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Solid-phase extraction with a dibutylmelamine-imprinted polymer as triazine herbicide-selective sorbent

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Abstract

Triazine herbicide-selective polymer spheres were prepared by molecular imprinting using dibutylmelamine (DBM) as a template in suspension polymerization, and were utilized in solid-phase extraction (SPE) of atrazine. Atrazine-selective SPE was successfully demonstrated with a recovery of ca. 97% and an enrichment factor of 50, proving the good aptitude of DBM as the template species for developing a specific sorbent for triazine herbicides. It is also noteworthy that DBM-imprinted polymers have no possibility of disturbance in agrochemical analyses even if DBM remained in the polymer, which may occur by insufficient washing at the stage of removing the template to yield the binding sites, increasing the availability of imprinted polymers for practical applications.

Keywords: Solid-phase extraction; Molecular imprinting; Sorbents; Triazines; Herbicides; Dibutylmelamine

1. Introduction

Analysis of a triazine-herbicide atrazine in the environment is one of the most important issues in the field of environmental analytical chemistry [1]. Currently, gas chromatography (GC) and high-performance liquid chromatography (HPLC) are known to be the most reliable methods for assaying herbicides, and studies on the chromatographic determination of herbicides are eagerly conducted. In parallel, efforts have also been made to establish techniques for sample enrichment and pre-purification of herbicides in the environment, which are performed prior to chromatographic analysis. For the sample preparation, solid-phase extraction (SPE) currently seems to be the most common tactic because only simple procedures are needed and a small amount of solvent is used. Conventional SPEs utilize normal-phase, reversed-phase, ion-exchange and affinity type adsorbents. The affinity type adsorbents in which antibodies and other biomolecules are immobilized are suitable for trapping specific chemical species, while the others can be used more generally.

Recently, molecularly imprinted polymers have attracted considerable attention as synthetic affinity media useful for SPE, because such features are appropriate for SPE as the tailor-made selectivity for analytes, the significantly low costs for the preparation, and the workability in organic solvents [2–4]. A number of recent publications have shown the effectiveness of the molecularly imprinted polymer-based SPE [5–18]. SPEs of triazine herbicides have also been reported to be successfully performed using molecularly imprinted polymers prepared in the presence of complexes of atrazine and methacrylic acid (MAA) [16–18]. After the polymer synthesis,

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atrazine is removed from the polymer to generate MAA-based binding sites complementary to atrazine. Although the atrazine-imprinted polymers displayed sufficient affinity and selectivity, employing the analyte itself as the template is problematic because it leaves the possibility of the presence of residual atrazine in the resultant polymers, which may leak out of the polymer and interfere in the analysis of atrazine.

We have recently reported on the preliminary study of dummy-molecule imprinting for developing triazine herbicide-selective polymers [19], whereby alkylmelamines instead of atrazine are used as the template species for the polymer synthesis, which are capable of intermolecular interaction with MAA in a similar fashion, and have no influences on the analysis of atrazine even if it cannot be eliminated from the polymer before application use. However, utility of the dummy-imprint polymer for the SPE applications has not been examined yet, which prompts us to carry out the dummy-imprint polymer-based SPE. In this paper, we report on the synthesis of atrazine-selective polymer spheres using dibutylmelamine (DBM) as a dummy template compound, and the demonstration on SPE using the dummy imprint polymer.

2. Experimental

2.1. Materials

Empty polypropylene columns for SPE, stop cocks, solvent reservoirs and adapters were purchased from Wako Pure Chemicals (Osaka, Japan). Acetonitrile, chloroform, dichloromethane and methanol were obtained from Katayama Chemicals (Osaka, Japan). Atrazine and DBM were kindly donated by Nissan Chemical Industries (Tokyo, Japan). Other chemicals were purchased from Wako. MAA and ethylene glycol dimethacrylate were purified by distillation prior to use. The compounds investigated are shown in Fig. 1.

