Atom Transfer Radical Polymerization Synthesis of Hybrid Cellulose-Synthetic Adsorbents for Valorisation of Olive/Olive-Oil Production Residues

By

Angelina Janet Raoula Mupiga Caldeira

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Supervised by

ULT supervisor	**IPB supervisor**
Dr Mohamed MEZNI	Dr Rolando Carlos PEREIRA SIMOES DIAS

Dedication

This work is wholeheartedly dedicated to my beloved mother, who, as a single mother, sacrificed everything for me and my success, who has been my source of inspiration and gave me the strength when I thought of giving up who continually provide her moral, spiritual, emotional, and financial support.

To my brother, my sister, and my friends for their encouragement to finish this work.

Moreover, lastly, I dedicated this work to the Almighty GOD. Thank you for the strength, the guidance, and the power mind, the protection and for giving me healthy life. All of these, I offer to you.

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Abstract

Olive leaves represent a huge amount of by-products in the production of olive and olive oil. Olive leaves, as pomace and olive mill wastewater, contain some compounds which interest food, cosmetics, and pharmaceutical fields like. To obtain these compounds, some steps must be achieved: extraction, separation, and purification. Ultrasound extraction method were performed to obtain olive leaves extract by using two different solvents (EtOH/Water and Ethyl acetate). These extracts were used to perform sorption experiences with a newly synthesized adsorbent. The synthesis of the adsorbent was performed via ATRP. SEM, FT-IR, HPLC-DAD and UV-visible spectrophotometer were used to get more information about the extracts and the adsorbent material.

In olive leaves extracts obtained with the two different solvents, some phenolic compounds were identified: hydroxybenzoic acid, Verbascoside, Luteolin-7-glucoside, and Oleuropein. The synthesis via ATRP using microcrystalline cellulose, Bromoisobutyryl bromide, and 4-Vynilpyridine gave conclusive results. 95.3% of poly 4-Vynilpyridine were found in the final product by gravimetrical analysis; the synthesis gave a percentage of grafting equal to 2045.3%. The sorption experience performed with the olive leaves by using the newly adsorbent material allowed to recover between 30% and 70% of identified polyphenols contained in olive leaves extracts.

Keywords: hybrid materials, cellulose synthetic, ATRP grafting, adsorbents

Resumo

As folhas de oliveira representam uma grande quantidade de subprodutos na produção de azeite. As folhas de oliveira, o bagaço e o efluente de moinho (água ruça), contêm alguns compostos de interesse para as indústrias alimentares, cosméticas e farmacêuticas. Para a obtenção desses compostos, algumas etapas devem ser realizadas, como a extração, separação e purificação. O método de extração por ultrassom foi realizado para obtenção do extrato de folhas de oliveira, utilizando dois solventes diferentes (EtOH / Água e Acetato de Etilo). Esses extratos foram usados para realizar experiências de sorção com um adsorvente -sintetizado. A síntese do adsorvente foi realizada atrâves de uma polimerização via ATRP e o produto final foi caracterizado por SEM, FT-IR, O HPLC-DAD e o espectrofotômetro UV-vis foram utilizados para analisar o extrato das folhas de oliveira.

Nos extratos de folhas de oliveira obtidos com os dois diferentes solventes, alguns compostos fenólicos foram identificados: ácido hidroxibenzóico, Verbascoside, Luteolin-7-glucosídeo e Oleuropeína. A síntese via ATRP usando celulose microcristalina, brometo de bromoisobutiril e 4-vinilpiridina deu resultados conclusivos. 95,3% de poli 4-Vinilpiridina foram encontrados no produto final por análise gravimétrica; a síntese deu uma percentagem de enxertia igual a 2.045,3%. A experiência de sorção realizada com as folhas de oliveira, utilizando material sintetizado como adsorvente, permitiu recuperar entre 30% e 70% dos polifenóis identificados contidos nos extratos de folhas de oliveira.

Palavras-chave: materiais híbridos, celulose sintética, enxerto ATRP, adsorventes

Résumé

Les feuilles d'olivier représentent une énorme quantité de sous-produits dans la production d'olive et d'huile d'olive. Les feuilles d'olivier, comme les grignons et les eaux usées des moulins à olives, contiennent des composés qui intéressent les domaines de l'alimentation, les cosmétiques et pharmaceutiques. Pour obtenir ces composés, certaines étapes doivent être réalisées : extraction, séparation et purification. Une méthode d'extraction par ultrasons a été réalisée pour obtenir un extrait de feuilles d'olivier en utilisant deux solvants différents (EtOH/eau et acétate d'éthyle). Ces extraits ont été utilisés pour réaliser des expériences de sorption avec un adsorbant nouvellement synthétisé. La synthèse de l'adsorbant a été réalisée via ATRP. SEM, FT-IR, HPLC-DAD et spectrophotomètre UV-visible ont été utilisés pour obtenir plus d'informations sur les extraits et le matériau adsorbant.

Dans les extraits de feuilles d'olivier obtenus avec les deux différents solvants, certains composés phénoliques ont été identifiés : acide hydroxy benzoïque, Verbascoside, Lutéoline-7-glucoside et Oleuropéine. La synthèse via ATRP utilisant de la cellulose microcristalline, du bromure de bromoisobutyryl et de la 4-Vynilpyridine a donné des résultats concluants. 95,3 % de poly 4-Vynilpyridine ont été trouvés dans le produit final par analyse gravimétrique ; la synthèse a donné un pourcentage de greffage égal à 2045,3 %. Les expériences de sorption réalisées avec les feuilles d'olivier en utilisant le matériau nouvellement adsorbant a permis de récupérer entre 30% et 70% des polyphénols identifiés contenus dans les extraits de feuilles d'olivier.

Mots clés : matériaux hybrides, cellulose synthétique, greffage ATRP, adsorbants

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Nomenclature

AcOH – Acetic acid

ACN – Acetonitrile

ATRP – Atom Transfer Radical Polymerization

BiBB – Bromoisobutyryl bromide

CuBr – Copper (I) bromide

DMAP – Dimethyl aminopyridine

DMF – Dimethylformide

DMSO – Dimethyl sulfoxide

EtOAc – Ethyl acetate

EtOH – Ethanol

FT-IR – Fourier Transform InfraRed

HPLC-DAD – High Performance Liquid Chromatography Diode Array Detector

MCC – Microcrystalline Cellulose

MeOH – Methanol

PMDETA – Pentamethyldiethylenetriamine

Reillex 402 – Poly(4-vinylpyridine) cross linked with divinylbenzene 2%

Reillex 425 – Poly(4-vinylipyridine) cross linked with divinylbenzene 25%

SEC – Size Exclusion Chromatography

SEM – Scanning Electron Microscope

SPE – Solid Phase Extraction

TEA – Triethylamine

4VP – 4 Vinyl pyridine

General introduction

During the olive oil/olive production process, a large quantity of by-products is produced. These by-products can be a problem for the environment because they are composed of compounds that can be toxic to the environment.

In order to solve this problem, research works have been done in order to recover these by-products. Indeed, this waste is a source of useful compounds for humans. It has been shown that these by-products are a source of compounds having an antioxidant activity that allows treating skin diseases, preventing premature aging of the skin, and treating serious illnesses such as cancers or even Alzheimer's. For centuries plants have provided humankind with useful. In the last century, the use of natural medicine has expanded, and much research has been performed on active components of herbs. The compounds which provide this antioxidant character are called polyphenols. These phenolic compounds contained in plants can be used in many fields like health, cosmetics, food, etc. The valorisation of these compounds is made by some chemical processes such as Extraction, Adsorption, or membrane separation. In this sense, a lot of research has already been carried out using several types of adsorbent materials such as synthetic polymers and activated carbon.

This work has as objective the identification of phenolic compounds contained in olive leaves extract. Also, the synthesizing of hybrid material as adsorbent for retention and the recovery of polyphenols contained in olive leaves extract. To develop a new type of adsorbent material, it has been decided to combine natural and synthetic polymers. The choice of the natural polymer fell on Cellulose. Cellulose is a cheaper polymer because of its availability; Cellulose comes from plants.

The thesis is organized into six (6) chapters:

- Chapter 1 presents the general concept of the topic.
- Chapter 2 explains the process of the synthesis of the adsorbent with the different steps and the discussion about the obtained results.
- In chapter 3, the extraction experiences are described, and a discussion about the identification of obtained compounds is made.

- Chapter 4 explains the different adsorption experiences performed using the olive leaves extracts or polyphenols standard.
- Chapter 5 mainly explains the process of the recovery of phenolic compounds after the adsorption.
- In Chapter 6, adsorption isotherm used to predict adsorption experience are represented.

Chapter 1 : Bibliographical review

1.1. Extraction of polyphenols from olive leaves

Olea europaea L, commonly named Olive tree, is a member of *Oleaceae* [1]. This plant is essentially native to the Mediterranean climate; it is planted in tropical and subtropical regions. It can be found predominantly in Portugal, Greece, Italy, Spain, France, Australia, Cyprus, Jordan, the USA, Morocco, Turkey, and Tunisia [1]. The main product extracted from Olive trees is olive, olive and olive oil hold an important place in the diet of Mediterranean countries. Olive oil is widely studied for its antioxidant benefits, particularly given to a large number of phenolic compounds present in olive oil [1] [2].

1.1.1. Olive leaves

The production of olive/olive oil generates a certain amount of waste. Among this waste, there are leaves, pomaces, and wastewater. Leaves are some residues of the harvest. Pomaces are by-products of the olive oil production process; pomace is composed of skins, pulp residues, and kernel fragments. The wastewater is composed of the water contained in the cells of the olive, the water used for both dilutions of olive paste, the washing water, and those related to the treatment process [3]. The only aqueous discharge is composed of washing water; it is an important source of antioxidants. Indeed, for a liter of wastewater, 1.2 grams of Hydroxytysol and 0.4 grams of flavonoids are obtained. In addition, other compounds such as Tyrosol, caffeic acid, and vanillic acid are obtained from wastewater [3].

Olive leaves, illustrated in *figure 1*, represent 4.5 million of tons of the olive oil industry per year. Olive leaves also contain polyphenol compounds [4], especially in Oleuropein. Moreover, olive leaves are within easy reach either from the olive orchard or from the residues that remained after agricultural and industries by-products. You should know that the concentration of polyphenolic compounds in olive leaves depends on the quality of the leaves, origin, maturity stage, climate, tree/leaf time, storage, the harvest time/period, the extraction method, the variety of olive tree and on the cultivator [1], [5].

Figure 1: Olive leaves

1.1.2. Phenolic compounds

Phenolic compounds or Polyphenols are a group of heterogeneous compounds. These compounds mainly come from the plant. Indeed, plants synthesize these compounds to control oxidative stress and protect them from external threats [6]. There are close to 10000 identified polyphenols [7]. As their name is, they have at least two phenolic groups in their structure. Their structure is complex. It is based on linkage between hydroxyl groups (polar phase) and one aromatic ring, and they have a high molecular weight.

There are synthetic and natural polyphenols. Natural polyphenols group together a vast collection of chemical substances. There are four main families of phenolic compounds:

- Phenolic acids: it is an organic compound with a carboxylic function and a phenolic hydroxyl. The name “phenolic acid” is used to characterize a derivative of benzoic acid and cinnamic acid.
- Flavones: it is the base molecule of the flavonoids. Flavones are colorless and water-soluble.
- Chlorogenic acids: it is produced by plants like coffee plants, potatoes, artichokes, endive, etc.
- Quinones: Do series of dienes comprise a benzene core on which four atoms of hydrogen are replaced by two atoms of oxygen, making two carbonyl bonds.

Polyphenols are a source of antioxidants. It is this property that makes us pay attention to polyphenols. The phenolic compounds from plants are waste from agriculture and the production of oil or natural beverage.

