INSTITUTO POLITÉCNICO DE BRAGANÇA
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Preparation of Molecularly Imprinted Polymers with Boronate Affinity and Their Application for the Valorization of Winemaking Residues

Ilyes Chouari

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Supervisors:

Professor Rolando Dias

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I dedicate this work to my family, for always helping and supporting my decisions.

ABSTRACT

In recent years, there has been growing interest in finding innovative and sustainable solutions for managing agricultural and food industry waste products. Winemaking, a prominent sector of the food and beverage industry, generates substantial residues and byproducts that often pose disposal challenges. These residues, including grape skins, seeds, and pomace, contain valuable components such as polyphenols, tannins, and organic acids, which, if properly extracted and utilized, could lead to economic and environmental benefits. This study explores a novel approach to address this challenge by employing molecularly imprinted polymers (MIPs) with boronate affinity for the selective extraction and separation of these valuable compounds from winemaking residues.

MIPs are synthetic polymers designed to exhibit selective binding capabilities towards specific target molecules. The choice of boronate-based templates in the MIP synthesis process is crucial, as boronate functional groups have shown remarkable affinity for polyphenolic compounds, which are abundant in winemaking residues. The preparation of MIPs involves the polymerization of functional monomers in the presence of a template molecule, forming cavities in the polymer matrix that are complementary in shape and chemical functionality to the target compound. Subsequent removal of the template leaves behind imprinted sites that specifically recognize and bind the desired molecules.

The MIPs developed in this study demonstrate a high degree of selectivity and affinity for polyphenols, tannins, and organic acids commonly found in winemaking residues. This selective binding property enables efficient extraction and separation of these valuable components from the complex matrix of winemaking waste. The process of using MIPs to recover these compounds not only contributes to the valorization of winemaking residues but also aligns with the principles of green chemistry by reducing waste generation and minimizing the use of hazardous chemicals.

The valorization of winemaking residues through MIP-based extraction processes offers numerous potential applications. Polyphenols extracted from these residues can find use in the food and beverage industry as natural antioxidants, colorants, and flavor enhancers. Additionally, tannins extracted from grape seeds can be employed in the tanning industry, while organic acids can be repurposed for various industrial applications, including pharmaceuticals and cosmetics.

Furthermore, the utilization of MIPs in winemaking residue valorization contributes to the sustainability of the wine industry, reducing its environmental

footprint by diverting waste from landfills and incineration. The recovered valuable compounds can generate additional revenue streams for wineries, thereby improving economic viability. Moreover, this approach aligns with global sustainability initiatives by promoting circular economy practices and reducing the environmental impact associated with waste disposal.

In conclusion, the development and application of boronate-affinity MIPs for the valorization of winemaking residues represent a promising and sustainable approach to address waste management challenges in the wine industry. This innovative strategy enables the efficient extraction and separation of valuable components from winemaking waste, creating opportunities for economic gains and environmental benefits. The integration of MIP technology in the valorization of agricultural and food industry byproducts underscores the importance of research-driven solutions in advancing sustainable practices across various sectors.

This expanded abstract highlights the significance and potential impact of the study, providing a comprehensive overview of the research's objectives, methods, and potential applications

Resumo

Nos últimos anos, tem havido um crescente interesse em encontrar soluções inovadoras e sustentáveis para o gerenciamento de resíduos da indústria agrícola e alimentícia. A produção de vinho, um setor proeminente da indústria de alimentos e bebidas, gera resíduos e subprodutos substanciais que muitas vezes representam desafios de descarte. Esses resíduos, incluindo cascas de uva, sementes e bagaço, contêm componentes valiosos como polifenóis, taninos e ácidos orgânicos, que, se extraídos e utilizados corretamente, podem trazer benefícios econômicos e ambientais. Este estudo explora uma abordagem inovadora para enfrentar esse desafio, utilizando polímeros molecularmente impressos (MIPs) com afinidade por boronato para a extração seletiva e separação desses compostos valiosos dos resíduos da produção de vinho.

Os MIPs são polímeros sintéticos projetados para exibir capacidades seletivas de ligação com moléculas-alvo específicas. A escolha de modelos baseados em boronato no processo de síntese de MIPs é crucial, pois os grupos funcionais de boronato têm mostrado afinidade notável por compostos polifenólicos, que são abundantes nos resíduos da produção de vinho. A preparação de MIPs envolve a polimerização de monômeros funcionais na presença de uma molécula-modelo, formando cavidades na matriz polimérica que são complementares em forma e funcionalidade química ao composto-alvo. A remoção subsequente do modelo deixa sítios impressos que reconhecem e se ligam especificamente às moléculas desejadas.

Os MIPs desenvolvidos neste estudo demonstram um alto grau de seletividade e afinidade por polifenóis, taninos e ácidos orgânicos comumente encontrados nos resíduos da produção de vinho. Essa propriedade de ligação seletiva permite a extração e separação eficientes desses componentes valiosos da matriz complexa dos resíduos da produção de vinho. O processo de usar MIPs para recuperar esses compostos não apenas contribui para a valorização dos resíduos da produção de vinho, mas também está alinhado com os princípios da química verde, reduzindo a geração de resíduos e minimizando o uso de produtos químicos perigosos.

A valorização dos resíduos da produção de vinho por meio de processos de extração baseados em MIPs oferece inúmeras aplicações potenciais. Os polifenóis extraídos desses resíduos podem ser utilizados na indústria de alimentos e bebidas como antioxidantes naturais, corantes e realçadores de sabor. Além disso, os taninos extraídos das sementes de uva podem ser utilizados na indústria de curtumes, enquanto os ácidos orgânicos podem ser reaproveitados para diversas aplicações industriais, incluindo farmacêuticos e cosméticos.

Além disso, a utilização de MIPs na valorização de resíduos da produção de vinho contribui para a sustentabilidade da indústria vinícola, reduzindo sua pegada ambiental ao desviar resíduos de aterros e incineração. Os compostos valiosos recuperados podem gerar receitas adicionais para as vinícolas, melhorando assim a viabilidade econômica. Além disso, essa abordagem está alinhada com iniciativas globais de sustentabilidade, promovendo práticas de economia circular e reduzindo o impacto ambiental associado ao descarte de resíduos.

Em conclusão, o desenvolvimento e a aplicação de MIPs com afinidade por boronato para a valorização de resíduos da produção de vinho representam uma abordagem promissora e sustentável para enfrentar os desafios de gestão de resíduos na indústria vinícola. Essa estratégia inovadora permite a extração e separação eficientes de componentes valiosos dos resíduos da produção de vinho, criando oportunidades para ganhos econômicos e benefícios ambientais. A integração da tecnologia MIP na valorização de subprodutos da indústria agrícola e alimentícia destaca a importância de soluções baseadas em pesquisa no avanço de práticas sustentáveis em diversos setores.

Este resumo expandido destaca a importância e o impacto potencial do estudo, fornecendo uma visão abrangente dos objetivos, métodos e aplicações potenciais da pesquisa.

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Abbreviation List

MIP: Molecularly imprinted polymer

NIP: Non imprinted polymer

SPE: Solid phase extraction

FRP: Free radical polymerization

CRP: Controlled radical polymerization

UV: Ultraviolet

PDMS: Poly(dimethylsiloxane)

FTIR: Fourier Transform Infrared Spectroscopy

SEM: Scanning electron microscopy

BET: Brunauer–Emmett–Teller

SLS: Statistic Light Scattering

TEM: Transmission electron microscopy

(4VBA): 4-vinylphenyl boronic acid

EGDMA: Ethylene glycoldimethacrylate

AIBN: Azobis(isobutyronitrile)

MIP_CA: Molecularly imprinted polymer Caffeic

MIP_Cat: Molecularly imprinted polymer Catechol

MIP_FA: Molecularly imprinted polymer Ferulic Acid

NIP_CA: No binding imprinted polymer Caffeic

NIP_Cat_FA: No binding imprinted polymer Catechol and Ferulic Acid

I. Objectives

This research aims at the synthesis of Molecularly Imprinted Polymers (MIPs) with boronate affinity via free radical polymerization of 4-vinylphenylboronic acid with crosslinkers (e.g. ethylene glycol dimethacrylate, in the presence of different template molecules, namely Ferulic acid, Caffeic acid and Catechol, that are often found in wine and winemaking residues. Thermal polymerization will be used for the preparation of these MIPs.

The use of other functional monomers jointly with the 4-vinylphenylboronic acid will be explored. This approach will enhance the specific interactions of the MIPs with the cis-diol compounds present in winemaking residues (e.g. diatomaceous earth). The final goal is the efficient valorization of these winemaking residues through the purification and fractionation of polyphenols which will be used later in cosmetic, food or pharmaceutical industries.[1], [2]

II. State of the Art

II.1 Generalities on molecularly imprinted polymers

Molecularly imprinted polymers (MIPs) are synthetic materials that mimic natural antigen–antibody mechanisms. The operational mechanism of MIP to rebind the template molecule is similar to that of an antibody when recognizing a specific antigen in the biological system.

These materials are prepared using functional monomer(s), crosslinker(s), a template and an initiator. This latter sparks the polymerization reaction between the monomer(s) and the cross-linker(s) around the template molecules, leading to a highly cross-linked three-dimensional network polymer.[3]

After washing the resulting material with adequate solvents (usually able to break the specific bonds established with the template), specific cavities embedded in the polymeric network are obtained. These cavities possess a complementary shape and size to the target analyte. In essence, a molecular memory is imprinted in the polymer, which is now capable of rebinding[4]

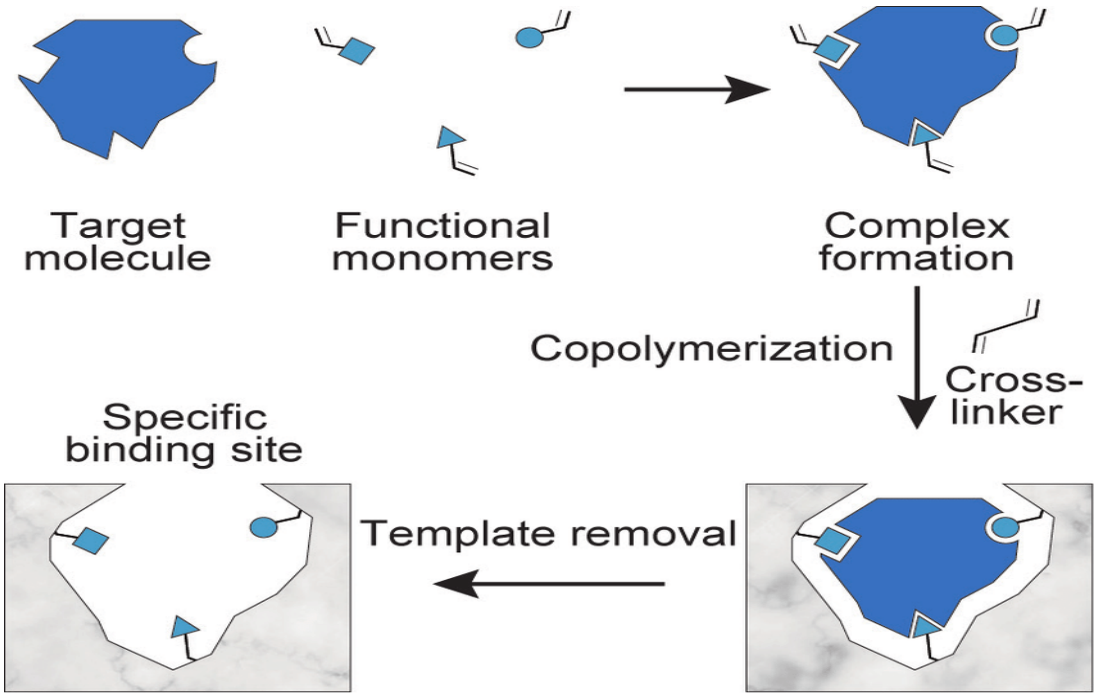


Figure 1: Molecularly imprinted polymer synthesis (adopted from International journal of molecular sciences ISSN 1422-0067)

Synthesized MIPs are stable and show resistance to a wide range of pH values, temperatures, and solvents. Additionally, MIPs are able to interact with the target molecule in a selective way. These materials have several advantages, such as the relatively ease of preparation and high thermal and chemical stabilities. MIPs have been used for a wide range of analytes, including pharmaceuticals drugs, emergent pollutants, agri-food products, virus, protein biomarkers, to mention a few[5]

II.1.1 History

MIPs first gained attention in 1993 when they were given the moniker of "antibody mimics", and ever since, they have been extensively researched for their potential to extract or trap chemical contaminants, to use in immunological assays, and to create sensors.

A close examination of Chemical Abstracts has revealed that the first time the term “imprinted polymer” was reported specifically in a paper that dates to 1984. The paper was written by K. Mosbach and B. Sellergen. However, it is worth noting that since 1973, Wulff had been publishing papers within a series titled “Enzyme-Analog Built”.

The major difference between The Mosbach group and Wulff resides in the fact that the former focused on the use of non-covalent interactions between the host and target, while the latter had a tendency to use covalent binding to generate or create the imprint. It is safe to say that the distinction between the two methods is obvious in the chemistry required to remove the templating molecules from the MIP.

MIP technologies were founded in Sweden based on Sellergen and Mosbach as the principals. It was later bought by a purifications-separations firm called Biotage AB. MIP Diagnostics was founded Piletsky K. Haupt, who was known for having published extensive work in the field of MIP [6]

II.1.2 Mechanisms of interaction

There are two types of molecularly imprinted interactions: covalent and non-covalent interactions. The molecular imprinting process is often based on the covalent and non-covalent attachment of appropriate monomers with functional groups to template molecules.[3], [7]

The fundamental ideas of the molecularly imprinted polymer structure were developed primarily through covalent interactions. In covalent interactions, the binding sites can be used in a stoichiometric ratio to the template molecule, however in non-covalent interactions, the binding constants are typically quite low, necessitating the presence of the binding site monomers in significant excess for the imprinting process[8]

The covalent bonding properties of the boronic acid groups are excellent. Commercially available 4-vinylphenyl boronic acid polymers are used in alkaline aqueous solution selective affinity chromatography to separate substances containing cis-diols. In water or alcohol, the boronic ester moieties can be easily split apart. The template can be removed from an imprinted microporous polymer in this normally quick reaction[9]

II.1.3 Production pathways of molecularly imprinted polymer

II.1.3.1 Free radical polymerization

Free radical polymerization (FRP) is a method of formation of polymers using free radicals generated in chemical reactions. The method is mainly used to synthesize polymers from monomers containing carbon double bonds**.** Free radical polymerization is one of the most useful and lucrative fields of chemistry ever discovered. [10]Recent years have seen a

tremendous increase in research in this area. Some FRP examples include emulsion and suspension polymerizations which are widely used on an industrial scale[11]

FRP is the most employed method to synthesize MIPs. FRP can be performed in a wide range of temperatures since it can be initiated thermally as well as photochemically. Additionally, it can be carried out in solution or in bulk and it is not water sensitive[12]. Moreover, it is very tolerant to functional groups in the monomers. FRP involves three distinct stages: Initiation, propagation, and termination. These stages can be explained as follows:

- Initiation: the initiator decomposes generating free radicals, which are very active because they have unpaired electrons.
- Propagation: once the initiating radical are formed through the initiation reaction, the propagation step takes place. These radicals attack the monomers which in turn attack other monomer molecules transferring its active center to the attacked molecule. This process leads to the chain growth of the polymer.
- Termination: At this point, the chains of large molecules stop growing, and the polymerization comes to an end when the active center is disabled.

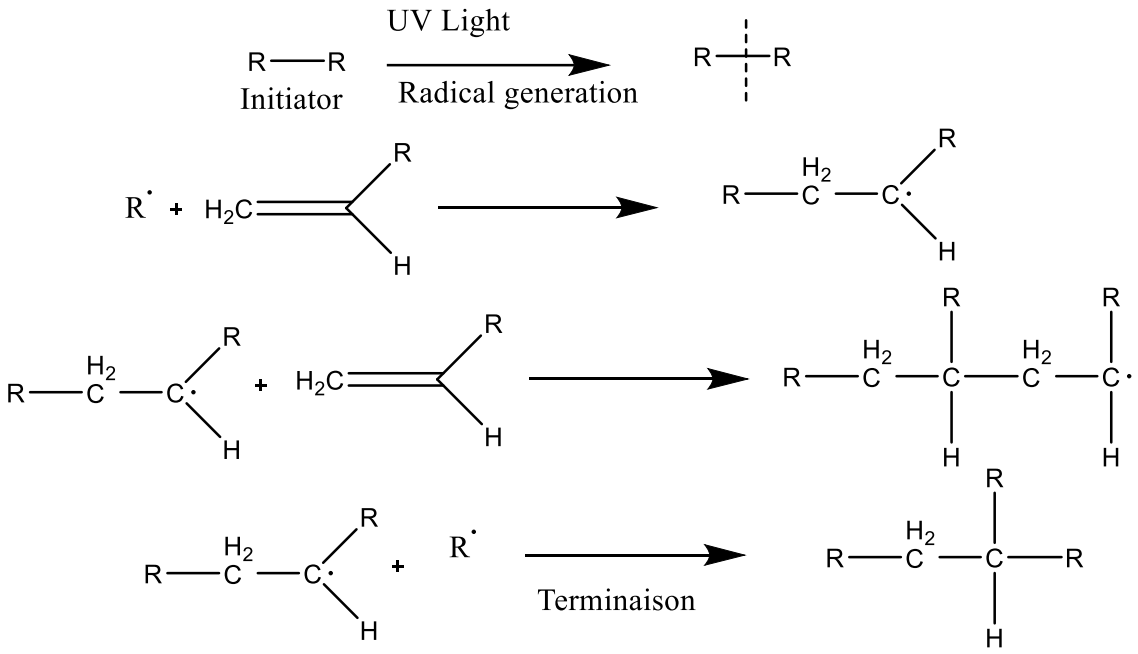


Figure 2: Free radical polymerization (FRP).

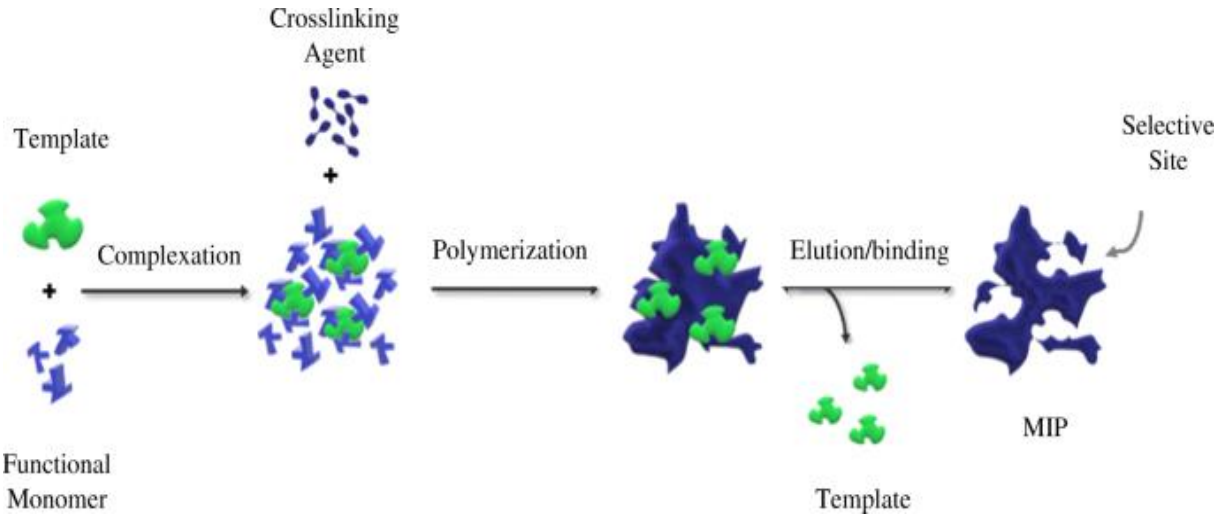


Figure 3: Synthesis steps of a MIP (adopted from: a historical perspective and the development of molecular imprinting polymer)

II.1.3.2 Controlled radical polymerization:

The molecular mass of synthetic polymers may be reliably controlled using controlled radical polymerization (CRP), which also offers numerous opportunities for molecular design, such as the creation of hybrid nanoscale polymeric structures. CRP consists in a reversible reaction of the polymer growth radical with fragments of the initiator or a special additive (X) for the quadratic chain termination (interaction of P.n + P.m). This reaction results in a macromolecule with a labile terminal group (Pn-X) that can dissociate when exposed to heat or radiation or when interacting with another active species. The chain growth is continued by the initial macroradical, which is regenerated[13] CRP processes can be divided into three main types:

1. Dissociation-recombination processes: Stable Free Radical Polymerization (SFR).
2. Degenerate chain transfer: Reversible Addition Fragmentation Chain Transfer (RAFT).
3. Atom transfer: Atom Transfer Radical Polymerization (ATRP).

