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ORIGINAL ARTICLE

Synthesis of Molecularly Imprinted Polymer as a Solid Phase Sorbent for Pesticide Dursban

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ABSTRACT

This study describes the synthesis of molecularly imprinted polymers (MIPs) by using an Anti-ChE OPs, namely dursban, as a template. Non-covalent bulk polymerization was successfully applied to synthesis different imprinted and non-imprinted polymers with MAA, MMA, AA, and 4-vpy as monomer in selected porogens (chloroform, toluene, and acetonitrile). In order to evaluate the template binding of the polymers, equilibrium binding experiments was carried out. High binding amount of imprinted polymers compared to non-imprinted polymer was due to effective imprinting or encoding of dursban template shape in the polymer matrixes. From this study, the dursban imprinted polymers prepared using acidic MAA as a functional monomer showed excellent molecular binding ability for dursban. This is because the hydrogen binding interaction between dursban and MAA may be formed between sulfur, oxygen, chlorine, and nitrogen groups of dursban and carboxyl group of MAA. The results shows the use of chloroform as porogen, with a poor hydrogen binding power, significantly affects the binding extend of the MIPs. MAA and chloroform were found to be the most suitable monomer and porogen for the preparation of appropriate dursban molecularly imprinted polymers. This study has shown the possibility of synthesizing and using molecularly imprinted polymers as sorbent for an Anti-ChE OPs.

Keywords: *Molecularly imprinted polymers, Dursban imprinted sorbent, Anti-ChE OPs, Non-covalent bulk polymerization*

INTRODUCTION

Development of sample preparation and clean up methods is a challenge for most of the scientists, working on analysis of occupational and environmental contaminants. Although, several sample preparation and clean up methods such as solid phase extraction (SPE) [1-7], super critical fluid (SFE), and solid phase micro extraction (SPME) have been emerged to sample preparation of some environmental and occupational contaminants [8], recently, considerable efforts and trends have been made to develop new approaches such as solid phase extraction using tailor-made sorbents, namely, molecular imprinted polymers [9] and solid phase micro extraction using polymer coated fibers. In the molecular imprinting technology, specific nano-sized cavities have been formed in polymeric materials,

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Table 1. Compositions of the pre-polymerization mixture for MIPs preparation [a]

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

[a] The molar amounts of dursban: 1 mmol

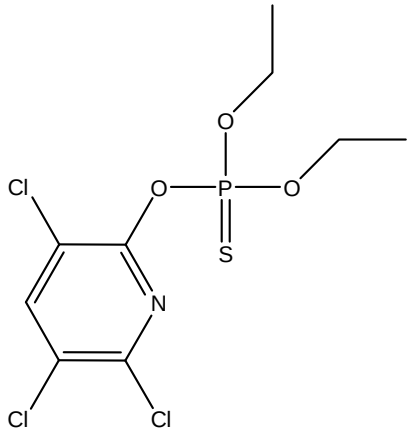

Fig 1. Chemical structure of dursban.

which often have an affinity and a selectivity approaching similar to antibody-antigen systems [10]. Molecular imprinting process is being increasingly applied for the generation of artificial antibodies [11, 12]. In molecular imprinted technology functional monomers are arranged in a complementary configuration to the template molecule, then, cross-linker material and porogenic solvents are also added and the whole mixture is cured to give a porous material containing nono-sized imprint sites. Template removal will leave vacant imprinted sites by washing, which are available for rebinding of the template or its structural analogue [13]. The stability, ease of preparation and low cost of these materials make them particularly attractive [14]. Other remarkable advantages of MIPs compared to biosystems like antibodies, are their reusability and compatibility with organic phases [15]. In recent years, the molecular imprinted polymers for occupational and environmental contaminants have been a subject of many investigations with interesting application in chemical analysis. Most of the MIP applications have focused on extracting compounds from biological and environmental samples [16]. MIPs are widely used as SPE sorbent for sample preparation e.g. atrazine [17], fenuron [18] monocrotophos [19] and terbutylazine [20].

In addition to MIP usages in sample clean-up methods, these synthetic materials have been used for capillary electrochromatography [21], chromatography columns [22], sensors [23], and catalyze system [24].