2.2. Preparation of molecularly imprinted SPE column

DBM-imprinted polymer spheres were prepared following the procedures reported elsewhere [16]. Into 45 g of chloroform were dissolved 1.74 g (7.3 mmol) of DBM, 9.38 g (109 mmol) of MAA, 41.0 g of ethylene glycol dimethacrylate and 940 mg of 2,2′-azobis(2,4-dimethylvarelonitrile). This organic phase was poured into 219 ml of water in which poly(vinyl alcohol) (partially hydrolyzed: 1.04 g, completely hydrolyzed: 0.56 g) was dissolved. The mixture was stirred (300 rpm) at 50°C for 6 h, and stirred at room temperature for 15 h. The resultant particles were filtered to remove particles of inappropriate size for chromatographic or SPE use. The polymer (32–63 μm) was washed by Soxhlet extraction using methanol (200 ml, 5.3 times/h), and dried under vacuum. The dummy molecular imprinting is summarized in Fig. 2.

2.3. Chromatographic tests

The polymer was packed into a stainless steel column (100×4.6 mm I.D.) and washed with methanol–acetic acid (7:3, v/v) by a chromatographic pump. Flow-rate was 1.0 ml/min. Sample size was 20 μl and concentration was 1.0 m*M*. Detection was carried out using UV absorption at 254 nm.

2.4. Solid-phase extraction

The 2.0-g amount of dry particles was packed into a 6.0-ml polypropylene SPE column. The column was attached with a stop cock and a reservoir at the bottom end and the top end, respectively. The polymer was rinsed with methanol and then with water. A model sample was prepared; atrazine (**1**), asulam (**9**), thiram (**10**), propyzamide (**11**) and iprodione (**12**) (50 μg each) were dissolved in 50 ml of acetone, and poured into 450 ml of water. The aqueous sample was loaded into a SPE column at a flow-rate of ca. 7 ml/min. After the loading, air was passed through for drying the solid phase for 1 h. The column was washed with 10 ml of dichloromethane, followed by the extraction with methanol. Contents in the wastes of the sample loading step and washing step, and the extracts were analyzed by HPLC using a Supelco LC-8-DB reversed-phase column (150 mm×4.6 mm I.D.) and acetonitrile–ammonium acetate buffer (0.1 *M*, pH 6.0) (47:53, v/v) as the eluent. The procedure is summarized in Fig. 3.