An antioxidant is a molecule that stops or reduces the oxidation of other molecules. Humans need dioxygen, but oxygen can create some compounds that can be toxic for cells integrity. These toxic compounds are maned reactive oxygen species or free radicals. That is why antioxidants are necessary. The human organism has its own antioxidants, but many severe human illnesses are due to oxidative stress, and an application of an external source of antioxidants can help in fighting cell oxidative cells and their consequences. For many years synthetic antioxidants were used, but it has been reported as dangerous for human health. That is why natural antioxidants pay attention [6].

There are several types of polyphenols from plant by-products. After recovering polyphenols from the by-products, it is necessary to carry out certain analyses to spot the presence of the compounds that interest us and to determine their quantity in the solution. These analyses are named Identification and Quantification. A lot of techniques can be used to achieve it, but in this work, we will focus only on High-Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD).

High-Performance Liquid Chromatography (HPLC) is a general analytical method. The HPLC has a stationary phase and a mobile phase. These phases are the meeting of spherical micro-particles or are porous monolithic materials that decrease the pressure drop of the column; a strong pressure is therefore applied to the mobile phase to have a suitable flow.

The apparatus of the HPLC, shown in *figure 2*, is constituted by a mobile phase (solvents), a degasser, a pump, an injector, a thermostatically controlled column, and a detector. The chromatographic analysis does not intend to determine the total composition of a sample, at least rarely. Its objective is to spot the presence or to dose a compound, and to do it, the choice of a suitable detector and the suitable solvents is necessary.

As the main detector, it can be cited: Spectrofluorometric detector, refractometric detector, and spectrophotometric detectors. And in the last type of detector, the diode array detector is the one that interests us. This detector is used in absorption spectroscopy using the UV-visible; it allows to obtain a chromatogram and a lot of spectral information, both, that can identify separate compounds [6].

The choice of solvent depends on the type of stationary phase; the mobile phase (solvent) must be less polar than the analyte. And to help to choose the solvent, Snyder's triangle can be used. As solvents, it can be cited: Methanol, Acetonitrile, Trifluoroacetic acid, Phosphoric acid [6], [8], [9].

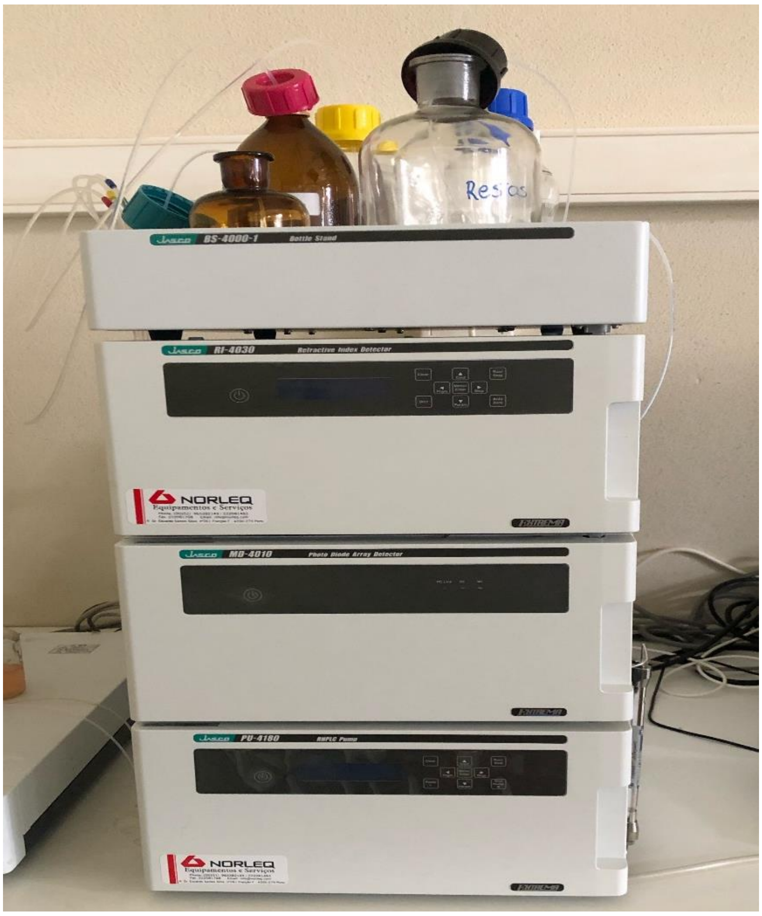


Figure 2: HPLC apparatus

1.1.3. Extraction of polyphenols from olive leaves

This work is mainly focus on the Ultrasound extraction method. Other methods were use for comparative purpose.

- **Soxhlet extraction method**

The Soxhlet extractor has three main sections. First, we have the boiler and reflux to circulate the solvent, then a thimble to retain the solid to be extracted, and the siphon mechanism to empty the thimble periodically.

The sample is placed in a thimble that is gradually filled with condensed fresh extractant from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates it from the thimble and unloads it back into the distillation flask.

The operation is repeated until complete extraction is achieved. This performance makes Soxhlet a hybrid continuous-discontinuous technique [10].

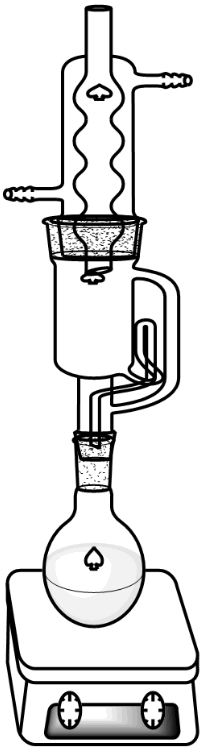


Figure 3: Soxhlet extractor

- **Ultrasound extraction method**

This method is one of the simplest and the lowest cost extraction methods, and it demands a short processing time. This extraction method is used with plant matrix because of cell walls rupturing due to the sound waves near the vegetal tissue.

The mixture of plant and solvent was sonicated directly for a specific time. The choice of the solvent is specific to the plant material, and it is crucial to choose it well. After sonication, the mixture was filtered to get the concentrated extract.

In this work, olive leaves were used with organic solvent such as Ethanol/Water and Ethyl acetate to perform the ultrasound extraction [11].

1.2. Sorption experiences

The general term is “Sorption.” This term was introduced by J. W. McBain. This term includes selective transfer and/or into the bulk of a solid or a liquid.

In chemistry, adsorption is a surface phenomenon by which the atoms, ions, or molecules are fixed on a solid surface from a gaseous, liquid, or solid solution phase. This process is based on the interaction between the species that are adsorbed and the surface adsorbent.

1.2.1. Adsorption/Desorption

The adsorption and the desorption are complementary reactions. The adsorption takes place first, and after it is the desorption.

The adsorbed solute is called *adsorbate,* and the solid material is the *adsorbent*. During the adsorption, the adsorbent becomes saturated or nearly saturated with the *adsorbate*, and to recover the *adsorbate* and allow *the adsorbent* to be reused, it is generated by desorbing.

Adsorption is a low-cost technology allowing the facile regeneration of active solvents [12]. The type of forces between the fluid molecules and the solid molecules allows classifying the adsorption; there are two types of adsorptions: physical or chemical.

Physical adsorption or van deer Wall’s adsorption: this method is made by a drop of the temperature. The method involves weak bonds between the adsorbed species and the adsorbent. These bonds are like those involved in liquefaction. This adsorption is reversible, and the equilibrium is obtained when the rates of the adsorption and desorption are equal. The physical adsorption begins as a monolayer, becomes multi-layered, and then capillary condensation occurs.

Chemical adsorption involves high binding energies between the adsorbed species and the adsorbent. The activated adsorption involves the formation of chemical bonds between the adsorbent and the adsorbate in a monolayer, often with a release of heat much larger than the heat of vaporization. This adsorption is often irreversible.

Commercial adsorbents rely on physical adsorption; catalysis relies upon chemical adsorption.

Table 1: Comparison between Physical and Chemical adsorption

Physical adsorption	**Chemical adsorption**
Low heat of adsorption	High heat of adsorption
Nonspecific	Highly specific
Monolayer or multilayer. No dissociation of adsorbed species. Only significant at relatively low temperatures.	Monolayer only. May involve dissociation. Possible over a wide range of temperature.
Rapid, non-activated and reversible. No electron transfer although polymerization of sorbate may occur.	Activated, may be slow and irreversible. Electron transfer leading to bond formation between sorbate and surface.

The adsorption can be achieved in two ways: batch or continuous process. The two processes must be done to make a comparison between them. Furthermore, some factors can influence adsorption. Therefore, we must consider the kinetics of the adsorption and the isotherm.

Several experimental adsorption isotherms can be cited, but the commonly used are eight in number. Among the eight, it is included the five major classifications of adsorption isotherms by *Brunauer, Deming, Deming,* and *Teller* (BDDT). The adsorption isotherms are represented by a plot of the amount adsorbed versus the relative pressure.

In the following equations, the recurrent parameters are q_e, C_e and q_m. They are successively the amount adsorbed at the equilibrium, the concentration at the equilibrium and the maximum amount adsorbed.

- *Linear adsorption Isotherm:* this isotherm model is described by the Henry Law.

$$q_e = K_{HE} C_e \tag{1}$$

With K_{HE} the Henry constant

- *Freundlich adsorption isotherm is* used for heterogeneous surfaces with high and low adsorption affinity regions.

$$log\, q_e = log\, K_F + \frac{1}{n} log\, C_e \qquad (2)$$

With K_F and n, characteristic constant of Freundlich isotherm

- *Langmuir adsorption isotherm* (Type I) can be described by the equation below.

$$\frac{c_e}{q_e} = \frac{1}{q_m K_L} + \frac{c_e}{q_m} \qquad (3)$$

With K_L the Langmuir constant

- *Type II:* refers to reversible and unrestricted multilayer physical adsorption on nonporous (e.g., metal; alumina) or macroporous solids, displaying a point of inflection that is attributed to the formation of a monolayer.

- Type II and III can both be described by the BET adsorption model given by the following equation.

$$\frac{q_e}{q_m} = \frac{c_{BET} \cdot x}{[1-x] \cdot [1+x(c_{BET}-1)]} \qquad (4)$$

With c_{BET} and x constants

- *Type III:* is characterized by the convexity towards the partial pressure axis, starting at the origin.
- *Type IV:* is analogous to Type II isotherm, having an inflection (or knee) as the monolayer formation.
- *Type V:* is commonly observed for flat, homogeneous adsorbents.
- *High affinity*: is used typically for very strong adsorption interactions [48].

Desorption is the reverse phenomenon of adsorption. The adsorbed molecules stand out from the adsorbent. It is made possible by increasing the temperature or by decreasing the pressure. This process is complementary to adsorption. The desorption allows the recovery of the adsorbed molecules and the adsorbent.

The adsorption/desorption is an important phenomenon in many physical and chemical processes (capture of pollutants, gas separation, measurement of the specific surface, porosity study, etc.).

1.2.2. Adsorbent

An adsorbent is a material optimized for a certain type of adsorption. There is no list that can be used to define an adsorbent, but there are a very large number of materials that may have different properties. To be suitable for commercialization, an adsorbent should have:

These properties are:

- High selectivity to enable sharp separations
- High capacity to minimize the amount of the adsorbent needed
- Favorable kinetic and transport properties for rapid adsorption
- Chemical and thermal stability
- Hardness and mechanical strength to prevent corrosion and crushing
- The free-flowing tendency for ease for filling or emptying vessels
- High resistance to fouling for long life
- No tendency to promote undesirable reactions
- The capability of being regenerated
- Relatively low cost

Most solids can adsorb species from liquids and gases. But only a few of them have sufficient selectivity and capacity to make them serious candidates for commercial adsorbents.

An adsorbent is in the form of spherical granules or rods. The specific surface of an adsorbent must be elevated; it means that the pores of the adsorbent must be lower. The International Union of Pure and Applied Chemistry gives a definition of adsorbent depending on diameter particles:

- Micropore is < 20Å
- Mesopore is between 20 and 500Å
- The macropore is > 500Å

The table 2 shows certain adsorbents and their nature.