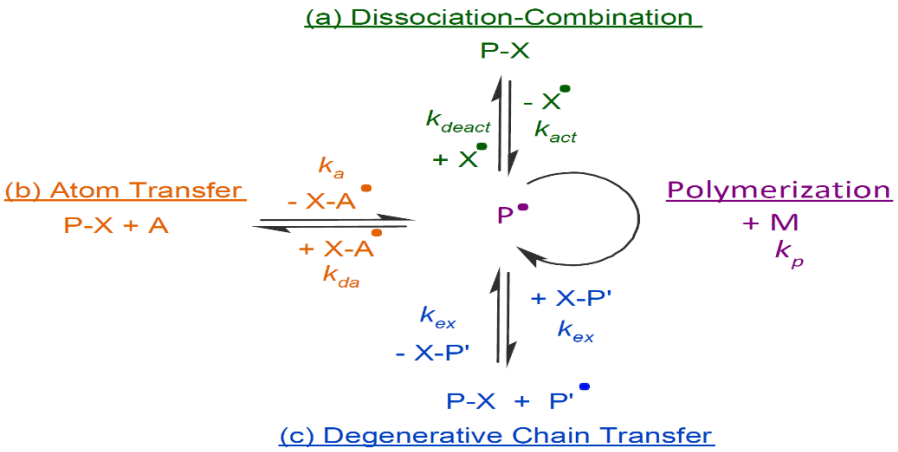


Figure 4: Main steps of controlled radical polymerization (adopted from V. Mishra and R. Kumar, *Journal* of Scientific Research, Vol. 56, 141-176)

II.1.3.3 Other production methods

Soft lithography

This technique, which aims to create a surface-imprinted substance, is typically used to detect big molecules that would be prevented from entering cavities beneath the surface. The soft lithography is both inexpensive and appropriate for biological applications. An inorganic siloxane backbone and organic methyl groups connected to silicosiloxanes give poly(dimethylsiloxanes) (PDMS) a special set of characteristics[14].

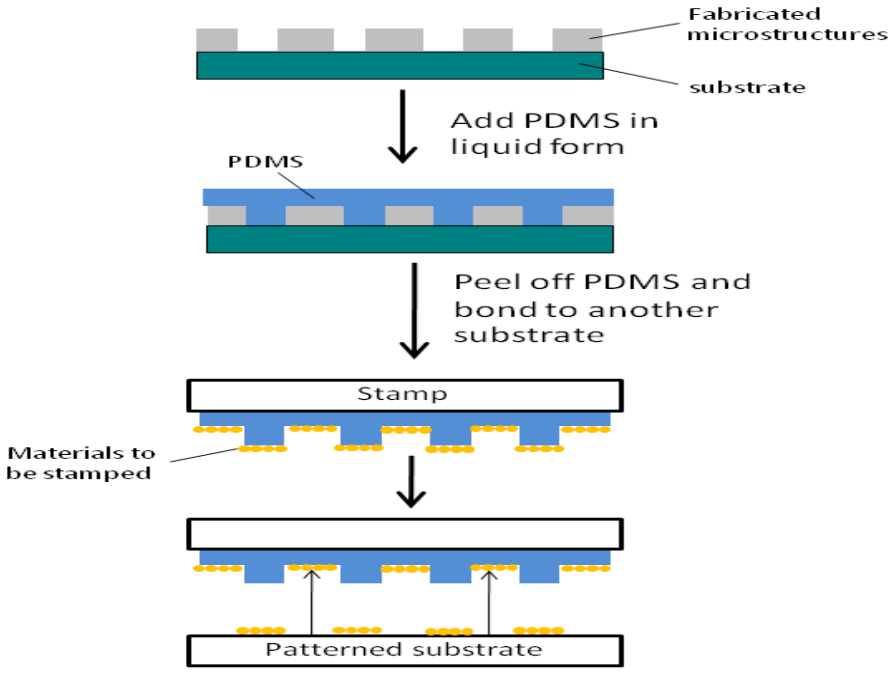


Figure 5. Schematic illustration of soft lithography process (adopted from review on micro and Nanolithography techniques and their application ENGINEERING JOURNAL Volume 16 Issue 1)

Phase inversion

The phase inversion process eliminates the synthetic aspect of MIP production, typically at a cost of less homogeneous binding sites due to the absence of significant polymer cross-linking. The major advantage is simplicity and faster production. Phase inversion is a process in which initially homogeneous polymer solution is transformed from a liquid state to a solid state in a controlled manner. This method is the most used and it helps to fabricate polymeric porous membrane with a large form of structure. Phase inversion is the most widely used technique for fabricating polymeric membranes. It takes place in an initial polymer solution that starts to separate in a polymer-rich phase and a polymer-poor phase that becomes thermodynamically [11], [15]

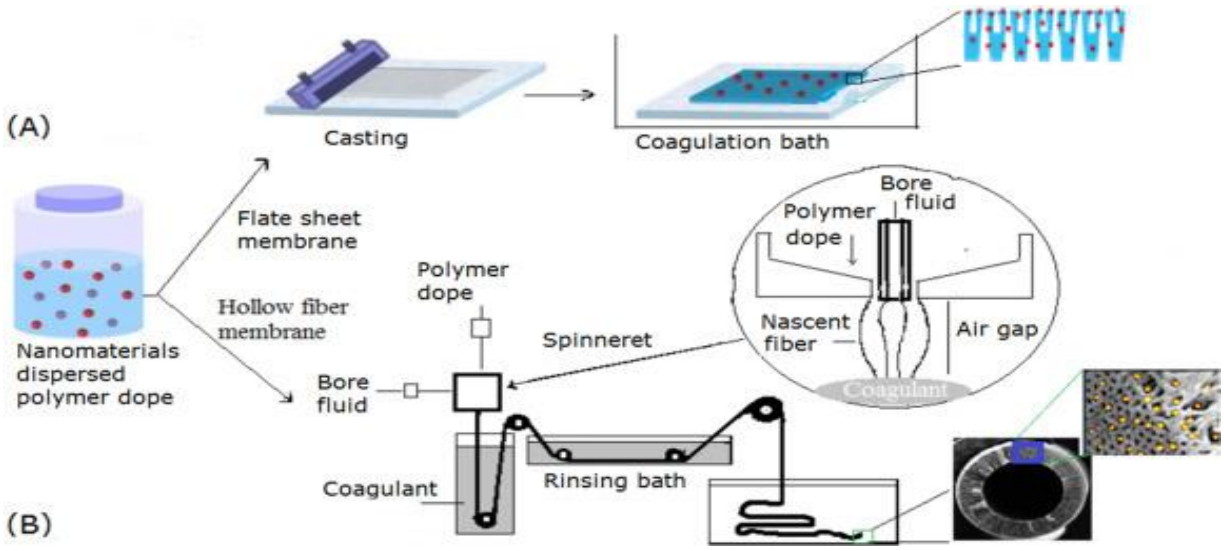


Figure 6: Phase inversion technique for fabricating polymeric membranes (adopted from Sahar Zare, Ali Kargari, in Emerging Technologies for Sustainable Desalination Handbook, 2018).

II.2 Characterization methods of MIPs

II.2.1 Sorption tests:

These sorption tests are divided into batch and continuous tests. Below we explain the main tests performed in each category.

Solid phase extraction (SPE)

The use of MIPs as sorbents enables the pre-concentration, cleaning, and selective extraction of the target analyte, which is crucial, especially when the sample is complex, and contaminants may cause quantification problems. In line with standard SPE practices, a cartridge is filled with a modest quantity of imprinted polymer. After that the material is conditioned with a selected solvent (should match the solvent of the analyte) and then the sample loading is performed. Consequently, eluting solvents are passed through the MIP

cartridge. Various solvents of selected compositions are usually used in the elution step to obtain different fractions[15]

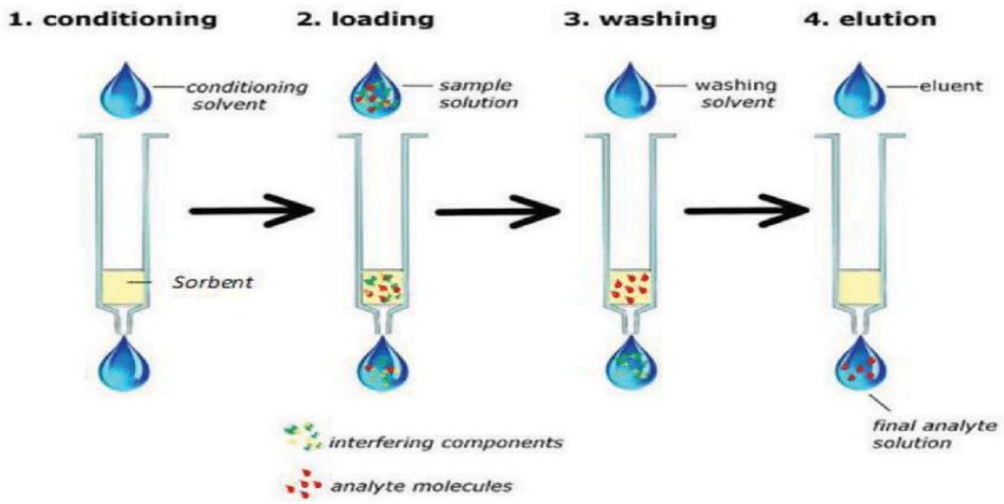


Figure 7: Principal steps of solid phase extraction (adopted from International Journal of Environmental Analytical Chemistry 99(50):1-52)

Batch technique

Batch adsorption is commonly used to test the synthesized MIPs. It consists of extracting the solute from a liquid phase into the solid one (adsorbent) by shaking the mixture of the two phases until equilibrium is attained. After this, the liquid phase is analysed by UV to determine the adsorption capacity (*Q)* which can be expressed as:

$$Q = \frac{(C_0 - C_E)\ V}{m}$$

where C_0 (mM) and C_E (mM) are the initial and equilibrium concentration of the phenolic compound, respectively. *V* (mL) is the volume of the testing solution and *m* (mg) is the mass of the adsorbent.

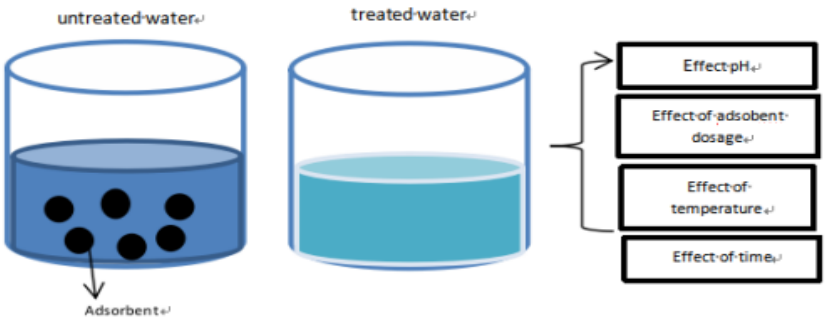


Figure 8: batch sorption process (adopted from: review on batch and column adsorption of various adsorbent towards the removal of heavy metal)

Frontal analysis

In this test, a stainless-steel column is filled with the synthesized material, then analyte solution is pumped through the column and the outlet is analyzed by a detector (in case of polyphenols a UV detector is suitable) allowing the obtention of the breakthrough curve shown in figure 9.

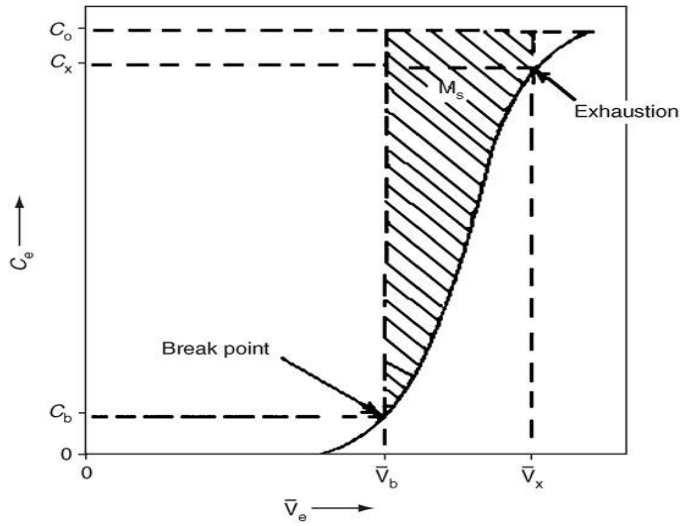


Figure 9: Typical breakthrough curve obtained at the outlet of a column during frontal analysis.

II.2.2 Physical- chemical characterization:

The table below comprises the main techniques used to characterize the synthesized MIPs.

Table 1: Different analytical techniques for MIP characterization.

Analytical technique	**Utilization**
FTIR: Fourier Transform Infrared Spectroscopy	FTIR allows the determination of the functional groups of a liquid or solid sample. This is vital in the characterization of MIPs as it permits to verify the integration of the functional monomer(s) and crosslinker into the final polymeric network.
SEM: Scanning electron microscopy	Through a flux of electrons, the SEM technique allows the determination of the shape and size of the synthesized MIP using the backscattered and the secondary electrons resulting from the interaction with the sample
BET: Brunauer–Emmett–Teller	(BET) theory is used to measure the surface area of solid or porous materials. It gives important information on their physical structure as the area of a material's surface affects how that solid will interact with its environment

Gravimetric	Mainly used to determine the yield of the polymerization reaction.
SLS: Statistic Light Scattering	Allows the measuring of absolute molecular weight through the light scattered by the particles.
TEM: Transmission electron microscopy	Transmission electron microscopes produce images that are incredibly enlarged by using an electron particle beam to view specimens.

Besides the afore mentioned techniques, high liquid chromatography (HPLC) is also used to in the current work to analyze mixtures of different phenolic compounds and the extracts of winemaking residues. HPLC is an analytical method for separating, identifying, and quantifying mixture components. The liquid sample is combined with solvent that is pumped from a solvent reservoir. A detector, which produces an electronic output in the form of a chromatograph, receives the solvent sample mixture after it has passed through an HPLC column. The chromatographic packing material required for the separation is present in the column. Since the column hardware keeps this packing material in place, it is known as the stationary phase. The mobile phase leaves the detector and can either be disposed of properly or recollected.

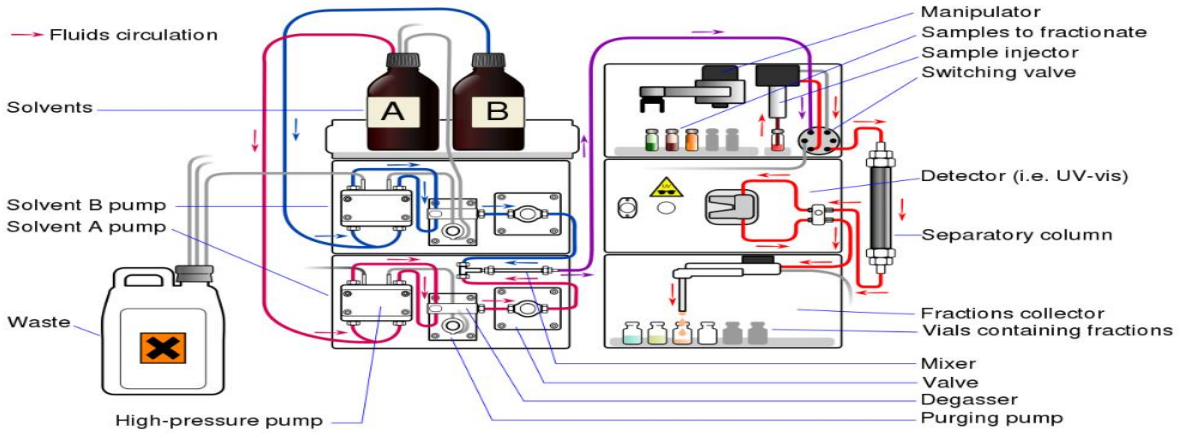


Figure 10: Schematic view of HPLC (adopted from Clark, Jim. Gas-Liquid Chromatography journal)

II.3 Applications of MIPs:

MIPs are versatile materials, meaning that they can be used in a wide range of applications and to target different types of molecules. Below, a brief summary of the main application fields of MIPs are presented.

Table 2: Fields of application of the MIPs

Application	Example	Reference
Biological and pharmaceutical fields	Pentamidine has been selectively removed from diluted human urine using MIPs.	[15], [16]
Environmental analysis	The identification of hazardous compounds is made possible using MIPs. For instance, a MIP has been produced to extract the bentazone (a pesticide) from water samples.	[15], [16]
Bromatological and toxicological analysis	MIPs are used to isolate the human cancer-causing mycotoxins *Aspergillus and Penicillium*. The synthesized MIP was capable of selectively capturing nicotine and its metabolite cotinine.	[15], [16]
Food industry	The MIP also made it possible to extract caffeine from food samples while selectively removing quercetin from a complex matrix, such as red wine.	[15], [16]

II.4 Molecularly imprinted polymer based on boronate affinity

Cis-diol containing compounds are a class of molecules that attract the academic community of different areas such as bioanalysis, food industry, and environmental analysis. Cis-diol biomolecules are significant target molecules in emerging scientific fields like proteomics and metabolomics as well as in crucial applications like single-cell analysis, cancer cell targeting, and disease detection[17], [18].

Despite their importance, cis-diol compounds are difficult to analyze (especially is biological matrices) because of the high interference caused by other compounds. In this context, boronate affinity materials have gained more attention in recent years owing to their special ability to bind cis-diol compounds[19]

The effectiveness of boronate affinity materials is dependent in the conditions of pH and temperature [20], [21]. Figure 11 shows different boronic acid ligands that are commercially available.

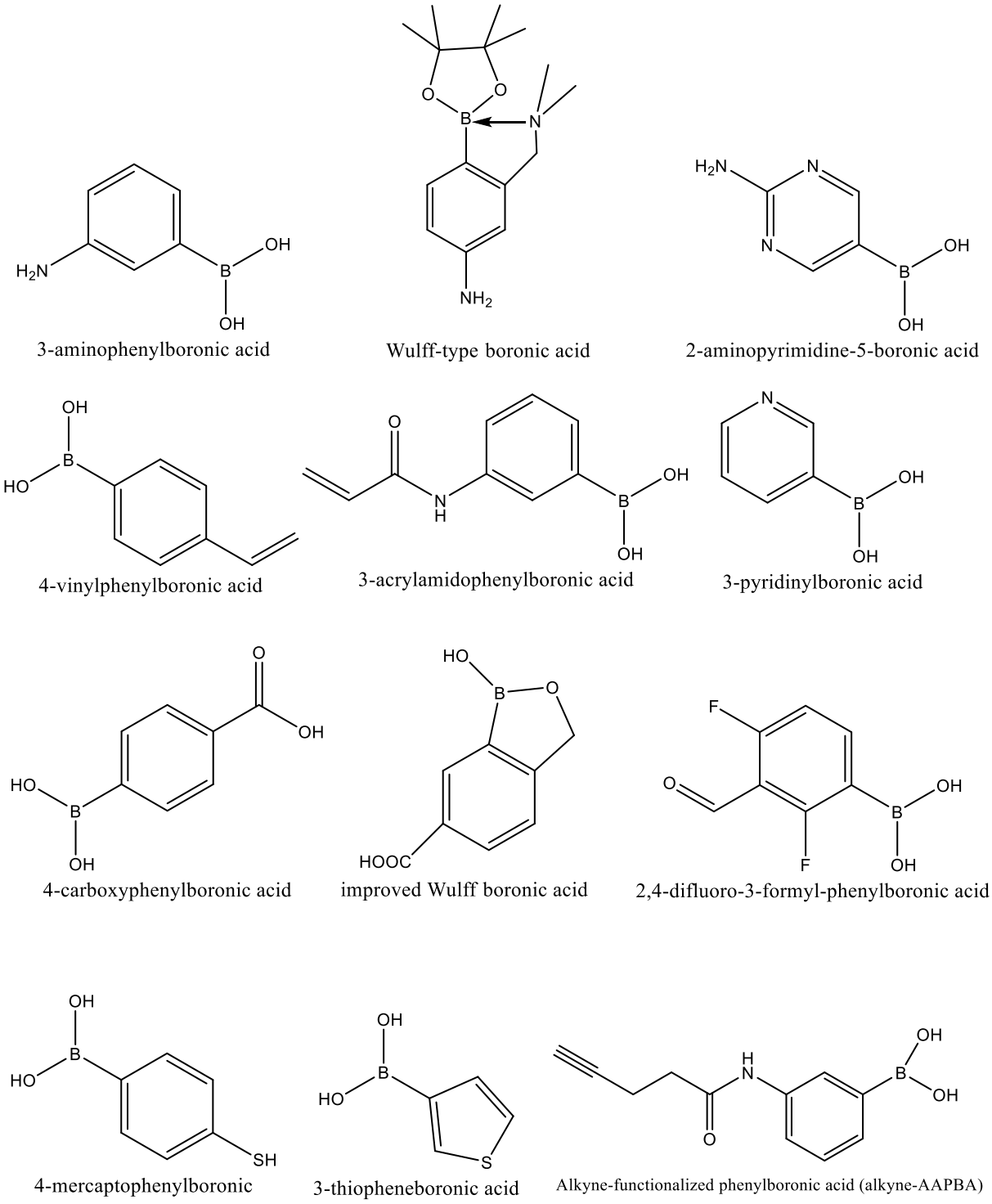


Figure 11: Commercially available boronic acid monomers

In an alkaline environment, boronate affinity materials and cis-diol compounds can combine to generate cyclic esters. When the environment turns acidic, the reversible process goes in the other direction, causing the cyclic ester to dissociate. The tetragonal boronate anion (sp3) is formed when the surrounding pH is greater than or equal to the pKa value of the boronic acid. This allows it to react with cis-diols to generate five- or six-membered cyclic esters. The boronic acid/cis-diol complex dissociates when the surrounding pH is significantly lower than

the pKa value of the boronic acid because this latter entirely switches back to trigonal form (sp2)[19], [22]

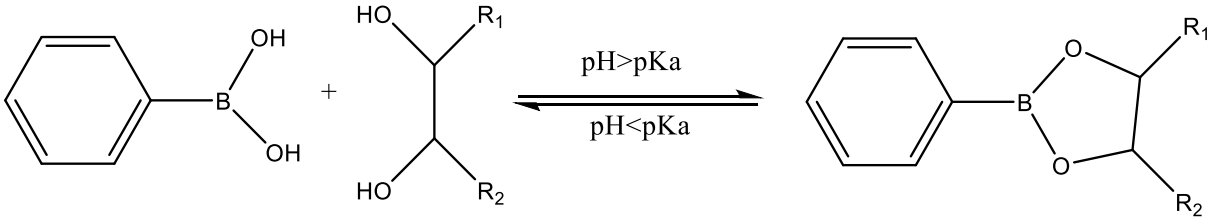


Figure 12: Interaction between the boronic acid and the cis-diol moieties under the effect of pH.

