

# Synthesis of caffeic acid and *p*-hydroxybenzoic acid molecularly imprinted polymers and their application for the selective extraction of polyphenols from olive mill waste waters

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## Abstract

Using caffeic acid and *p*-hydroxybenzoic acid as templates, two molecularly imprinted polymers (MIPs) were prepared that were used for isolation of polyphenols from olive mill waste water samples (OMWWs) without previous pre-treatment. For the preparation of the caffeic acid MIPs 4-vinylpyridine, allylurea, allylaniline and methacrylic acid were tested as functional monomers, ethylene glycol dimethylacrylate (EDMA), pentaerythritol trimethylacrylate (PETRA) and divinylbenzene 80 (DVB80) as cross-linkers and tetrahydrofuran as porogen. For *p*-hydroxybenzoic acid 4-vinylpyridine, allylurea and allylaniline were tested as functional monomers, EDMA and PETRA as cross-linkers and acetonitrile as porogen. The performance of the synthesized polymers was evaluated against seven structurally related compounds by means of polymer-based HPLC. The two polymers that presented the most interesting properties were further evaluated by batch rebinding and from the derived isotherms their capacity and binding strength were determined. Using solid-phase extraction (SPE), their ability to recognize and bind the template molecule from an aqueous solution as well as the pH dependence of the binding strength were explored. After establishing the best SPE protocol, an aqueous model mixture of compounds and a raw OMWWs sample were loaded on the two best polymers. The result of the consecutive use of the two polymers on the same sample was explored. It was concluded that acidic conditions favour the recognition abilities of both polymers and that they can be used for a quick and efficient isolation of the polyphenol fraction directly from raw OMWW.

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## 1. Introduction

The need for separation of specific compounds from complex mixtures, industrial or biological, has led to an increase in the synthesis and use of molecularly imprinted polymers (MIPs), which in fact act as biomimetic materials [1]. The synthesis of a MIP is based on the polymerization of a certain monomer, containing functional groups complementary to the one present on the target molecule, thus forming adequately strong covalent or non-covalent bonds. A cross-linker is added to co-polymerize

with the monomer and produce a rigid polymer network with the desired physicochemical properties. The polymerization takes place in solution, usually initiated by a free radical initiator. After the polymerization is complete the template is removed under mild extraction conditions (e.g. Soxhlet extraction using a polar solvent) and the cavities left are complementary to the template in terms of size, shape and functionality, thereby serving as recognition sites for the template used.

MIPs have been extensively used for the imprinting of pharmaceuticals, pesticides, carbohydrates, peptides and other molecules of biological interest [2]. Comparatively little attention has been paid on phenolic compounds, which are nonetheless of great interest for both food and pharmaceutical industry, mainly because of their antioxidant properties. At the same time, they are among the main pollutants found in liquid waste of the food industry (e.g. wine production, olive

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oil production, etc.). They are water soluble and when found in increased concentrations present phytotoxic properties [3,4].

Among the wide group of polyphenols, *p*-hydroxybenzoic acid has attracted some attention and has been successfully imprinted [5–8] using 4-vinylpyridine, acrylamide and methacrylic acid. Protocatechuic acid has also been used as template for the synthesis of an acrylamide-based MIP for the separation of structurally related compounds from plant material [9]. As far as caffeic acid is concerned, a MIP using methacrylic acid as monomer has been synthesized but it aimed to the separation and purification of the structurally related chlorogenic acid [10]. Except for the work of Dmitrienko et al. [6], where the recognizing ability of the polymer was evaluated in aqueous solutions, the rest of the cited publications refer to the behaviour of the polymer in an organic solvent, mainly acetonitrile. Even though in all of the above works successful imprinting of phenolic compounds has been proved plausible, the authors feel the need of further testing such polymers under aqueous conditions given the polyphenols' high occurrence in aqueous waste. Their efficient removal from aqueous waste is of great environmental interest and at the same time of economical value since these type of materials can be reused many times without significant loss of properties.

The present paper deals with the synthesis of MIPs using caffeic acid and *p*-hydroxybenzoic acid as template molecules. More emphasis has been put on the synthesis of an effective caffeic acid MIP, since *p*-hydroxybenzoic acid MIPs have been more extensively investigated, as mentioned above. The produced MIPs were evaluated for their efficiency in recognizing the template molecules primarily in an organic solvent by means of polymer-based HPLC and secondly in an aqueous environment by means of SPE. Especially, their recognition ability in an aqueous environment was assessed by using a mixture of seven structurally related compounds. Finally, raw olive mill waste water samples were also applied on the polymers in order to evaluate their efficiency towards such a complex aqueous mixture. Summarizing, the goal was to propose an alternative method to conventional liquid–liquid extraction used so far, for the isolation of polyphenols from olive mill waste waters.