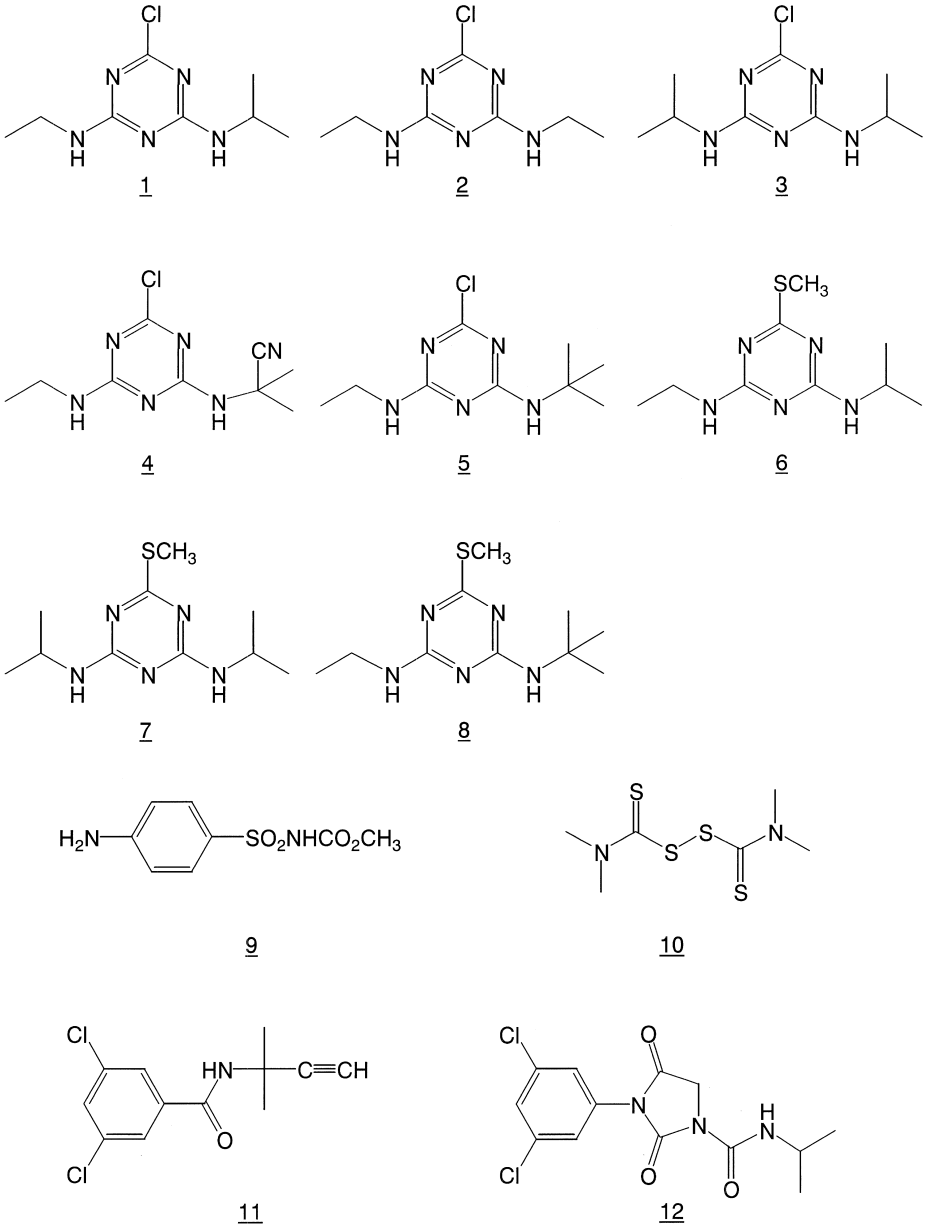

Fig. 1. Compounds investigated.

3. Results and discussion

3.1. Preparation of imprinted polymer

Triazine herbicide-selective polymers have been prepared by both bulk polymerization and suspension polymerization in our laboratory [16,20]. In the former case, a resultant block polymer was crushed and ground, then utilized as polymer particles. In the latter case, polymer spheres can be obtained with a certain range of diameter. In the suspension polymerization, however, the conditions are unfavorable for molecular imprinting because MAA, added as the functional monomer for binding site formation,

