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ARTICLES

An Experimental Investigation of the Molecularly Imprinted Polymers as Tailor-Made Sorbents of Diazinon[1]

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Abstract—Diazinon imprinted sorbent can be a useful tool for selective enrichment, clean-up, and purification methods. In this study, investigation of synthesis and evaluation of diazinon imprinted polymers has been performed using equilibrium binding experiments. It is possible to use molecularly imprinted polymers as sorbents for anti-choline esterase (**Anti-ChE**) organophosphate pesticides (**Ops**). It has been found that MAA monomer is most suitable for the preparation of appropriate diazinon molecularly imprinted polymers (**MIPs**). The type of porogen also influences the binding results. The best porogen for diazinon imprinting is chloroform due to its poor hydrogen bonding capacity.

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Anti-choline esterase organophosphate pesticides (Anti-ChE OPs), which are likely to be the most used insecticides in developing countries, are used widely in agriculture, public health, and domestic fields for controlling insects [1]. Although Anti-ChE OPs structures differ, the mechanism, of their toxic action is identical and is associated with the inhibition of the nervous tissue cholinesterase enzyme (ChE), responsible the termination of the biological activity of the neurotransmitter acetylcholine [2].

Over the years, assays of blood or plasma ChE activity have been widely used for confirming and assessing occupational and non-occupational exposures to Anti-ChE OPs [3]. However, these methods suffer from serious drawbacks, which have been highlighted in previous publications[4, 5]. Furthermore, since the metabolic products of the most Anti-ChE OPs are nonspecific, the exposure to a group of Anti-ChE OPs is usually assessed by the determination of the metabolites [4–6]. Sensitive, more accurate, and reliable methods for assessing and monitoring occupational and non-occupational exposures to Anti-ChE OPs via determination of OPs in biological samples is therefore important.

Although several analytical methods have been developed to detect and measure some Anti-ChE OPs in biological samples [7, 8], most of them need lengthy procedures, expensive and sophisticated equipments with well-trained operators. Consequently, new approaches such as solid-phase extraction, supercritical fluid extraction (**SFE**) [9–11], accelerated solvent extraction (**ASE**) and solid-phase microextraction (**SPME**) [12]. Clean-up methods based on molecularly imprinted polymers (**MIPs**) [13, 14] are needed to overcome these problems in monitoring, assessment, evaluation and controlling of OPs effects.

Through the recent decades, considerable efforts have been made to synthesize artificial materials mimicking biological recognition systems, namely MIPs, for clean-up and separation methods. In molecular imprinting technology functional monomers are arranged in a complementary configuration to the template molecule, then a cross-linker and a porogenic solvent are added and the whole mixture is cured to give a porous material containing nascent imprinted sites. Template will be removed from the imprinted sites by washing, liberating these sites for rebinding of the template or its structural analogue [15]. The molecular imprinting technique is a useful tool for the preparation of materials with synthetic recognition sites that have a predetermined selectivity for analytes [16]. These imprinted sites are similar to the binding sites of antibodies and other biological receptor molecules. The stability, ease of preparation and low cost of these materials make them particularly attractive [17]. Other remarkable advantages of MIPs compared to bimolecules such as antibodies are their reusability and compatibility with organic phases [18]. In addi-

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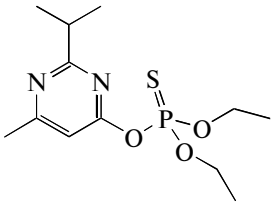

Fig. 1. Chemical structure of diazinon.

