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Removal of Atrazine Herbicide from Contaminated Water Using Molecularly Imprinted Nano-polymer

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Abstract

In the present work, molecularly polymeric nanoparticles were synthesized by miniemulsion polymerization procedure using Atrazine (ATZ) as imprinting molecule. Three different monomers and two different crosslinkers were investigated. The MIPs obtained were subsequently characterized in detail with rebinding investigation by HPLC-DAD UV. The results indicated that the polarity of both monomer and crosslinker play an important role for affinity and retention behavior to ATZ. The affinities of the polymers to ATZ increased with the decreasing polarity of crosslinker. It is illustrated that the monomer is much more important than the crosslinker, and that the hydrophobic interaction and π -π interaction between analytes and the MIP play a key role in the release process. This work focuses on synthesis of nano-particles and application of toxic contaminant material in water purification and using miniemulition polymerization method. Molecularly imprinted nanospheres (nanoMIPs) adsorbent for micropollutants were prepared using non-covalent templating technique. MIP nanoparticles has particle size about 140 nm were synthesized in toluene by using ATZ as a template, Itaconic acid (ITA) as functional group, and EDMA as the crosslinker polymer. The efficiency of the non-covalent molecular imprinting was examined by rebinding experiments and quantified by HPLC-UV. When compared with the non-imprinted polymer, the MIP shows an excellent affinity towards ATZ in the aqueous solution with imprinted factor (IF) 4.15 and the maximum capacity was about 20µg/mg.

Key words: Molecularly imprinted polymer (MIP), Atrazine (ATZ), Nano-polymer

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Introduction

Natural water sources are much contaminated with various pesticides because of their wide use in commercial and agricultural applications. These chemicals eliminated directly into the ground are rapidly drained into groundwater. At present, the atrazine used for plant protection has overtaken several hundred **(Sherma, 1995)** and the European Union has restricted its used but other countries are still used with the maximum allowable concentration for a single herbicide to 0.1 mg/L in drinking waters. Triazine herbicides such as Atrazine are broad-spectrum herbicide that comprises the major proportion of agricultural herbicide used in today's agricultural industry. These compounds are considered hazardous because they potently inhibit acetylcholine esterase and formed are potent mutagens **(Hashimoto et al., 2002).** Particularly atrazine is one of the common compounds of many commercially available herbicides and has been classified by the Environmental Protection Agency as a compound likely to be carcinogenic to humans. Because of its toxicity, combined with its wide use, detection of this environmental pollutant requires the development of sensitive and selective analytical methods in order to monitor the pesticide residue levels and to control the bioaccumulation process.

Identification and quantification of pesticides is routinely carried out by high performance liquid chromatography with either mass spectrometry or diode array detection **(Nunes et al., 1998 and Nogueira et al., 2004)**, or gas chromatography coupled to atomic emission or mass spectrometry detection **(King, et al., 2002 and Nunes et al., 1998).** Many of the above mentioned determination methods are accurate and selective, but require relatively expensive instrumentation and high consumption of reagents, which render them expensive or time consuming. Moreover, since those pesticides are usually found at trace amounts in water, extraction or preconcentration steps must be performed prior to analysis **(Marty et al., 1995)**. Such methods are laborious, time-consuming and should be carried out in the laboratory. The present requirement of "in situ" analysis and/or monitoring at real time has triggered the development of alternative methods able to minimize reagents consumption and time of analysis, while maximizing sample throughput, without deteriorating the quality of the measurements.

Molecularly imprinted polymers (MIPs) are crosslinked polymers with specific binding sites for a template molecule. These binding sites are tailor-made by the copolymerizing crosslinker with a functional monomer in the presence of the template molecule **(Haupt, 2003)**. After removal of the template from the polymer, the recognition sites, in terms of size, shape and functional groups, are complementary to the template molecule **(Mosbach, 1994)**. MIPs possess advantages of physical robustness, rigidity, resistance to elevated temperatures

and pressures, and inertness towards acids, bases, metal ions and organic solvents compared to biomolecules **(Owens et al., 1999 and Zhu et al., 2002).** MIPs have been extensively used in biosensors, to mimic enzyme catalysis, for solid-phase extraction (SPE) and as HPLC stationary phase **(Haupt and Mosbach, 2000 and Sanbe et al., 2003)**. Nowadays, MIPs are being performed in different research fields including enzyme mimic catalysis **(Añtuna-Jiménez et al., 2014),** sensing devices **(El Gohary et al., 2015)**, and drug delivery **(Ruela et al., 2014)**, or selective separation applications by applying MIPs as stationary phases in liquid chromatography **(Li et al., 2014)**, capillary electrochromatography **(Ye and Li, 2014). 2014),** or solid-phase-extraction **(Ji et al., 2014)**.