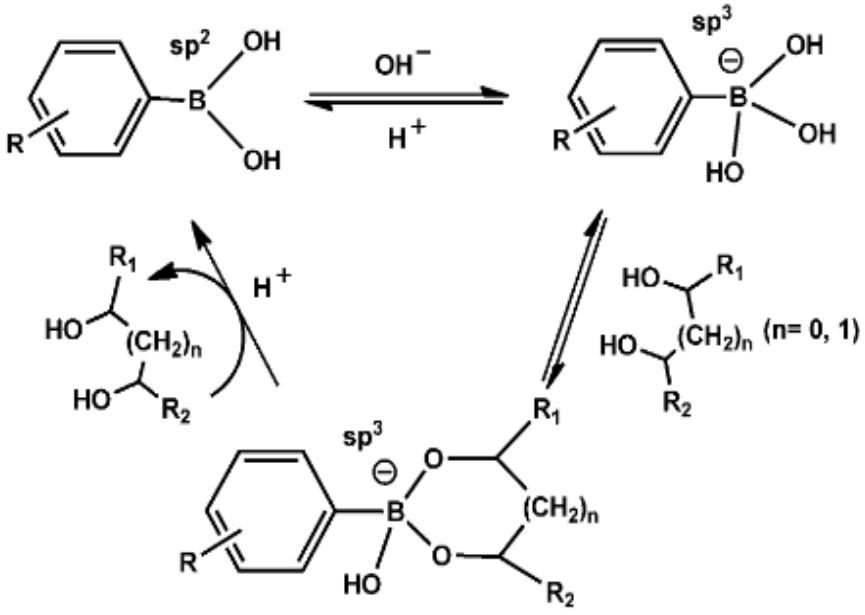


Figure 13: Tetragonal boronate anion (sp3) to trigonal configuration (sp2) when associating and dissociation with a cis-diol compound. (Adapted from Synthesis and Applications of Boronate Affinity Materials: From Class Selectivity to Biomimetic Specificity).[19]

II.5 Winemaking residues:

The extracts of winemaking residues have been reported in the literature because they function as multipurpose ingredients or additives in various industries. These residues have a high fiber content and antioxidant substances that could be used in a variety of food, cosmetic, and pharmaceutical applications. Both seeds and grape pomace are rich in bioactive components, but anthocyanins phenolic compounds found in the skins as well as flavanols concentrated in the seeds are of particular significance. Grape pomace extracts can be used and incorporated primarily in food, medicinal, and cosmetic goods. They come in liquid, concentrate, or powder form[23], [24]

Grapes are also regarded as one of the main sources of phenolic compounds, and due to their anti-aging properties, polyphenolic extracts from grape seeds are increasingly used as active components in commercial cosmetics products. Studies on cell lines employing epidermal keratinocytes or dermal fibroblasts reveal that polyphenols have positive effects on human skin[24]

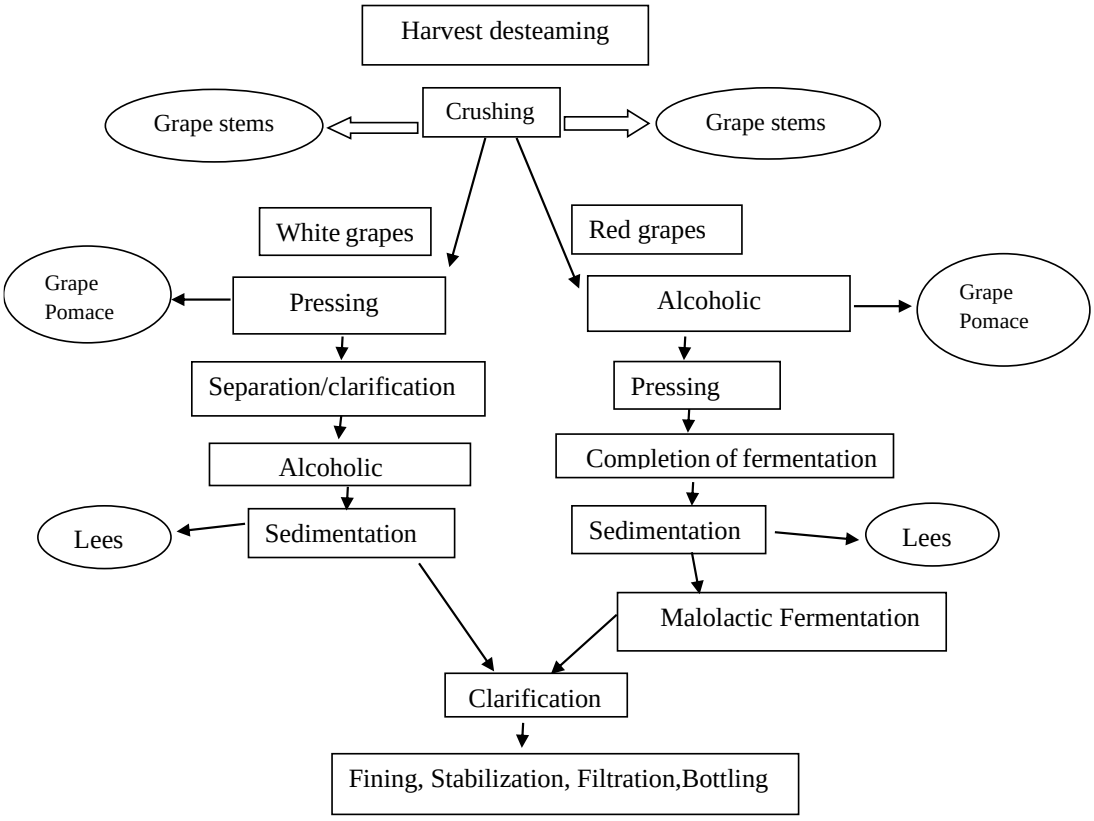


Figure 14: Different residues from winemaking industry

II.6 Polyphenols: definition and properties.

Antioxidant molecules called polyphenols are present in a variety of foods, including green tea, chocolate, grapes, and wine. Antioxidant, anti-inflammatory, and anti-cancer capabilities are all features of polyphenols. Flavonoids, stilbenes, phenolic acids, and lignans are main classes of polyphenols which are primarily found in vegetables and fruits[25], [26]

Vegetables, fruits, cereals, tea, coffee, and other plants all naturally contain polyphenols. These compounds are described as substances that have at least one hydroxyl group on an aromatic ring. [27], [28]

They can range in structure from simple molecules to complex polymers with high molecular weights. Chemicals with a benzene ring structure and two or more phenolic hydroxyl groups are known as polyphenols, and depending on their structural makeup, they can be divided into flavonoids, phenolic acids, stilbenes, lignans or tannins [29]

Kaempferol, quercetin, myricetin and isorhamnetin appear the fore-most representatives of flavonols. The main stilbenes present in grapes, wine and in winemaking residues is resveratrol [30], [31]

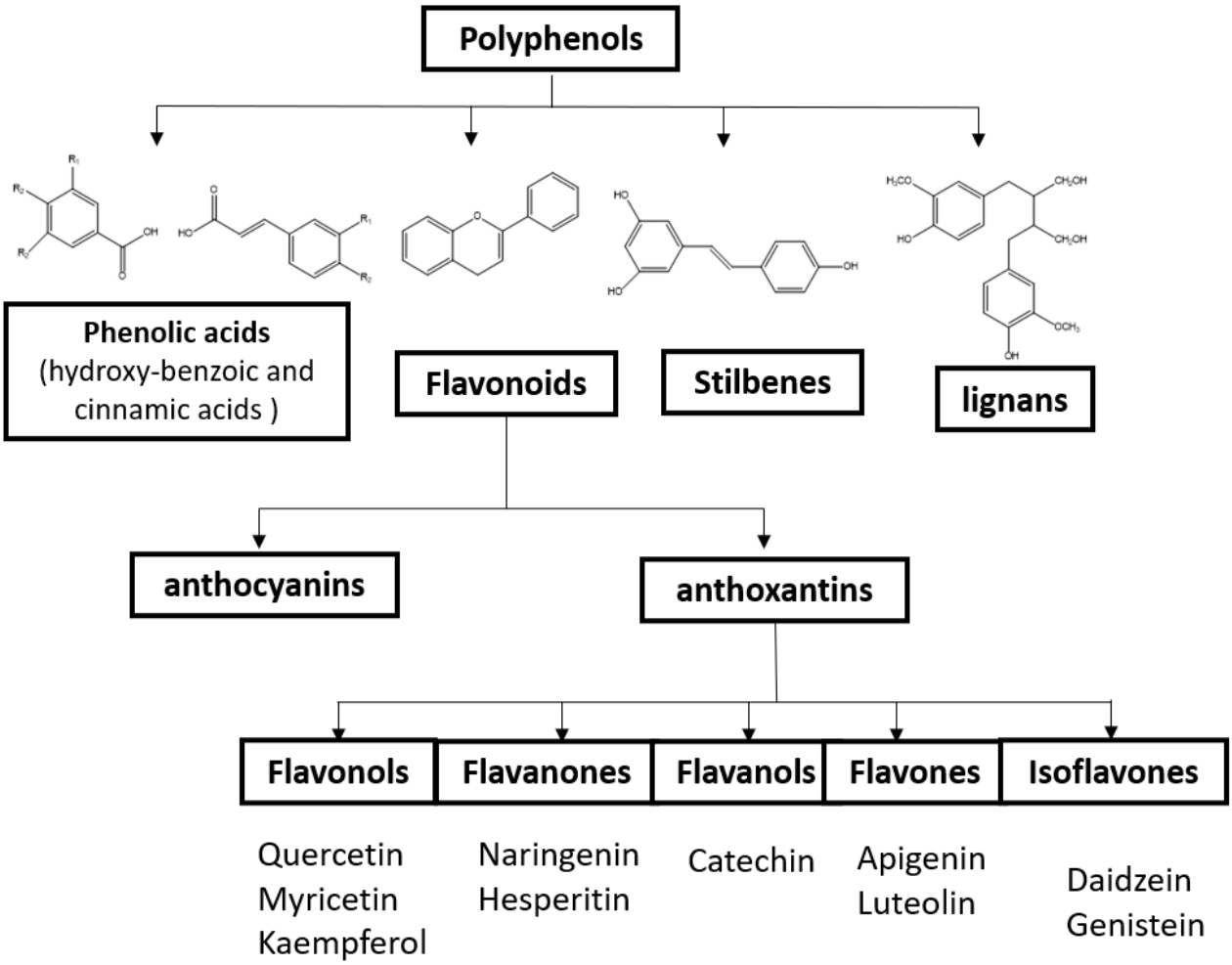


Figure 15: General classification of polyphenols.

III. Results and discussion:

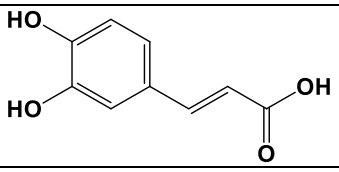
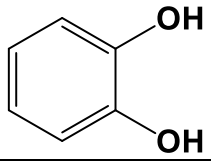
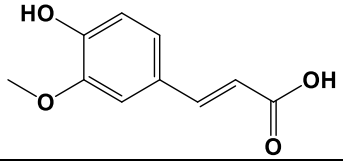
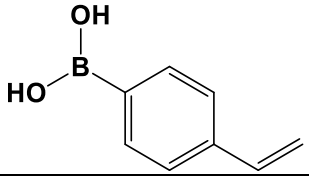
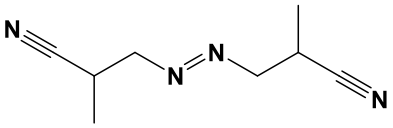
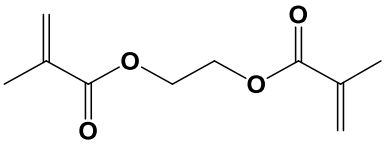
III.1 Materials

In order to conduct different adsorption tests and syntheses, different reagents and solvents were used. Details about these chemicals are specified in this section.

III.1.1 Reagents:

Both cis-diol and non-cis-diol compounds were used to molecularly imprint the polymers. The cis-diol compounds were caffeic acid and pyrocatechol, whereas the non-cis-diol compound was ferulic acid. The functional monomer used in the synthesis of the materials is 4-vinylphenylboronic acid (4VPBA). The crosslinker is ethylene glycol dimethacrylate (EGDMA) and the initiator is azobis(isobutyronitrile) (AIBN).

Table 3: List of reagents used in this work.

Reagents	Nomenclature	Molecules	Purity	Origin
Caffeic acid (template)	3,4-Dihydroxycinnamic acid		purity ≥ 99%	Acros Oragnics
Catechol (template)	1,2-dihydroxybenzène		purity ≥ 99%	Acros Oragnics
Ferulic acid (template)	4,Hydroxy-3-methoxycinnamic acid		purity ≥ 99%	Acros Oragnics
4VPBA	4-vinylphenylboronic acid		purity ≥ 95%	Sigma-Aldrich
AIBN	Azobis(isobutyronitrile)		purity ≥ 98	Fluka
EGDMA	Ethylene glycoldimethacrylate		purity ≥98%	Sigma-Aldrich
Isopropanol	-	$\underline{CH_3}CH(\underline{OH})\text{-}CH_3$	Purity ≥98%	Fisher Chemical
Ethanol	-	$CH_3\text{-}CH_2\text{-}OH$	Purity ≥ 99.8%	Fisher Chemical
Methanol	-	$\underline{CH_3OH}$	Purity ≥ 99.9%	Fisher Chemical
Acetic acid	-	$CH_3\text{-}CO\text{-}OH$	Purity ≥ 99.7%	Fisher Chemical
Ultra-pure water	-	H2O	-	-

III.1.2 Equipments :

- FTIR: FTIR was used to analyse the synthesized MIPs and NIPs after being washed and dried under vacuum. The apparatus used was a PerkinElmer Spectrum Two, and the characterization was carried out in attenuated total reflectance (ATR) mode between 4000 cm−1 and 450 cm−1
- SPE: Solid phase extraction measurements were carried out using packing extraction cartridges containing the MIP or NIP particles (e.g., 150 mg of dried products).
- HPLC: Analyses were performed using a High-performance Liquid Chromatography coupled to Diode-Array Ultraviolet Detector (HPLC-DAD). The latter was supplied by KNAUER and consisted of a P6.1 L gradient pump equipped with a degasser, a 6.1 L autosampler, a column thermostat CT2.1 and a diode array detector 6.1 L. KNAUER ClarityChrom® was the software that allowed the control of the HPLC-DAD system. The chromatographic analysis was performed using an Ascentis® C18 (SUPELCO®) column with a particle size Molecules 2022, 27, 6406 14 of 16 of 5 μm and dimensions 25 cm × 4.6 mm. A gradient of solvents was used as a mobile phase varying from A 100% (90/10 water/ACN) to B (10/90 water/ACN) 100% for 45 min.
- UV: Loading, washing and elution steps were monitored using P9 double Beam UV – Visible spectrophotometer from VWR.

III.2 Methods:

III.2.1 Materials synthesis

We start the synthesis of different MIPs with different templates which are catechol, ferulic acid and caffeic acid with the same functional monomer 4-vinylphenylboronic acid (4VPBA) in a solution of water pH 8.5 with isopropanol in the proportions of 15%-85%. After dissolving the monomers and the templates, the crosslinker EGDMA and the initiator AIBN were added and the whole mixture was purged with argon (NIPs were synthesized the same way but with the absence of the template).The flasks were left to react in an oil bath at 60°C for 24h. Due to the nature of the covalent interaction between the 4VPBA and the template, the polymerization temperature will heavily influence the process of imprinting since it will enforce the covalent bonding between the cis diol and the boronic moiety. At the end of the synthesis, the MIPs/NIPs were collected and washed by methanol/ acetic acid (9:1) then with methanol to remove the unreacted reagents and until no template was detectable by UV. Finally, pure ethanol was used to wash the materials which were left to dry in the vacuum oven over night (pression 0,5 bar, 60°c). The fractions that were used in the synthesis process of the different templates are: YM, YI, YCL, YFM.
YM: mass fraction of functional monomer and crosslinker in the solution YI: mole fraction of initiator comparatively to functional monomer and crosslinker. YCL: mole fraction of crosslinker in the mixture functional monomer and crosslinker. YFM/T: mole ratio between the functional monomer to the template molecule.

III.2.2 Adsorption tests

III.2.2.1 Individual adsorption

Batch sorption tests were carried out by preparing three separate solutions of 0,1 mM catechol, 0.1 mM caffeic acid and 0.1 mM Ferulic acid, using EtOH-Water (8:2) as a solvent. For each compound, a mother solution was prepared in a concentration of 2 mM. The batch sorption test consisted in weighing 50 mg of the synthesized material and then adding 1 ml of the phenolic compound solution which had been prepared earlier. The mixture is left to equilibrate for 24 hours in an orbital shaker. The table below shows the mass of each material used in the sorption tests.

Table 4: Experimental weights of the of synthesized materials

Materials	Mass
MIP_Catechol	51,1 mg
MIP Caffeic	51,2 mg
MIP Ferulic	49,5 mg
NIP_Caffeic	49,4 mg
NIP Catechol + Ferulic acid	51,6 mg

The choice of using a solution with a concentration of 0.1 mM was due to its response in the UV-vis range. After cleaning the materials with a solution of MeOH-AcOH (8:2) for several times using solid phase extraction and then with a solution of pure MeOH. Prior to the sorption tests, the materials were conditioned using a solution of EtOH-H_2O (8:2). In some cases, the materials were difficult to clean, thus another efficient cleaning solvent (AcOH-MeOH (1:1)) was used to ensure the proper elimination of any eluting compounds from the materials. Other concentrations of 0.25 mM and 0.5 mM were also employed in these sorption tests. The pH was changed to assess its effect on the binding capacity of the materials in alkaline and acid conditions. The binding capacity (Q) was calculated following equation 1 which practically is simplified into equation 2 where UV_0 and C_0 (in mM) correspond respectively to the absorbance and the concentration of the initial solution. UV_e corresponds to the absorbance of the solution after 24 hours, V is the volume of the batch solution (in ml), and m is the mass of the material (in mg)

$$Q = \frac{(C_0 - C_e) \times V}{m} \qquad (1)$$

$$Q = \frac{(UV_0 - UV_e) \times V \times C_0}{UV \times m} \qquad (2)$$

III.2.2.2 Competitive sorption

To assess the selectivity of the materials, solid phase extraction was used. To this end, a solution containing three different compounds (ferulic acid, caffeic acid and catechol) was loaded into the materials at a concentration of 0.25 mM at different pH conditions (acid, neutral and alkaline). The outlets of the SPE cartridges were analysed by HPLC since it is not possible to do so by UV (the templates have overlapped responses). Before starting this set of experiments, a cleaning step of the materials using MeOH-Water (pH= 2) (1:1) was

conducted, and UV spectroscopy allowed to check that the materials are exempt of any interfering compounds.

Method optimization of HPLC

In order to separate the peaks of each compound, different methods of HPLC were tried to select the most suitable one which give the highest peak resolution. An injection volume was set to 50 µL. The mobile phase consisted of a gradient of solvents varying from A (Water-ACN (9:1)) to B (Water-ACN (1:9)). The stationary phase column (25cm * 4,6 mm) filled with particles of C_{18} with a size of 5 µm. The solution mixtures containing the three templates were injected following the methods listed below. The correspondent chromatogram is shown for each method.