Table 2: Nature of some type of adsorbents

Adsorbent	Activated Alumina	Silica Gel	Activated Carbon	Molecular-sieve Carbon	Molecular-sieve zeolites	Polymeric adsorbents
Nature	Hydrophilic, amorphous	Hydrophilic, Hydrophobic, amorphous	Hydrophilic, amorphous	Hydrophobic	Polar-hydrophilic, crystalline	------

The most important characteristics of an adsorbent are its specific surface area. This specific surface area is measured by adsorbing gaseous Nitrogen using the well-accepted BET method. The BET equation (5) is the most used method for the monolayer and the specific area values [3].

$$\frac{x}{V(1-x)} = \frac{1}{V_m c_{BET}} + \frac{x(c_{BET}-1)}{V_m c_{BET}} \qquad (5)$$

$$x = \frac{P}{P_0} \qquad (6)$$

With P total pressure, P_0 vapor pressure of adsorbate at test temperature, V volume of gas adsorbed at STP, V_M volume of monomolecular layer of gas adsorbed at STP and c_{BET} constant related to the heat of adsorption.

In this work, a tailored adsorbent will be synthesized with cellulose as a base, a polymeric adsorbent. The good adsorbent for polyphenols must combine hydrophilic and hydrophobic properties. Therefore, synthetic polymers are used to make this adsorbent. Synthetic polymers are used to control hydrophilic and hydrophobic moieties or to improve the selectivity and efficiency by the inclusion of specific functional groups [12], [13].

FTIR analysis provides important information concerning the formation and the final composition of polymers [14]. The FTIR does not directly record the Spector of the source in the spectral domain, but it records an interferogram in the interferometer domain. To obtain the frequency space, the Fourier transform must be applied to the interferometric space. The introduction of the Fourier Transform in IR spectrometry improved the potential of the IR spectrometry to provide the solution in a wide range of the analytical problem. Fourier transform infrared spectrometers correspond to a single beam optical assembly which has as an essential part an interferometer placed between the source and the sample. The Fourier Transform infrared spectrometer performs simultaneous analysis of the entire spectral band from interferometric measurements. The plot of this analysis is Absorbance versus wavenumber.

1.3. Synthesis of adsorbent

1.3.1. Atom transfer radical polymerization

The radical processes are most tolerant of functional groups and impurities. The development of the Controlling Radical Polymerization has been a long-standing goal in polymer chemistry. With time, there has been the development of new Controlling Radical Polymerization methods such as Stable free Radical Polymerization, Nitroxide Mediated Polymerization, Reversible Addition Fragmentation Chain Transfer, Degenerative Transfer and Atom Transfer Radical Polymerization [15].

The atom transfer radical polymerization (ATRP) is a living/controlled polymerization; it was discovered in 1995 by SAWAMOTO and MATYJASZEWSKI independently [16]. The origin of the name Atom Transfer Radical Polymerization is the Atom transfer step. The Atom Transfer Radical Addition and the transition metal-catalyzed telomerization reaction are the roots of the ATRP [17], [18].

The ATRP is one of the most areas of chemistry which is developed rapidly and is a versatile process that allows achieving a controlled radical polymerization involving a solubilized initiator. ATRP is a useful tool for the synthesis of complex architecture, and it is applicable to various monomers [15], [16]. The ATRP is a multi-component system composed of monomers, initiators, and catalysts. The ATRP process uses alkyl halides as initiators [15], [16].

The typical monomers of the ATRP are Styrene, Methacrylate, Methacrylamide, and acrylonitrile. They are used because they contain substituents that stabilize the propagating radicals [19].

In the ATRP, the main role of the initiator is to determine the number of the growing chains as it is visible in the *figure 4*. The alkyl halides are used as initiators, and when the halide is a Bromide or a Chloride, the control of the molecular weight is better. The α-Bromoesters and the α-Bromoamides are typical ATRP initiators [15], [20].

In ATRP, the most important component is the catalyst because it is the key to the ATRP. His role is to determine the position of the atom transfer equilibrium and dynamics of the exchange between active and dormant species [15].

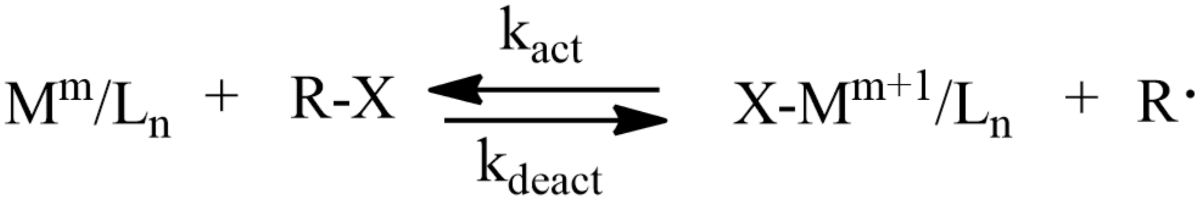


Figure 4: ATRP mechanism

ATRP can be used for the modification of polymer in grafting another polymer on the previous one. This grafting process affords different polymer architecture (comb or brush). This process has been mainly achieved by three processes: *grafting to*, *grafting from,* and *grafting through* [21]–[23].

- Grafting to

Grafting onto is a method that allows obtaining polymers brushes. It involves the reaction of complementary functional groups located on the surface with the end-functionalized polymer molecules. It is a combination of ATRP and *click* reactions. In this method, the backbone chain is used with functional groups that are distributed randomly along the chain. The graft polymer originates from the click reactions between the functional backbone and the end-groups of the branches that are active; it is possible when the backbone is chemically modified.

The *grafting to* have a major advantage which is the characterization of the final polymer can be made via various chemical and physical methods. But it is a limited process because the polymer chains to be grafted must first diffuse through the existing polymer film to reach the reactive sites on the surface zi[21].

- Grafting from

Grafting from is a technique of polymerization that is initiated by attached initiating groups at the surface of the substrate [21]. In this method, active sites able to initiate the functionality are introduced; to make it possible, the macromolecular backbone is chemically modified.

Indeed, the radicals are formed along the backbone of the polymer by various chemical initiators or irradiation. In this method, chain scission occurs, and the polymer backbone can be destroyed because it is an uncontrolled method. This method renders block-copolymerization impossible because it kills the end-groups. By the way, it is impossible to predetermine the lengths of the grafts [16].

- Grafting through

This method is the simplest method to synthesize a graft polymer with well-defined side chains. In this method, the surface is functionalized with a polymerizable group which can participate in polymerization as a monomer [24].

This method allows for the branches to be added heterogeneously or homogeneously based on the reactivity ratio of the terminal functional group on the macromolecule to the monomer. The *grafting through* method can be employed using any known polymerization technique. Living polymerizations give special control over molecular weight, molecular weight distribution, and chain-end functionalization.

The application of ATRP in the industry is limited because of metal catalysts. Indeed, the ATRP uses a large quantity of metal catalyst and to remove the catalyst several methods, and the cost of these methods is high [25]. This is one of the reasons why it is necessary to obtain a well-controlled polymerization process as Surface Initiated Polymerization. But this problem can be worked around; there is the solution as the prolongation of the polymerization time and the increase of the monomer dosage. Indeed, these changes can increase the degree of grafting [26].

As said above, ATRP, like each chain polymerization, needs an initiator. For this work, a macro-initiator will be produced. Its role will be to immobilize the α-bromoisobutyryl bromide in the cellulose backbone. Then, the grafting of synthetic polymers can be made. They will be synthetic functional brushes; in this work, 4-vinyl pyridine will be used to modify the cellulose. The choice of these monomers is based on previous work made in this domain. These works show the 4-Vinylpiridine as the perfect monomer used to graft cellulose to obtain the perfect adsorbent material.

1.3.2. Hybrid synthetic Cellulose materials

The natural polymer cellulose is untimely connected with man history. The modern science and technology of cellulose began with *Anselme Payen*. In 1838, he found that the fibrous component of all plant cells had a unique chemical structure; he named this fibrous component *cellulose*. Cellulose is the main molecule of cells walls of the plants; it is produced by plants essentially, but we can find it in some bacteria and animal tunicates. Cellulose is the most abundant renewable organic molecule present in the biosphere. In 1930, the macromolecular nature of cellulose was demonstrated. Cellulose, shown in the *figure 5*, is a carbohydrate polymer constructed from the β-D-glucopyranose units [27], [28].

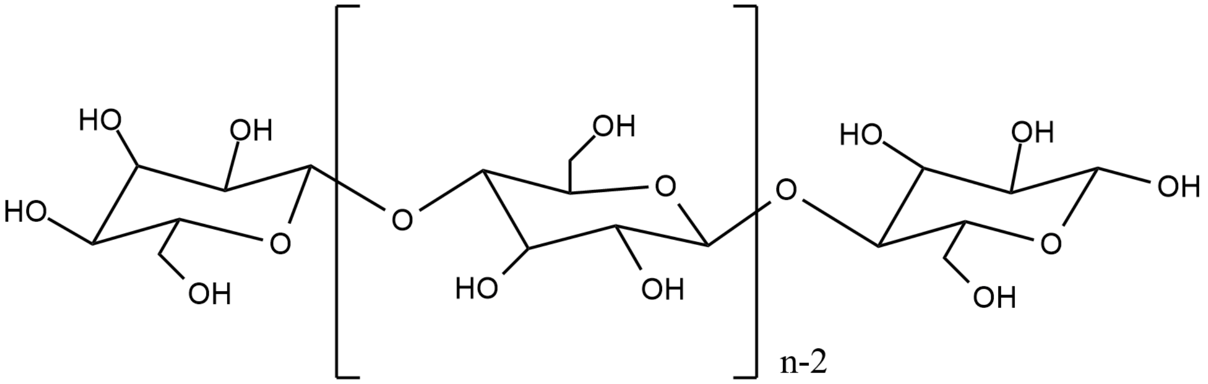


Figure 5: Semi-structural cellulose

Cellulose has many useful properties such as hydrophilicity, chirality, biodegradability, and high functionality [27]. The application of natural polymers like cellulose and its derivatives is increasing because of their abundance, biodegradability, and their nontoxicity. Indeed, much attention has been paid to cellulose and its derivatives due to its properties.

(Cellulose has advantages and disadvantages. One of these disadvantages is the polar and hydrophilic nature of this compound. And to fix this problem, surface modification has been used. Indeed, the surface of the cellulose has highly active and attractive hydroxyl groups. And these hydroxyl groups can have various chemical reactions with organic compounds, inorganic compounds, and synthetic polymers.)

Table 3: Cellulose properties

Chemical Formula	**$(C_6H_{10}O_5)_n$**
Molar mass (g/mol)	162.1406±0.007
Melting temperature (°C)	>150
Volumic mass (kg/m^3)	From 70 to 400
Higher heating value (MJ/kg)	17.3
Solubility parameter (MPa)	32.02

To use the cellulose as well we want, it is better to modify the cellulose. The cellulose can be modified by a lot of reactions, and in this way, it will be obtained certain derivatives of the cellulose.

- Modification of the cellulose with organics and inorganics materials: by this way, it obtained cellulose esters, desoxycellulose, cellulose ethers, and the heterocyclic-Nano cellulosic derivatives, which are the main derivatives obtained by this way.
- Modification of the cellulose by grafting polymers: there are many methods like Single-Electron Transfer Living Radical Polymerization (SET-LRP), Nitroxide-Mediated Radical Polymerization (NMP), Reversible Addition-Fragmentation Chain Transfer (RAFT), ATRP, and others.
- Modification of the cellulose by different nanoparticles and other minerals [27]

The general ATRP reaction on cellulosic materials representing in figure 6, show two steps. The fist step is the synthesis of the macroinitiator based on cellulose by using a halide (X= halogen) and the second step is the grafting of monomers (R and R' are groups, rest of the structure).