Material and Method

2.1. Chemicals

Atrazine (ATZ) was purchased from Carbone Scientific Co. Ltd (London, UK). Sodium dodecyl sulfate (SDS), Hexadecyl trimethyl ammonium bromide, Dimethyl myristyl ammonium propane sulfonate, Tween 85 surfactant, Methacrylic acid (MMA), Itaconic acid (ITA) and Styrene (ST), ethlene glycoldimethacylate (EGDMA) and divinylbenzene(DVB), hexadecane, toluene, methanol and chloroform were purchased from Sigma Aldrich (Taufkirchen, Germany). The other surfactants were obtained from their respective producer. Azobisisobutyronitrile (AIBN V-59) was purchased from Wako Chemicals (Neuss, Germany). All solutions were prepared using ultrapure water (Milli-RO 5 Plus, Milli-Q185 Plus, Millipore, Eschborn, Germany).

2.2. Apparatus

A Sonics Vibra-Cell VCX130 ultrasonicator was used for obtaining nano-particles, a Malvern Master Sized 2000 for particle size analysis and a Merck-Hitachi HPLC for ATZ analysis.

2.3. HPLC operating conditions

A previous method (**Rustander, 2005**) was modified by increasing the flow rate from 0.25 to 0.5 ml/min. As samples was measured by HPLC equipped with autosamplers. separation was performed Betabasic C18 column (150 mm ×2.1 mm, 5um was film thickness). Detection was performed by UV 230 nm. The mobile phase comprised 90 % acetonitrile and 1% (10mM NH_4PO_4, pH 6,9) by volume and had a flow rate of 0.5 mL min^{-1}.

2.4. Preparation of miniemulsion nano-polymers

The preparation of the Nano-MIP was carried out via miniemulsion polymerization (**Vaihinger, 2002)** by mixing 6.0 g of a mixture of 0.482 mg (0.483 mmol) of MAA and 5.55 gm (1.1 mmol) of EGDMA, 323 mg of hexadecane (1.11 mmol) and 120 mg (0.625 mmol) of AMBN (oil phase) and 295.8 mg of ATZ (1.37 mmol) with 100 mL of water containing 576 mg (2 mmol)

of dissolved SDS (aqueous phase). The two phases were dispersed by stirring vigorously for 1 h at room temperature followed by sonicating for 4 min in 30 s run, 30 s break cycles. The polymerization was carried out at 80 ^{o}C overnight (15 h). The particle sizes of diameters between 100 nm and 200 nm were selected. The template was removed from nano-particles by dialysis first in water (one cycle), then methanol (10 cycles) and finally methanol /HAc 95/5 (5 cycles). Each cycle was at least 8 h. the nano-polymers were washed with methanol and left dry at room temperature and reused for binding experiment.

2.5. Rebinding of ATZ

To investigate the selectivity of the MIP adsorbents, MIP synthesized for ATZ and its corresponding NIP were used to study the binding capacity of ATZ by a saturation binding experiment. The binding isotherms of ATZ to MIP and NIP were measured at several concentrations in the 1-50 mg L^{-1} range as shown in Figure 2. The binding of ATZ was calculated using the Langmuir type adsorption isotherm by fitting equation $q=a_1C/ (1+b_1C)$ where q (μmol. g^{-1}) is adsorption capacity, C is the concentration of free adsorbate and a1, b1 are numerical parameters. **(Katti et al., 1992 and Sajonz et al., 1998)**. The imprinted factor parameter is used to characterize the molecular recognition abilities of imprinted membrane. The imprinted factor (IF) reflects the strength of the interaction between the cavities of imprinted polymer and temple molecule. It is obtained by measuring saturation binding amount of template in moleculary imprinted polymer (q_{MIP})and Non imprinted Polymer(q_{NIP})by the following Equation $IF=q_{MIP}/q_{NIP}$. **(Baggiani et al., 2004).** 25 mg of different nano-polymer materials were contacted with 5mL of ATZ in methanol with initial series stander solution with concentrations ranging from 1 to 50 mg L^{-1}. The different mixtures of nano-polymers were stirred for 1h at a room temperature. After binding process was completed. The mixtures were centrifuged and filtered with a 0.45 um filter. Initial and final ATZ concentrations were measured by HPLC-DAD UV and amount of ATZ bound to the MIP and NIP was calculated by subtracting the concentration of the free ATZ from the initial concentration.

