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Molecularly imprinted polymers for triazine herbicides prepared by multi-step swelling and polymerization method
Their application to the determination of methylthiotriazine herbicides in river water

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Abstract

Uniformly-sized, molecularly imprinted polymers (MIPs) for atrazine, ametryn and irgarol were prepared by a multi-step swelling and polymerization method using ethylene glycol dimethacrylate as a cross-linker and methacrylic acid (MAA), 2-(trifluoromethyl) acrylic acid (TFMAA) or 4-vinylpyridine either as a functional monomer or not. The MIP for atrazine prepared using MAA showed good molecular recognition abilities for chlorotriazine herbicides, while the MIPs for ametryn and irgarol prepared using TFMAA showed excellent molecular recognition abilities for methylthiotriazine herbicides. A restricted access media-molecularly imprinted polymer (RAM-MIP) for irgarol was prepared followed by *in situ* hydrophilic surface modification using glycerol dimethacrylate and glycerol monomethacrylate as hydrophilic monomers. The RAM-MIP was applied to selective pretreatment and enrichment of methylthiotriazine herbicides, simetryn, ametryn and prometryn, in river water, followed by their separation and UV detection via column-switching HPLC. The calibration graphs of these compounds showed good linearity in the range of 50–500 pg/mL ($r > 0.999$) with a 100 mL loading of a river water sample. The quantitation limits of simetryn, ametryn and prometryn were 50 pg/mL, and the detection limits were 25 pg/mL. The recoveries of simetryn, ametryn and prometryn at 50 pg/mL were 101%, 95.6% and 95.1%, respectively. This method was successfully applied for the simultaneous determination of simetryn, ametryn and prometryn in river water.

Keywords: Molecularly imprinted polymer; Triazine herbicides; Methylthiotriazine herbicides; Environmental analysis; Restricted access media; Sample preparation; Solid-phase extraction

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1. Introduction

Pretreatment and enrichment processes are indispensable for trace analysis of target analytes in biological and environmental samples even by LC–MS and LC–MS/MS because of ultra-low levels of the analytes and high levels of many interferences. Although solid-phase extraction (SPE) is now often used in those processes as an alternative to laborious liquid–liquid extraction, conventional SPE sorbents such as C_{18}, ion-exchange and size-exclusion phases are lacking in selectivity towards target analytes. In order to overcome this drawback, a lot of affinity SPE sorbents have been developed. These include immuno- and chemo-affinity sorbents and molecularly imprinted polymers (MIPs) [1,2]. The first ones are prepared by binding an antibody produced for a target analyte to a solid support and are able to selectively extract the analyte and its structurally related compounds from complex matrices with high recovery. However, production of the antibody is expensive, laborious and time-consuming in addition to its low stability against heats and chemicals.

On the other hand, MIPs are obtained by polymerization of a functional monomer and cross-linker in the presence of a target molecule (template molecule). Removal of the template molecule from the resulting polymer leaves cavities complementary in size and shape to the target molecule. Consequently, MIPs can rebind the target molecule as antibody mimics and can also

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recognize its structurally related compounds selectively. MIPs offer some advantages compared to immunosorbents: easy, low-cost and quick preparation in addition to thermal and chemical stabilities. Therefore, MIPs are expected as an alternative affinity SPE sorbent to immuno-sorbents [3,4]. However, for the utilization of a MIP for SPE, especially on-line SPE in trace analysis, a few points should be taken into account. Since it is difficult to remove the template molecule completely from the resulting MIP even by thorough washing, leakage of a trace amount of the template molecule remaining in the MIP prevents the accurate and precise assay of an analyte in trace analysis. Therefore, a MIP has been prepared using its structurally related analogue as an alternative template molecule [5–7]. Secondly, surface hydrophobicity of a MIP causes non-specific adsorption of serum proteins in biological samples and hydrophobic interferences in environmental samples. Therefore, we have developed a uniformly sized MIP selectively modified with hydrophilic external layer, so called restricted access media-molecularly imprinted polymers (RAM-MIPs), using a multi-step swelling and polymerization method combined with a hydrophilic surface modification technique [8]. The RAM-MIPs were applied for direct injection assays of drugs in plasma or serum by column-switching HPLC [7,9,10]. Furthermore, it has been reported that those could be effective for removal of the interferences present in environmental water [11,12].