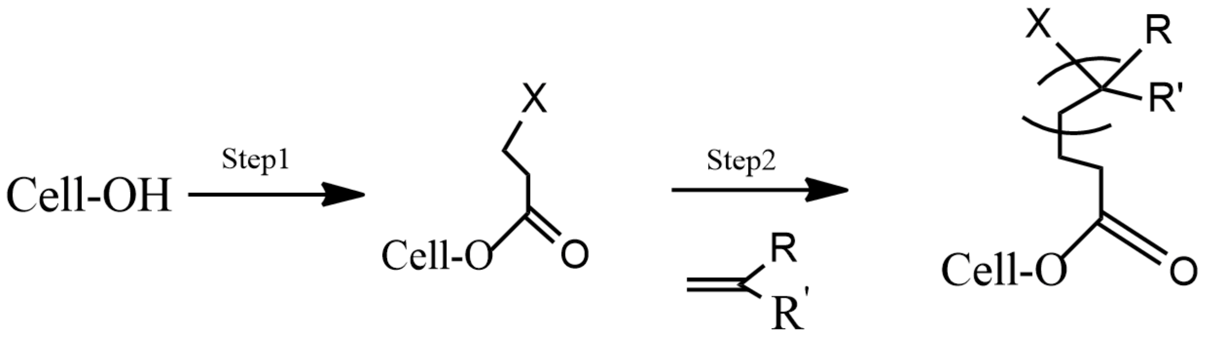


Figure 6: General ATRP reaction on cellulose

The use of ATRP for the grafting of cellulosic materials allows tailoring the amount grafted just by altering the initiator-to-monomer ratio in bulk. ATRP does not destroy the cellulose backbone [29].

Cellulose and its derivatives are excellent membrane materials, but they are some drawbacks such as low mechanical strength, poor weather-ability, and biodegradation. But the membranes of cellulose with synthetic polymer grafts are known to suffer minimum fouling.

Generally, polymers are divided into two classes: thermoplastics and thermosetting. In recent decades, natural fibres have been the alternative reinforcement in polymer composites, and many scientists and researchers are attracted by these composites due to their advantages. These advantages are low cost, low density, comparable specific tensile properties, non-abrasive to the equipment, reduced energy consumption, less health risk, renewability, recyclability, and biodegradability [30].

The chemical modification of cellulose is a sustainable alternative for many durable, oil-based synthetic polymer materials [28]. The modified cellulose can be used for the development of polymeric membranes. The first time that polymer was used in the industry to obtain a polymeric membrane, the cellulose nitrate was used [31].

The recommended polymer to produce membranes with applications in industrial and biomedical processes such as adsorption, catalysis, etc.… is cellulose acetate [31]. The synthesis of hybrid cellulose-synthetic adsorbents will be done by ATRP. Indeed, polymers will be grafted on the backbone [32].

To modify the cellulose surface, some polymers such as 4-vinyl pyridine and styrene can be used. The choice of these monomers is due to some properties that they have. 4-vinyl pyridine is hydrophilic and has characteristics of a weak base and provides a pH-sensitive amphoteric material [33]; styrene is cheaper than 4-vinyl pyridine; both can be used in adsorption or extraction process [34], [35]. Acrylic acid was chosen for its ability to generate anionic compounds with high pH sensitivity. (2-dimethylamino) ethyl methacrylate was chosen for obtaining cationic compounds for its hydrophilicity. And the last one, N-isopropyl acrylamide, was chosen for obtaining compounds exhibiting temperature stimulation [7], [14], [36].

Chapter 2 : Olive leaves extractions

2.1. Introduction

Olive leaves are harvest waste, and they constitute a huge amount of olive production waste. Just like the other olive trees products and olive and olive oil production waste, olive leaves can contain phenolic compounds. These polyphenols have an antioxidant activity that interests several industry sectors. It is, therefore, to valorize these production wastes that the olive leaves will be used to extract potential polyphenols.

The preparation of the extracts of olive leaves will be made using organic solvents and using different extraction methods (ultrasound, Soxhlet). To verify the presence of polyphenols in the olive leaves extracts, the HPLC-DAD will be used to identify the extracts.

2.2. Experimental section

To have the olive leaves extract, five extractions were performed with three different solvents:

- The first, second, and the third extractions by using EtOH/Water 80/20 v/v and ethyl acetate and deionized water (Ultrasound method)
- The fourth and the fifth extractions by using EtOH/Water 80/20 and ethyl acetate with a different method: Soxhlet extraction

2.2.1. Chemicals

To proceed the extraction, olive leaves were collected in Bragança (Mirandela, Trás-os-Montes, Portugal) during autumn 2020, ethanol (EtOH) was bought from PanReac, ethyl acetate (EtOAc) purchased from Fischer scientific, and deionized water was used. Acetonitrile (ACN) and acetic acid (AcOH), also purchased from Fischer Scientific, were used to prepare the mobile phase of the HPLC-DAD analysis.

2.2.2. Extractions

The olive leaves were collected and directly pulverized in a blender. The crushed olive leaves, presented in *figure 7*, were stored in a plastic bag in the cold room of a refrigerator until their utilization.

Figure 7: Olive leaves crushed

Only the ultrasound extractions were performed using a ratio of 1 g of ground olive leaves for 10 mL of solvent. The three first extractions were performed by Ultrasound and the others by Soxhlet extraction.

The Ultrasound extractions were made as follows: 10 g of ground olive leaves for 100 mL of solvent. The first one was used as solvent EtOH/Water 80/20 v/v, and it was in an ultrasonic bath for 1 minute at room temperature.

The second extraction was performed in the same way by using Ethyl acetate instead of EtOH/Water. And it was for 60 min at 40˚C. The third extraction was performed with the same condition as the second one by using deionized water as solvent.

After the ultrasound bath, the mixtures were filtered through a nylon filter and stocked in the dark (cold room) until their utilization. The fourth and fifth extractions (Soxhlet extractions) were performed with a different ratio. Indeed, around 15mg of olive leaves were weighed and put in a capsule; 200mL of solvent was used (ethyl acetate and EtOH/Water 80/20). After the extraction, the extracts were taken and stocked in the dark.

The experimental data stated above are reported in the *table 4*.

Table 4: Experimental data of extraction experiences

Extraction	**Methods**	**Solvent**	**Ratio**	**Mass of Olive leaves**	**Time**	**Temperature**
1	Ultrasound	EtOH/Water 80/20	1g/10mL	10.2126g	1 min	Room temperature
2	Ultrasound	Ethyl acetate	1g/10mL	10.1960g	60 min	40˚C
3	Ultrasound	DI Water	1g/10mL	10,7308g	60 min	40˚C
4	Soxhlet	Ethyl Acetate	-	15.8726g	-	-
5	Soxhlet	EtOH/Water 80/20	-	15.3187g	-	-

2.2.3. Characterization of extracts

To know if the obtained extracts contain some polyphenols, the characterization by HPLC-DAD was performed. The analysis was made at room temperature with a flow rate of 1mL/min. Two solvents were used as mobile phase: (A) acetonitrile/water 10/90 v/v and (B) acetonitrile/water 90/10 v/v. The two solvents were at pH=3; the pH was adjusted by using acetic acid. The linear gradient method was used, starting with 100% of (A) and ending with 100% of (B).

The Jasco MD-4010 photodiode array (PDA) detector was used to perform the analysis with a Nucleosil® C18 column L × I.D. = 15 cm × 4.6 mm and 5µm particle size.

MATLAB was used to treat the obtained data in order to obtain 3D HPLC chromatograms.

2.3. Results and discussion

The extracts were stocked until their utilization. Because of the lower temperature, certain extracts (Soxhlet extracts and Ultrasound extract with deionized water as solvent) started to precipitate. These extracts were centrifugated and lyophilized to have a product without solvent and dehydrated (a powder).

The percentage of extraction was calculated after the lyophilization. The results are reported in *table 5*. After lyophilization, the Soxhlet extraction with Ethanol/Water 80/20 as solvent allowed to get around 3 grams of powder of extract, it represents 24.67% of the extraction. The lowest percentage of extraction was got with Ethyl acetate as solvent.

Table 5: Lyophilization data

Extraction	**Methods**	**Solvent**	**Volume of solvent (mL)**	**Mass of olive leaves (g)**	**Mass after lyophilization (g)**	**% Of Extraction**
1	US	DI water	100	10	1.3455	13.45
2	Soxhlet	Ethyl acetate	200	15.8726	1.4005	8.82
3	Soxhlet	EtOH/water (80/20)	200	15.3187	3.7796	24.67

The other extracts were analyzed by HPLC-DAD to identify the phenolic compounds contained in these extracts. The HPLC chromatograms are shown below, and to discuss the results obtained, some reference polyphenols were used to identify characteristic peaks in the HPLC chromatograms of olive leaves extracts.

The olive leaves can contain many polyphenols. It can have phenolic compounds from secoiridoids and flavonoids families like it can have simple phenolic and pentacyclic triterpenoids compounds [37], [38].

The concentration of polyphenols depends on several factors such as le quality of the leaves, the age of the trees, or the period of the harvest [1], [5]. The presence or absence of polyphenols in the extracts depends on the extraction method and/or the solvent used.

2.3.1. HPLC-DAD of extract made with EtOH/Water 80/20

The *figure 8,* the 3D chromatogram of the extract made by ultrasound with the mixture of ethanol and water 80/20 v/v as the solvent, shows few peaks. The comparison between 3D chromatograms of commercial polyphenols allowed to identify certain of these few peaks. Four peaks have been identified, among these peaks there are two simple phenols, one flavonoid and one secoiridoid.

The identified peaks are numerated from 1 to 4; the peaks correspond to the following compounds: (1) hydroxybenzoic acid, (2) Verbascoside, (3) luteolin-7-glucoside, (4) oleuropein. The chemical structures of these phenolic compounds are shown in the *figure 10*.

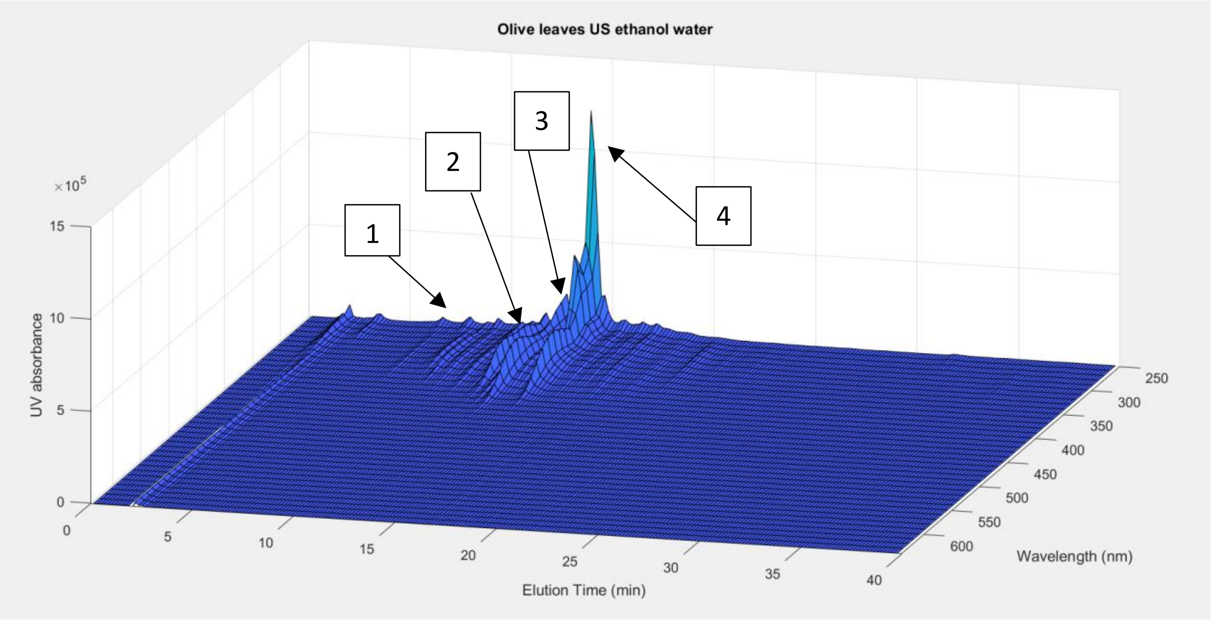


Figure 8: HPLC chromatogram of Olive leaves extract with EtOH/Water 80/20

2.3.2. HPLC-DAD of extract made with Ethyl acetate

In this extract made by ultrasound with ethyl acetate as the solvent, the 3D HPLC chromatogram represented in *figure 9*, the comparison with 3D HPLC of commercial polyphenols as reference allowed to identify only three peaks contrary to the other extract.

