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Water compatible stir-bar devices imprinted with underivatised glyphosate for selective sample clean-up

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ABSTRACT

This paper reports the development of stir bars with a new MIP based coating, for the selective sorptive extraction of the herbicide glyphosate (GLYP). Molecular imprinting of the polymer has directly been carried out employing underivatised GLYP as the template molecule. Due to the poor solubility of the target compound in organic solvents, the MIP methodology has been optimised for rebinding in aqueous media, being the synthesis and the rebinding steps carried out in water:methanol mixtures and pure aqueous media. The coating has been developed by radical polymerisation initiated by UV energy, using *N*-allylthiourea and 2-dimethyl aminoethyl methacrylate as functional monomers and ethylene glycol dimethacrylate as the cross-linker. Mechanical stability of the coating has been improved using 1,3-divinyltetramethyldisiloxane in the polymerisation mixture. Under the optimised conditions, the MIP has demonstrated excellent selectivity for the target compound in the presence of structural analogues, including its major metabolites. The applicability of the proposed method to real matrices has also been assessed using river water and soil samples. Registered mean recoveries ranged from 90.6 to 97.3% and RSD values were below 5% in all cases, what confirmed the suitability of the described methodology for the selective extraction and quantification of GLYP.

1. Introduction

Glyphosate (GLYP) is an organophosphorus, broad-spectrum, non selective herbicide that has been worldwide employed during the last decades for both urban and agricultural uses [1,2]. It is a very polar compound, non-volatile and resistant to photodegradation. In the environment, it is mainly catabolised by soil microorganisms to aminomethylphosphonic acid (AMPA) and glyoxylate through the enzyme glyphosate oxidoreductase [3]. AMPA is known to be the principal metabolite of GLYP, however, there are other bacteria having enzymes capable of cleaving the C–P bond of the herbicide and thus producing other metabolites such as sarcosine [3].

The herbicide effect of GLYP occurs via the shikimate pathway which is present in plants but not in animals, this compound thereby exhibiting low toxicity to the latter [4]. Nevertheless, it has been found that GLYP-based herbicides can potently affect the cardiovascular system in mammals; adjuvants present with GLYP in pesticide formulations may be primarily responsible for this effect [5]. In contrast, GLYP based formulations do not appear to present significant genotoxic risk after exposure to normal doses [6].

GLYP and AMPA have been included in Annex III of the 2008/105/EC Directive on environmental quality standards in the field of water policy as "substances subject to review for possible identification as priority substances or priority hazardous substances". The maximum acceptable concentration for glyphosate in drinking water varies according to the countries and legislations: 280 μg/L in Canada [7], 0.7 mg/L USEPA [8] or 0.1 μg/L in Spain [9]. There seems to be no doubt that, in order to determine these targets at these trace levels, sensitive analytical methods are required.

The analytical determination of GLYP and AMPA can be considered particularly challenging mainly due to characteristics related with their chemical nature as, for instance, their high polarity, insolubility in most organic solvents, small molecular size [10] and amphoteric behaviour [11]. Moreover, the lack of chromophores or fluorophores in their chemical structure renders impossible their determination by optical techniques [12]. Accordingly, in order to enable UV or fluorescence detection, most of chromatographic methods that focus on these targets include derivatisation steps with reagents such as 9-fluorenylmethyloxycarbonyl chloride (FMOC-Cl) [13], *o*-phthalaldehyde (OPA), 2-mercaptoethanol

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(MCE) or *p*-toluenesulfonyl chloride (PTSCl) among others [10,14,15]. Both gas and liquid chromatography have been employed for the analysis of these pesticides, however, owning to their low volatility, GC techniques always require a previous derivatisation procedure in order to get sufficiently volatile and thermally stable compounds to be detected by mass spectrometry [16,17]. During the last decades, LC–MS has been mainly employed for the analysis of GLYP and AMPA in food [18,19], alcoholic beverages [20], environmental [4,21–23] and biological samples [14,24]. Even if LC–MS is employed, derivatisation of GLYP continues being considerably beneficial not only for a better separation of target compounds by reversed-phase chromatography, but also for analyte preconcentration in sample pretreatment [21]. In any case, direct determination of underivatised GLYP has also been performed by hydrophilic interaction chromatography (HILIC) using a zwitterionic type mixed-mode Obelisc N stationary phase [18,25]. This column allows for the chromatographic retention and subsequent analysis of GLYP without the need of any derivatisation procedure.

In most cases, prior to GLYP and AMPA analysis, preconcentration and clean-up steps are highly desirable [2,12]. In some instances, matrix complexity leads to making the decision of using a clean-up step. In this regard, soils with high organic matter content [21] natural water [26] and drinking water samples [27], have, for instance, been pretreated with different SPE sorbents in order to minimise matrix effect. Due to the ionic character of the target, most frequently, commercial cartridges based on anion exchange resins have been employed to directly extract GLYP [27,28], whereas apolar/reversed phase cartridges have been used for the extraction of the derivatised compound [4,29–32]. More recently, other materials such as TiO_2 or ZrO_2 have been studied as selective sorbents for phosphonic group containing herbicides [12,33]. Moreover, due to its adsorption characteristics, TiO_2 has also successfully been employed as binding phase for passive sampling of GLYP and AMPA in an aquatic environment [1]. Additionally, imprinted polymers have also been reported to be promising materials as sorbents [34,35], sensors [36] or passive samplers [37] of these targets with increased selectivity.