Several MIPs for triazine herbicides have been prepared and applied for their selective extraction in environmental samples [13–18]. However, they could not be used as an on-line SPE sorbent for direct injection assays of the analytes in biological and environmental samples. In this study, we prepared the RAM-MIP for irgarol, having a methylthiotriazine skeleton, as an alternative template molecule by a multi-step swelling and polymerization method and hydrophilic surface modification technique. Furthermore, we utilized it for the simultaneous determination of methythiotriazine herbicides, ametryn, prometryn and simetryn in river water as an on-line SPE sorbent combined with column switching HPLC.

2. Experimental

2.1. Materials

Ethylene glycol dimethacrylate (EDMA), 2-(trifluoromethyl) acrylic acid (TFMAA) and 4-vinylpyridine (4-VPY) were purchased from Tokyo Kasei (Tokyo, Japan). Glycerol dimethacrylate (GDMA) and glycerol monomethacrylate (GMMA) were gifts from Fuso (Osaka, Japan). Polyvinyl alcohol (degree of polymerization = 500, saponification value = 86.5–89 mol%) and potassium peroxodisulfate were purchased from Nacalai Tesque (Kyoto, Japan). 2,2′-Azobis(2,4-dimethylvaleronitrile) (ADVN), methacrylic acid (MAA), atrazine, propazine, simazine, ametryn, prometryn and simetryn were purchased from Wako (Osaka, Japan). Terbuthylazine and irgarol were purchased from AccuStandard (New Haven, CT, USA) and Riedel-de Haën (Seelze, Germany), respectively. The structures of triazines used in this study are illustrated in Fig. 1.

Other reagents and solvents of an analytical-reagent grade were used without further purification. Water purified with a Nanopure II unit (Barnstead, Boston, MA, USA) was used to prepare eluents and sample solutions.

2.2. Preparation of MIP and RAM-MIP

MIPs were prepared followed by a multi-step swelling and polymerization method and RAM-MIPs were prepared by the method followed by *in situ* hydrophilic surface modification of the MIPs as reported previously [8]. Similarly, non-imprinted polymers (NIPs) and RAM-NIPs were prepared for comparison.

A water dispersion of uniformly sized, polystyrene seed particles (0.497 g/mL) was admixed with a microemulsion prepared

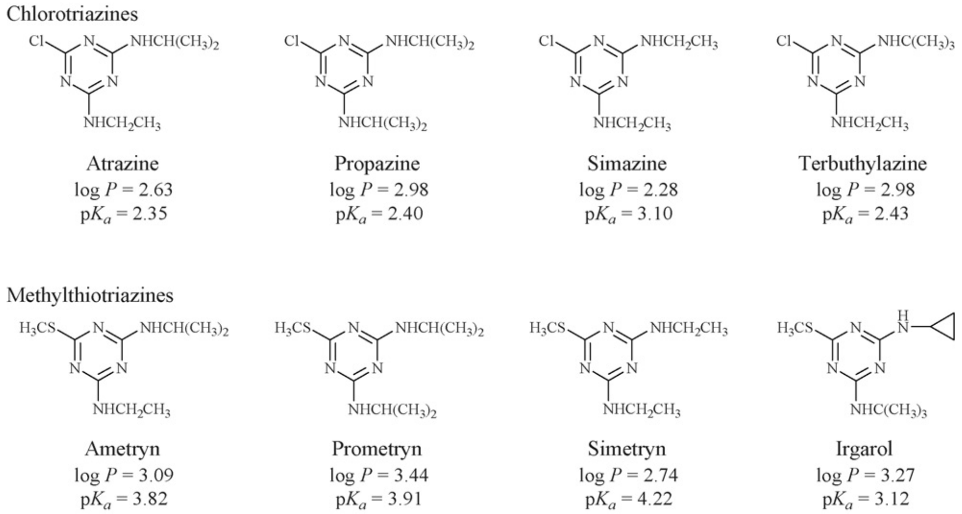

Fig. 1. Structures of triazines used in this study. The log P and pK_a values calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris in SciFinder Scholar 2006. http://www.cas.org/SCIFINDER/SCHOLAR/index.html.