispenser (Merck Millipore) to reach a resistivity of 18.2 MΩ.cm.

Sample preparation
The stock solution of parathion was prepared in ethanol. Then the aqueous solution of samples (100 µM, 50 µM, 10 µM, 1 µM, 100 nM and 0 nM) was diluted with ultra-pure water in gradient.

Sensor fabrication
Firstly, QCM was soaked in piranha solution (9 mL of conc. sulphuric acid (98%) and 3 mL of hydroperoxide (30%)) for 5 minutes. Then, the crystal was washed thoroughly with ultra-pure water until the water was neutral (pH = 7). It was subsequently flushed with nitrogen flow followed by air-drying for few hours. After cleaning, the crystal is ready for coating without any other treatment.

In a 1.5 mL Eppendorf tube, to a solution of ethanol (178.2 µL), parathion (11.8 µL, 0.34 M), MAA (10.0 µL) and DVB (50.0 µL) was added 1.0 mg of AIBN. The mixture was mixed by vortex for few minutes and bubbled with nitrogen for 5 minutes. Then the sealed tube was incubated at 60 ^oC in water-bath for 10 minute until the mixture turned to be slightly muddy. 10.0 µL of the semi-polymerized solution was casted on the gold surface of QCM. The QCM was incubated in oven at 60 ^oC overnight to make sure the polymerization was complete. Non-imprinted polymer coated-QCM (NIP@QCM) was prepared in the same way without the template molecule. The sensor was soaked in 30 mL of ethanol with gentle shaking. The solvent was renewed every 6 hours for 4 times. Then the sensor was stored in ultra-pure water with gentle shaking for 1 day.

Characterization of MIP and NIP
The polymer (MIP or NIP) in the tube was soaked in ethanol under the same conditions to remove unreacted reagents and target and then dried in vacuum oven. The dried polymer (1 part) was mixed with dried KBr (100 parts). The mixture was ground and the resulted powder was pressed to a transparent pellet. FT-IR spectroscopy was obtained with an ALPHA FTIR Spectrometer (Bruker). The characteristic peaks for the MIP (cm^{-1}) included: 3018 (alkene), 2960 (alkane), 2925 (carboxylic acid), 1700 (C=O); NIP (cm^{-1}): 3019 (alkene), 2960 (alkane), 2926 (carboxylic acid), 1699 (C=O).

Analytical procedure
The detection procedure was monitored on-line with flow rate of 50 µL after the sensor was prepared and installed properly, followed by few pulses of maximum flow rate to remove air bubbles. After each analysis, desorption step was performed in ethanol (30 mL) with gentle shaking. The solvent was renewed every 6 hours for 4 times. Then the sensor was stored in ultra-pure water with gentle shaking for 1 day.

Results and Discussion

Design strategy and sensor fabrication
The design of the sensor is shown in Figure 2a. In the first step, after necessary processes of QCM, the MIP was coated onto the gold electrode surface of the quartz crystal by free radical polymerization. The polymer system includes 4 parts: 1) MAA as functional monomer is used to build the framework of MIP and the carboxylic acid is served as the recognition element via hydrogen-bonding donor; 2) DVB as a cross-linking reagent is used to reinforce the stability of the rigid framework which contributes to the solid "memory" of MIP. DVB also

contributes to recognition by spatial hindrance and hydrophobic interaction to those hydrophobic domains of target including π-π interaction; 3) AIBN is used as initiator; 4) Parathion as the template molecules were mixed in the reaction solution and imbedded in the polymer to create many close-fit cavities with the same size and shape as themselves. By this design, the three-dimensional "memory" of the template was established. Then, in the second step, the template and unreacted reagents were removed by proper repeated washing to generate the cavity specifically for the template. Finally, the sensor was prepared and ready for detection after equilibrium process. The NIP was prepared as a negative control under the same conditions without template. MIP and NIP were characterized with FT-IR spectroscopy.