Method 1

Table 5: HPLC optimization method 1

Time (min)	H2O/ACN (90/10)	H2O/ACN (10/90)
0	100	0
7	85	15
15	77.7	22.3
20	68.8	31.2

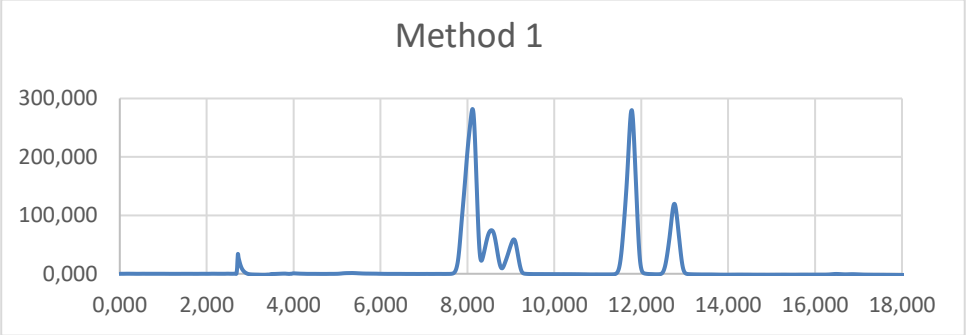


Figure 16: Chromatogram obtained using method 1

Method 2

Table 6: HPLC optimization method 2

Time (min)	Water-ACN (9:1)	Water-ACN (1:9)
0	100	0
7	90	10
8	90	10
8.5	85	15
10	85	15
12	77	23
14	70	30
15	65	35

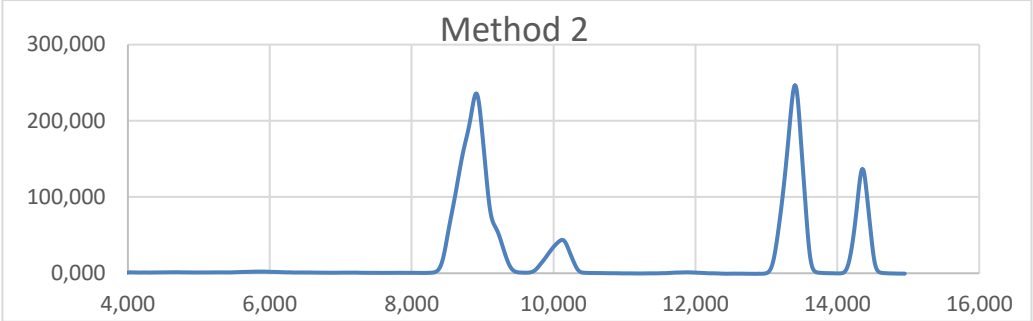


Figure 17: Chromatogram obtained using method 2

Method 3

Table 7 : HPLC optimization method 3

Time (min)	Water-ACN (9:1)	Water-ACN (1:9)
0	93.8	6.3
5	93.8	6.2
10	87.5	12.5
20	81.3	18.8
25	75	25

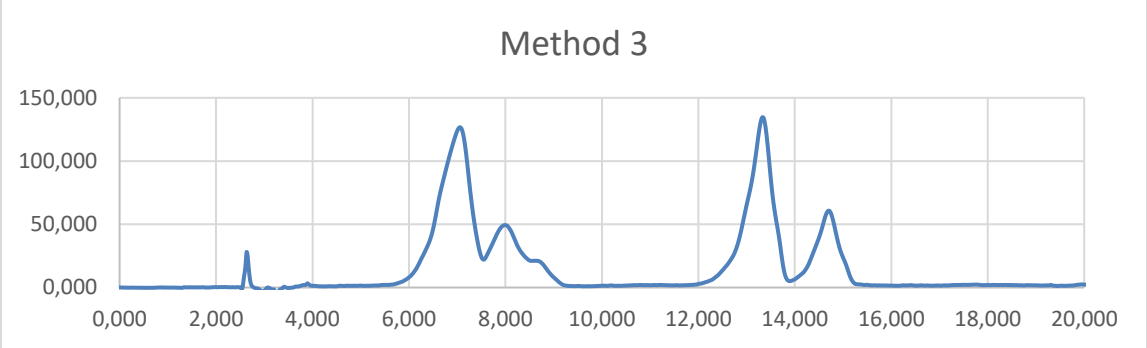


Figure 18: Chromatogram obtained using method 3

Method 4

Table 8: HPLC optimization method 4

Time (min)	Water-ACN (9:1)	Water-ACN (1:9)
0	100	0
45	0	100

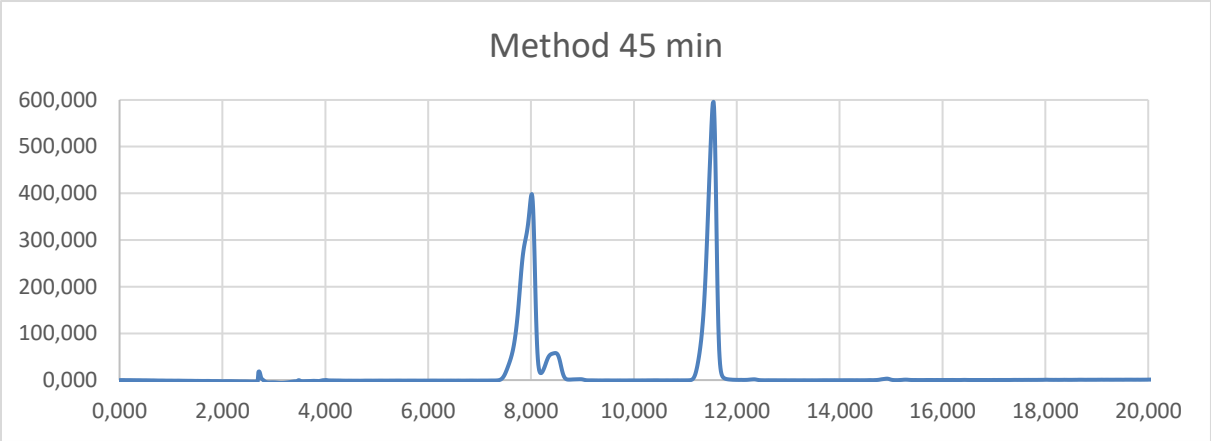


Figure 19: Chromatogram obtained using method 4

After trying different methods with changing the time and the percentage of the solvents we choose method 4 (45 minutes).

III.2.3 Chemical characterization:FTIR analysis

All the Materials (MIP_CA , MIP_FA, MIP_Cat, NIP_CA, NIP_Cat_FA) were analysed by FTIR between 400 cm^{-1} and 4000 cm^{1} in their solid state, all the FTIR results are nearly the same for the five different materials , we made the choice of MIP caffeic material as a representative material that displays the peaks we reached in all the adsorption tests.

III.3 Results

III.3.1 Rationale of materials synthesis

In this work we aim to synthesize materials, molecularly imprinted polymers, able to bind to cis-diol molecules present in the winemaking residue (diatomaceous earth). The main monomer for the synthesis of those materials is 4-vinylphenylboronic acid. In addition to the use of monomers, the use of crosslinker EGDMA also is a vital factor to create the three-dimensional cavities that are complementary to the target molecule. The table below explains the different factors affecting the molecularly imprinted polymers and discusses the use of the high or low range.

Table 9: Parameters describing the synthesis of the materials.

Parameter	Definition
Y_M (%)	Mass fraction of the polymerizable monomers mixture (including functional monomer(s) and crosslinker(s)) in the polymerization solution.
Y_{CL} (%)	Mole fraction of the crosslinker in the polymerizable monomer's mixture.
$Y_{T/FM}$	Mole ratio between the template and a functional monomer.
Y_I (%)	Mole ratio between the initiator and the polymerizable monomers.

Table 10: Variables that affect the final attributes of the materials and their respective advantages while using the high or the low range.

Parameter	High range	Low range
Crosslinker content (Y_{CL})	Geometrical stability of binding sites. High mechanical resistance (e.g. Y_{CL} > 80 %).	Enhancement of the functional groups density and increase of interaction possibilities (e.g. Y_{CL} < 50 %).
Composition of the functional monomers' mixture	Exploiting multiple interactions between complex templates and different functional monomers (e.g. 4VP + styrene for hydrogen bonding and π-π stacking).	Simplicity of polymerization recipe, avoiding heterogeneity of polymer networks due to different incorporation rates for the diverse functional monomers (e.g. single functional monomer).
Total monomers' concentration (Y_M)	Good polymer yield; fast polymerization (e.g. Y_M > 25 %).	Control of particle size and morphology with enhancement of mass transfer; control of reaction temperature; solubilization of initial reactants (e.g. Y_M < 10 %).
Template content ($Y_{T/FM}$)	Increasing stereospecific cavities for molecular recognition (e.g. $Y_{T/FM}$ > 1/3).	Increase the functional groups of the material while decreasing its specificity (lesser stereospecific cavities). Avoiding solubility problems, polymerization inhibition, material cleaning difficulties and high price caused by template inclusion (e.g. $Y_{T/FM}$ =0 for NIPs).
Solvent hydrogen bonding capacity and polarity	Good solubility of monomers and templates (e.g. inclusion of DMF or DMSO).	Decreasing competition with template/functional monomers self-assembly Higher control on polymer particles morphology and size through phase separation (e.g. 100% ACN).
Polymerization temperature and initiation mechanism	High polymer yield and diverse initiation possibilities (e.g. T>60 °C with thermal initiators).	Improve the template/functional monomers self-assembly (e.g. e.g. T<30 °C with photo-polymerization).

III.3.2 Synthesized materials:

III.3.2.1 Materials yield:

Table 11: Summary of synthesized materials

Material	Template	YM (%)	YCL (%)	Yi (%)	YFM/T molar	Solvent	Yield (%)
MIP_CA	Caffeic acid	15,25	50	4,04	1,25	52.1% EtOH40.6% Isopropanol+7.1% water pH=8.5	35, 49
MIP_FA	Ferulic Acid	30,83	50	2,50	1,25	52.1% EtOH40.6% Isopropanol+7.1% water pH=8.5	22,05
MIP_Cat	Catechol	30,83	50	2,50	1,25	52.1% EtOH40.6% Isopropanol+7.1% water pH=8.5	22,05
NIP_CA	-	15,26	50	4,04	1,25	52.1% EtOH40.6% Isopropanol+7.1% water pH=8.5	34,24

NIP_FA_Cat	-	30,83	50	2,5	1,25	52.1% EtOH40.6% Isopropanol+7.1% water	21,84

After washing and drying, the yields of the materials have been calculated using the Formula:

$$Yield = \left(\frac{weighed\ mass}{m4VBA + m\ EGDMA}\right)$$

III.3.2.2 FTIR analysis

Regarding the MIPs and NIPs synthesized with the monomer 4VBPA, their FTIR spectra are plotted in Figure The presence of crosslinker EGDMA is evidenced by the peaks 1716 cm^{-1} and 1135 cm^{-1} which are assigned respectively to the stretching of C=O and aliphatic ether C-O. The boronic acid moiety has two distinctive bonds. The first one is B–O and it usually reflected by a peak around 1430–1355 cm^{-1}. The second one is correspondent acid function (-OH) that absorbs infrared light at 3450 cm^{-1}.

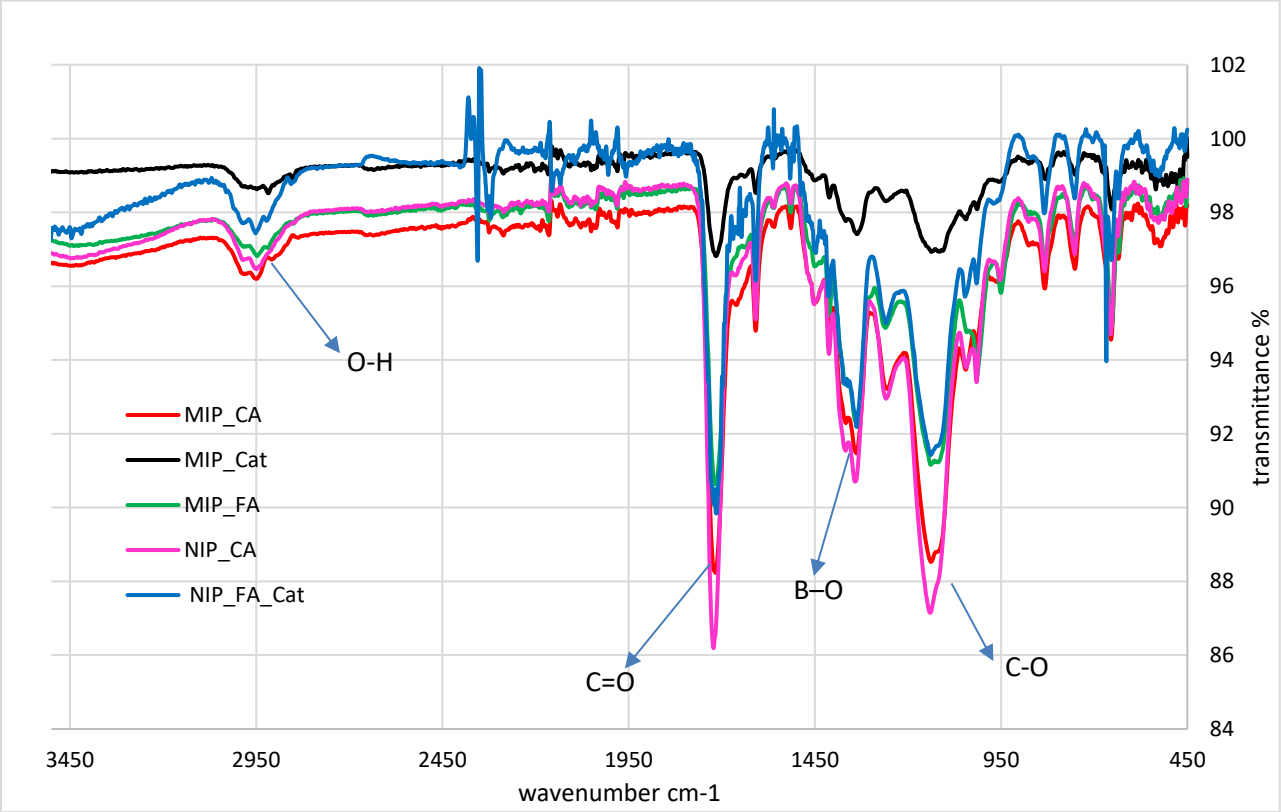


Figure 20: FTIR spectra of the synthesized materials.

III.3.3 Sorption experiments

III.3.3.1 Individual compound

During the solid-phase extraction analysis, we found out that the most efficient way of cleaning is to use a solution of MeOH/water pH =2 (8:2) for the different materials. The cleanliness of the material was verified by UV. The figure below shows an example of UV response for the different materials upon the cleaning step.

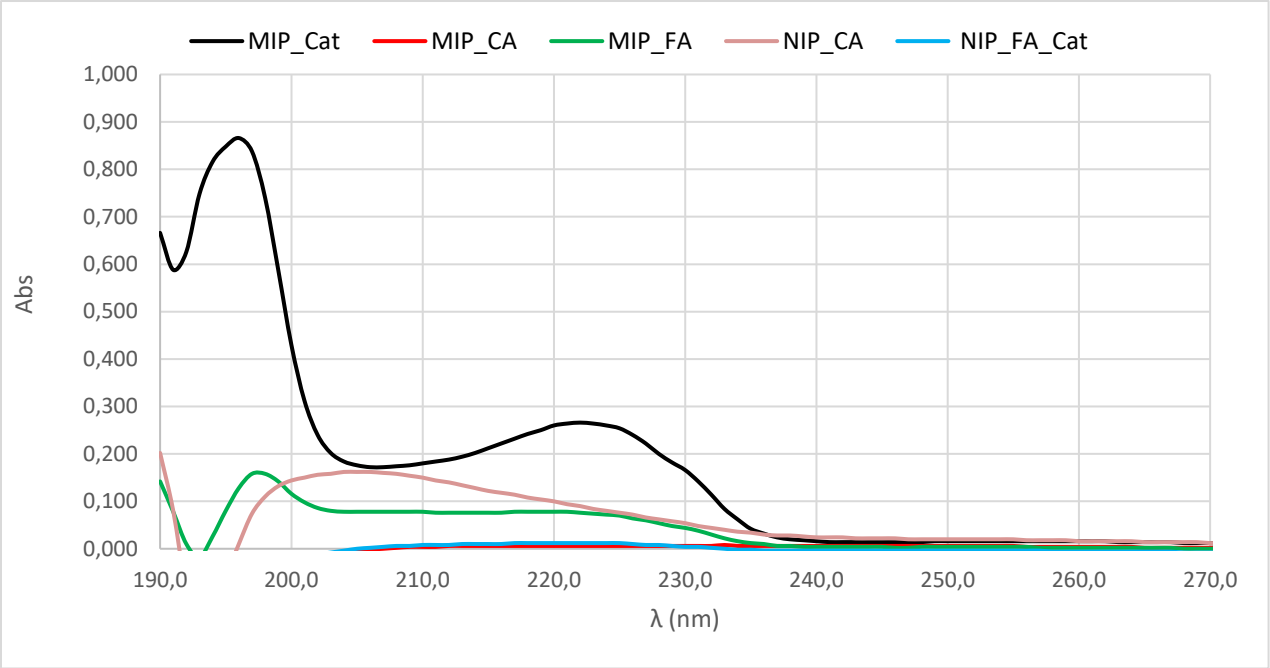


Figure 21: UV response after the cleaning of different materials using MeOH/H2O (pH =2; HCl)

At the beginning, the concentration of 0,5 mM of caffeic acid gave a saturated response in the UV. Thus, we used a concentration of 0,25 mM for all the molecules (Ferulic Acid, Catechol, and Caffeic) to permit the proper monitoring of the sorption experiments. Before starting the SPE tests, the materials were conditioned using a mixture of water and ethanol (80/20). The pH of the water used in this mixture was adjusted into alkaline acid or left neutral. This allowed the assessment material's retention and its dependence on the solution's pH.

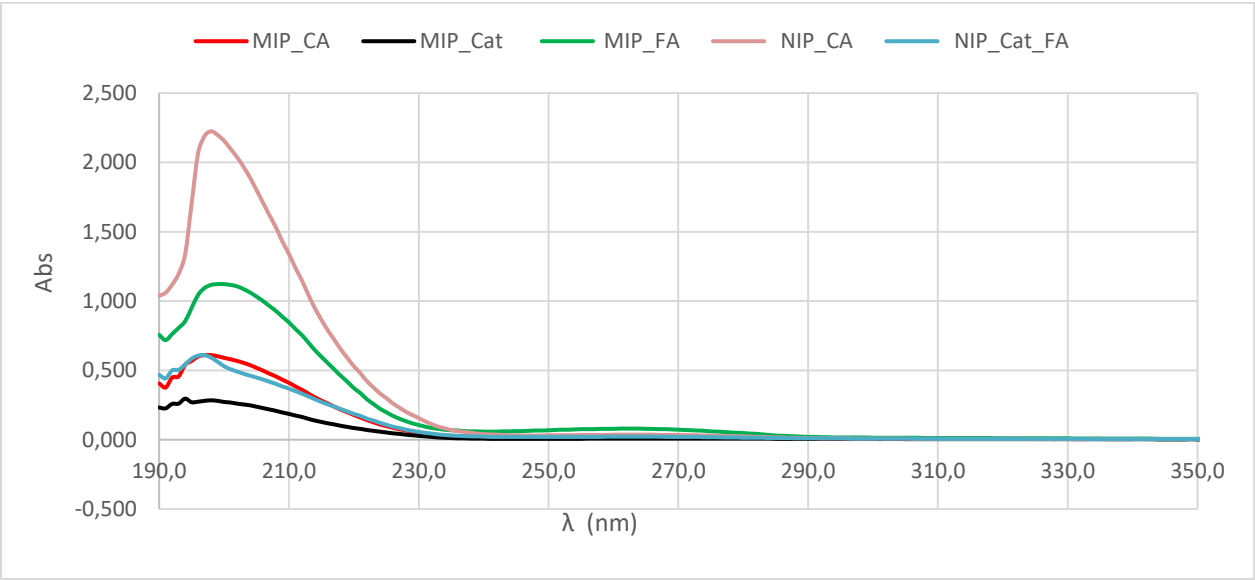
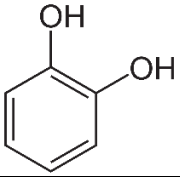
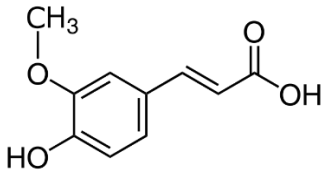
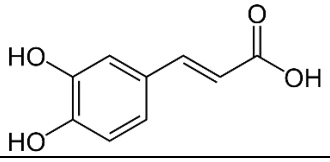


Figure 22: UV response of the materials during the conditioning step: Example of EtOH/Water (pH=2).

Table 12. Summary of the conducted experiments to study the effect of pH on the retention of the materials. (+) signifies that that the experiment was conducted at those conditions while (-) means that no experiment was made.

Template	Chemical structure	pH = 2	Neutral pH	pH = 8.5
Catechol		+	+	+
Ferulic acid		-	+	+
Caffeic acid		-	+	+

As shown in table 1, for the different templates, the solid-phase extraction tests were carried out in acid and alkaline conditions. For the catechol molecule, the SPE test was also carried out at neutral conditions of pH and in different concentration.

III.3.3.1.1 Sorption experiments using Ferulic Acid

After doing the cleaning step and verifying through UV that the materials are exempt of any interfering compounds, the materials were conditioned with the appropriate solvent. Consequently, the loading step took place using a solution of 0.25mM of ferulic acid at neutral and alkaline conditions. The quantity of retained ferulic acid was calculated using the UV responses of the loading solutions shown in figures X and XX (UV figures). The amount retained of ferulic acid per material at both alkaline and neutral conditions is shown in figure (bar graph).

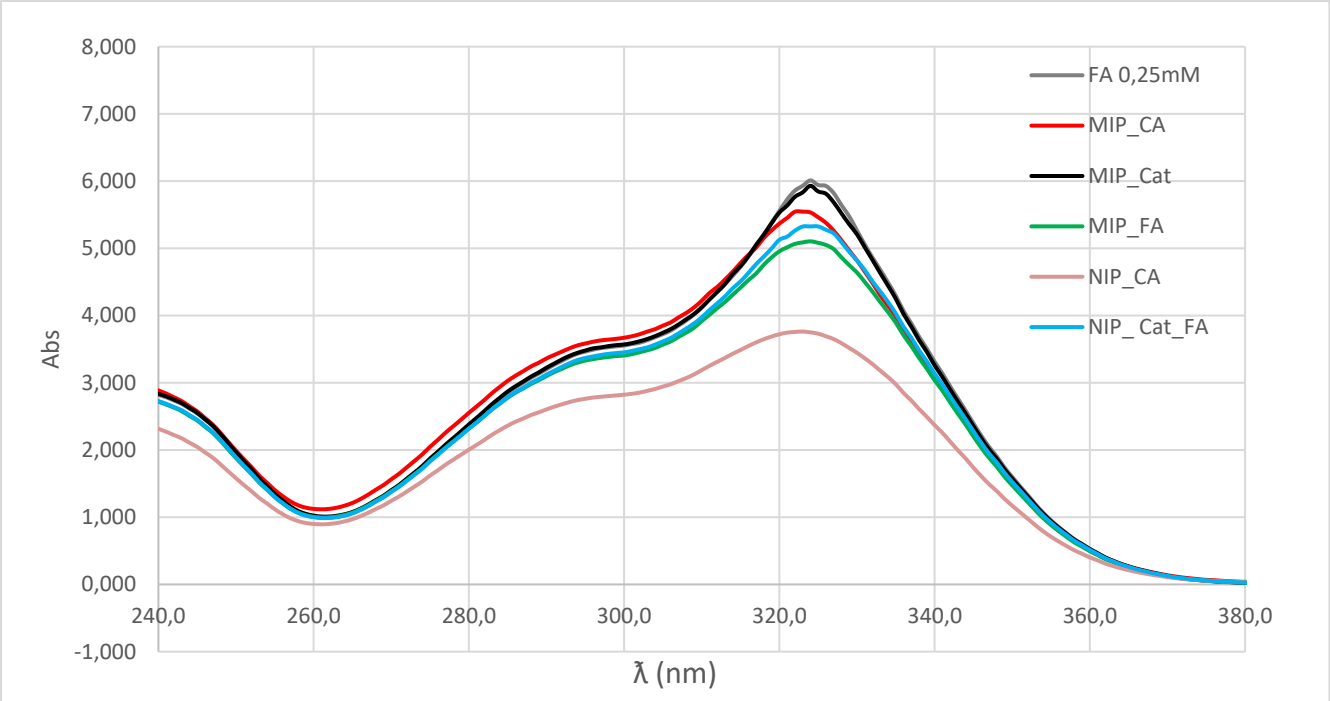


Figure 23: UV response of the fractions collected at outlets of the SPE cartridges after passing 0,25 mM Ferulic acid in neutral condition.