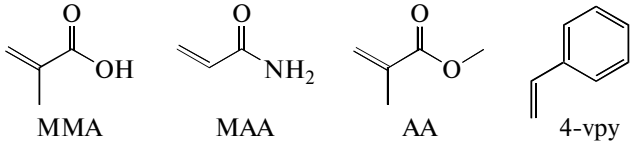

Fig. 2. Chemical structures of the functional monomers used for polymer preparation.

tion to MIP use in sample clean-up methods, these synthetic materials have been used in capillary electrochromatography [19], chromatography columns [20], sensors [21], and catalytic system [22]. To our knowledge, there are few reports for imprinted polymers designed to bind Anti-ChE OPs. The purpose of this work was to synthesize an appropriate tailor-made MIP adsorbent for binding of an Anti-ChE OP. In this study, diazinon (O,O-diethyl-O-(6-methyl-2-(1-methylethyl)-4-(pyrimidinyl) phosphorothionate) methylcarbamoylmethyl phosphorodithioate) as an Anti-ChE OP (Fig. 1), which is commonly used to control insects [23], was chosen as the MIP template.

EXPERIMENTAL

The analytical standard of diazinon was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Acrylamide (**AA**), methacrylic acid (**MAA**), methylmethacrylate (**MMA**) and ethylene glycol dimeth-acrylate (**EDMA**) were purchased from Merck (Hohenbrunn, Germany). 4-Vinyl pyridine (4-vpy) was obtained from Sigma-Aldrich Inc. (USA). Figure 2 shows molecular structures of functional monomers used for polymer preparations. 2,2'-Azobis(2-methylpropionitrile) (**AIBN**) was obtained from ACROS (New Jersey, USA). Acetonitrile, methanol, chloroform and toluene were HPLC or analytical grade. Ultra pure water used for HPLC analysis was provided from a Direct-Q3 Water Purification System (Millipore Corporation, USA).

Chromatographic determination of diazinon. Chromatographic experiments were performed on JASCO LC-2000 series (Hachioji, Japan) high performance liquid chromatograph equipped with PU-2080 Pump, CO-2060 Column Oven, AS-2055 Auto Sampler and UV/VIS 2075 Detector set at 245 nm. The column was reversed phase–C_{18} (250 × 4.6 mm i.d; Supelco, USA). The mobile phase was acetonitrile/methanol/water = 60 : 20 : 20 (V/V/V) containing 5 μL of H_3PO_4. The flow rate was set at 1.2 mL/min. The column temperature was fixed at 40°C. The injection volume was 10 μL.

Synthesis of imprinted and non-imprinted polymers. For polymer preparation, non-covalent bulk polymerization was employed as a more versatile approach than the alternative covalent protocols [24, 25]. In brief, in 25 mL test tube, a predetermined quantity of a functional monomer (6 mmol) and diazinon as template (1 mmol) were dissolved in the selected porogen (5 mL). The compositions of the pre-polymerization mixture are presented in Table 1.

Each pre-polymerization mixture was shaken on a digital shaker set at a speed of 100 rpm at room temperature for 1 h. This period was needed to ensure the formation of the complex between diazinon and monomers. Following the shaking period, EDMA (20 mmol) and AIBN (40 mg) were added to each pre-polymerization mixture. Then, the mixture was purged with nitrogen for 6 min and sealed under N_2 atmosphere. The polymerization process was carried out at 60°C in a thermostated water bath. After 18 h, the bulk polymers were ground and particles ranging from 53–106 μm were extracted repeatedly with methanol: acetic acid (9:1) using Soxhlet extractor until no diazinon was detected in the effluent. After that particles were washed with methanol to remove acetic acid. Finally, polymer particles were washed with 20 mL of acetonitrile and dried at 58°C. Non-imprinted polymers (**NIPs**) were synthesized using the same procedure but without the addition of diazinon.