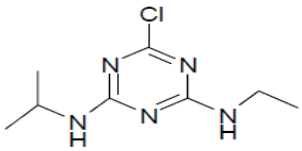
2.6. Effect of types of monomer

The choice of monomer is an important when desiring to increase selectivity. In order to obtain the best result, three monomers were assayed: MAA, ST and ITA. The optimium results were obtained with MAA; the selectivity was lower than that obtained with ST, and selectivity was the highest with ITA. Once, it had been decided that ITA was the best monomer; the influence of type of this monomer was assessed.

Result and Discussion

An imprinted polymer was prepared by miniemulsion polymerization using ATZ as a template and MAA, ST and ITA as the function monomers,

together with a corresponding non-imprinted polymer. This polymers has been matched to ATZ. The chemical structure of ATZ is illustrated in Figure 1.



Atrazine

Fig.1 the chemical structure of atrazine.

3.1 Binding performance of MIP

Binding property of ATZ imprinted polymer adsorption experiments were performed on MIP and NIP particles to evaluate the binding affinity of ATZ. The binding capacity of ATZ on MIP was an important to estimate how much MIP was required to bind a specific of ATZ from solution. The results showed that the MIPs had higher or stronger binding capacity than NIP when the concentration of ATZ was higher than 1 mg L^{-1}, but the adsorption amount of MIP ITA (14.422 mg g^{-1} was 4.15 fold over that of NIP (3.47mg g^{-1}) when the ATZ concentration was 25 mg L^{-1} (Fig. 2). The result confirmed that the arrangement of ITA in MIP was inherently different from that of NIP. However, as the water solubility of ATZ was low, a higher concentration of ATZ than 33 mg L^{-1} in water solution could not prepared and adsorption of MIP did not reach the saturation point even at 50 mg L^{-1} of ATZ **(Geng et al., 2015)**.

According to Table 1 the calculation of IF of studied monomers shown IF of MIP ITA has the best binding properties (4.15) than other studied monomers

Table 1 Imprinted factor for different monomers with ATZ.

Polymer	Template	Monomer	Crosslinker	Molar ratio	IF
MIP ITA	ATZ	ITA	EGDMA	1:4:20	4.15
MIP MMA	ATZ	MMA	EGDMA	1:4:20	1.23
MIP ST	ATZ	ST	EGDMA	1:4:20	1.07

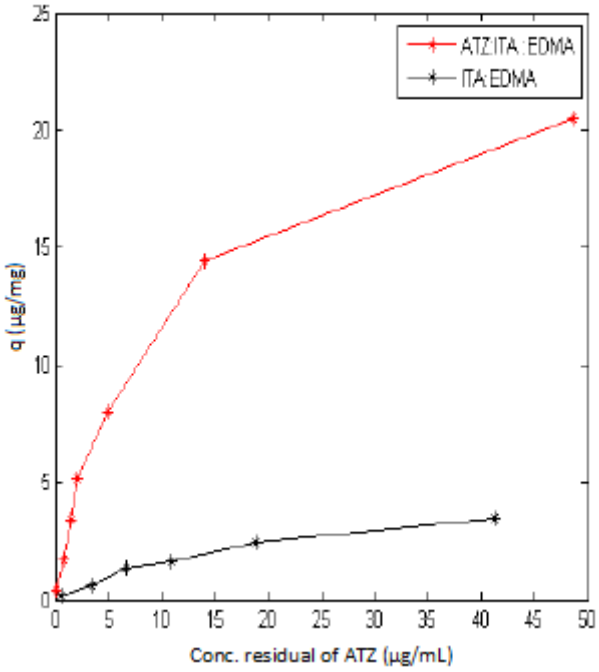


Fig.2 Adsorption isotherm plot of MIP (ATZ: ITA:MAA) and NIP (ITA:MAA)

Conclusion

In this work, New Molecularly imprinted polymers nanospheres were successfully synthesized the target analyte ATZ from environmental water samples with using MMA, ST and ITA by miniemulsion polymerization for ATZ. So, all chosen monomers and crosslinkers could successfully be used as monomers and crosslinkers respectably to make miniemulsion polymerization. The successful key to produce nanoparticles with using anionic surfactant. So, SDS as anionic surfactant was successfully used to produce nanoparticles. All attempts to use cationic, zwitterionic and non-ionic surfactants were unsuccessful. The resultant MIPs showed good recognition towards ATZ over the non-imprinted polymers. Designing MIPs that have selective binding in water is difficult, but the ITA MIP bound ATZ stronger compared to the NIP. This confirms that molecular imprinting has indeed taken place in the MIP, but the other MIPs did not show any selectivity compared to the NIPs. The imprinted factor was calculated for ITA (4.15), followed by MAA (1.23) and ST (1.07). The maximum capacity of the best polymers was at least 20 µg/mg. So the best binding properties consider with ITA monomer than other studied monomers. Further work will focus on the determination of selectivity experiments of MIP toward ATZ in the presence of similar triazine compounds and also the using MIP –Solid phase extraction (SPE) to remove ATZ from contaminated samples whether aqueous or soil environmental samples.