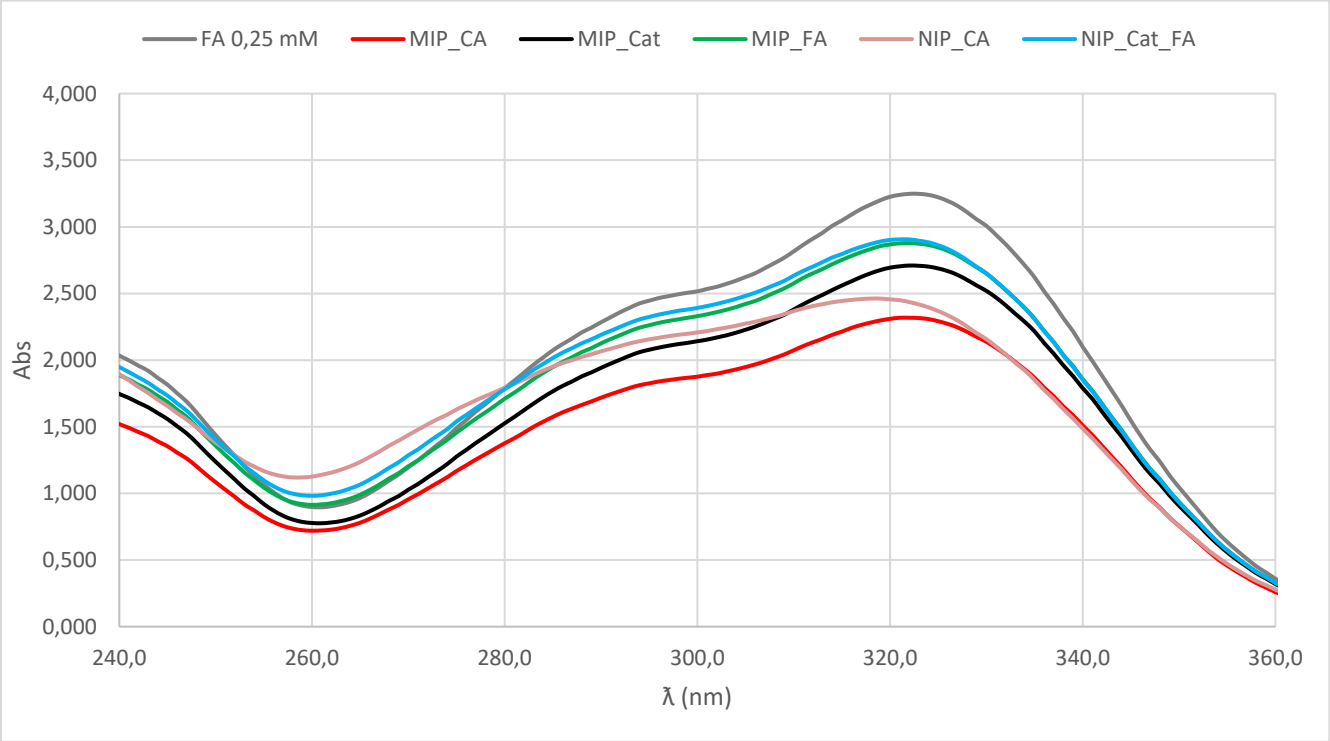


Figure 24: UV response of the fractions collected at outlets of the SPE cartridges after passing 0,25 mM Ferulic acid in alkaline condition.

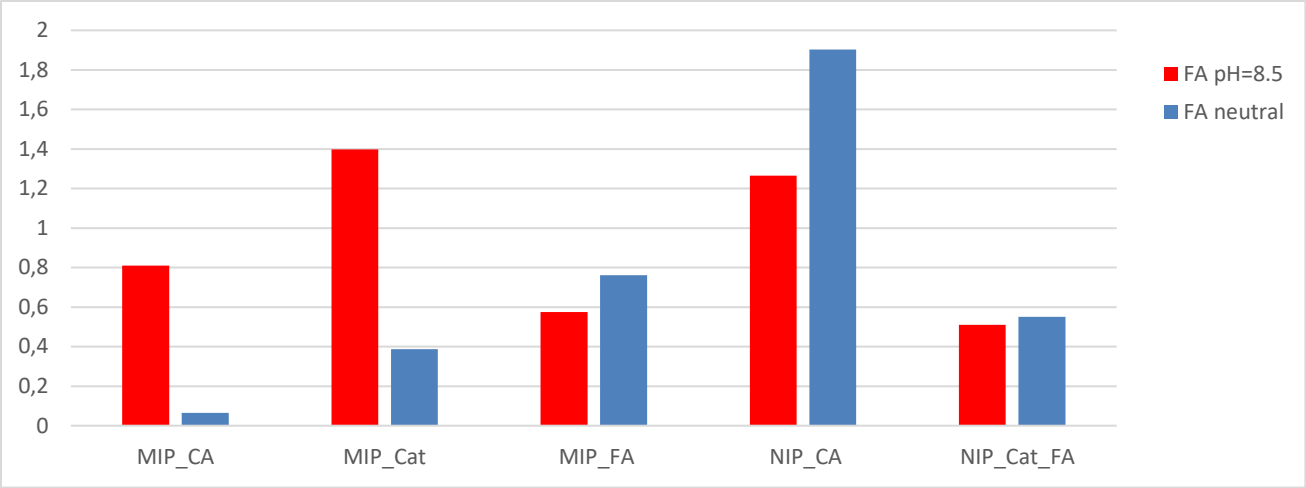


Figure 25: Retained quantities of ferulic acid using a concentration of 0,25 mM at alkaline and neutral pHs.

The retention of FA by the NIPs is higher in a neutral pH than in alkaline one. Although MIP_FA was made using FA as a template, it doesn't seem to retain it preferentially since the corresponding NIP has a higher retention for FA.

The binding capacity of MP_Caff and MIP_cat for FA is higher than that of MIP_FA. Such outcome is due to the structural similarity between FA and CA that makes it difficult for the MIPs to differentiate between the two compounds. At a pH of 8.5, the binding capacity of MIP_Cat and MIP_Caff intensified which is expected since the molecular imprinting process was carried out in alkaline solution, thus, and upon applying the same pH of the synthesis, the materials regain "memory" and the imprinted cavities are available again to bind to the FA. It should be highlighted that the interactions that exist between FA and the materials are not based on boronate affinity, since the chemical structure of FA does not allow it (non-presence of cis-diol function).

III.3.3.1.2 Sorption experiments using Caffeic acid

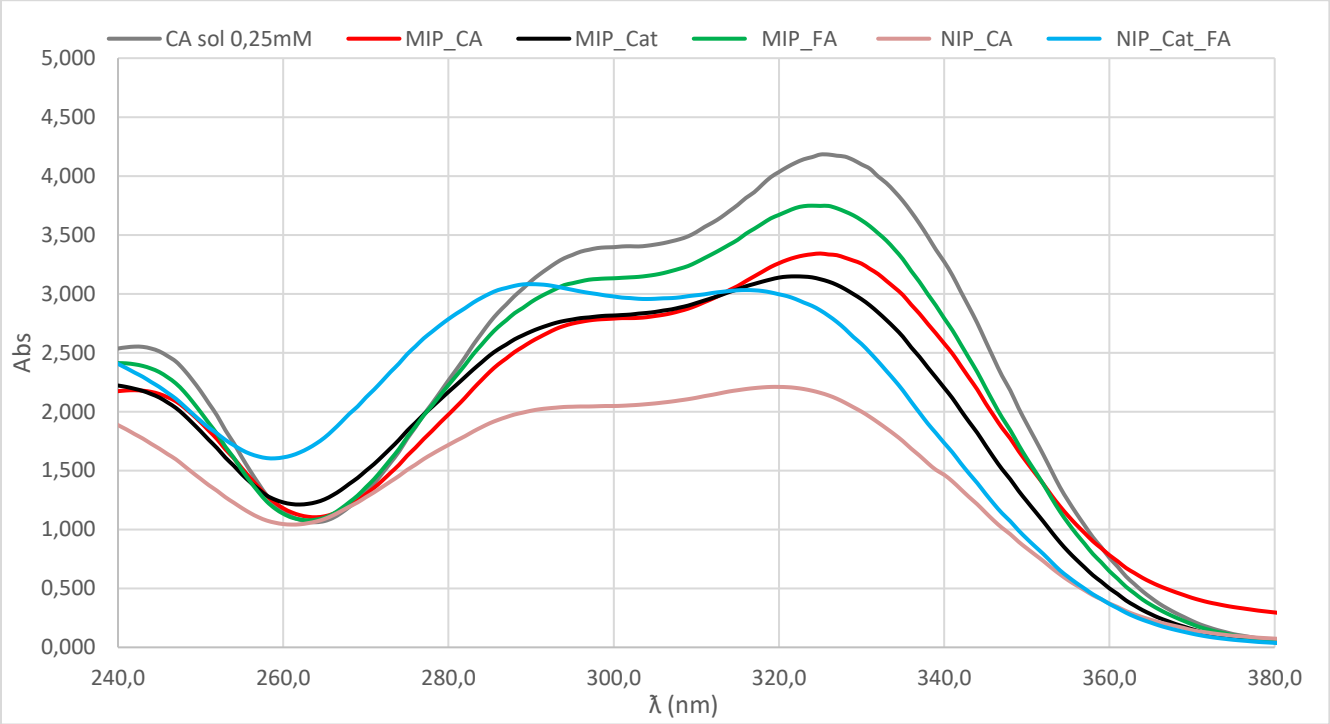


Figure 26: UV response of the fractions collected at outlets of the SPE cartridges after passing 0,25 mM caffeic acid in neutral condition.

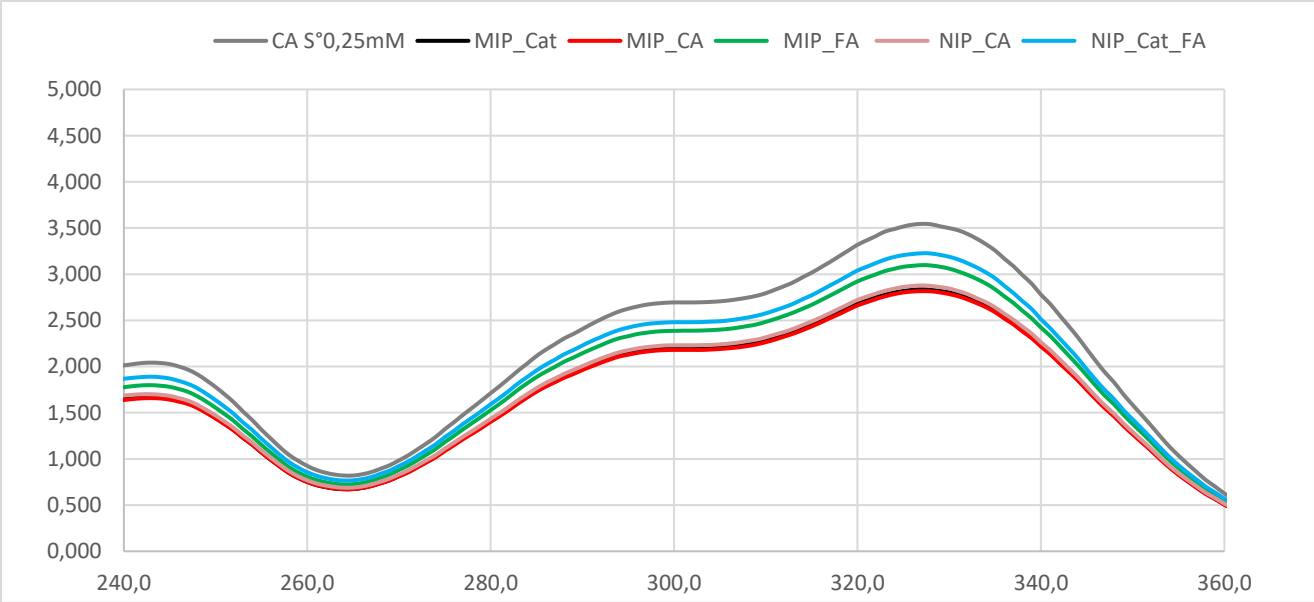


Figure 27: UV response of the fractions collected at outlets of the SPE cartridges after passing 0,25 mM caffeic acid in alkaline condition.

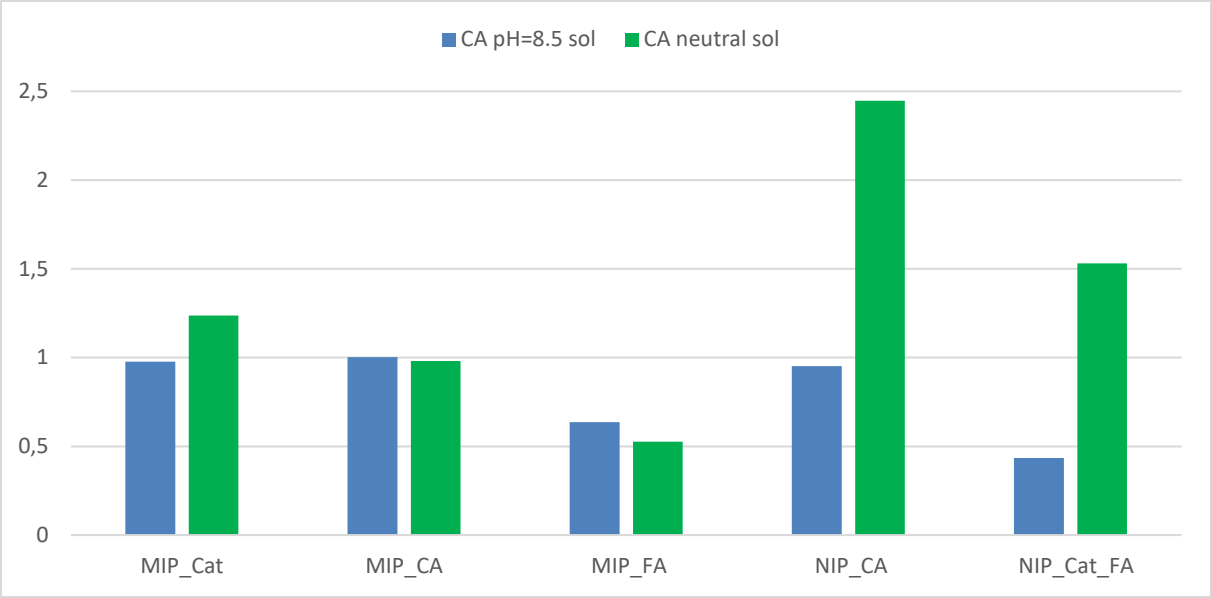


Figure 28: Retained quantities of caffeic acid using a concentration of 0,25 mM at alkaline and neutral pHs.

The comparative plot between the different pH conditions shows an important retention with the NIP_CA both conditions. MIP caffeic retains more in alkaline condition than neutral condition.

III.3.3.1.3 Sorption experiments Catechol

With the catechol molecules we tried different amount of concentration and pH at different conditions: acidic, neutral, and alkaline.

Neutral condition :

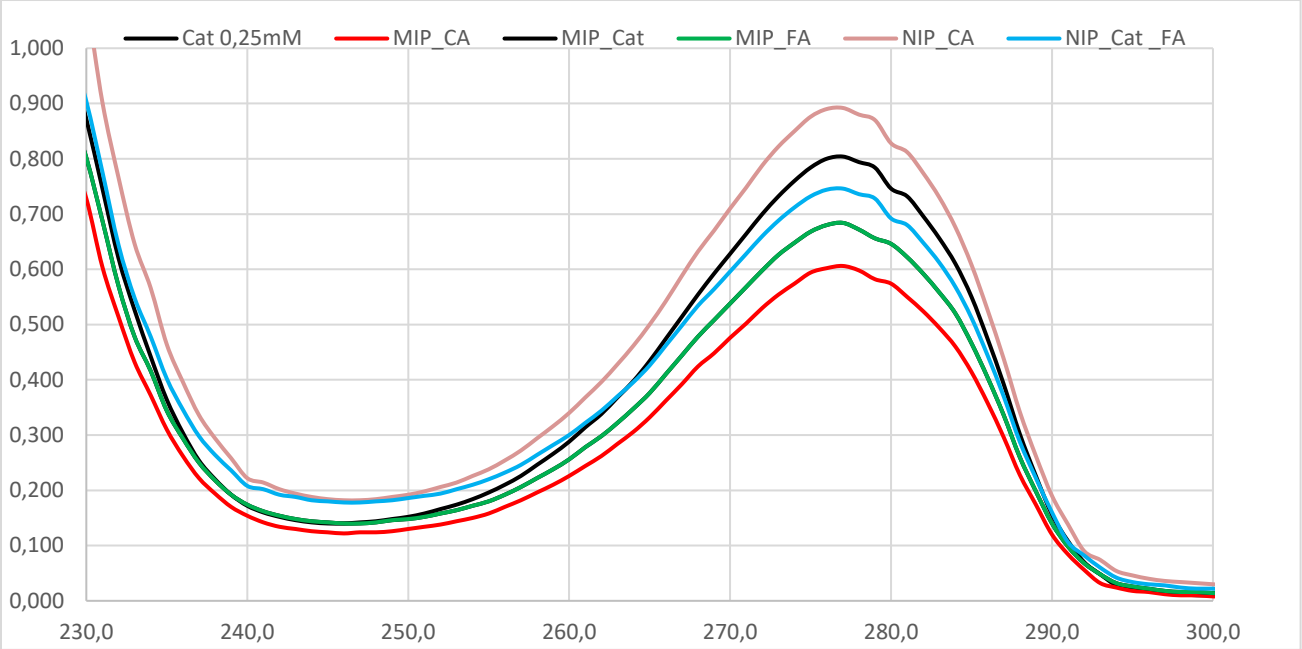


Figure 29: UV response of the fractions collected at outlets of the SPE cartridges after passing 0,25 mM catechol in neutral condition.

Alkaline conditions:

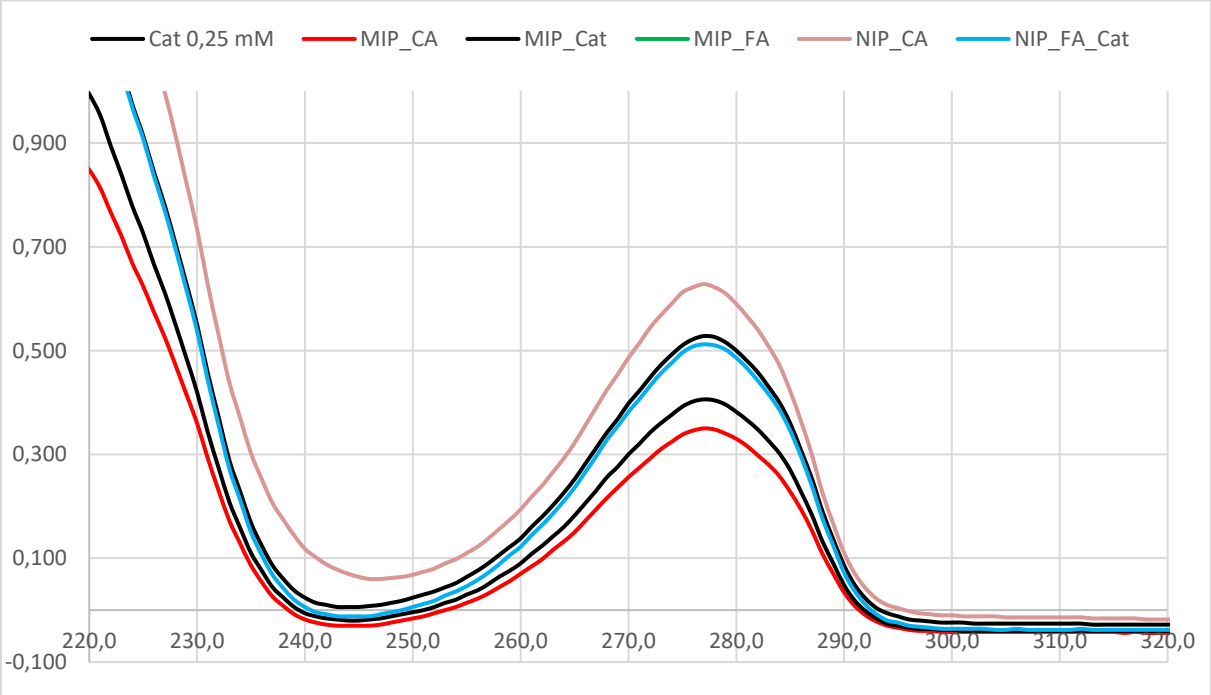


Figure 30: UV response of the fractions collected at outlets of the SPE cartridges after passing 0,25 mM catechol in alkaline condition.