Among these compounds there is one simple phenol, one flavonoid, and one secoiridoid. Numerated from 1 to 3, the identified peaks correspond to the following compounds: (1) Verbascoside, (2) luteolin-7-glucoside, (3) oleuropein. The chemical structures of these compounds are also shown in the *figure 10*.

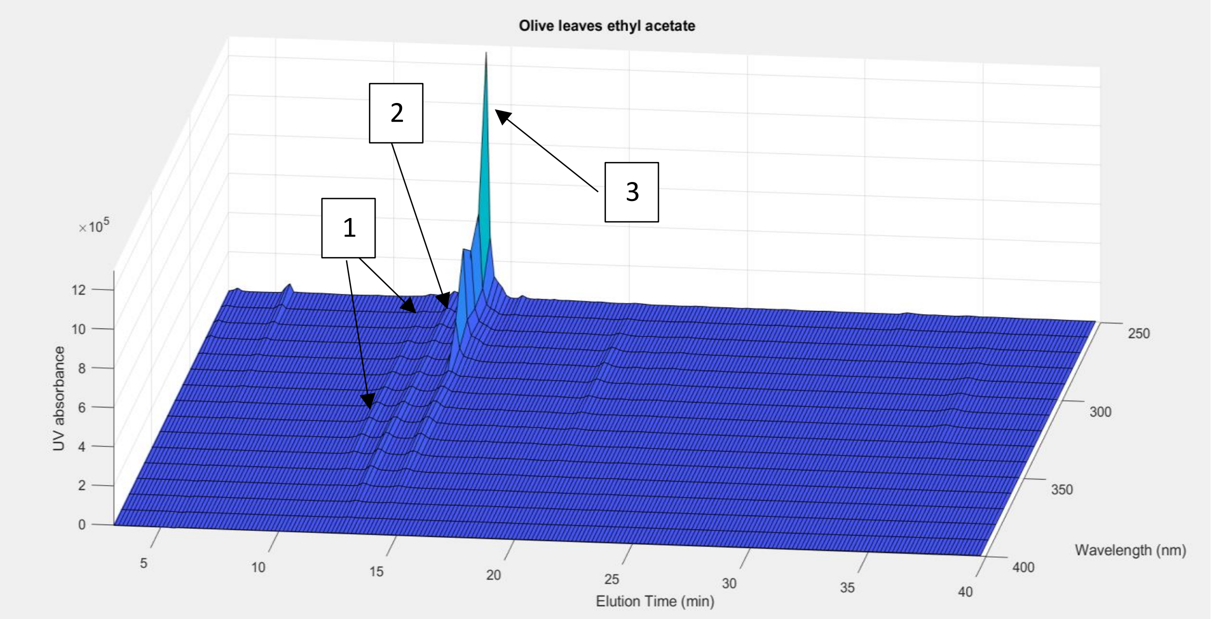


Figure 9: HPLC chromatograms of Olive leaves extract made with ethyl acetate

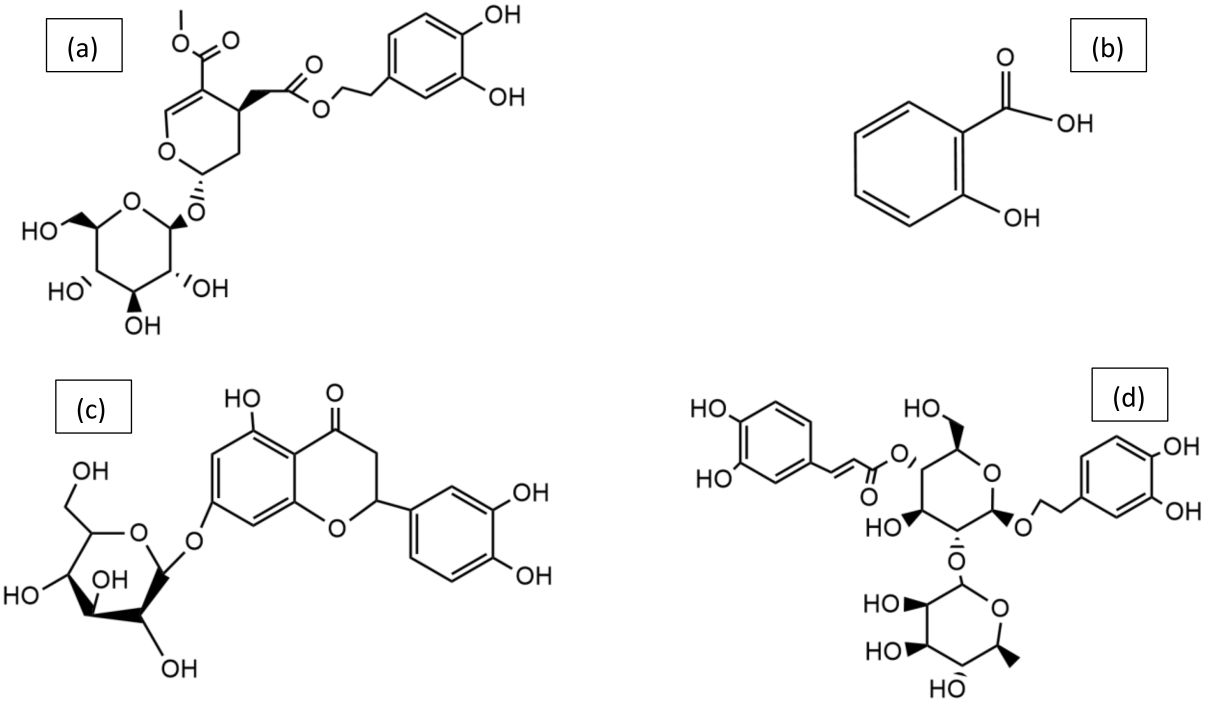


Figure 10: Structures of (a) Oleuropein (b) Hydroxybenzoic acid (c) Luteolin-7-glucoside (d) Verbascoside

2.4. Conclusion

The synthesized adsorbent was made to catch phenolic compounds by using its functionalized surface. These phenolic compounds come from olive leaves. To have the polyphenols, extractions were made by using organic solvents and different methods (Soxhlet and Ultrasound).

Among the five extractions performed, only 2 (the two first Ultrasound extractions) were used to perform the sorption experience. The other was centrifuged and lyophilized to obtain powders. These powders can contain a mixture of polyphenols and chlorophyll.

Before using the extracts for the sorption experience, and identification of phenolic compounds contained in these extracts was made. The identification was based on the reference polyphenols in the database: identification by comparison.

The two extracts contain the phenolic compounds (Verbascoside, luteolin 7-glucoside, and oleuropein). But only the extract with a mixture of ethanol and water contains the hydroxybenzoic acid.

Chapter 3: Synthesis of polymeric adsorbent via Atom Transfer Radical Polymerization

3.1. Introduction

To synthesize a polymeric adsorbent by grafting synthetic polymers on a biopolymer, the ATRP method was chosen. This choice has been made due to the efficiency of this method. The biopolymer chosen (cellulose) has a good rapport as an adsorbent, but to graft any synthetic polymers, its backbone must be modified.

The synthesis will be made in two (2) steps. The first step will consist of the immobilization of hydroxide sites of the cellulose by synthesizing a macroinitiator based on cellulose and bromide (a-Bromoisobutyryl Bromide). The second step will therefore be the grafting of synthetic polymers (Poly 4-Vinylpyridine). The grafting will be done onto the immobilized sites.

To check the smooth running of each step, a characterization of obtained products at the end of each step of the synthesis will be done. The method of characterization used is the Fourier Transform InfraRed spectroscopy (FT-IR). A complementary analysis will be done to see the topography of the adsorbent particles: Scanning Electron Microscopy (SEM).

3.2. Experimental section

The synthesis of the polymeric adsorbent was made in two steps:

- The synthesis of the bromide macroinitiator: MCC-Br
- The grafting of Poly-4-vinyl pyridine P4VP: MCC-P4VP

3.2.1. Chemicals

For the synthesis of the adsorbent, the following chemicals were used: microcrystalline cellulose (MCC, powder, 20µm, cotton linters), triethylamine (TEA, 99.5% purity), a-Bromoisobutyryl Bromide (BiBB, 98% purity), Dimethyl aminopyridine (DMAP, purity^{3}99%), 1,1,4,7,7-Pentamethyldiethylenetriamine (PMDETA), and the Copper (I) Bromide (CuBr, 97%

purity) were purchased from Sigma-Aldrich. 4-Vinylpyridine (4VP) was provided by Alfa Aesar. N, N-Dimethylformamide (DMF), Dimethyl sulfoxide (DMSO), Acetic acid (AcOH), Acetonitrile (ACN), and Methanol (MeOH) were bought from Fischer Scientific, and Ethanol (EtOH) was bought from PanReac,

During the experiences, all the chemicals were used directly; without any treatment, and throughout the experiences, deionized water was used.

3.2.2. The synthesis

3.2.2.1. Synthesis of the bromide initiator

The synthesis of the macroinitiator was made as follows: 500 mg of MCC and 50 mL of DMF were put together and were dissolved by sonication in an ultrasonic bath. The following chemicals were added successively in the dispersion: 4 mL of TEA, 2 g of DMAP. 4 mL of BiBB was added dropwise at 0˚C in an ice bath. The *figure 11* shows how the macroinitiator is obtained.

The reaction continued for 24 hours at room temperature then EtOH was added to end the reaction. The MCC-Br was washed with DMF until colorless and dialyzed with deionized water for six days. The MCC-Br was obtained by centrifugation and dried using the vacuum oven at 40˚C.

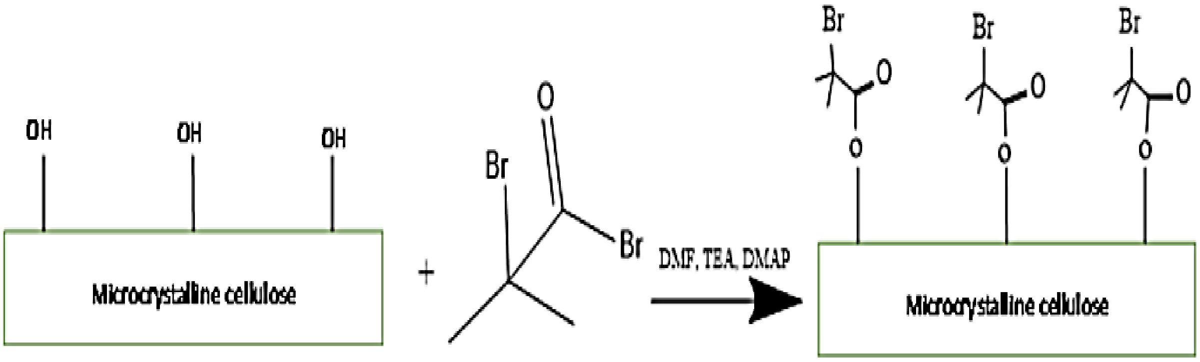


Figure 11: Macroinitiator synthesis equation

3.2.2.2. Grafting of 4-vinyl pyridine

The synthesis was performed as follows. The chemicals were added successively: 100 mg of MCC-Br, 67.5 mL of DMSO, 7.29 mL of 4VP, 35,27 µL of PMDETA, and 24.24 mg of CuBr. Everything was passed in an ultrasonic bath to dissolve the solid phase in the liquid phase. The obtained solution was purged with a flow of Argon dry for 15 minutes, then was put in an oil bath at 80˚C for 24 hours.

