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Application of multivariate analysis to the screening of molecularly imprinted polymers (MIPs) for ametryn

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Abstract

Among the solid-phase extraction (SPE) techniques, a novel system for a triazine herbicide named ametryn, has been developed based on a molecular imprinted polymer (MIP) phase. Through this method, the synthesis of the complementary to ametryn MIP was accomplished and the factors influencing its efficiency have been optimized. Through the optimization process, the type and the amounts of functional monomer and solvents, template amount, cross-linker, initiator as well as the polymerization temperature were considered to be evaluated. Based on the obtained results, the optimum conditions for the efficient polymerized sorbent, considering the recovery efficiency were solvent: acetonitrile, 6.41 mL; monomer: methacrylic acid, 5.41 mmol; template: 1.204 mmol; cross-linker: 27.070 mmol; initiator: 2.03 mmol; temperature: 40.86 °C. The optimum molar ratio among the template, monomer and cross-linker for ametryn was 1:4.49:22.48. The reversed-phase HPLC-UV was used for the ametryn determination, using an isocratic solvent delivery system (acetonitrile: H_2O, 60:40), flow-rate of 0.8 mL min^{-1} and a UV wavelength of 220 nm. In line with the obtained results, using central composite design (CCD) can increase the precision and accuracy of synthesis and optimization of MIP to ametryn and possibly other similar analogues.

Keywords: Molecular imprinted polymers; Solid-phase extraction; Central composite design; Herbicides; Triazine; Ametryn

1. Introduction

The most widely used group of herbicides, since their discovery in the 1950s, is triazines including ametryn, atrazine, simazine, cyanazine and propazine. Triazinic herbicides were considered as systemic toxicants, causing a variety of acute health effects. On the other hand, the analysis of herbicides, in order to monitor, assess, evaluate and control their effects in the environment, is one of the most important fields in analytical chemistry [1]. In occupational and environmental assessment, a large number of samples are needed. Consequently, inexpensive and rapid analytical techniques are required [2]. In some efforts to reduce the cost, time and use of organic solvents in the organic trace analysis, many researchers have developed miniature methods, requiring fewer sample amounts and thus, less extracting solvents to carry out their analyses. In addition, new approaches such as solid-phase extraction (SPE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) and solid-phase microextraction (SPME) using polymer-coated fibers have been investigated, demonstrating more promising approaches in the environmental analysis [3]. Other approaches, which are gaining popularity for the sample clean-up, include the immunoaffinity chromatography [4–6] and the molecular imprinting polymers (MIPs).

In the MIP technique, functional monomers and cross-linkers are polymerized in the presence of a template molecule, which is followed by the template removal from the resultant polymer network to leave a template-fitted cavity. The molecular imprinting technology is less expensive than the antibody production and may offer an alternative when the cost of the antibody production is prohibitive or the antibody performance is a problem.

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In addition, the molecular imprinted polymers are highly resistant to the organic solvent effects, unlike antibodies (or other biological receptors). Subsequently, the molecular imprints may have applications to the analysis of highly lipophilic compounds (such as PCBs or pesticides) either in a sample clean-up step or in a detection method [7]. The molecular imprinted polymers for triazinic herbicides have been a subject of many investigations in the recent years with interesting application in chemical analysis [8–11]. MIPs are used for SPE and chromatographic separation [1,12–18].

For a general use of the molecular imprinting technology, the class of the imprintable compounds needs to be extended and the existing recognition elements need to be improved in order to meet the requirements in the given application. The large number of compositional (monomers, cross-linkers, porogenic solvents, template to monomer ratios, etc.) and operational (initiation method, polymerization temperature and time) variables, present even in a relatively simple MIP preparation, coupled with the fact that they are dependent with each other, make it an extremely difficult task to optimize an MIP. In this development, a key factor is the identification and the optimization of the main factors, affecting the material structure and the molecular recognition properties.