Acidic conditions:

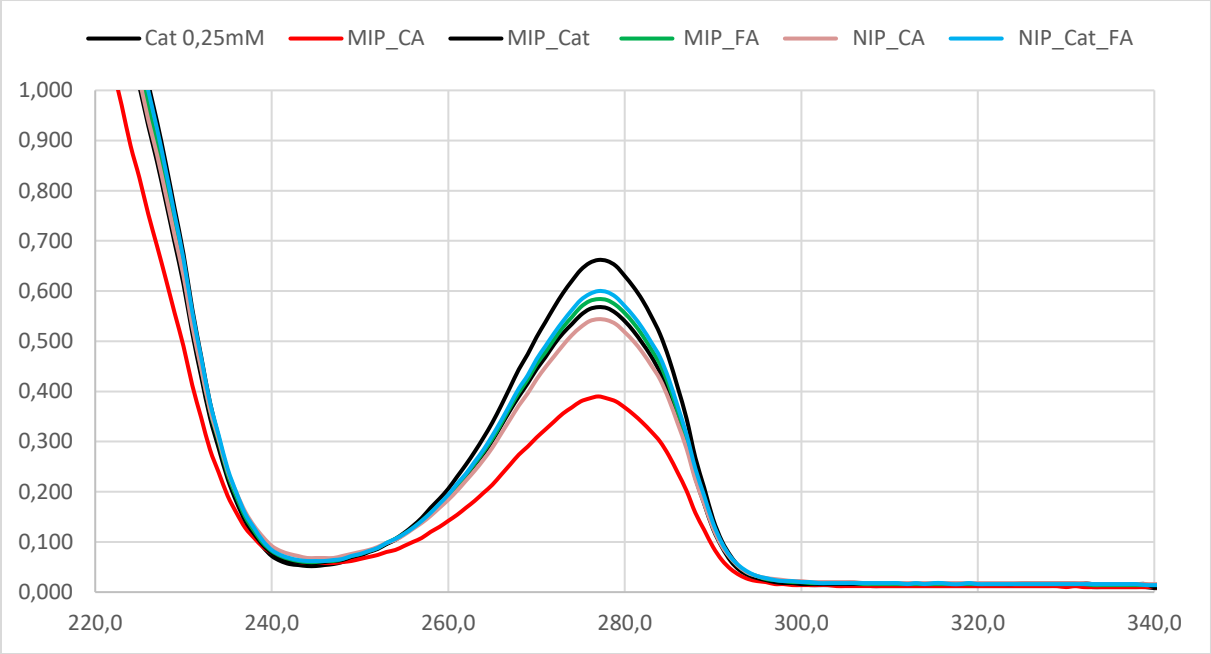


Figure 31: UV response of the fractions collected at outlets of the SPE cartridges after passing 0,25 mM catechol in acidic condition.

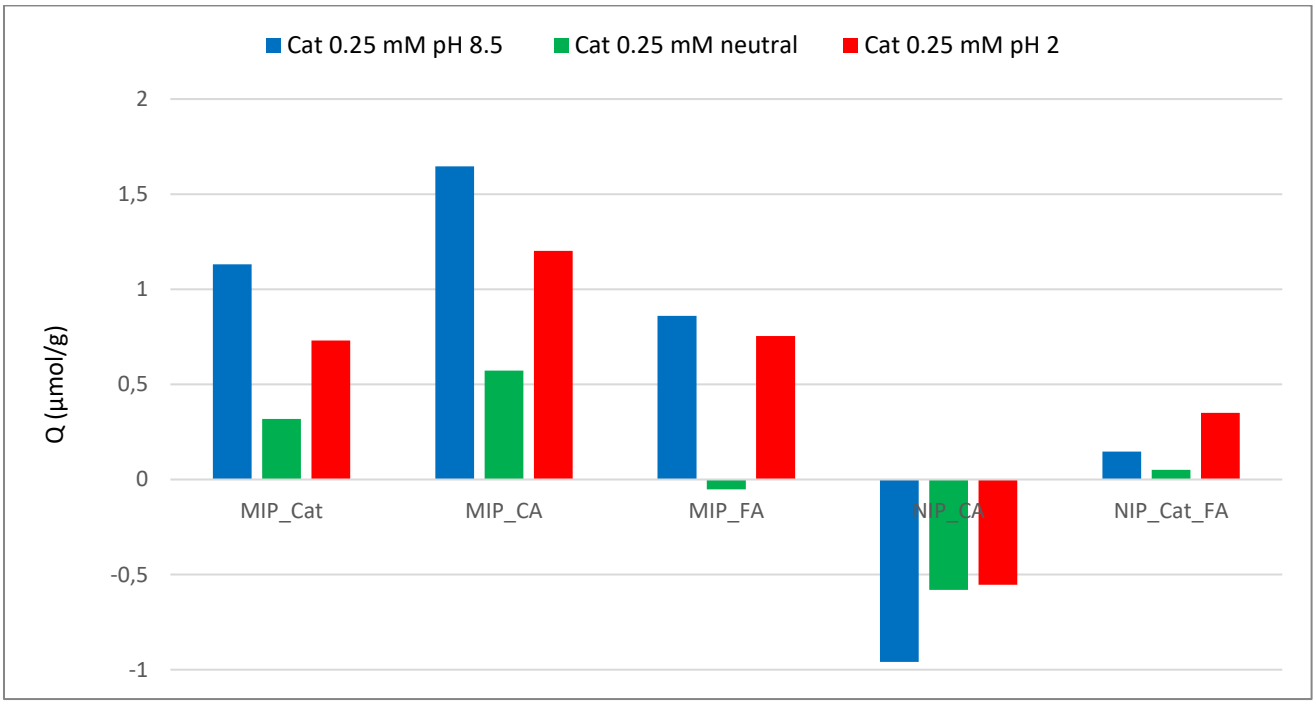


Figure 32: Retained quantities of catechol using a concentration of 0,25 mM at acid, alkaline and neutral pHs.

The plot shows a comparison between three different pH conditions: alkaline, neutral, and acidic with the same concentration of 0.25mM of catechol. For the MIP catechol and MIP Ferulic, we noticed a better retention in alkaline condition.

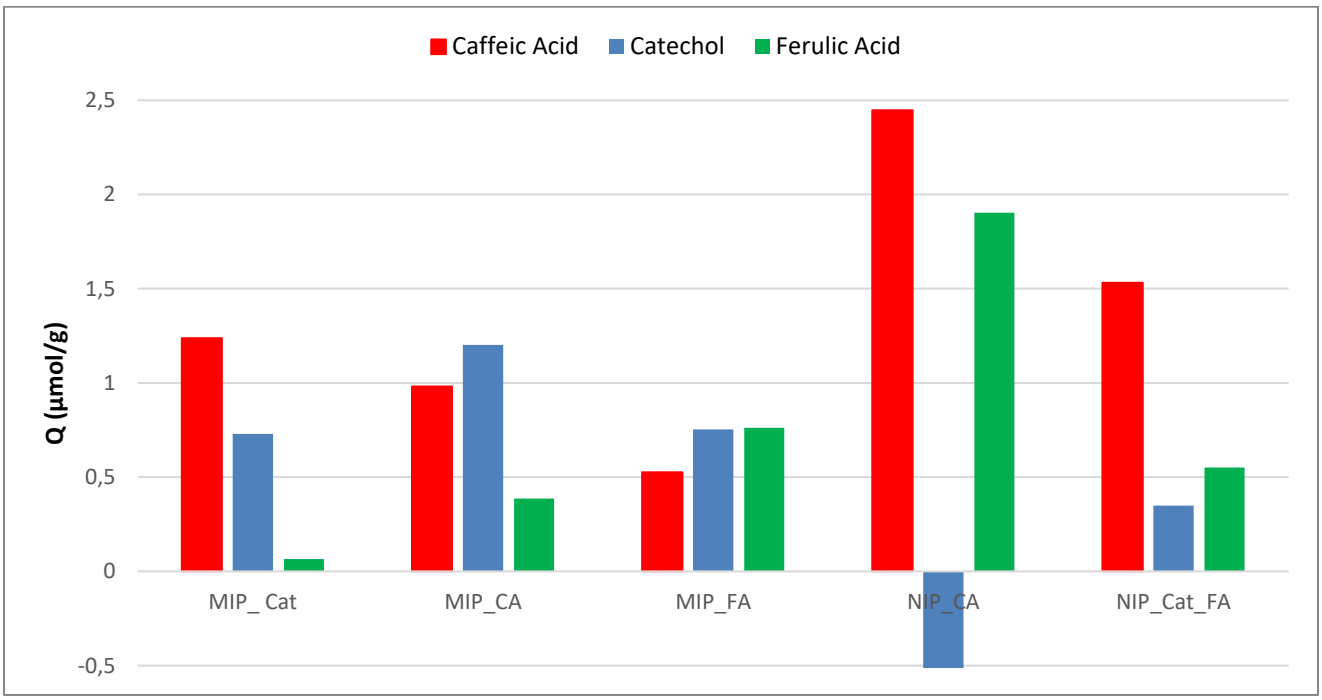


Figure 33: Summary of the retained quantities of catechol, caffeic acid and ferulic acid at the same concentration 0.25mM and at neutral pH.

A comparative plot between the different molecules loaded with a concentration of 0.25mM in neutral condition shows that Caffeic acid has been retained more than the Ferulic acid and the catechol by the MIPs and NIPs especially by NIP_CA and MIP_Cat.

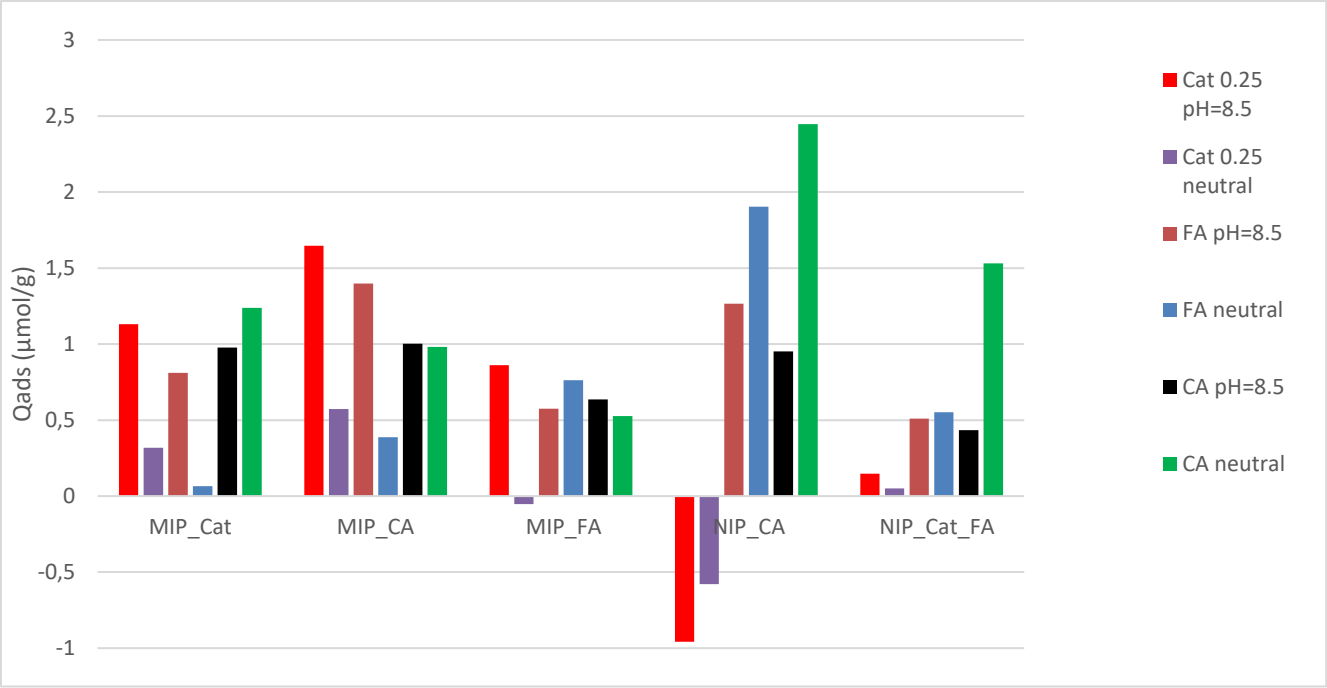


Figure 34: Summary of the SPE results using catechol, ferulic and caffeic acids at neutral and alkaline conditions (all the concentrations are 0.25mM).

Catechol is being retained at alkaline conditions by the MIPs whereas its retention by the NIPs is relatively low. This can be explained by the preferential interactions between cis-diol functions of the templates (excluding Ferulic acid) and the boronate moiety of the 4VPBA. The cyclic esters that form during the alkaline conditions between the boronic materials and the cis-diol templates, disintegrate under acidic conditions. Comparing the retention of MIP_Catechol to NIP_Catechol we can conclude that there is an apparent imprinting effect towards catechol. Regarding caffeic acid, there is no significant apparent imprinting effect. At neutral conditions, NIPs have higher retention for Ferulic and Caffeic acid than the MIPs which can be explained by the non-specific binding of these templates with the materials. This non-specificity is mainly due to the pH conditions that don't promote the boronate affinity.

At an alkaline pH, the binding capacities of MIP_Caff and MIP_cat for ferulic acid is significant (compared to a neutral pH). Such output does not support the theory of the boronate affinity since ferulic acid does not contain any cis-diol function. However, this result can be also explained by the cleanliness of the MIPs which could have been contaminated before the test was performed.

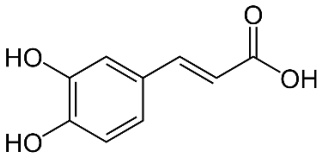
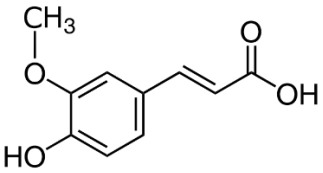
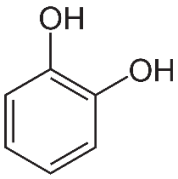
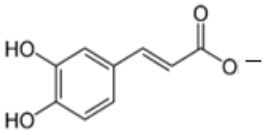
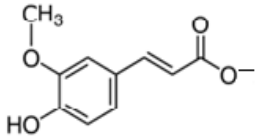
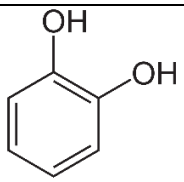
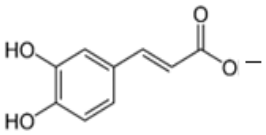
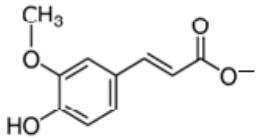
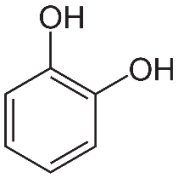
Regarding the caffeic acid, the MIPs do not appear to recognize this molecule since their binding capacities for this compound are close to the ones for ferulic acid.

The catechol molecule appears to be the most retained by the MIPs. This can be attributed to the relatively molecular size of catechol which allow it to diffuse it easily through the particles of the material.

Overall, a discernible pattern explaining the materials' behaviour is not easy to find, and further experiments need to be conducted. However, it is suspected that besides the effect of

pH on the boronate affinity of the materials, the phenolic compound is also affected since they ionize at certain pH level (see table below) which may influence the material's binding capacity.

Table 13 : Influence of pH on the structural change of each molecule.

Cis-Diol ionisation	Caffeic Acid	Ferulic Acid	Catechol
pKa	pKa1 = 4.62 pKa2 = 8.6 pKa3 = 11.2	pKa1 = 4.58 pKa2 = 8.92	pKa1 = 9.45 pKa2 = 12.1
pH = 2	 (Not ionized)	 (Not ionized)	 (Not ionized)
pH = 7	 (Ionized)	 (Ionized)	 (Not ionized)
pH = 8.5	 (Ionized)	 (Ionized)	 (Not ionized)

III.3.3.2 Competitive sorption

The end goal of the present work is to synthesize a polymer material which could selectively retain a cis-diol polyphenol even if this latter exists in a mixture of compounds with similar chemical structure. To test this hypothesis, sorption tests using the synthesized MIPs and NIPs were carried out in a solution containing a mixture of compounds. The sorption test consisted in loading the mixture solution of Ferulic Acid, Catechol and Caffeic Acid (0.25 mM each) into the material by means of a SPE apparatus. Different pH conditions were used, and the outlets were collected and analysed by HPLC. UV-Vis spectroscopy is not suitable to analyse these collected fractions since the compounds absorb in the same regions of the UV-vis spectrum resulting in overlapped responses.

Neutral conditions

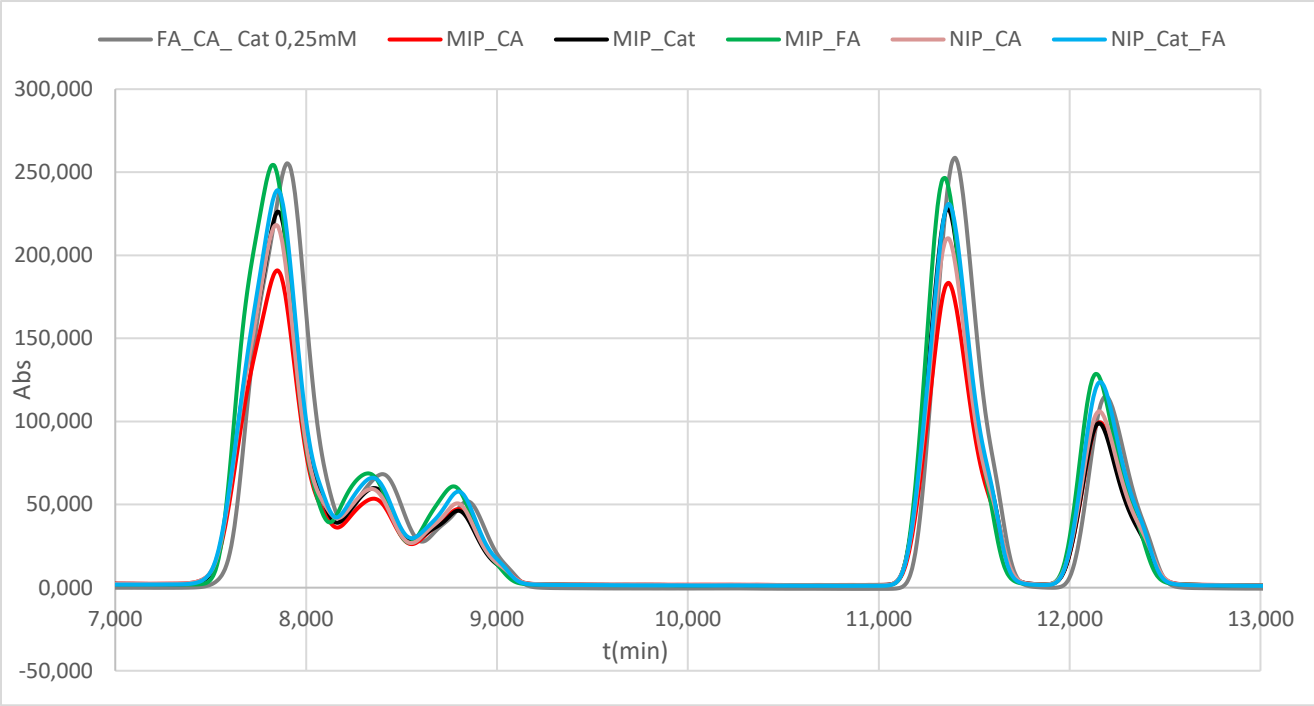


Figure 35 : UV response of the fractions collected at outlets of the SPE cartridges after passing 0,25 mM catechol, caffeic acid and Ferulic acid at neutral condition.

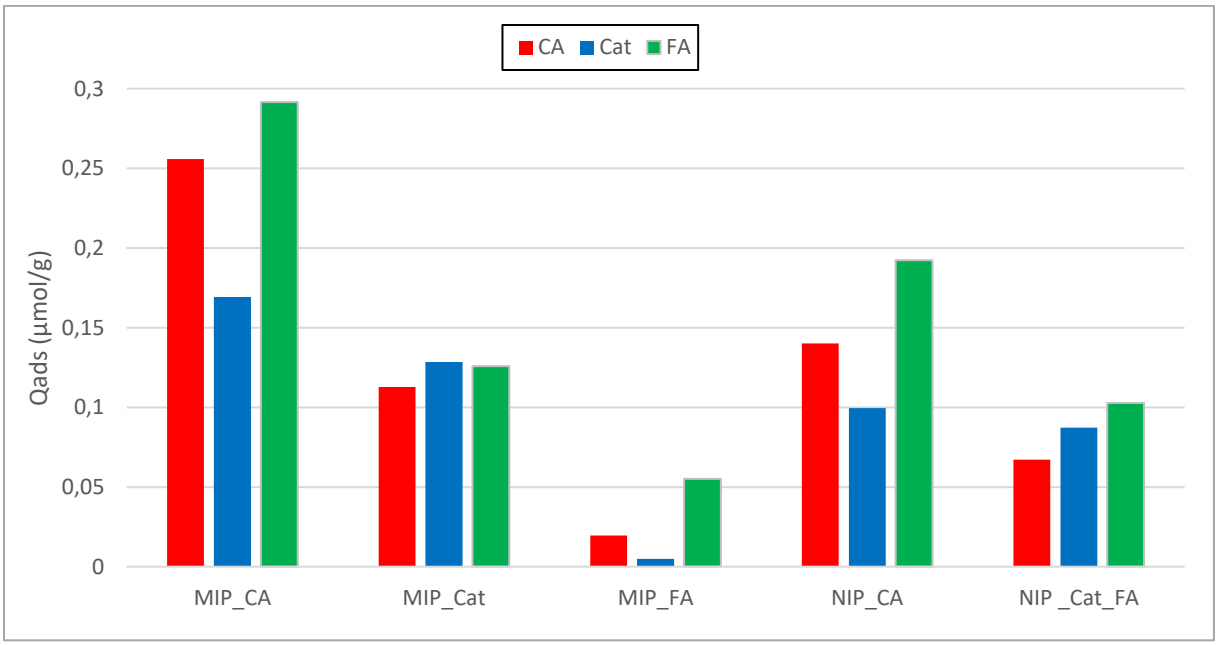


Figure 36: Retention results of mixture of molecules at neutral conditions

At neutral conditions, and after loading the mixture solution of the three molecules, the retention for ferulic acid (lacks cis-diol moiety) was the highest. These results demonstrate that at a neutral pH, the boronic acid monomer units are not activated (lack proper ionization), hence the materials do not bind catechol and caffeic acid (cis-diol compounds) selectively. In

fact, at a neutral pH, other non-specific interactions prevail such as π-π stacking and hydrophobic leading to the retention of ferulic acid.