The obtained product was washed three times with EtOH then dialyzed with deionized water for ten days. The MCC-P4VP was obtained by Vacuum oven drying.

The grafting was made on the immobilized site as the *figure 12* shows.

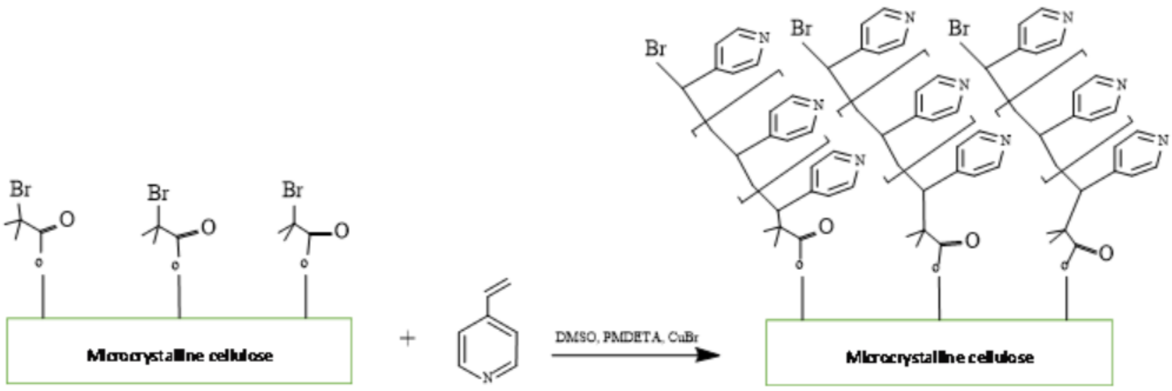


Figure 12: Grafting equation of 4VP

3.2.3. Determination of P4VP mass

To determine the mass of P4VP in the final product, the gravimetric weight difference was performed by using an analytical balance. After the drying, MCC-P4VP the final product was weighed to have the mass of the synthesized material. This mass allowed to determine the percentage of the grafting by determining the mass of P4VP in the material. The mass of P4VP was obtained by doing a simple calculation of subtraction; the mass of MCC-Br was subtracted of mass of the material. The percentage of grafting was also obtained by a simple calculation.

3.2.4. Characterization of the synthesized material

The characterization of the obtained products was performed to determine the structure of the product and see if the reaction worked. This analysis was performed by Fourier Transform Infrared Spectroscopy (FT-IR) Directly using the ATR method. The equipment used to perform this analysis is a Perkin Elmer model Spectrum two™. The obtained spectrum after performing the FT-IR allowed to identify characteristic peaks of characteristic groups in the structure of the material.

Beside the FT-IR analysis, another analysis was performed in order to see the topographic surface of the material and the composition of the adsorbent samples. It was performed by the International Iberian Nanotechnology Laboratory using a FIB/SEM system HELIOS NANO LAB 450S. To obtain the SEM images, an electron beam of 3 keV, a beam current of 25 pA, and field-free lens mode was used.

3.2.5. Measurement of bromide content in the bromide macroinitiator

These measurements were performed by ICP-MS (Inductively Coupled Plasma – Mass Spectrometry) using a Thermo Scientific™ iCAP™ Q instrument at LAQV/REQUIMTE – Laboratory of Applied Chemistry (Trace Element Analysis unit), Faculty of Pharmacy of the University of Porto (FFUP). To perform these analyses, MCC-Br was first dissolved in dimethyl sulfoxide at 70 ◦C at a concentration of 0.675 mg/mL as in previous related work [39].

3.3. Results and discussion

3.3.1. Mass of synthesized adsorbent material

The gravimetric method used shows some results, the resumes are resumed in the *table 6*. In previous work[39] using the same initiator and the same monomer in different proportions (50 mg of MCC-Br and 3.25 mL of 4VP), the measured content of P4VP in the final product was 90%. In this work, for 100 mg of bromide initiator (MCC-Br) and 7.29 mL of 4-vynil pyridine (4VP), 2145.3 mg of MCC-P4VP was obtained. After subtracting the mass of the bromide initiator, the mass of poly 4-vynil pyridine was obtained; 2045.3 mg .

The poly 4-vynil pyridine represent 95.3% of the final product, 5.3% more than in previous work. With these results, the percentage of grafting was calculated by using the equation below. Indeed, a percentage of grafting of 2045.3% was obtained.

$$\%Grafting = \frac{m_{MCC\text{-}P4VP} - m_{MCC\text{-}Br}}{m_{MCC\text{-}Br}} * 100 \qquad (7)$$

Table 6: Results of gravimetrical analysis

Mass of MCC-Br used (mg)	100
Mass of MCC-P4VP obtained (mg)	2145.3
Mass of P4VP (mg)	2045.3
%Grafting (%)	2045.3
%P4VP (%)	95.3

3.3.2. FT-IR characterization

On the following figures (*figure 13* and *figure 14*) the transmittances are negatives due to the use of a software to adjust the baseline. The software used is ORIGINLAB.

3.3.2.1. FT-IR characterization of the bromide initiator

The MCC-Br was ground and analyzed by FT-IR spectroscopy. The analysis was performed directly in ATR mode. The obtained spectra are shown in *Figure 13*. The spectra of MCC were used to compare with the spectra of MCC-Br. We can see at first sight the disappearance of the characteristic peak of hydroxide group OH (~3329 cm-1). Indeed, the immobilization of BiBB was made through reaction with hydroxide groups in the cellulose microcrystalline, which explains the disappearance of this characteristic peak and the appearance of another characteristic peak. At 1742 cm-1 in the MCC-Br FT-IR spectra, we can observe a peak attributed to the valence vibration of carbonyl group C=O, which results from the reaction between the hydroxide group from MCC and BiBB. The measurement of Br content in MCC-Br reported that the concentration of Br was 135 mg/g. This is in the same range as previous related work [39], [40]. At 649 cm-1, we can also see the peak attributed to the elongation of C-Br.

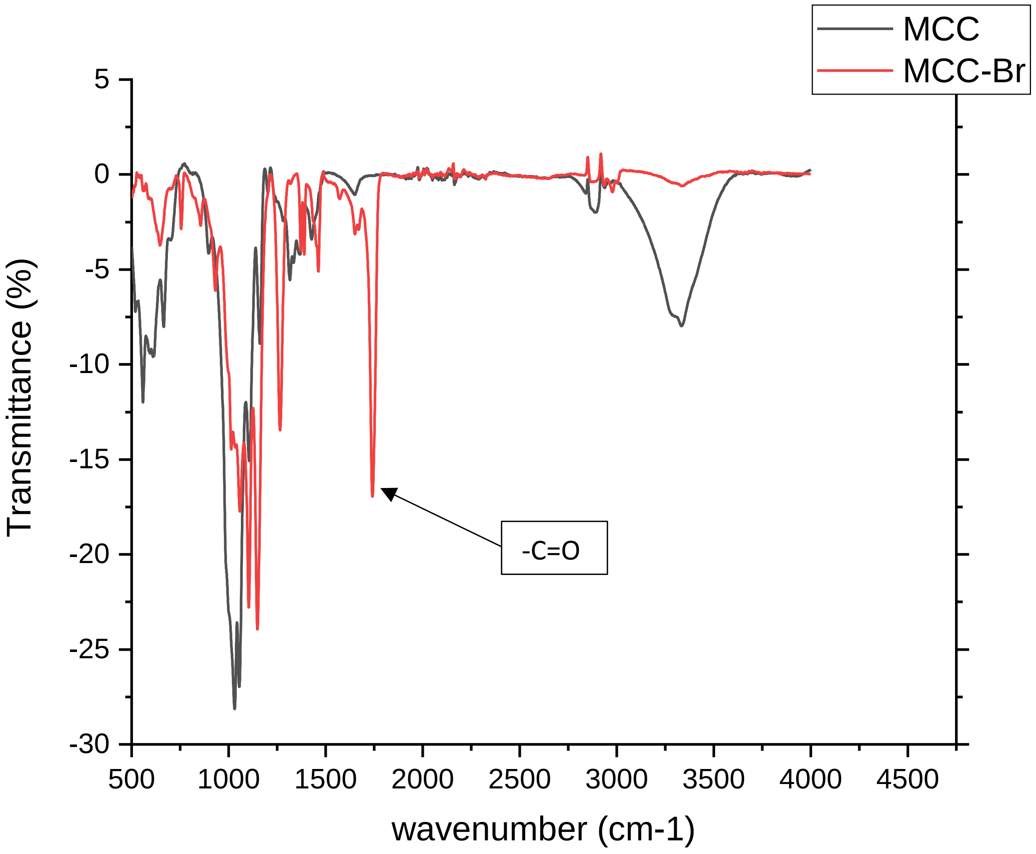


Figure 13: FTIR spectrum of MCC and MCC-Br

3.3.2.2. FT-IR characterization of the synthesized adsorbent

The MCC-P4VP was also ground and analyzed by FT-IR spectroscopy. The analysis was also performed directly in ATR mode. Like with the native cellulose and the bromide initiator, a comparison was made to see if the grafting of polymers worked. The comparison was made between the FT-IR spectrum of MCC-Br and the FT-IR spectrum of MCC-P4VP. The obtained spectra are shown in *figure 14*. By comparing, we can see the appearance of the characteristic peaks attributed to the C=N (at 1413 cm-1) and C=C (at 1595 cm-1) in the pyridyl group from 4VP, which indicates the success of the grafting of 4VP on MCC-Br. The peaks appear at the same range of wavenumber than previous work[39].