The procedural optimization can be achieved in a traditional trial and error manner or with the assistance of chemometrics. Even using combinatorial methods under the best conditions, a few of the compositional variables can be explored. The complexity of these problems makes the application of chemometric methods an ideal opportunity for the design and the evaluation of the MIPs [19]. The chemometric approach is based on the use of an optimum set of experiments (experimental design), which allows the simultaneous variation of all the studied experimental factors [20]. Rather than making every combination in an *n*-dimensional matrix, these methods allow one to vary multiple parameters simultaneously. The utilization of the multivariate analysis of the 'response factor' (e.g. fractional binding of the template) in the polymers, which are synthesized and screened, allows the optimum polymer composition to be predicted. This approach has recently been used in several studies successfully. Navarro-Villoslada et al. [21] used this approach to optimize six key factors in the MIP preparation for the bisphenol A polymers. In that research, the polymers were synthesized as mini-MIPs and screened by HPLC, leading to a rapid and cost-effective identification of the optimized polymers. Kempe and Kempe [22] synthesized beaded polymers by suspension polymerization in mineral oil for their multivariate study and, then, screened for rebinding by radioligand counting methods. In this way, a more extensive screening became feasible and more sophisticated response parameters could be defined, if required using, e.g. competitive binding formats. Davies et al. [23] applied chemometrics to the optimization of highly selective and group selective polymers for sulphonamides. To achieve this aim, they used more sophisticated response factors, based on a multi-analyte competition assay, to estimate the rebinding ability of the synthesized polymers and to select the optimum sulphonamide structures to use as template and for the rebinding studies. The chemometric optimizations of the template/monomer/cross-linker ratios led to the polymers that demonstrated either specificity or group selectivity depending on the experimental design. This methodology can open a new approach for the synthesis of tailor-made phases, called MIPs.

The aim of this work was the optimization of the main factors, affecting the material structure and the molecular recognition properties of the molecular imprinting polymers by a chemometric approach. Triazinic herbicide ametryn was chosen as the model system of this study. The triazinic herbicides are suitable models for these studies because they are relatively inexpensive and stable. As a consequence, the gram quantities necessary in a typical reaction can be handled without extraordinary precautions.

2. Experimental

2.1. Reagents

Ametryn, with greater than 98.2% purity, was obtained from Riedel-de-Häen (Seelze, Germany), 4-vinylpyridine, methacrylic acid (MAA, functional monomer) [79-41-4] and ethylene glycol dimethacrylate (EGDMA, co-monomer) [97-90-5] were purchased from the Merck Company, Germany. 2,2′-Azobisisobutyronitrile (AIBN, initiator) [78-67-1] was obtained from the Acros Company, USA. All solvents (acetic acid, acetonitrile, dichloromethane and methanol) were of analytical reagent grade (Merck, Germany). Ultra pure water was obtained from a Purite Purification System. Stock standard solutions ($1\,g\,L^{-1}$) were prepared by weighing the solutes, their dissolution in acetonitrile and their storage at $-18\,^{\circ}C$. The standard solutions were obtained with the dilution of the stock solutions in acetonitrile.

2.2. Equipment

All measurements were performed by a reversed-phase HPLC system from the Knauer Company (Germany), consisting of a K-1001 series high-pressure pump, a K-2006 photo diode-array detector and a VS injection valve, equipped with a 20-μL loop. The analytes were separated on a Chromolith Performance RR-C_{18}e 100 mm × 4.6 mm i.d. (Merch KGa A, Germany) and column guards (Chromolith Guard Cartridge Kit RP-C_{18}e and 5 cm × 4.6 mm i.d., 5 μm), using isocratic elution as follows: 60% acetonitrile and 40% purified water. Ametryn was monitored at 220 nm and quantified with external calibration using the peak area measurements ($R^2 = 0.9998$). Each sample was repeated three times to assure the chromatogram reproducibility. The flow-rate was set at $0.8\,mL\,min^{-1}$. The system was linked with a LaserJet 1200 series printer for recording the chromatograms, using a 1456-1 Chromogate Data System, Version 2.55. For the polymer synthesis, the used apparatus included soxhlets and a heater unit, a liquid extraction unit (S&S, Germany), a reactor heater system (Memmert, Germany), a nitrogen supply system, an ultrasonic shaker (Tecna-6, Italy), a syringe-filtration unit (FH-0.45 μm, Millipore Corp., USA), PTFE filters (0.2 μm, Sartorius, Germany),