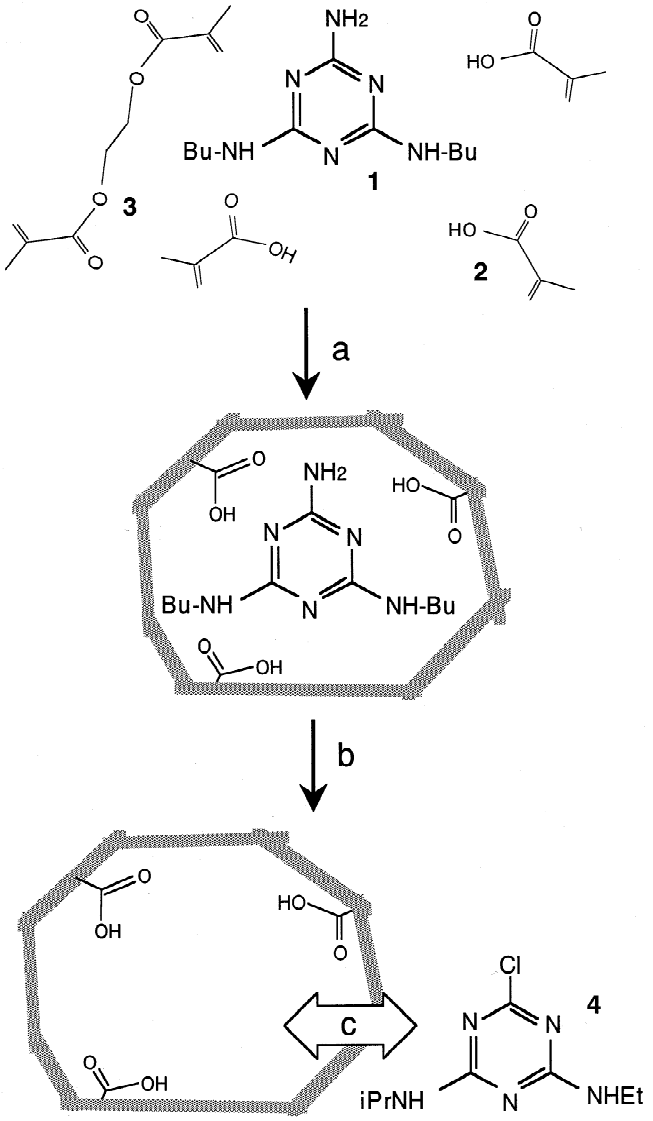

Fig. 2. Schematic representation of molecular imprinting of a dummy template molecule **1**: (a) crosslinking of ethylene glycol dimethacrylate **3** is carried out in the presence of a template molecule **1** and a functional monomer methacrylic acid **2**; (b) **1** is extracted from the crosslinked polymer network (represented by thick wavy lines); (c) the resultant cavity complementary to **1** also works as a selective binding site for atrazine **4** because of topographical, chemical analogy.

distributes also to the aqueous phase, and water dissolved in the organic phase (chloroform) interferes with the imprinting process. Actually, it has been reported that the polymer prepared by suspension polymerization exhibits lower affinity to atrazine, though more MAA was used compared with the recipe for bulk polymerization [16]. In this study, however, the bulk polymer was not adopted because it resulted in extremely high pressure when packed in SPE columns in our preliminary studies, probably due to irregular shape and size, even after careful grinding and sieving. Therefore, dummy-imprint

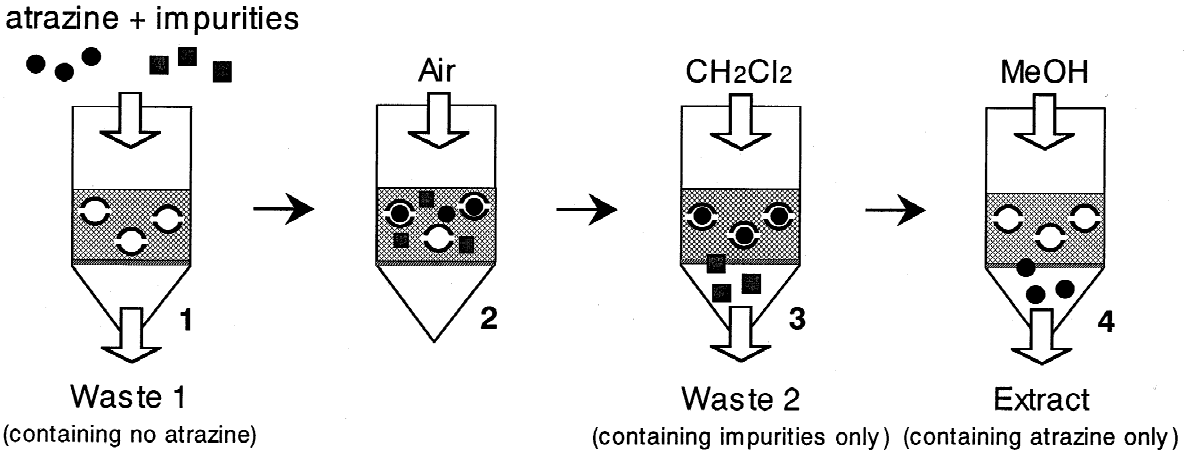

Fig. 3. The procedures of the solid-phase extraction: 1, loading of aqueous sample solution; 2, air passage for drying; 3, selective washing with dichloromethane; 4, extraction with methanol.

polymers were prepared by suspension polymerization using excess (15 equiv.) of MAA to the template in this study.