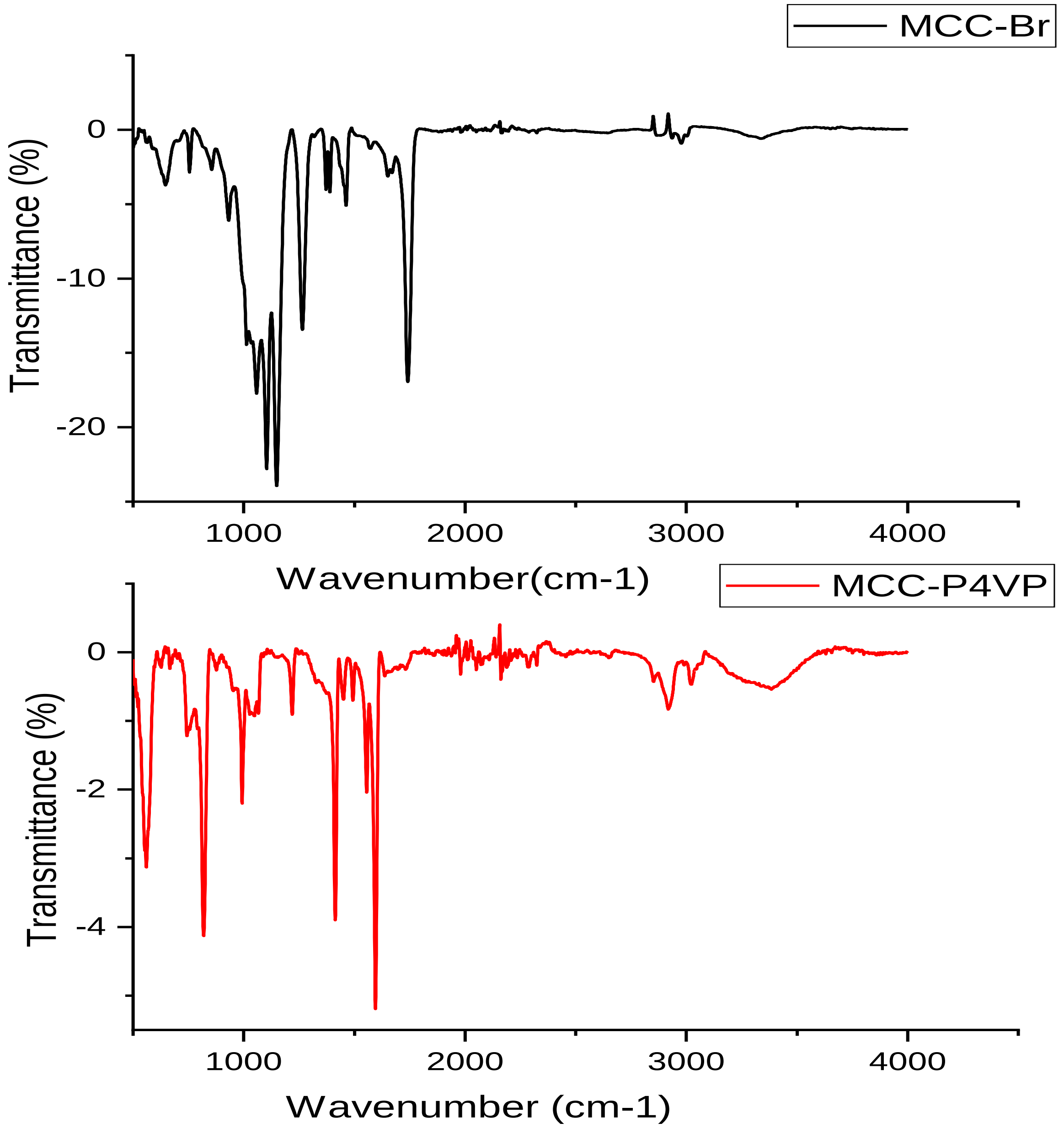


Figure 14: FT-IR spectrum of MCC-Br and MCC-P4VP

3.3.3. SEM analysis

SEM analysis gave a view of particles which allows the measurement of the particle size. The results illustrated in the *figure 15*. The native cellulose particles have a characteristic dimension around 20 µm the obtained results show that the MCC- P4VP present particles have dimensions in the range of 50 µm. The observed increase in particle size can be explained by the ATRP grafting of this synthetic polymer in the MCC. These results are in the same range as previous related work [39].

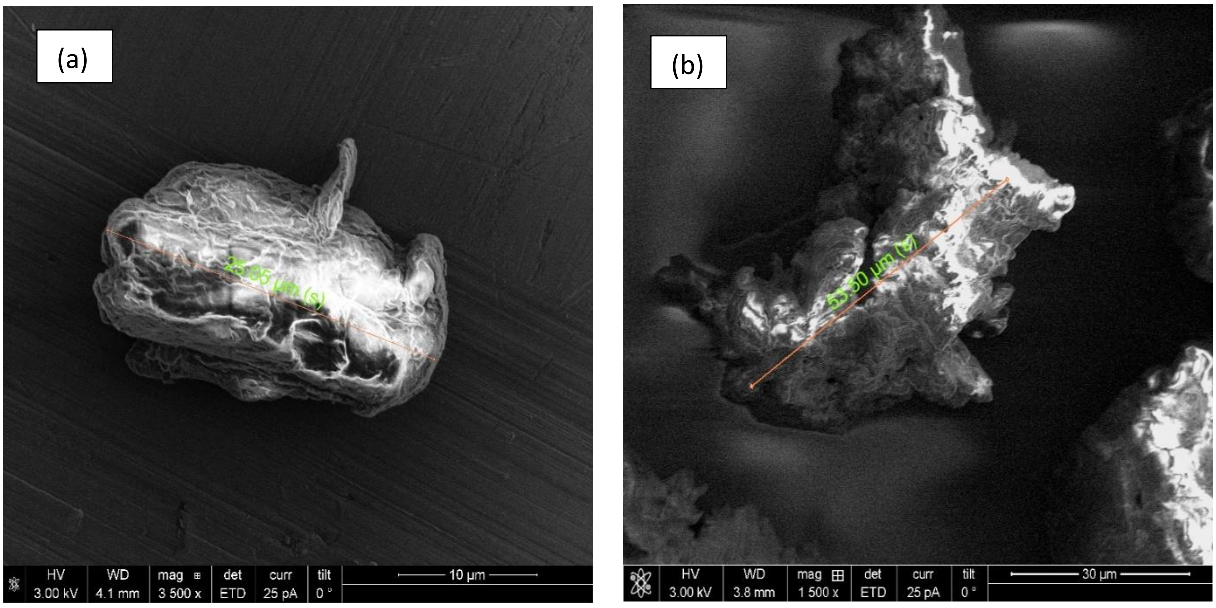


Figure 15: SEM images showing the morphology of some particles addressed in this research. (a) Native microcrystalline cellulose particles (MCC). (b) ATRP synthesized hybrid cellulose-poly(4-vinylpyridine) particles (MCC-P4VP) [39]

3.4. Conclusion

The Atom Transfer Radical Polymerization allowed the obtaining a synthetic hybrid polymer by grafting a synthetic polymer (P4VP) on a biopolymer (cellulose). Indeed, the synthesis was made in two steps. The first step was the immobilization of BiBB on the cellulose backbone to obtain the bromide initiator used to achieve the second step. This step was confirmed by performing the FT-IR analysis, which allowed to detection of the characteristic peak of -C=O from BiBB and by measuring the concentration of Br in the bromide initiator.

The second step, for this part, allowed the grafting of P4VP on the cellulose backbone by using immobilized sites by the synthesis of the macroinitiator (MCC-Br). Just like the first one, the second step was confirmed by an FT-IR analysis which allowed to visualize the appearance of characteristics peaks as -C=N and -C=C from the pyridyl group belonging to 4VP.

In addition to FT-IR, another analysis was performed to confirm the success of the grafting. Indeed, this analysis (SEM) showed the increase of the size of cellulose particles after the ATRP, which is also a confirmation of the success of the grafting.

Finally, some analyses as SEC and the BET experience were not performed. Indeed, the SEC is a chromatography technic that separates dissolved molecules according to their size; in the synthesis of MCC-P4VP, no free polymers were found. That explains why no results were obtained by performing this experience. The BET Experience, for its part, cannot be performing cause the MCC-P4VP does not have any pores but a functionalized surface; the adsorption of nitrogen gas cannot be used to evaluate this functionalized surface.

The hybrid synthetic material (MCC-P4VP) was used to perform adsorption to target polyphenols contained in olive leaves extract.

Chapter 4 : Sorption experiences

4.1. Introduction

The adsorbent was synthesized to achieve the adsorption of polyphenols contained in olive leaves extracts. So, it is for this reason that adsorption reactions will be performed.

Firstly, to obtain an idea of the behavior of adsorbents, adsorption experiences were performed with reference polyphenols. Other adsorbents (commercials adsorbents) were also used to compare the adsorbent between them.

Secondly, adsorption experience by saturation and desorption was performed with these adsorbents (commercials and synthetic adsorbents). For these experiences, olive leaves extracts were exclusively used. Each adsorbent will therefore be saturated by olive leaves extracts before being washed by different solvents to elute phenolic compounds retained in each adsorbent.

Finally, analysis (UV spectrometer and HPLC-DAD) was done to calculate the number of polyphenols adsorbed and to identify the polyphenols retained in the adsorbents during the loading (saturation experience).

4.2. Experimental section

Two different types of sorption were performed:

- Batch experiences: these experiences were performed with reference polyphenols which were chosen (Catechol, Vanillic acid, and Rutin).
- Saturation experiences: these experiences were performed with the olive leaves extracts.

4.2.1. Chemicals

Commercial polyphenols (vanillic acid, rutin, and catechol from Acros Organics and Sigma-Aldrich), olive leaves extracts were used to perform the sorption experience. Ethanol bought from PanReac, ethyl acetate, methanol, and acetic acid purchased from Fischer Scientific, and deionized water were used as solvents. The poly(4-vinyl pyridine) cross-linked with divinylbenzene 2% (Reillex 402) and cellulose microcrystalline (MCC) bought from Sigma Aldrich, poly(4-vinyl pyridine) cross-linked with divinylbenzene 25% (Reillex 425) purchased from Vertellus, and the synthesized MCC-P4VP and were used as adsorbent materials.

4.2.2. Batch adsorption experiences

- **Batch adsorptions**

The batch adsorption experiences were performed only with the commercial polyphenols. All the commercial polyphenols used were dissolved in three solvents: EtOH/Water (80/20), EtOH/Water (20/80), and Ethyl acetate. The *table 7* resumes the experimental data of the preparation of different commercial polyphenols solution. For Catechol and Vanillic acid, seven (7) solutions (0.1 0.25 0.5 1 1.5 2 and 2.5 mM) were prepared with each solvent. For the rutin, only 6 solutions were prepared (0.02 0.05 0.1 0.25 0.5 and 0.75 mM). Due to the non-solubility in the ethyl acetate, rutin solutions were just prepared in two solvents (EtOH/Water 80/20 and EtOH/Water 20/80).

Each experience was performed with a ratio of 10mg of adsorbent for 1mL of polyphenol solution.

For Catechol, 16.52mg were weighed and dissolved in EtOH/water (80/20) to obtain a solution with 2.5 mM of concentration. This solution was diluted with a solvent to obtain the other concentrations. Eight (8) samples were prepared as follows: 30mg of Reillex 402 were weighed in the flask for each sample, 3 mL of catechol solutions were put in the flask.

The same thing was made for the other adsorbent materials (Reillex 425 and MCC-P4VP). This procedure was repeated for the other solvents. The amount of each adsorbent used is reported in the *table 8*.

All the samples were left at room temperature for 24 hours under orbital stirring at 90 rpm.

The same procedure was used for the other standard polyphenols except for Rutin. Indeed, due to solubility problems, the solution of Rutin was prepared in solvents with adjusted pH. The two solvents used were prepared by adjusting the pH to 10 (by adding sodium hydroxide).

Table 7: Polyphenol's solutions preparation table

Polyphenols	Mass of polyphenols (mg)	Volume of solvent (mL)	Concentration (mM)
Catechol	16.52	60	2.5
Rutin	29.91	60	0.75
Vanillic Acid	25.22	60	2.5

Table 8: Summary of Batch experience data

Adsorbent	Mass of adsorbent (mg)	Volume of solution (ml)
Reillex 402	30	3
Reillex 425	30	3
MCC-P4VP	30	3

- **UV absorbance**

The initials UV absorbance was measured for all polyphenols solution at a different wavelength. The UV absorbance of Catechol and the Rutin was measured at 280 nm and the vanillic acid at 260 nm.

After the batch experience, the sample was taken and analyzed with UV equipment at the same wavelength. The absorbances read were used to calculate the amount adsorbed by each material following the equation below:

$$q = \frac{V(C_0 - Ce)}{m} \times 1000 \qquad (8)$$

Where q is the amount adsorbed (µmol/g), V is the volume of phenolic compounds (L), C_0 and C_e are respectively the initial concentration and the concentration at the equilibrium (mM), and m is the masse of adsorbent materials (g).

4.2.3. Saturation experiences

- **Saturation**

The saturation experience was performed by Solid Phase Extraction (SPE) by using the olive leaves extracts. To perform the SPE, a simulation of an adsorption column was made; three syringes with a capacity of 5mL and two filter discs were filled with 200mg of adsorbent materials (Reillex 402, MCC, and MCC-P4VP).

The columns were preconditioned by washing with 5mL of solvent (the same solvent used during extractions).

The olive leaves extract was diluted 20 times and has been read with the UV equipment. 1mL of the extract solution was loaded to the preconditioned column. Volumes of the extract solution were loaded in the column until the saturation of the materials. And these fractions were collected, and the absorbance of these fractions was read in the UV photometer equipment.

This experience was made in the same way for each extract.

- **UV absorbance**

The initial UV absorbance was measured for the diluted extracts at a wavelength of 280nm.

After the loading of all the volumes, all the samples were taken and analyzed with the UV spectrometer equipment at the same wavelength (280mn) to draw the saturation curves. The curves have for abscissa the volume of elution and for ordinate the UV absorbances.

- **HPLC-DAD**

After the elution of the phenolic compounds by washing the column, all the fractions were analyzed by HPLC-DAD using the same column, the same solvents, and the same conditions described in *Chapter 2* in order to identify the compounds eluted.