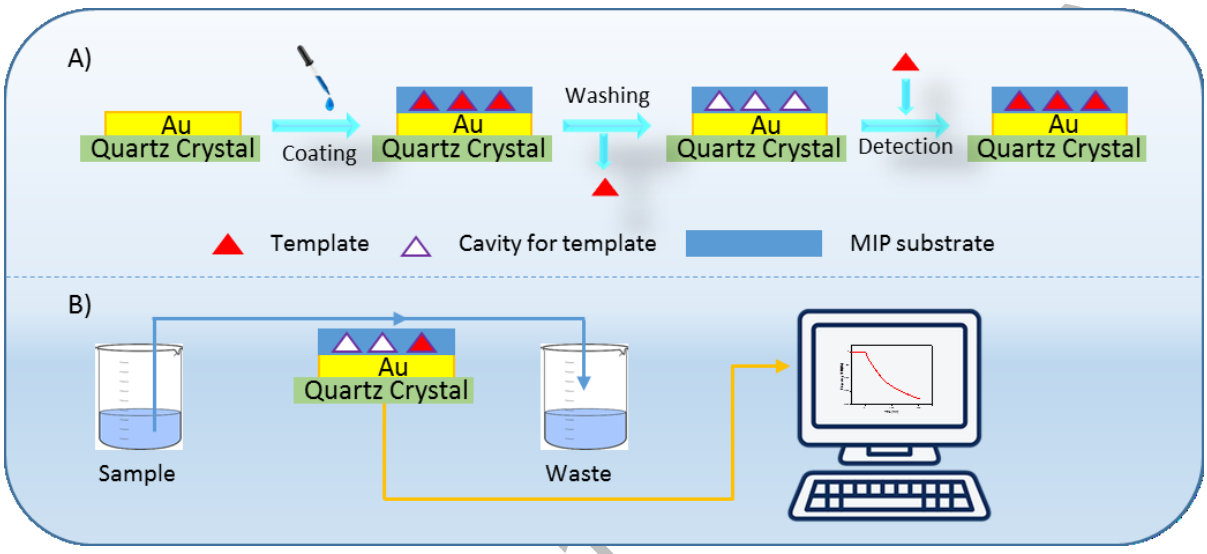

Figure 2. Schematic illustrations of fabrication of the MIP@QCM sensor and detection flow chart

Fast evaluation of the sensor

The QCM sensor is based on the piezoelectric properties of quartz crystals which could reflect mass and viscosity changes on the crystal surface. By the aid of the specific nature of MIP, the sensor of MIP@QCM could detect mass of the target captured in samples by reading the corresponding frequency changes according to Sauerbrey's equation in Equation 1. As shown in Figure 2b, in the detection step, the sensor was subjected to the detection of preseted target of MIP. The sample solution was pumped to pass through the surface of MIP@QCM sensor and the frequency shift was monitored in real-time. To rapidly evaluate the response to the target, we performed the detection of water samples in sequence in the interval of 30 minutes, including various concentrations of target from (0.1 to 100 μM or 0.029 to 29 ppm, b to e) as shown in Figure 3a. After 50 minutes of water-rinsing (a), the sensor was brought to resonant frequency (f_0). Then frequency of sensor decreased once it was treated with aqueous parathion solution. The frequency remained steady until 10 μM (2.9 ppm) of parathion. By increasing the concentration of parathion, the frequency shifts dramatically enhanced as shown in Table 1. Compared to MIP@QCM sensor, NIP@QCM sensor showed much smaller frequency shifts to the samples. MIP has larger capacity for target as it contained many cavities for binding target molecules, while NIP hold much less target molecules by the nonspecific bonding on the surface. The binding experiment illustrates that the MIP@QCM shows superior specific and sensitive response to the target compared with NIP@QCM.