Acidic condition pH = 2

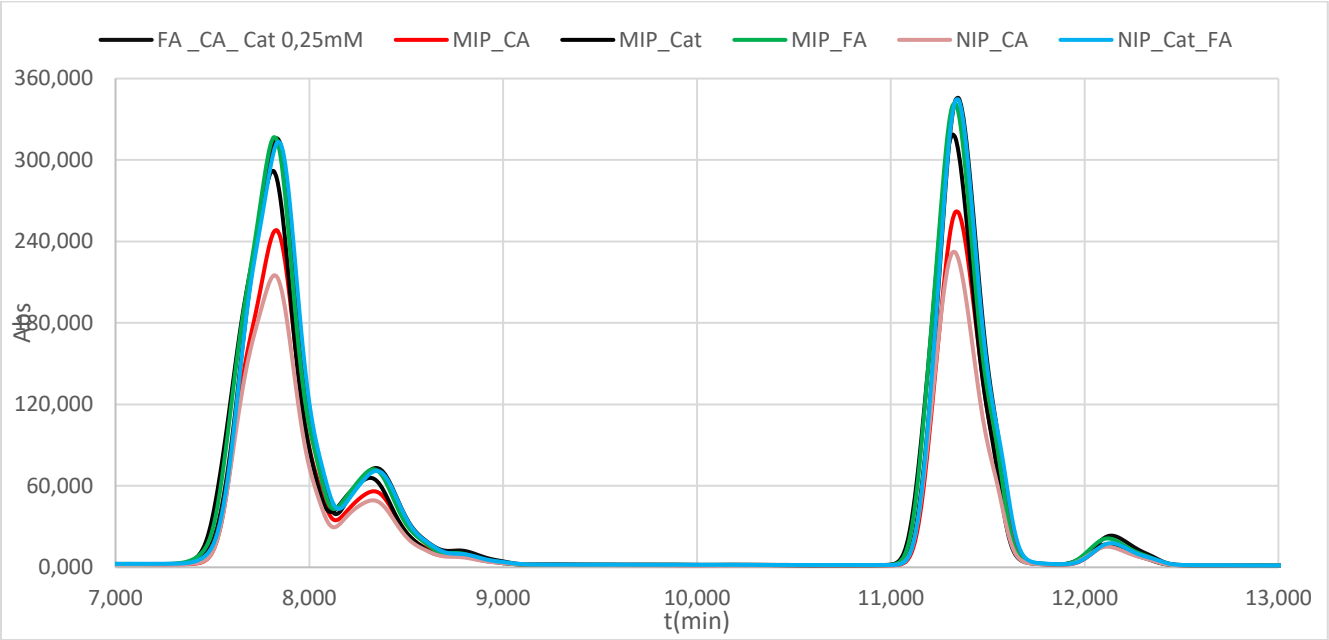


Figure 37: UV response of the fractions collected at outlets of the SPE cartridges after passing 0,25 mM catechol, caffeic acid and Ferulic acid at acidic condition.

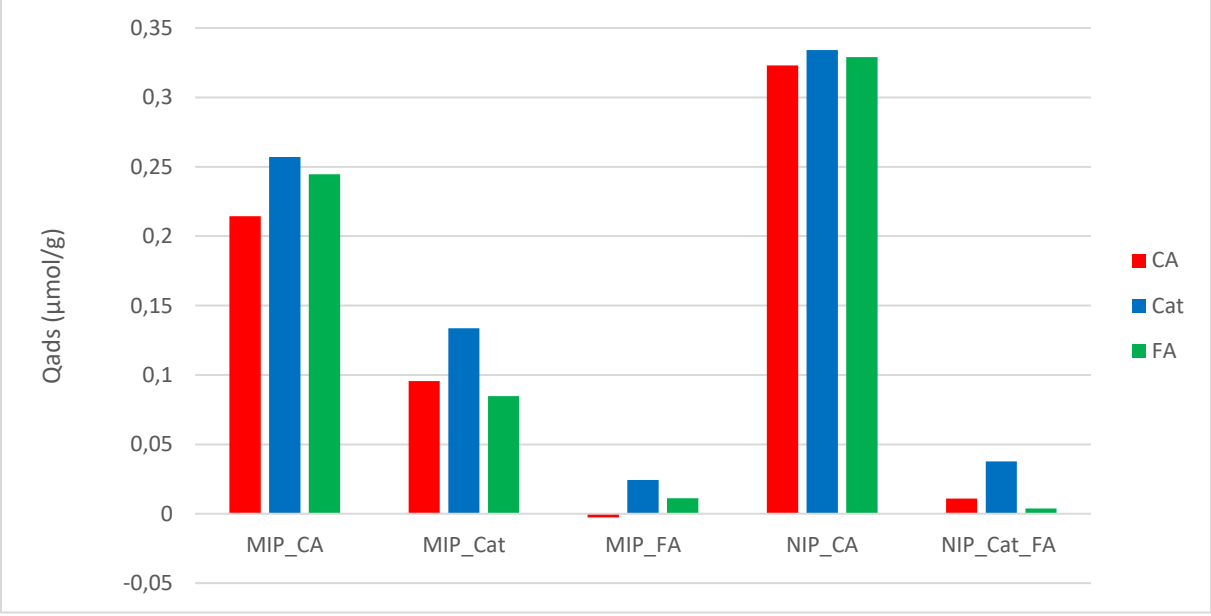


Figure 38: Retention results of mixture of molecules at acidic conditions.

At acidic conditions, the MIPs have the highest retention for catechol. Nonetheless, the other molecules are being retained approximately with the same intensity. This shows that at these conditions, there is no selectivity towards cis – diol molecules, and the high binding capacity

for catechol could be merely explained by the molecular weight that influences the diffusion of the compounds into the materials.

Alkaline condition pH = 8.5

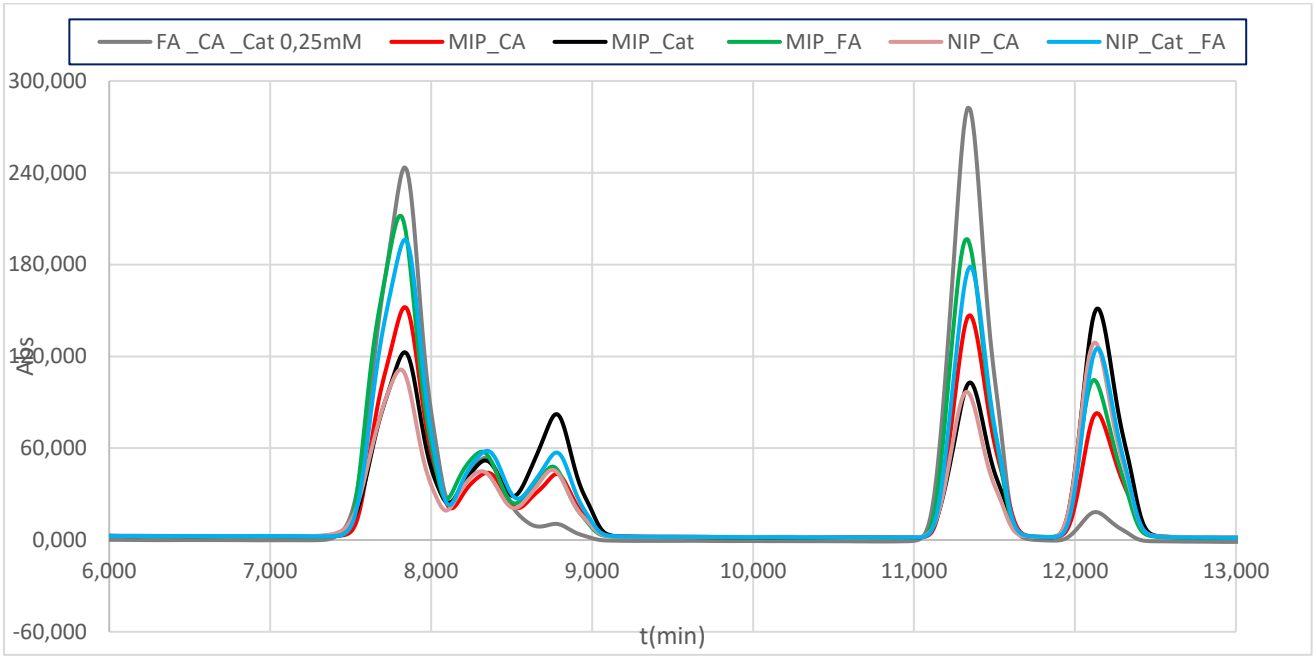


Figure 39: UV response of the fractions collected at outlets of the SPE cartridges after passing 0,25 mM catechol, caffeic acid and Ferulic acid at alkaline condition.

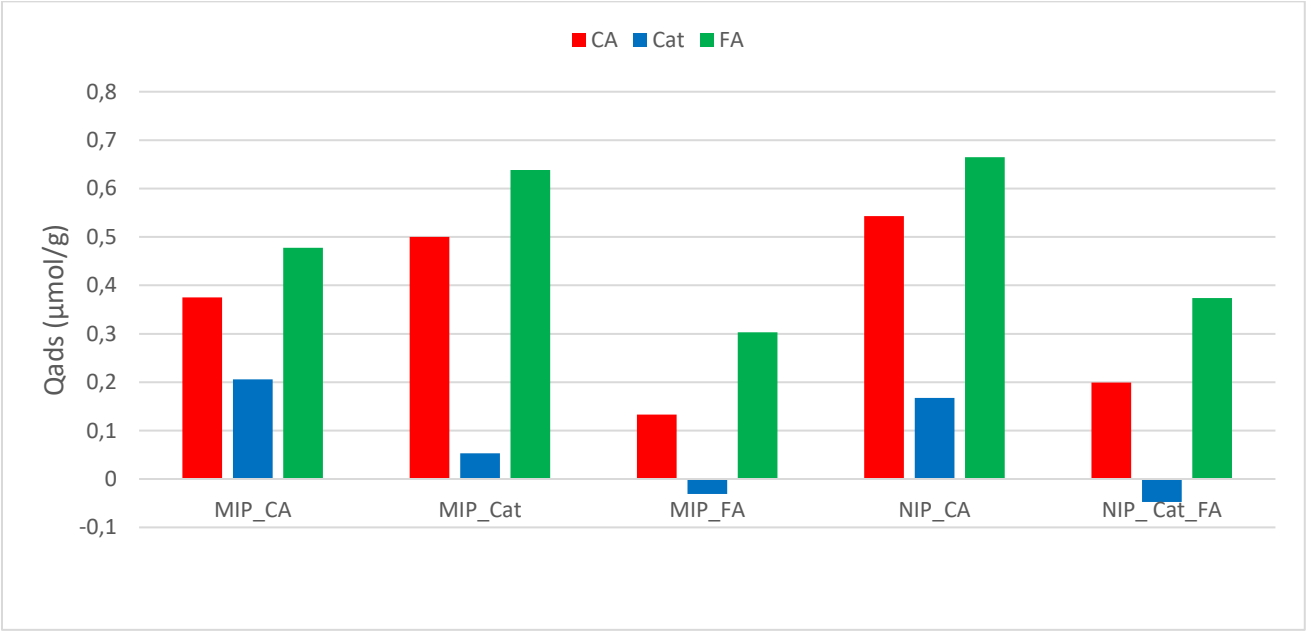


Figure 40: Retention results of mixture of molecules at alkaline conditions.

Compared to the previous acidic conditions, there is a significant difference in the retention of caffeic acid compared to catechol. Such results imply that at alkaline conditions the recognition capability of MIP caff and MIP cat is heightened.

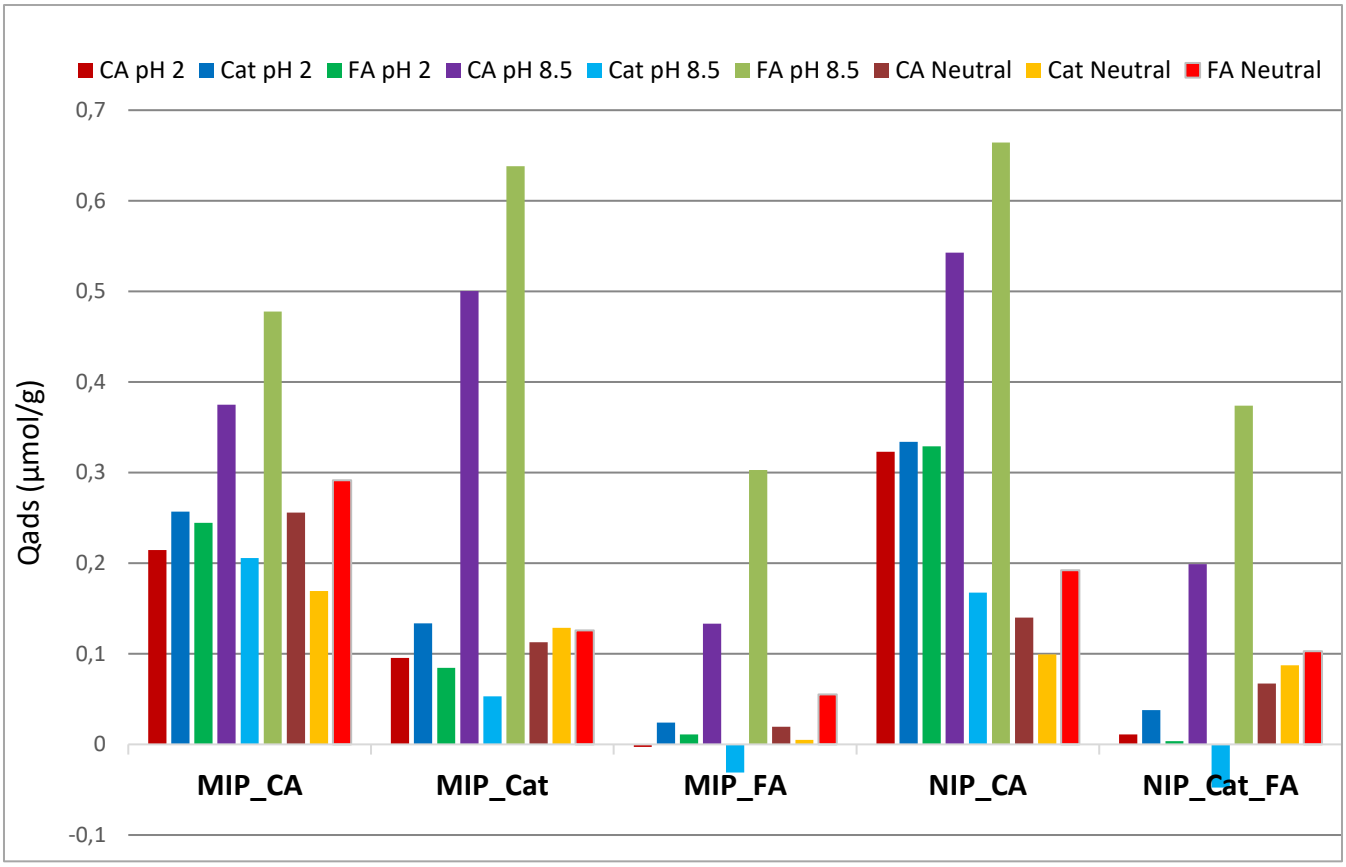


Figure 41: Retention results at different pH conditions for the different materials

All the materials show a high retention for ferulic acid especially at alkaline conditions which can be attributed to the non-specific interactions mainly hydrophobic and π-π stacking. MIP Catechol is retaining caffeic acid which contains the catechol fragment. For catechol the retention seems to be higher at acidic conditions. For ferulic acid and caffeic acid, the highest retention is observed at alkaline conditions.

III.3.4 Diatomaceous earth treatment

In this part, we show a real application of the synthesized materials in the valorisation of winemaking residues, specifically residual diatomaceous earth (RDE). This latter was supplied by Caves Campelo winery (Braga-Portugal). The extraction consisted in weighing an accurate mass of RDE and mixed with ethanol/water (80/20 v/v). The mixture was then put for one hour in an ultrasound bath and then left under stirring for two hours and in dark (wrapped with aluminium paper). Consequently, the mixture was filtered and the extract of RDE was obtained. This same extract was used to saturate a synthetic material followed by different steps of elution using selected solvents. MIP caffeic was the material of choice as it showed promising results in the SPE adsorption tests. The experiments were performed in both neutral and alkaline conditions and the eluted fractions were analysed by HPLC in a tentative to determine the retained/desorbed compounds.

III.3.4.1 Saturation of the material at a neutral pH

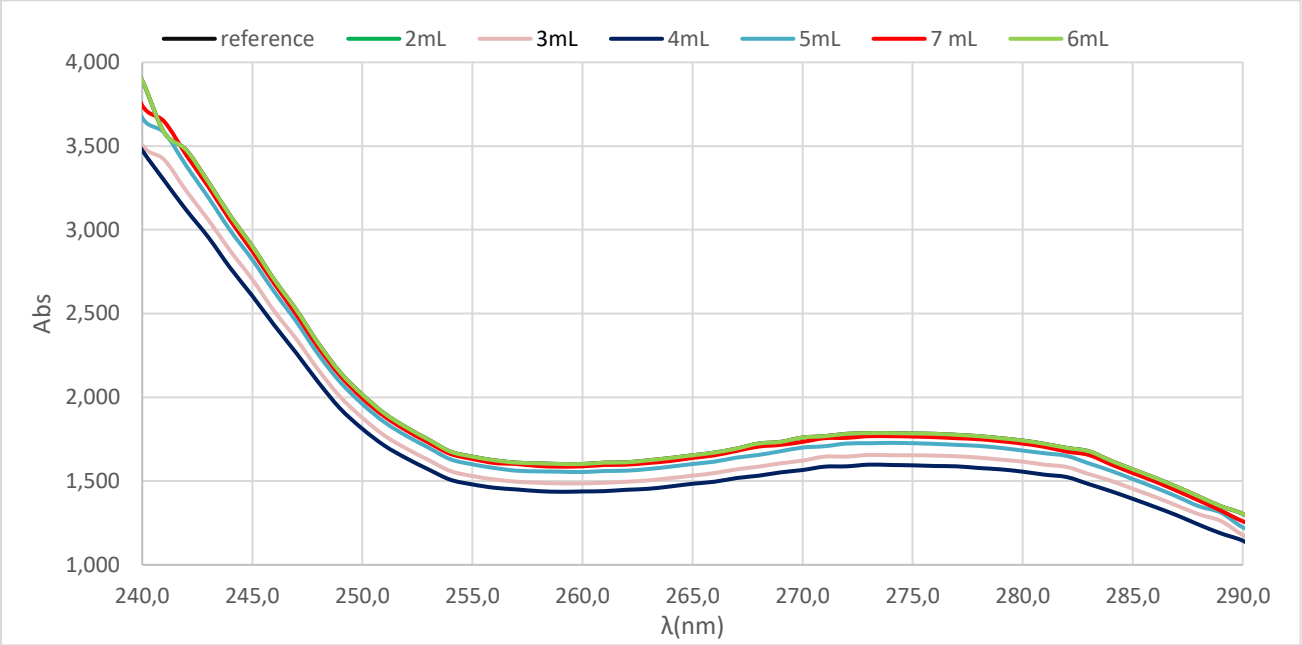


Figure 42: Saturation results for diatomaceous earth extract in neutral conditions.

At a neutral pH, the saturation of the materials was reached after only 7 ml which indicates that these conditions are not the optimal ones for the retention of components existing in the diatomaceous earth extract. This is explained by the activation pH of the synthesized material which corresponds to the pKa of the boronic acid moiety. At a pH > $pKa_{Boronic\_Acid}$, the synthesised polymer is able to form covalent bonds with cis-diol compounds present in the RDE. However, at a pH > $pKa_{Boronic\_Acid}$, the polymer's retention is due only to other intemrolueclar interactions (Van Der Waals forces, hydrophobic etc.).