3.2. Polymer evaluation by chromatography

As shown in Fig. 3, the procedure of SPE conducted in this study can be summarized as follows: (1) loading an aqueous sample, (2) drying the sorbent, (3) washing with an organic solvent of low polarity and (4) extraction with a more polar organic solvent. Non-specific hydrophobic interaction is utilized in step 2 for retaining all lipophilic compounds including triazine herbicides, while hydrogen bonding is operated in step 3 for ''specific washing'', retaining the target herbicides and releasing impurities. After that, the extraction will result in an enriched sample containing the desired herbicides only. Thus, the polymer adsorbent is required to be hydrophobic enough to retain atrazine under aqueous conditions and specific enough to selectively adsorb atrazine in less polar organic solvents. The DBM-imprinted polymer spheres were, therefore, examined by liquid chromatography using the polymer as stationary phase to ensure the binding performance of the polymer necessary for SPE use.

The retention of atrazine was examined under various eluent conditions. Fig. 4A shows the retention factors of atrazine when aqueous acetonitrile was used as the eluent. With an increase of water in acetonitrile up to about 20% (v/v), the retention of atrazine was decreased, suggesting that carboxylic residues are effective for retaining atrazine in acetonitrile. With over 50% of water, however, the retention factor drastically increased with the water content. When the water content was more than 80% (v/v), the elution of atrazine was not observed, suggesting that the polymer could adsorb atrazine in aqueous solution by hydrophobic interaction. The results also confirm that atrazine can be extracted from the polymer using appropriate polar and/or protic solvents. Fig. 4B shows the retention factors of atrazine when a mixture of acetonitrile and dichloromethane was used as the eluent. When acetonitrile was less than 30% (v/v), the elution of atrazine was not observed. It may be due to the complete adsorption to the polymer stationary phase or due to a too broadening peak shape. The results suggest that the polymer is capable of strong retention of atrazine in the washing step of SPE. In order to observe the elution of atrazine with an appropriate retention time, the content of acetonitrile in the eluent was increased from 30% (v/v) to 100% (v/v). The retention became shorter in accordance with the increase of acetonitrile content, as observed in atrazine-imprinted polymers previously reported [20].

The selectivity was also examine by comparing

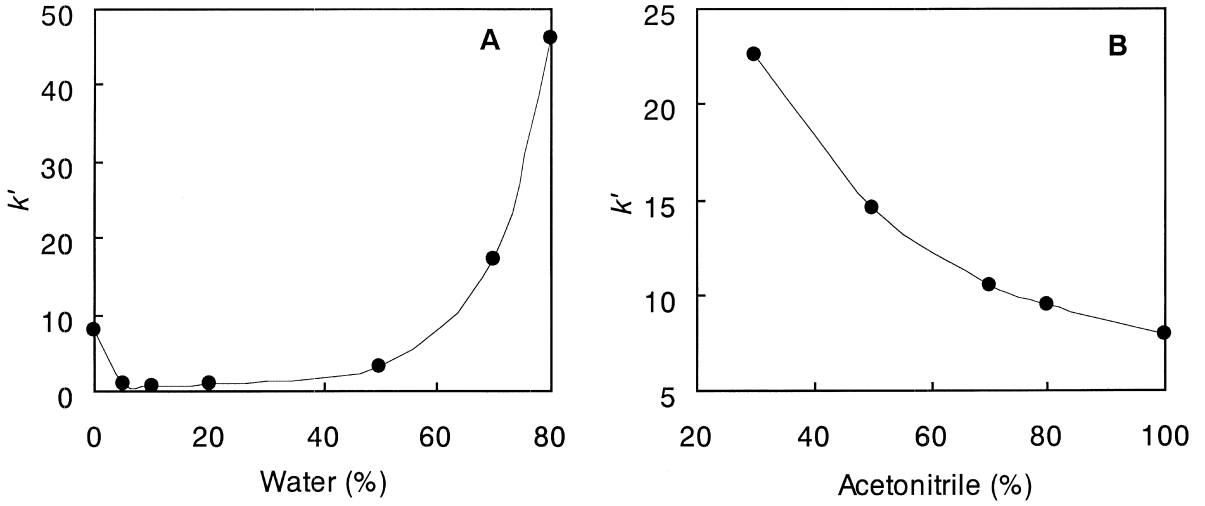

Fig. 4. The retention of atrazine: (A) using water–acetonitrile; (B) using acetonitrile–dichloromethane as the eluent.