## 2. Experimental

### 2.1. Materials

Gallic acid (GA), *p*-hydroxybenzoic acid (*p*-HBA), vanillic acid (VA), caffeic acid (CA) and vanillin (V) were of HPLC grade and were purchased from Fluka (Buchs, Switzerland). Tyrosol and veratric acid were a kind donation of Laboratory of Agroindustrial Chemistry, INP (Toulouse, France). 4-Vinylpyridine (4-VPy), methacrylic acid (MAA), allylurea and allylaniline monomers along with EDMA and DVB80 cross-linkers were provided by Sigma–Aldrich. PETRA cross-linker was purchased from Fluka. Azo-bis-dimethylvaleronitrile (ABDV) initiator was purchased from Wako (Neuss, Germany). Water (HPLC grade), methanol (MeOH; HPLC grade), acetonitrile (MeCN; HPLC grade) and tetrahydrofuran (THF; HPLC grade) were purchased from Merck (Darmstadt, Germany). Tri-

ethanolamine (TEA), ethyl acetate (EtOAc), acetic acid and phosphoric acid 85%, all of analytical grade, were provided by Fluka. For the HPLC evaluation of the polymers system, a system comprising of two LC-10ADVP Shimadzu HPLC pumps controlled by a SCL-10AVP Shimadzu pump controller (Kyoto, Japan), a manual Rheodyne injector with a 20  $\mu$ L loop (Cotati, CA, USA), a column oven and a Shimadzu UV-diode array detection (UV-DAD) system, model SPD-M6A were used. For data collection and peak area calculations the software Class-LC10 (Shimadzu) was used. HPLC evaluation was performed at 25 °C at a flow rate of 1 mL/min and the detector was set at 254, 280 and 325 nm. Each compound was injected at least twice. MeCN with 0.05% CH<sub>3</sub>COOH was used as elution solvent, in order to slightly reduce the long retention times. Acetone 1 mM in MeCN was used as void marker.

### 2.2. Synthesis of MIPs

Seven caffeic acid and four *p*-hydroxybenzoic acid imprinted polymers were synthesized. The composition of the pre-polymerization mixtures is described in Table 1. In all cases, the pre-polymerization mixture was dissolved in the porogen, which was THF for the caffeic acid-based MIPs and MeCN for the *p*-hydroxybenzoic acid MIPs, and was placed in a thick-walled glass tube with a narrow neck. The mixture was emerged in an ice-bath and purged with nitrogen gas for 5 min in order to remove oxygen and establish inert supernatant atmosphere. Afterwards, the glass tube was hermetically sealed, placed in a water bath at 40 °C and left for 24 h for the polymerization to proceed. The glass tubes were smashed for the removal of the polymer monolith. The collected polymers were slightly ground and subjected to Soxhlet extraction with MeOH in order

Table 1  
Composition of the produced polymers (ABDV 1%, w/w was used as the free radical initiator)

Polymer	Template (T)	Monomer (M)	Cross-linker (C)	Ratio T:M:C
MIP 1	CA	4-VPy	EDMA	1:4:20
NIP 1	–	4-VPy	EDMA	1:4:20
MIP 2	CA	4-VPy	PETRA	1:4:12
NIP 2	–	4-VPy	PETRA	1:4:12
MIP 3	CA	4-VPy	DVB80	1:4:20
NIP 3	–	4-VPy	DVB80	1:4:20
MIP 4	CA	Allylurea	PETRA	1:4:12
NIP 4	–	Allylurea	PETRA	1:4:12
MIP 5	CA	MAA	PETRA	1:4:12
NIP 5	–	MAA	PETRA	1:4:12
MIP 6	CA	MAA	EDMA	1:4:20
NIP 6	–	MAA	EDMA	1:4:20
MIP 7	CA	Allylaniline	EDMA	1:4:20
NIP 7	–	Allylaniline	EDMA	1:4:20
MIP 8	<i>p</i> -HBA	4-VPy	EDMA	1:4:20
NIP 8	–	4-VPy	EDMA	1:4:20
MIP 9	<i>p</i> -HBA	4-VPy	PETRA	1:4:12
NIP 9	–	4-VPy	PETRA	1:4:12
MIP 10	<i>p</i> -HBA	Allylurea	PETRA	1:4:12
NIP 10	–	Allylurea	PETRA	1:4:12
MIP 11	<i>p</i> -HBA	Allylaniline	EDMA	1:4:20
NIP 11	–	Allylaniline	EDMA	1:4:20

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