A major group of occupational and environmental contaminants, which are widely used in agriculture, public health, and domestic fields for controlling insects, are Anti-choline esterase organophosphate pesticides (Anti-ChE OPs). Although Anti-ChE OPs structures are different in nature, the mechanism by which these insecticides elicit their toxicities is identical, and is associated with the inhibition of the nervous tissue cholinesterase enzyme (ChE). This enzyme is responsible for the termination of the biological activity of the neurotransmitter, acetylcholine [25].

The sensitive, more accurate, and reliable methods for assessing and monitoring occupational and non-occupational exposures to Anti-ChE OPs via determination of OPs compounds in biological samples is therefore important. Although several analytical methods have been developed to detect and measure some Anti-ChE OPs in biological samples [26,27], most of them need lengthy procedures, expensive, and sophisticated equipments with well-trained operators for the instrumentation. Consequently, new approaches such as clean-up methods based on molecularly imprinted polymers (MIPs) are needed to overcome these problems in monitoring, assessment, evaluation and controlling of OPs effects. Up to now, there are few papers dealing with the OPs – based molecularely imprinting studies and based on our study, dursban as an Anti-ChE OPs were not used as a template in molecular imprinting technology. In the present study, the molecularly imprinted polymer was synthesized by using dursban (O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) (Fig. 1) as a template.

MATERIALS AND METHODS

Materials. The analytical standard dursban was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Acrylamide (AA), methacrylic acid (MAA), methylmethacrylate (MMA) and ethylene glycol dimethacrylate (EDMA) were purchased from Merck (Hohenbrunn, Germany). 4-vinyle 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

Table 2. Dursban binding by polymers in acetonitrile[a]

Polymers	Bound dursban by imprinted polymers (µg/g)	Bound dursban by respective non- imprinted polymers (µg/g)	IF
P1	6.99±0.86	1.92±0.24	5.07
P2	5.32±0.81	0.88±0.14	4.44
P4	5.84±0.45	4.68±0.72	1.16
P5	5.50±0.66	0.77±0.11	4.73
P6	2.70±0.30	2.18±0.23	0.52
P8	3.67±0.13	2.83±0.36	0.84
P9	1.08±0.13	0.54±0.02	0.54
P10	2.60±0.41	1.81±0.12	0.79
P11	1.36±0.15	1.01±0.09	0.35
P12	3.03±0.23	2.58±0.31	0.45

[a] All experiments were carried out at 10 µg/ml dursban in acetonitrile and 400 mg polymer. All experiments were performed in triplicate and results are reported as mean ± standard deviation.

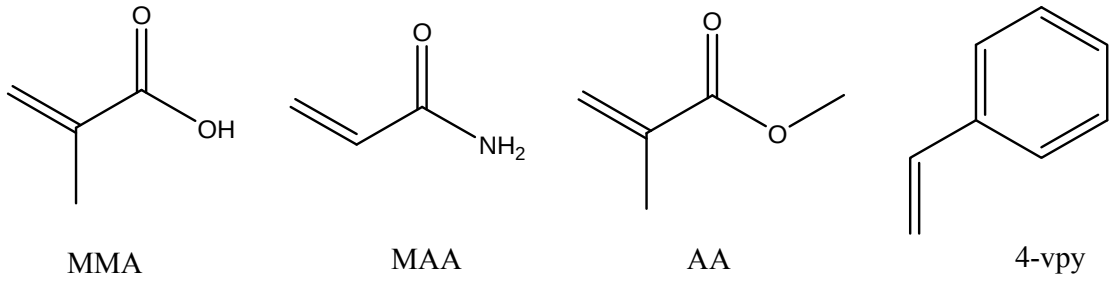

Fig 2. Chemical structures of the functional monomers used to polymer preparation

ACROS (New Jersey, USA). Acetonitrile, methanol, chloroform, and toluene were HPLC or analytical grade obtained from well-known companies. Ultra pure water used for HPLC analysis was provided from a Direct–Q3 Water Purification System (made in Millipore Corporation, USA).

Chromatographic analysis of dursban. Chromatographic experiments were performed on JASCO LC-2000 series (Hachioji, Japan) high performance liquid chromatography equipped with PU–2080 Pump, CO-2060 Column Oven, AS-2055 Auto Sampler and UV/VIS 2075 Detector set at 230 nm. The column was reversed phase–C18 (250×4.6 mm i.d; Supelco, USA). The mobile phase was acetonitrile/methanol/water=60:20:20 (V/V/V) containing 5 µL H_3PO_4. The flow rate was set at 1.2 ml/min. The column temperature was fixed at 30 ºC. 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 [28, 29]. As a brief, in 25 ml test tube, a predetermined quantity of a functional monomer (6 mmol) and dursban as template (1 mmol) was dissolved in the selected porogen (5 ml). The compositions of the pre-polymerization mixture have been presented in Table 1.