the retention factors of atrazine (**1**), simazine (**2**), propazine (**3**), cyanazine (**4**), terbutylazine (**5**), ametryn (**6**), prometryn (**7**), terbutryn (**8**), asulam (**9**), thiram (**10**), propyzamide (**11**) and iprodione (**12**) (Table 1). The selectivity is crucial for the washing step of SPE to effectively purify the target analyte. While triazine herbicides exhibited comparable retention, other agrochemicals showed almost no retention. The selectivity observed for atrazine and triazine herbicides is promising for the selective release of the impurities in the washing step of SPE. As described in the previous paper [16], the reference polymer prepared without the template showed the retention factors of **1** (2.5), **2** (2.3), **3** (3.0), **9** (0.2), **10** (0), **11** (0) and **12** (0), the specific binding ability described here could be induced by the imprinting process.

Table 1
Retention factors (k') of triazine herbicides and other agrochemicals

Sample	k'
Atrazine (**1**)	15.7
Simazine (**2**)	17.3
Propazine (**3**)	14.2
Cyanazine (**4**)	8.1
Terbutylazine (**5**)	15.7
Ametryn (**6**)	13.2
Prometryn (**7**)	9.8
Terbutryn (**8**)	9.2
Asulam (**9**)	0.3
Thiram (**10**)	0.0
Propyzamide (**11**)	0.0
Iprodione (**12**)	0.0

3.3. Solid-phase extraction

SPE was carried out by the same procedure as previously reported by us [16], as described in Fig. 3. A model sample was prepared by adding a mixture of herbicides (50 μg each) dissolved in 50 ml of acetone into 450 ml of water.

The waste 1 was analyzed to estimate the amount of adsorbed atrazine. In the waste 1, no atrazine was found, showing that all atrazine loaded to the column was adsorbed. After the washing with 10 ml of dichloromethane, the waste 2 was analyzed by HPLC. As shown in Table 2, atrazine was satisfactorily retained during the treatment with dichloromethane, while most of the other herbicides were eluted in certain degrees. The extraction with 10 ml of methanol resulted in a 96.8% recovery of atrazine (enrichment factor=50). Notably, the extract contained atrazine only, demonstrating the effectiveness of the dummy-molecule imprinted polymer as atrazine-selective adsorbent.

Table 2
Solid-phase extraction of atrazine

	Step[a]	Atrazine (μg)	Asulam (μg)	Thiram (μg)	Propyzamide (μg)	Iprodione (μg)
Load	1	50.0	50.0	50.0	50.0	50.0
Sorbed[b]	1	50.0	11.0	50.0	50.0	50.0
Washed	3	0.6	1.4	5.9	34.5	19.6
Extract	4	48.4	0.0	0.0	0.0	0.0

[a] The number shows to which step in Fig. 3 the procedure corresponds.
[b] The sorbed amount was obtained by subtracting the amount of each compound found in waste 1 (Fig. 3) from the amount loaded.

4. Conclusions

A molecularly imprinted polymer selective for triazine herbicides was prepared using the dummy template compound, DBM, and was applied for SPE of atrazine. SPE using the dummy-imprint polymer exhibited satisfactory selectivity, recovery rate and enrichment factor, though optimized conditions have not been investigated. In addition, the proposed SPE method presented exhibited no template contaminant interfering with the analysis after the SPE. Currently, the applicability of this technique to the analysis of real samples from river water, soil and agricultural products is investigated in our laboratory.

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