Table 1. Compositions of the pre-polymerization mixtures for MIPs preparation

Polymer name	Template amount, mmol	Functional monomers (6 mmol)	Porogen (5 mL)
P1	1	MAA	Chloroform
P2	1	MMA	Chloroform
P3	1	AA	Chloroform
P4	1	4-vpy	Chloroform
P5	1	MAA	Toluene
P6	1	MMA	Toluene
P7	1	AA	Toluene
P8	1	4-vpy	Toluene
P9	1	MAA	Acetonitrile
P10	1	MMA	Acetonitrile
P11	1	AA	Acetonitrile
P12	1	4-vpy	Acetonitrile

Table 2. Diazinon binding by polymers in acetonitrile

Polimers	Bound diazinon by imprinted polymers, μg/g	Bound diazinon by respective non-imprinted polymers, μg/g	IF
P1	19.25 ± 0.86	10.28 ± 1.45	8.94
P2	4.60 ± 0.55	0.88 ± 0.01	3.72
P4	18.86 ± 0.10	12.12 ± 0.41	6.74
P5	5.44 ± 0.66	0.12 ± 0.01	5.32
P6	2.81 ± 0.54	0.18 ± 0.02	2.63
P8	2.58 ± 0.35	2.61 ± 0.41	0
P9	4.88 ± 0.74	3.30 ± 0.55	1.58
P10	0.66 ± 0.09	0.30 ± 0.03	0.36
P10	6.02 ± 0.88	4.14 ± 0.64	1.88
P12	4.81 ± 0.68	4.08 ± 0.71	0.73

Note: All experiments were carried out with 2μg/mL diazinon and 100 mg/μL polymer. All experiments were performed in triplicate.

Equilibrium binding experiments. Evaluation of the binding properties of polymers was carried out by equilibrium binding experiments. The polymer particles (200 mg) of both MIP and NIP polymers were accurately weigh out in 18 mL vials and mixed with 2 mL of 25 μg/mL solution of diazinon in acetonitrile under mechanical shaking (100 rpm) in a shaking water bath at 25°C for 4 h. Then, polymer solutions were filtered using a syringe driven filter unit (0.45 μm). Following filtration, the supernatants were analyzed by the HPLC system using the method described above. The amount of template bound to the polymers (T_b) was calculated according to the following equation:

$$T_b\,(\mu g) = V(c_i - c_f), \quad (1)$$

where V, c_i, and c_f represent the volume of supernatant in each vial (mL), initial solution concentration and solution concentration after equilibrium binding period (μg/mL), respectively. In the present study, the amount of diazinon bound per gram of polymer (c_{mip}) was calculated as follows:

$$c_{mip}\,(\mu g/g) = T_b/\text{mass of polymer in grams}. \quad (2)$$

Also, the molecular imprinting factor (**IF**) was used to evaluate the imprinting effect. It was calculated according to the following equation:

$$IF = c_{mip} - c_{nip}, \quad (3)$$

where c_{mip} and c_{nip} represent the amount of diazinon bound per gram of imprinted and non-imprinted polymers. The average value of triplicate analyses was obtained and used for the following discussion.

RESULTS AND DISCUSSION

In this study, different imprinted polymers have been prepared with different types of functional monomers (MAA, MMA, AA, and 4-vpy) and porogens (chloroform, toluene and acetonitrile). Then, their binding amounts were examined with equilibrium binding experiments. All pre-polymerization mixtures were synthesized with exception of P3 and P7 and the respective non-imprinted polymers, $P3_{-NIP}$ and $P7_{-NIP}$, which was due to solubility difficulties of AA monomer in the porogenic solvent. AA monomer is highly water soluble and its solubility in non-polar solvents (chloroform and toluene) is limited. Table 2 depicts the diazinon binding by different MIPs and NIPs.

The variation of diazinon binding by polymers is high, reflecting that the number of binding sites in these sorbents is not equal. However, the amount of diazinon bound by MIP based on MAA monomer, namely P1 in Table 2, is considerably high compared to the other polymers. As mentioned elsewhere [9], the carboxylic acid-based monomers, principally MAA, have been most successful by far. This phenomenon is probably caused by their ability to interact with template in varios ways: as H-bond donors and H-bond acceptors. However, in this work the donor of hydrogen bonds is the carboxyl group of MAA, and the acceptors of hydrogen boud are sulfur, oxygen and nitrogen containing groups of diazinon. Consequently, the binding of diazinon by P1 polymer is high. P4 polymer also binds a higher amount of diazinon compared to other polymers under the conditions we carried out the equilibrium binding experiments. This can be probably attributed to the acidic nature of diazinon, whose pKa is <2.5 [26], which improves the interaction of diazinon molecules and 4-vpy monomers via acid-base interaction.