Each pre-polymerization mixture was shaken in a digital shaker set on speed 100 rpm at room temperature for 1 h. This period was to ensure the formation of the complex between dursban and monomers. Following the shaking period, EDMA (20 mmol) and AIBN (40 mg) were added to each pre-polymerization mixture. Then, the mixtures were purged with nitrogen for 5 min and sealed under a N2 atmosphere. The polymerization process was carried out at 60 ºC in a thermostated water bath. After 18 h, the bulk polymers were ground and polymer particles ranging from 53-106 µm, were collected. The particles were extracted repeatedly with methanol:acetic acid (20:1), using Soxhlet extractor until no dursban was detected in the effluent. Then, particles were washed with methanol to remove acetic acid. Finally polymer particles were washed with 20 ml acetonitrile and dried at 70 ºC. Non-imprinted polymers (NIPs) were synthesized using the same procedure without the addition of dursban.

Equilibrium binding experiments. Evaluation of the binding properties of polymers was carried out by equilibrium binding experiments. The polymer particles (400 mg) of both MIP and NIP polymers were accurately weighted out in 18 ml vials and mixed with 3 mL of dursban in acetonitrile (10 µg/mL), under mechanical shaking (100 rpm), at 27 ºC in a shaking water bath for 5 h. Then, polymer solutions were filtered using syringe driven filter units (0.45 µm). Following the filtration, the supernatants were analyzed by the HPLC system using the method described above. The amount of template bound to the polymers (Tb) was calculated according to the following equation:

$$T_b\,(\mu g) = V\,(C_i - C_f) \qquad (1)$$

Where V, C_i, and C_f represent the volume of supernatants in each vial (mL), initial solution concentration and, solution concentration after equilibrium binding period (µg/mL), respectively. In the present study, the amount of dursban bound per gram of polymers (C_{mip}) was calculated as follow:

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

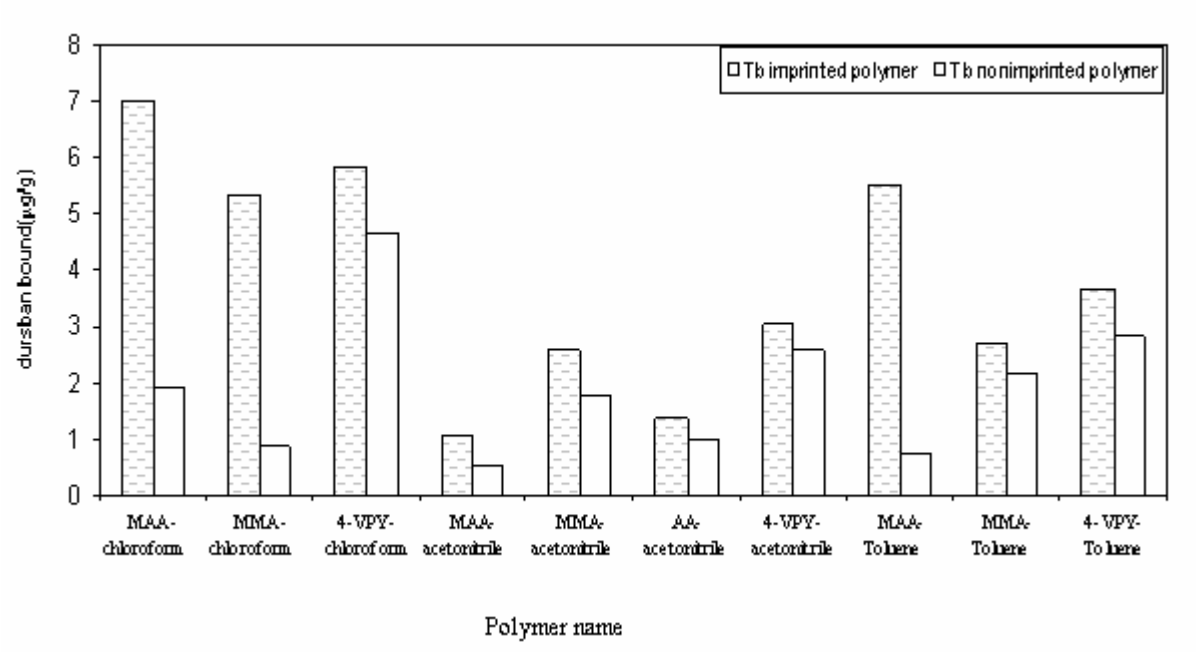

Fig 3. Influence of the type of porogen in the dursban binding of the MIPs synthesized with different functional monomers