III.3.4.2 Fractions' recovery resulting from the neutral saturation

The following solvents were used in the desorption phase to obtain the correspondent fractions abbreviated F1 to F7:

1- Neutral water: F1
2- Water at pH=2: F2
3-MeOH/Water (pH=2) 20/80: F3
4- MeOH/Water (pH=2) 40/60: F4
5- MeOH/Water (pH=2) 60/40: F5
6- MeOH/Water (pH=2) 80/20: F6
7- MeOH 100: F7

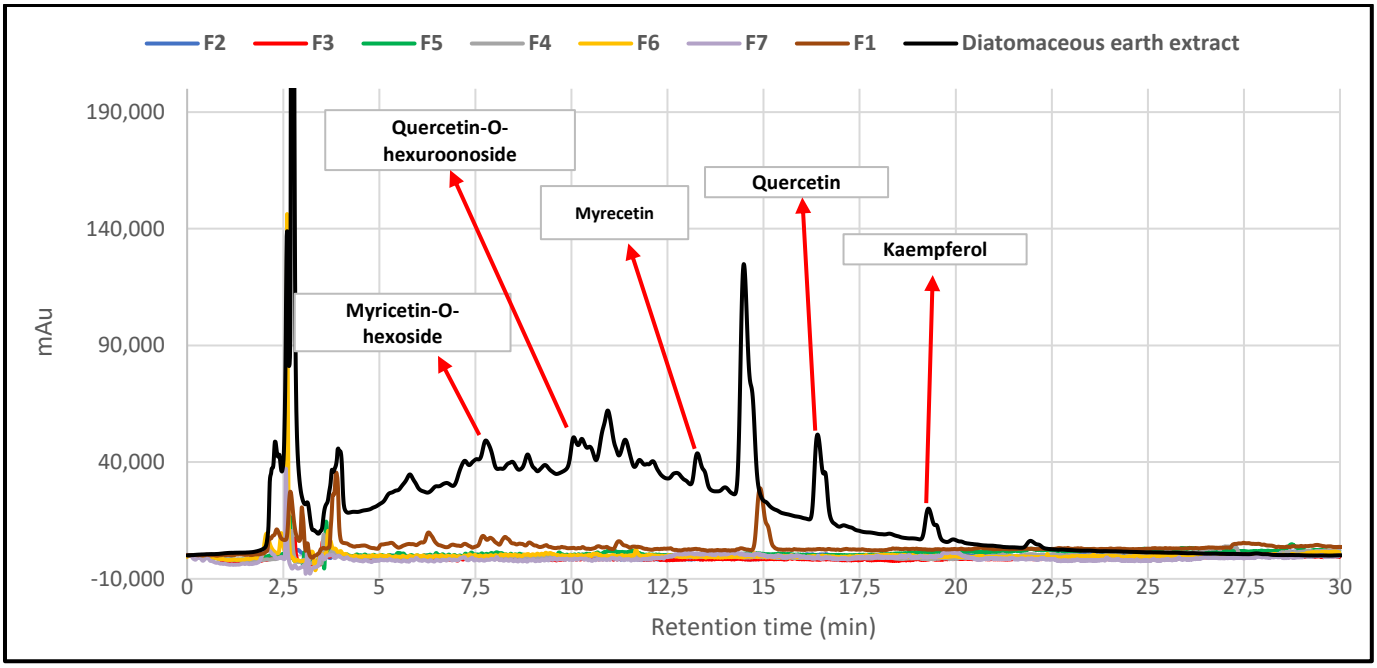


Figure 43: Chromatograms of the desorbed fractions (F1 to F7) and the original diatomaceous earth extract at neutral conditions.

Figure shows the chromatograms obtained of the different fractions resulting from the desorption of MIP caffeic acid at neutral conditions. Only the first fraction shows the isolation of the peak at 14.8min implying that at a neutral pH, the material is not capable of retaining most of the phenolic compounds that initially exist in the diatomaceous earth extract.

III.3.4.3 Saturation of the material at an alkaline pH

We added the ammonia buffer to the diatomaceous earth extract to adjust the pH to 8.5 to permit the activation of the boronic acid moieties of MIP_Caffeic.

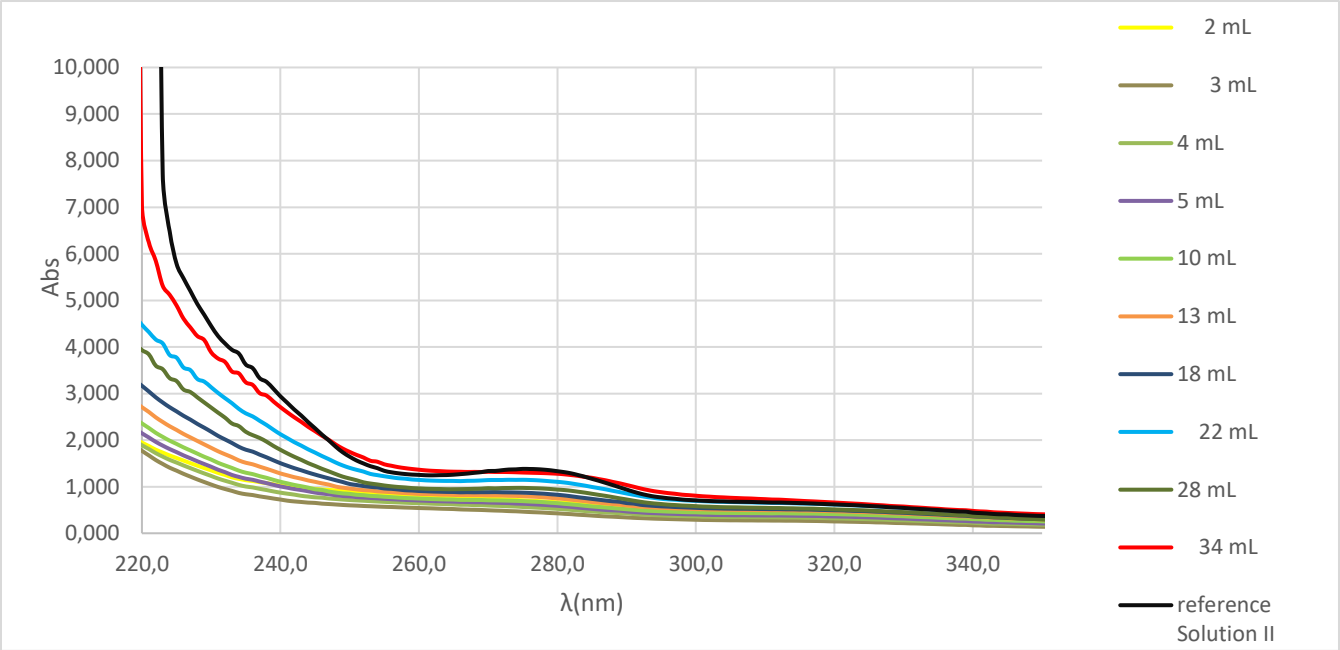


Figure 44: Saturation results in alkaline conditions for extract diatomaceous earth + ammonia buffer

The saturation was reached after 34 ml at alkaline conditions which indicates that these conditions are suitable for the retention of the components existing in the diatomaceous earth extract.

As explained previously, boronated affinity-based materials exert high binding capacity at a pH > $pKa_{Boronic\_Acid}$ which is evidenced by the extract volumes used to saturate the material at neutral and alkaline pHs.

III.3.4.4 Fractions' recovery resulting from the alkaline saturation

The following solvents were used in the desorption phase to obtain the correspondent fractions abbreviated F1 to F7:

1- Neutral water: F1
2- Water at pH=2: F2
3-MeOH/Water (pH=2) 20/80: F3
4- MeOH/Water (pH=2) 40/60: F4
5- MeOH/Water (pH=2) 60/40: F5
6- MeOH/Water (pH=2) 80/20: F6
7- MeOH 100: F7
8-MeOH/AcOH 90/10: F8

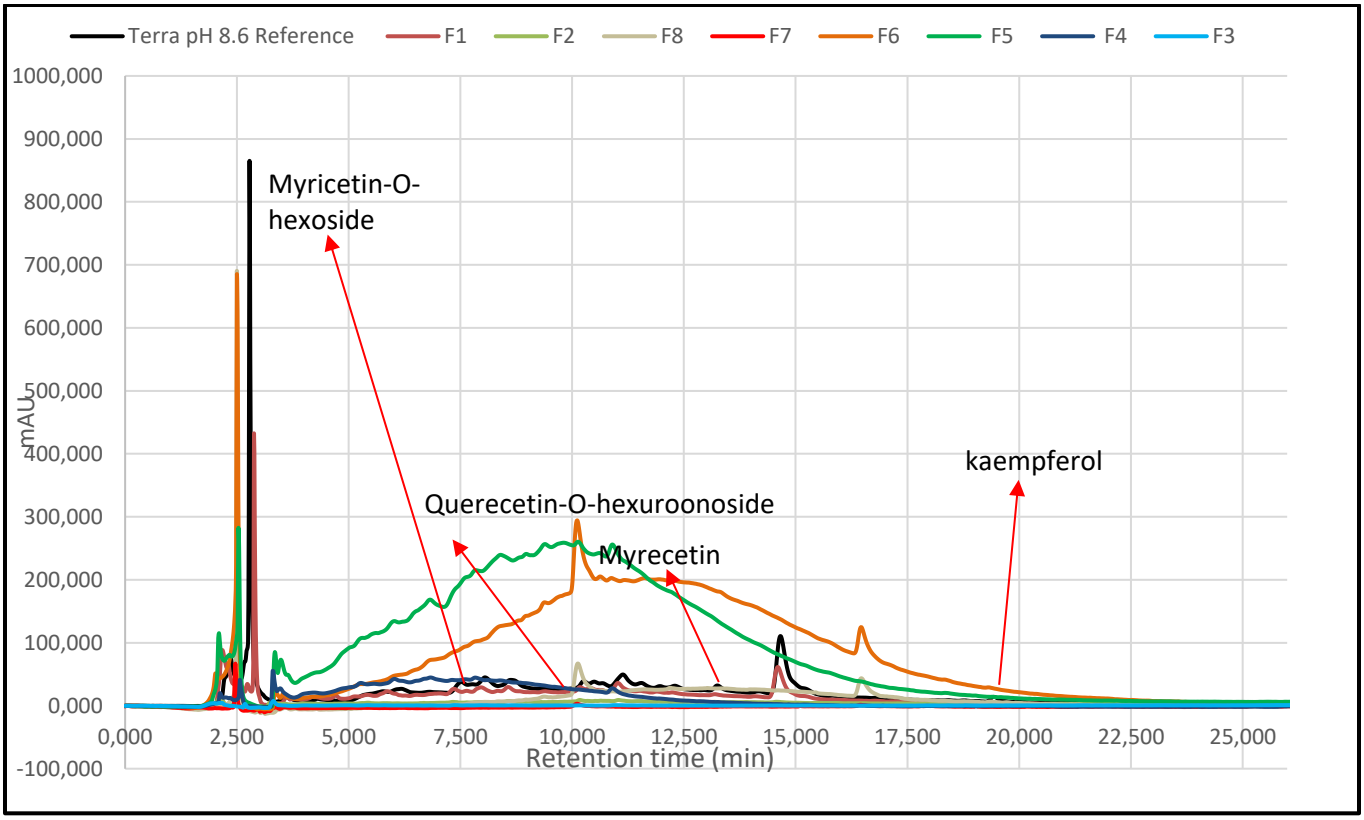


Figure 45: Chromatograms of the desorbed fractions (F1 to F8) and the original diatomaceous earth extract at alkaline conditions

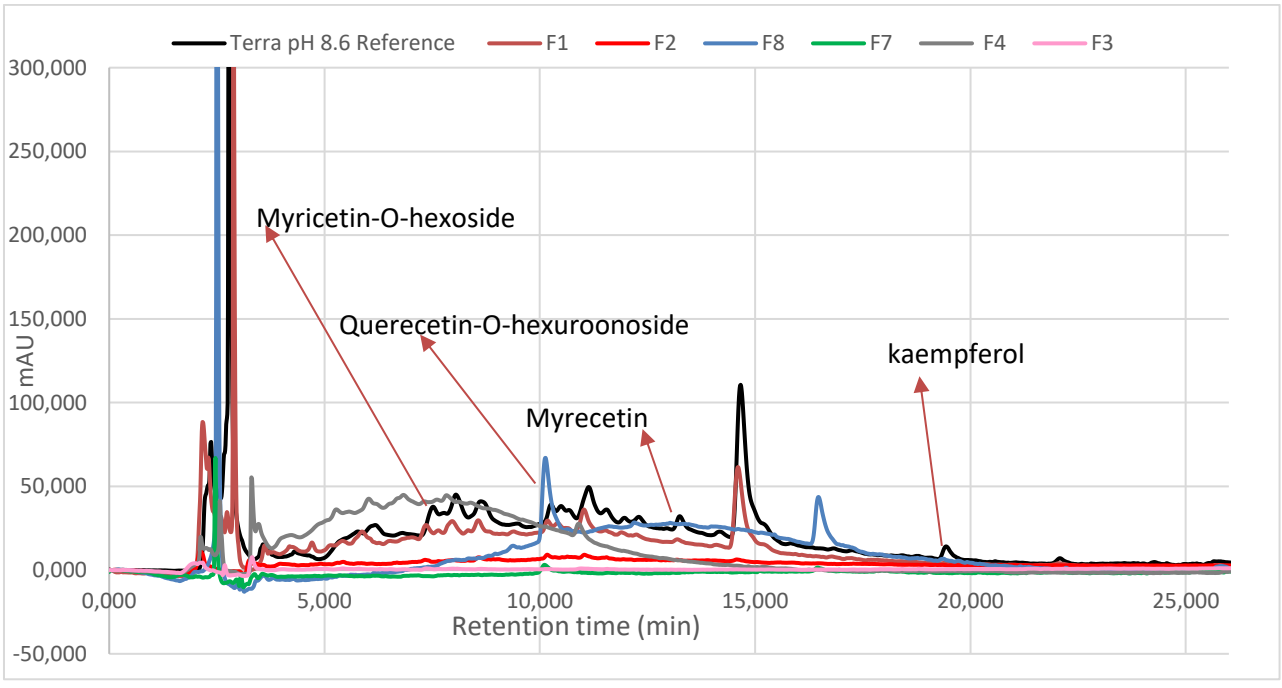


Figure 46: Chromatograms of the desorbed fractions and the original diatomaceous earth extract at alkaline conditions (without F5 and F6)

The figure shows the chromatograms of the different fractions resulting from the desorption of MIP caffeic that was previously saturated at alkaline conditions.

Compounds such as kaempferol, quercetin, myricetin, quercetin-O-hexuroonoside and myricetin -O- hexoside were identified in the diatomaceous earth extract. This identification was carried out by analysing the LC-MS data performed previously by our research group.[32]

In the first fraction (F1) which was obtained by passing neutral water through the MIP_Caffeic, we notice that different phenolic compounds have eluted such as myricetin-O-hexoside, myricetin and quercetin-O-hexuronoside. This shows that water is not the most suitable solvent to fractionate the phenolic compounds since no compound isolation was possible using water. Regarding F2 (water at pH=2) and F3 (MeOH-Water (2:8), pH=2), the peaks have very low intensity implying that these solvents are not able to desorb then retained phenolic compounds from the MIP_Caffeic. In the fourth fraction (F4), hydrophilic compounds have eluted from the material (retention times between 5 and 15 minutes). This output is interesting since it proves that the solvent mixture of MeOH/Water (pH=2) 40/60 permits the separation of the hydrophilic compounds in a single fraction, permitting to ease the process of the separation of the phenolic compounds. [33]

The ammonia buffer appears to affect the chromatographic analysis since in F5 and F6 there is an intense background effect which does not appear in the other chromatograms. A plausible explanation of this, is that mixtures of MeOH-Water 40-60 and 60-40 are eluting the retained buffer which hides the peaks of susceptible compounds. Nonetheless, in fraction 6, two isolated peaks around 10 min and 16.3 min corresponding respectively to quercetin-O-hexuronoside and quercetin. Furthermore, in fraction 7, these two peaks are more purified, suggesting that MeOH is suitable to isolate such compounds without eluting the interfering ammonia buffer. In fraction 8, one can notice that the peaks of quercetin-O-hexuronoside and quercetin increased in intensity signalling that they are almost totally eluted from the material. Both compounds contain cis-diol functions, which explains their desorption form the material when using high composition of MeOH (80% and more) which approximates the polarity of these compounds (increasing the water proportion in the eluting solvent would force these two compounds to stick more to the material).

To overcome the background effect created by the ammonia buffer, the eluted fractions were treated by silica particles. Briefly, 100 mg of silica gel (40-63 microns) were packed into SPE cartridges and then obtained fractions resulting from the alkaline saturation of MIP_Caffeic were passed through these silica particles in an attempt to retain the ammonia buffer.

III.3.4.5 Elution with silica treatment

The same experimental procedure was repeated to saturate MIP_caff and desorb the retained compounds. However, the resulting fractions were treated with silica and injected in to the HPLC allowing the obtention of chromatograms shown in figure 47. The solvents used in the desorption phase to obtain the correspondent fractions are as follows:

1- Neutral water: F1
2- Water at pH=2: F2
3- MeOH/Water (pH=2) 40/60: F4
4- MeOH/Water (pH=2) 60/40: F5
5- MeOH/Water (pH=2) 80/20: F6

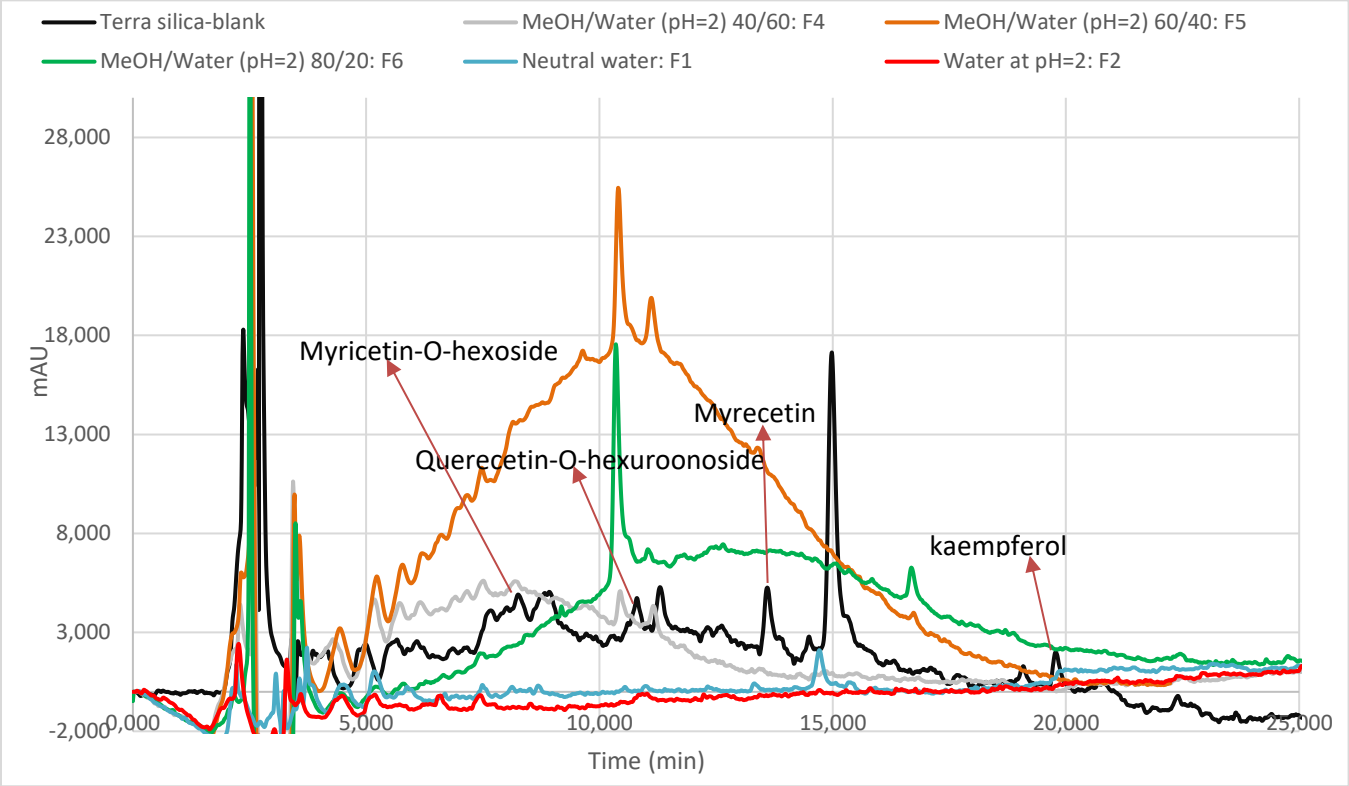


Figure 47: Chromatograms of the desorbed fractions (F1 to F6) and the original diatomaceous earth extract at alkaline conditions and through silica treatment.

Figure 47 shows the chromatograms obtained of the different fractions resulting from the desorption of MIP caffeic at alkaline conditions. It is clear that passing the fractions though silica gel particles, did not eliminate (or even diminish) the background effect of the buffer solution.

IV. Conclusions:

The present work aimed to develop materials able to capture certain phenolic compounds in hydroalcoholic media. Thus, boronic acid was chosen as a functional monomer to synthesize these materials since its working mechanism is suitable for such media. Molecularly imprinted polymers were used to achieve this goal in conjunction with free radical polymerization. The obtained materials were characterized by gravimetrically (to calculate the yield) and by FTIR (to confirm the incorporation of the monomers in the polymers). Furthermore, sorption tests were carried out to determine the binding capacity and the selectivity of the materials towards three phenolic compounds: catechol, ferulic acid and caffeic acid.

These materials were further used to treat residual diatomaceous earth in an attempt to purify some phenolic compounds. High-performance liquid chromatography (HPLC) was used to identify the resulting fractions. Quercetin-O-hexuronoside and quercetin were fairly isolated in some fractions demonstrating the applicability of these materials in real residues treatment.

III- References

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