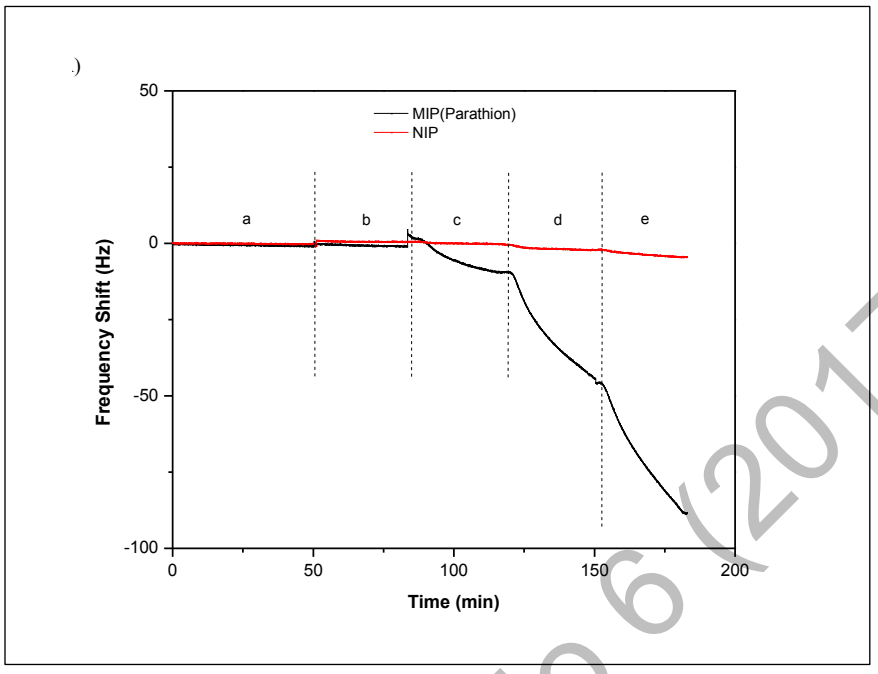

Figure 3. Evaluation of detection of MIP@QCM sensor for parathion against NIP@QCM. A) On-line frequency shift (Δf, Hz) responses of the sensor for water (a, 50 minutes) and parathion (0.1, 10, 100 µM, b-e) in the interval of 30 minutes. Flow rate: 50 µL/min.

Table 1. The frequency shifts for the corresponding samples.

Sample	**a**	**b**	**c**	**d**	**e**
Concentration of parathion (µM)	0	0.1	10	50	100
Concentration of parathion (ppm)	0	0.029	2.9	14.5	29
Δf of MIP(Hz)	-0.94	-0.97	-9.97	-37.95	-41.19
Δf of NIP(Hz)	-0.14	0.36	-0.36	-1.78	-2.6

Performance of the sensor

After the sensor was evaluated by using aqueous parathion solution, the sensor was further studied to establish the correlation between the frequency shift and sample concentrations. As shown in Figure 4, after 20 min of water flow, the sensor was treated with various concentrations of parathion for 60 min. Compared to the blank sample (0 µM of parathion in water), aqueous parathion solution showed obvious frequency shifts. The calibration curve of frequency shift *vs* concentration of parathion is shown as insert in Figure 4. The limit of detection (LOD) was calculated as 0.83 µM. Table 2 shows the frequency shifts for the corresponding samples.

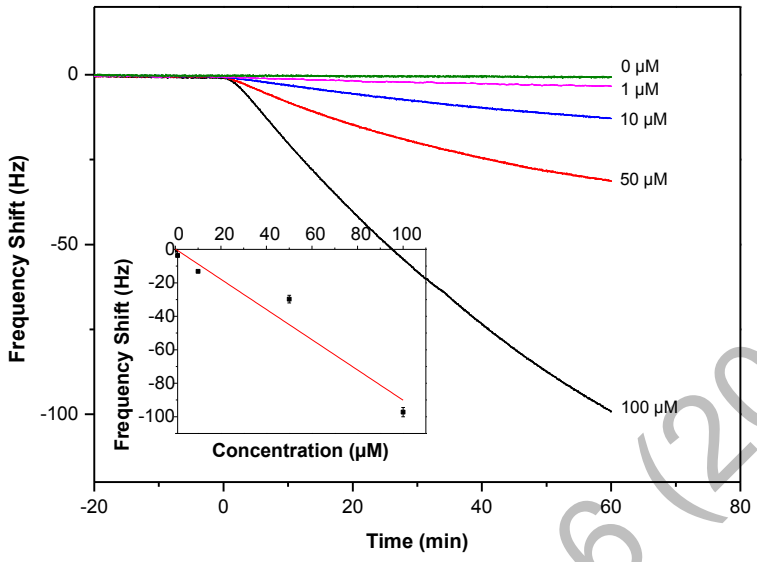

Figure 4. On-line detection of MIP@QCM sensor for parathion. A) On-line frequency (Δ*f*, Hz) responses of the sensor for parathion (0, 1, 10, 50, 100 μM). 60 minutes of incubation time for each sample. Flow rate: 50 μL/min. Insert: the linear relationship between the frequency shifts against parathion concentration.