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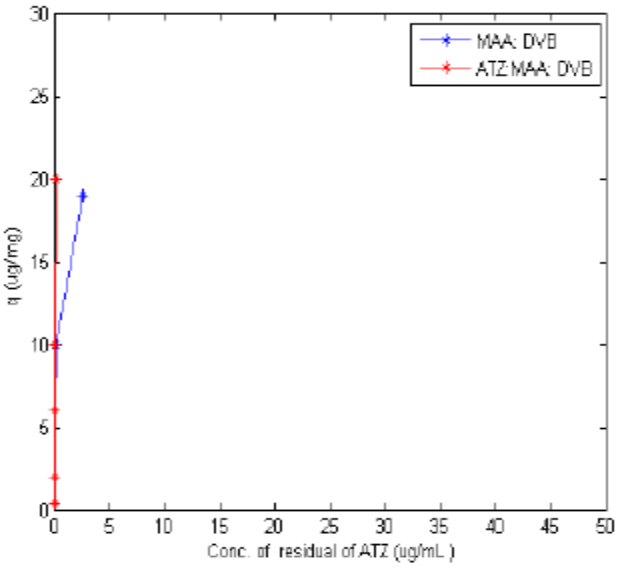
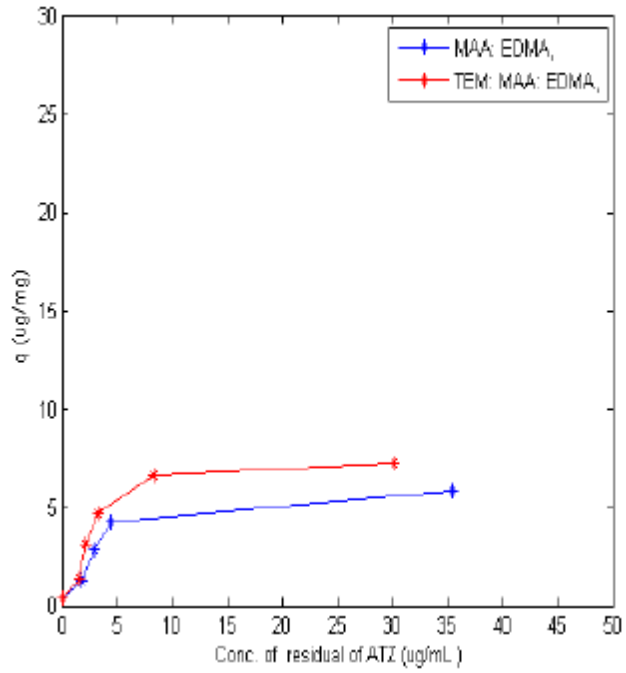
Supplementary information



7.1 Adsorbed amount of ATZ in ug per mg polymer of imprinted(red) and non-imprinted (blue) MAA:DVB nanoparticles



7.2 Adsorbed amount of ATZ in ug per mg polymer of imprinted(red) and non-imprinted (blue) MAA:EDMA nanoparticles

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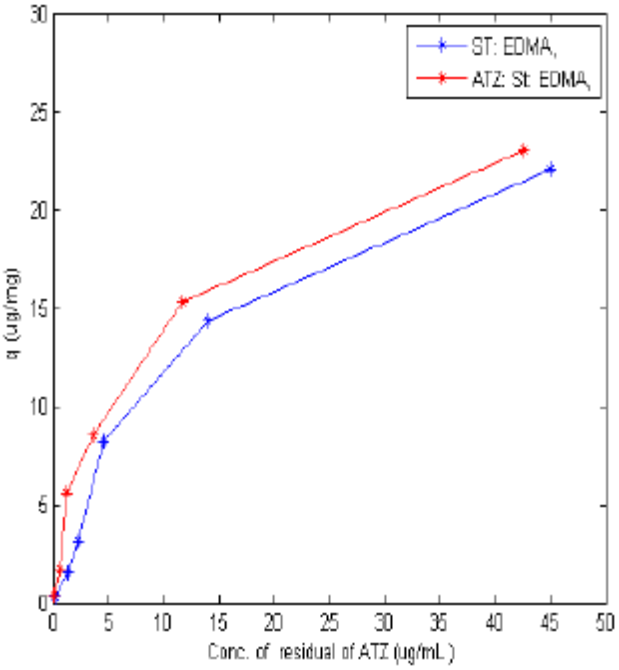

7.3 Adsorbed amount of ATZ in ug per mg polymer of imprinted (red) and non-imprinted (blue) ST:EDMA nanoparticles