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

$$IF = C_{mip} - C_{nip} \qquad (3)$$

Where C_{mip} and C_{nip} represent the amount of dursban bound per gram of imprinted and non–imprinted polymers. The average value of triplicate analysis was obtained and used for the following results and discussion.

RESULTS

In this study, different imprinted polymers were synthesized by the bulk polymerization method with MAA, MMA, AA, and 4-vpy as monomer in selected porogens (chloroform, toluene, and acetonitrile). It was observed that, AA monomer was not completely soluble in chloroform and toluene. Therefore, P3 and P7 pre-polymerization mixtures, in Table 1, were excluded from the polymerization process. AA monomer is water soluble and its solubility in chloroform and toluene is limited. However, other polymers were satisfactorily polymerized. In order to study the template binding of the dursban-imprinted polymers after polymerization, the imprinted polymers were incubated in the template solution and the template concentration of solution was estimated after 5 h. Table 2 depicts the amounts of dursban bound to the various polymers and respective non-imprinted polymers. IF values were calculated by subtracting the amount of free dursban concentration from the amount of dursban initially added to equilibrium binding experiments. As a rule of thumb, large IF indicates an appropriate imprinting effect. As shown in Table 2, the value of dursban bound by P1 is high compared with other polymers.

In this study, three different solvents were used as a porogen to evaluate the influence of porogen characteristic on the binding of dursban on the polymer. Fig. 3 shows the best binding capacity was achieved with chloroform as a porogen.

DISCUSSION

The difference in binding extent among imprinted polymers is due to the difference in the structure of polymers. Dursban binding to the imprinted polymers appeared higher than that of the nonimprited polymers. In the other word, the non-imprinted polymers exhibited less activity than molecularly imprinted polymers under the binding experiment conditions. Thus, a high binding amount of imprinted polymers was due to effective imprinting or encoding of dursban template shape in the polymer matrixes.

The dursban-imprinted polymers prepared using acidic MAA as a functional monomer showed excellent molecular binding ability for dursban. This is because the hydrogen binding interaction between dursban and MAA may be formed between sulfur, oxygen, chlorine, and nitrogen groups of dursban (Fig. 1) and carboxyl group of MAA. Consequently, the binding of dursban is high in P1 polymer. In agreement with previously mentioned [13] by far carboxylic acid-based monomers, principally MAA have been the most successful monomer for imprinting. This phenomenon probably attributes to their ability to interact in various ways with the template, for example as H-bond donors, H-bond acceptors.

This result shows the use of chloroform as a porogen, with a poor hydrogen bonding power, significantly affects the binding extent of the MIPs prepared with MAA. In these polymers, MAA monomers form hydrogen binding with the template. This observation agrees with the report of another author that, optimum results are often obtained when chloroform is used as a porogen, which provides polymers with little or no porosity or surface area [30]. The results obtained for template binding indicate the template interacts less favorably with the functional monomers in acetonitrile than other porogens. In non-covalent bulk polymerization approach, the binding ability of MIPs depends on the polarity of the porogens in polymerization. Generally, the weaker the polarity of porogen is, the stronger the binding ability of MIP is [31].

CONCLUSION

In the present study, non-covalent bulk polymerization and equilibrium binding experiments have been carried out to synthesize and develop a molecularly imprinted polymer for dursban. This study has shown the possibility of using molecularly imprinted polymers as sorbent for an Anti-ChE OPs. The effect of functional monomer and porogen types that have an important role on binding properties of dursban imprinted sorbent have been investigated. In summary, MAA monomer was found to be the most suitable monomer for the preparation of appropriate dursban molecularly imprinted polymers. The type of porogen also influenced the binding results. For successful dursban imprinting, the best porogen is chloroform due to its poor hydrogen bonding power affect. Dursban imprinted sorbent as well as other imprinting materials can be a useful tool for selective enrichment, clean-up methods, purification and so on.

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