The main goal of the present work was to develop a highly selective material capable of extracting GLYP in order to ease and simplify the extraction and clean-up steps from complex matrices. In this regard, a sorbent based on a molecularly imprinted polymer (MIP) would be highly desirable since it could meet the pursued objective. Here, in order to simplify the extraction step, a MIP has been coated on the surface of a magnetic stir-bar to selectively extract underivatised GLYP from aqueous extracts. This device has been designed to be compatible with water environments, minimising non-specific hydrophobic interactions thereby increasing extraction selectivity.

2. Materials and methods

2.1. Chemicals and reagents

The cross-linking monomers ethylene glycol dimethacrylate (EDMA), triethylene glycol dimethacrylate (TriEDMA), diethylene glycol diacrylate (DEDA), trimethylolpropane trimethacrylate (TRIM), the initiator benzophenone and the functional monomers acrylamide (AA), acrylic acid (AAc), 2-dimethyl aminoethyl methacrylate (DMAEM), 2-diethyl aminoethyl methacrylate (DEAEM), ethylene glycol methacrylate phosphate (EGMP) and *N*-allylthiourea (ATU) were purchased from Sigma-Aldrich (Madrid, Spain). Polyethylene glycol (PEG, average MW 20,000) and 1,3-divinyltetramethyldisiloxane (DVTD) were obtained from Alfa-Aesar (Barcelona, Spain).

The analytical standard of Glyphosate (GLYP; 98%) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) while aminomethyl phosphonic acid (AMPA; 99.5%) was acquired from Chemical Service (West Chester, USA). The other standards, that is, gluphosinate (GLU; 98.5%), glycine (GLY; 99.8% certified) and sarcosine (SAR; 98%) were purchased from Sigma-Aldrich.

9-Fluorenylmethyloxycarbonyl chloride (FMOC-Cl, 99.5%) was used for GLYP derivatisation and it was acquired from Sigma-Aldrich. FMOC solutions of 1000 mg L^{-1} were weekly prepared in HPLC grade ACN (Scharlab, Spain) and stored at −42 °C. All other reagents and solvents were analytical or HPLC grade and they were used as received. Every buffer solution was prepared with ultrapure water obtained from Elix20 reverse osmosis and Milli-Q water purification systems.

2.2. Polymer syntheses

The polymerisation mixtures were prepared dissolving 0.142 g of GLYP and the functional monomer(s) in the corresponding porogen amount (MeOH:H_2O, 90:10, v/v) (Table 1). Then, the crosslinker and the initiator benzophenone (2% wt.) were added and the mixture was purged with nitrogen for 5 min. Photochemical polymerisation was performed for 30 min irradiating the polymerisation mixture with a UV lamp (100 W) model UVAcube 100 from Dr. Hönle UV-technology (Gräfelfing, Germany). Non Imprinted Polymers (NIP) were synthesised using the same protocol but in the absence of the template in the polymerisation mixture. The resulting polymers were ground and wet-sieved in MeOH/water and subsequently washed with water to collect particles between 25 and 50 μm.

2.3. Binding capacity experiments

For binding capacity experiments, the synthesised polymers were packed first in HPLC stainless steel columns of 100 mm in

Table 1
Composition of polymerisation mixtures.

Polymer	Functional monomer(s) (mass)	Cross linker(mass)	T:FM:CL(ratio)	Porogen(% wt.)	Initiator(mass)
MIP-1	DEAEM (0.467 g)	EDMA (2.0 g)	1:3:12	100%	0.05 g
MIP-2	DMAEM (0.397 g)	EDMA (2.0 g)	1:3:12	100%	0.05 g
MIP-3	DMAEM (0.264 g) + ATU (0.191 g)	EDMA (2.0 g)	1:4:12	100%	0.05 g
MIP-4	DMAEM (0.529 g)	EDMA (2.0 g)	1:4:12	100%	0.05 g
MIP-5	DMAEM (0.264 g) + ATU (0.191 g)	EDMA (2.0 g)	1:4:12	50%	0.05 g
MIP-6	DMAEM (0.183 g) + ATU (0.133 g)	TriEDMA (2.0 g)	1:4:12	50%	0.05 g
MIP-7	DMAEM (0.245 g) + ATU (0.177 g)	DEDA (2.0 g)	1:4:12	50%	0.05 g
MIP-8	DMAEM (0.245 g) + ATU (0.177 g)	DEDA (2.0 g)	1:4:12	100%	0.05 g
MIP-9	DMAEM (0.264 g) + ATU (0.191 g)	EDMA (2.0 g)	1:4:12	150%	0.05 g
MIP-10	DMAEM (0.264 g) + ATU (0.191 g)	EDMA (2.0 g)	1:4:12	200%	0.05 g
MIP-11	DMAEM (0.397 g) + ATU (0.287 g)	EDMA (2.0 g)	1:4:8	100%	0.05 g
MIP-12	DMAEM (0.198 g) + ATU (0.144 g)	EDMA (2.0 g)	1:4:16	100%	0.05 g
MIP-13	DMAEM (0.132 g) + ATU (0.096 g)	EDMA (2.0 g)	1:4:24	100%	0.05 g