Table 2. The frequency shifts for the corresponding samples

Concentration of Parathion (μM)	**0**	**1**	**10**	**50**	**100**
Concentration of parathion (ppm)	0	0.29	2.9	14.5	29
Δ*f* of MIP(Hz)	-0.50	-3.34	-12.85	-31.35	-99.22

Selectivity of the sensor

Subsequently the selectivity of MIP (parathion) was evaluated against two analogues – paraoxon and methyl parathion. As shown in Figure 5, the frequency shift of parathion (-30.28 Hz) is much larger than that of methyl parathion (-16.29 Hz) and paraoxon (-11.02 Hz), which indicated MIP (parathion) has higher selectivity to the template (parathion) than to the two analogues. Comparing parathion with paraoxon, MIP (parathion) shows distinct affinities. Although the hydrogen-bonding to the sulphur atom of parathion should be weaker than to the oxygen atom of paraoxon, the dominant interaction is the three-dimensional matching between MIP (parathion) and parathion which is much better for parathion than for paraoxon because of the differences in lone-pair repulsions of sulphur and oxygen atom. Comparing parathion with methyl parathion, the dominant interaction is the differences in three-dimensional matching because of the sizes of ethyl and methyl group. The results indicated that the designed sensor had good selectivity to parathion. Table 3 shows the frequency shifts for the corresponding samples.

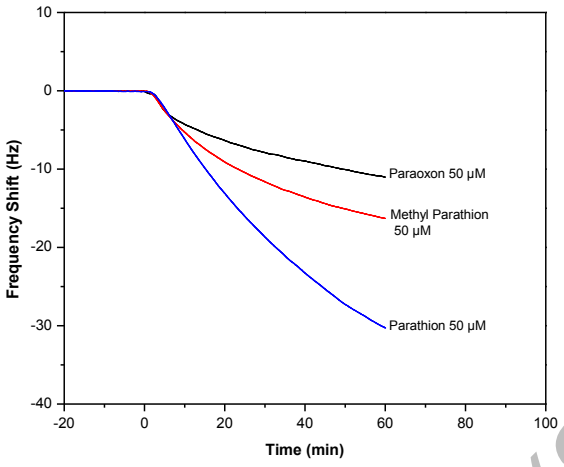

Figure 5. On-line detection of MIP@QCM sensor for parathion against paraoxon and methyl parathion. On-line frequency (Δf, Hz) responses of the senor parathion (50 µM) against paraoxon (50 µM) and methyl parathion (50 µM). 60 minutes of incubation time for each sample. Flow rate: 50 µL/min. The insets: structures of paraoxon, parathion and methyl parathion.

Table 3. The frequency shifts for the corresponding samples.

Targets	Paraoxon	Methyl Parathion	Parathion
Δf of MIP(Hz)	-11.02	-16.29	-30.28

Conclusion

We have developed a novel sensor based on QCM coated with MIPs. The MIP system used methyl methacrylic acid (MAA) as monomer, divinylbenzene (DVB) as cross-linking reagent and azobisisobutyronitrile (AIBN) as initiator. With parathion as the pre-set template, the selectivity experiment showed that the MIP-based QCM sensor could selectively detect parathion in water samples as the MIP offered specific cavity for the template molecule. The sensor has wide detection range (290 ppb ~ 29 ppm) and fast response (60 minutes) to parathion. The sensor is easy to prepare and can be potentially applied to other templates including organophosphorus compounds. The biosensor could potentially be useful for monitoring CTA targets in real water samples.

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