In this paper, three different solvents were used as porogens to evaluate the influence of porogen characteristic on the binding of diazinon by the polymer. Figure 3 shows the effect of porogen type on binding ability of MIPs prepared with MAA, MMA, AA and 4-vpy. This result shows the use of chloroform, a compound with poor hydrogen bonding power, significantly improves the binding ability of the MIPs prepared with MAA, a monomer forming hydrogen bonds with template. This observation agrees with the report of another authors that optimum results are often obtained when chloroform is used as a porogen, which provides polymers with little or no porosity or small surface area [27].

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In fact, the binding capacity of a molecularly imprinted polymer can be related to numerous factors such as type and amount of functional monomer(s), porogen, cross linker and so on. Thus, identification

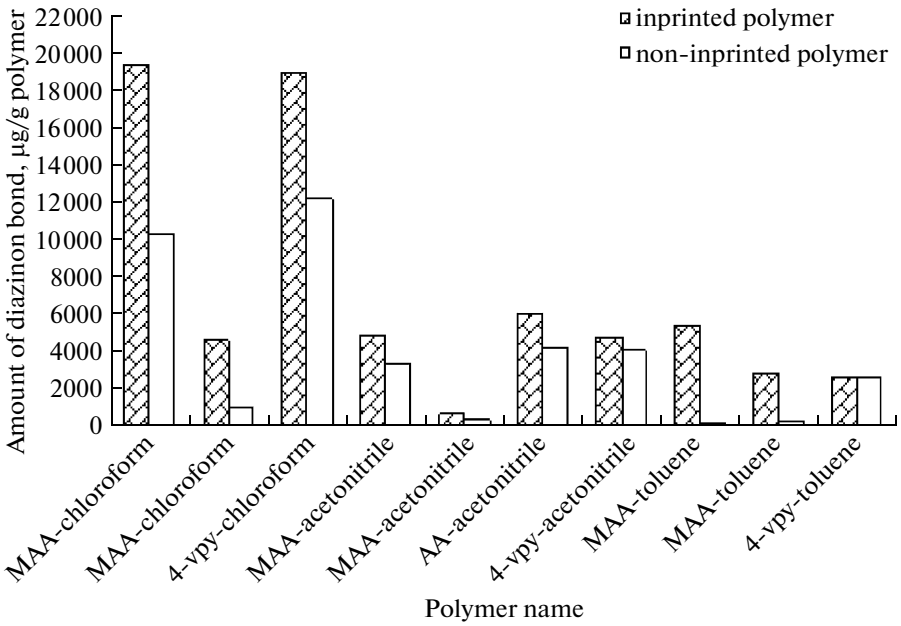

Fig. 3. Influence of the type of porogen in the diazinon binding by the MIPs synthesized with different functional monomers.

and optimization of main factors affecting the polymer properties is essential. In the present study, investigation of synthesis and evaluation of diazinon imprinted polymers has been carried out using equilibrium binding experiments. This study has shown that it is possible to use molecularly imprinted polymers as sorbents for Anti-ChE OPs. The effects of functional monomer and porogen type that have an important effect on binding properties of diazinon imprinted sorbent have been tested. The MAA monomer was found to be most suitable for the preparation of appropriate diazinon imprinted polymers. The type of porogen also influenced on the binding results. For successful diazinon imprinting, the best porogen is chloroform due to its poor hydrogen bonding capacity. Diazinon imprinted sorbent as well as other imprinted materials can be useful tools for selective enrichment, clean-up and purification methods.

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