4.3. Results and discussion

4.3.1. Batch adsorption experiences

To discuss the obtained results, two factors must be considered: the solvents and the materials. For each polyphenol's standards, two comparisons were made:

- Comparison between the solvents for a material
- Comparison between the materials for a solvent

The amount adsorbed was a plot depending on the concentration.

4.3.1.1. Catechol

To obtain the curves in the *figure 17* ad the *figure 18*, calculations were made to get the concentration at the equilibrium. These calculations were made by using the calibration curve represented in the *figure 16*. Indeed, the equation of the calibration curve was used to calculate the concentration at the equilibrium.

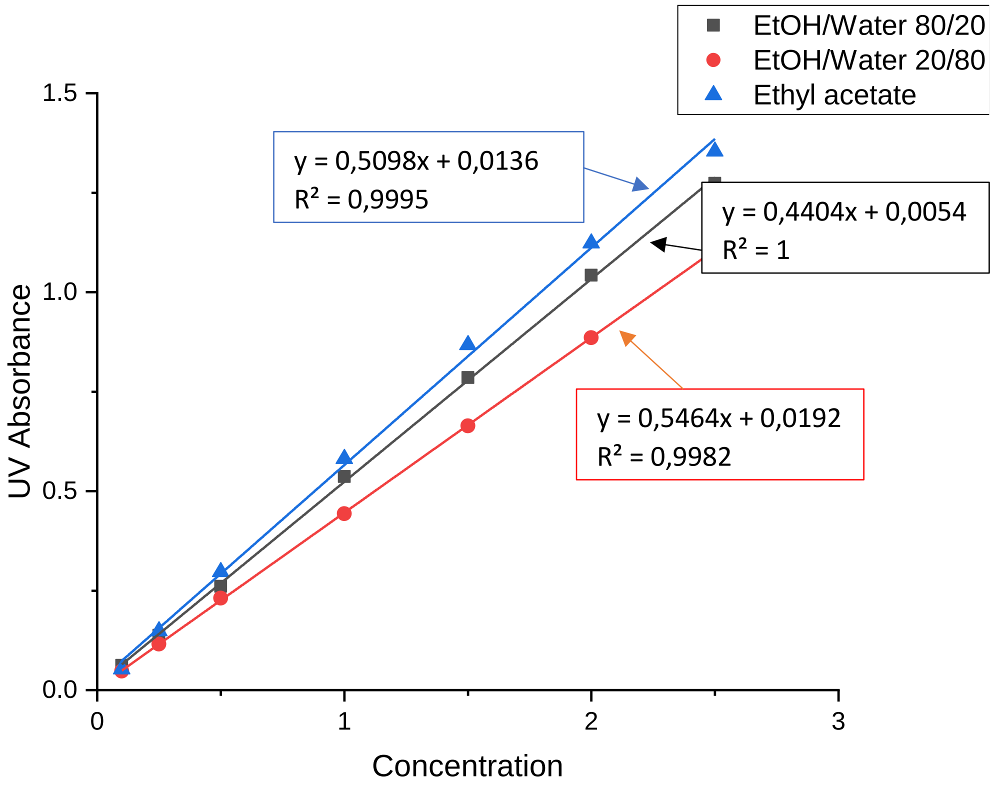


Figure 16: Calibration curves of Catechol

The isotherm curves obtained for this polyphenol allowed to see which material, between the three used (Reillex 402, Reillex 425, and MCC-P4VP), has a better behavior. The hydrophilic behavior of Catechol can be a factor in the adsorption. In the *figure 17*, for each material used the mixture of ethanol and water (EtOH/Water 20/80 v/v) gives better retention.

A comparison between the different materials was also made. Indeed, as each solvent have their behavior with the material and the polyphenols solution, the materials have also their behavior depending on the solvent used. The Catechol shows an affinity with the newly synthesized adsorbent (MCC-P4VP). In the *figure 18*, regardless of the solvent used, the best retention (the highest amount adsorbed) is obtained with the polymeric adsorbent.

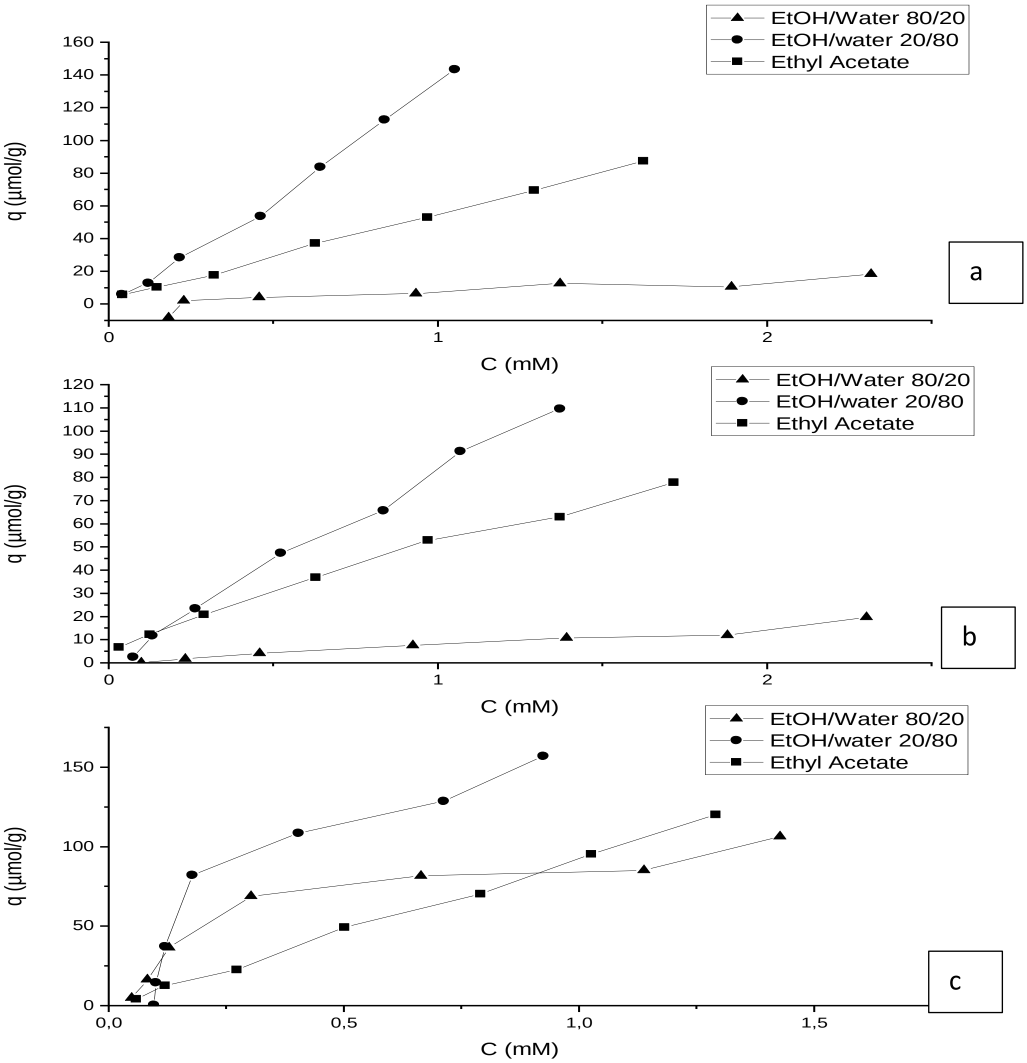


Figure 17: Catechol isotherms for each material (a) Reillex402 (b) Reillex425 (c) MCC-P4VP

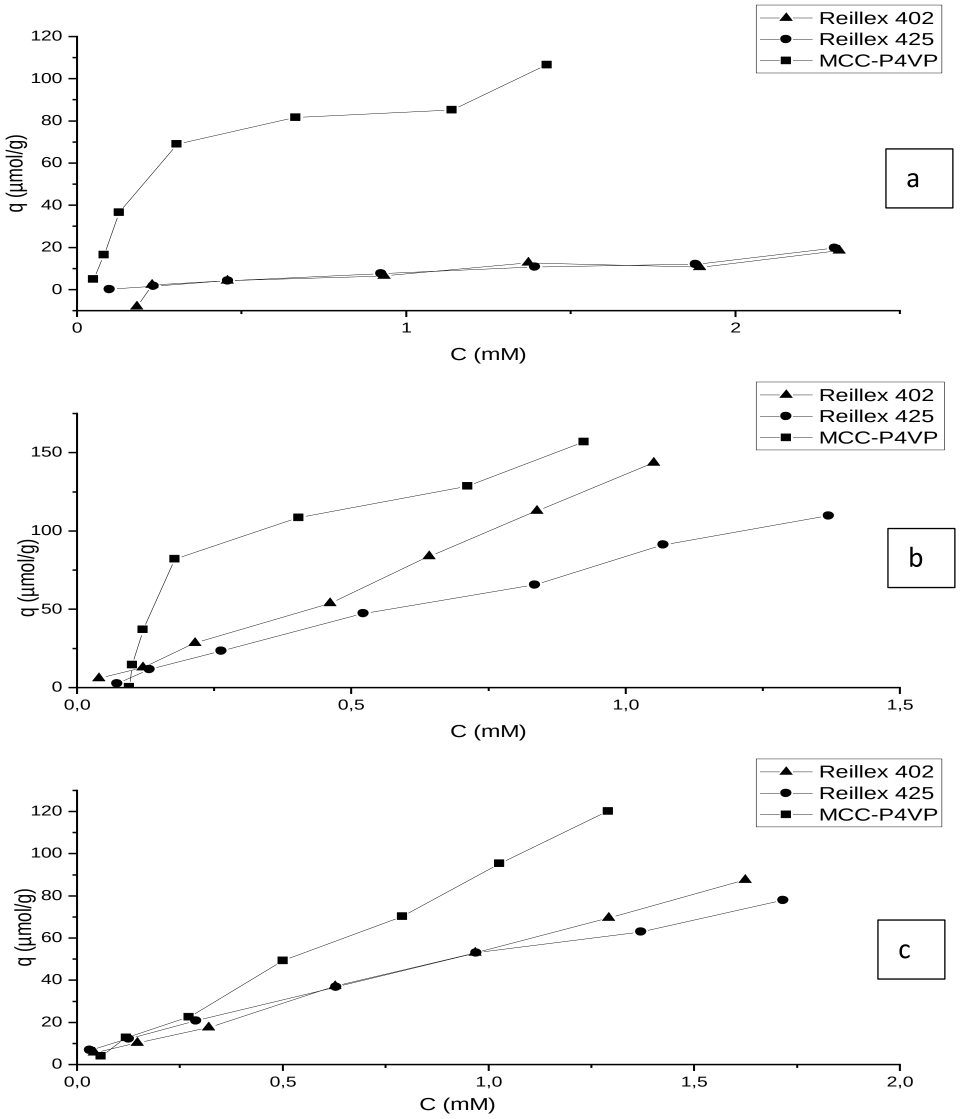


Figure 18: Catechol isotherms for each solvent (a) EtOH/Water 80/20 (b) EtOH/Water 20/80 (c) Ethyl acetate

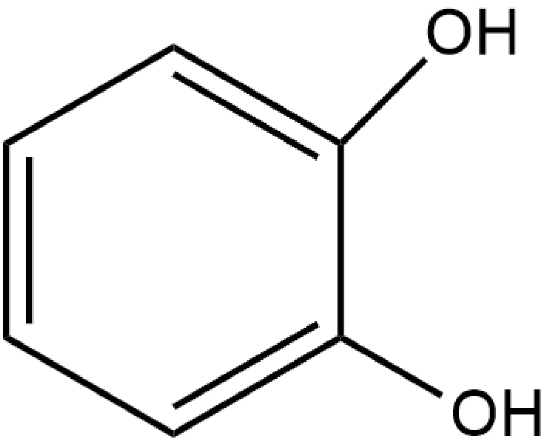
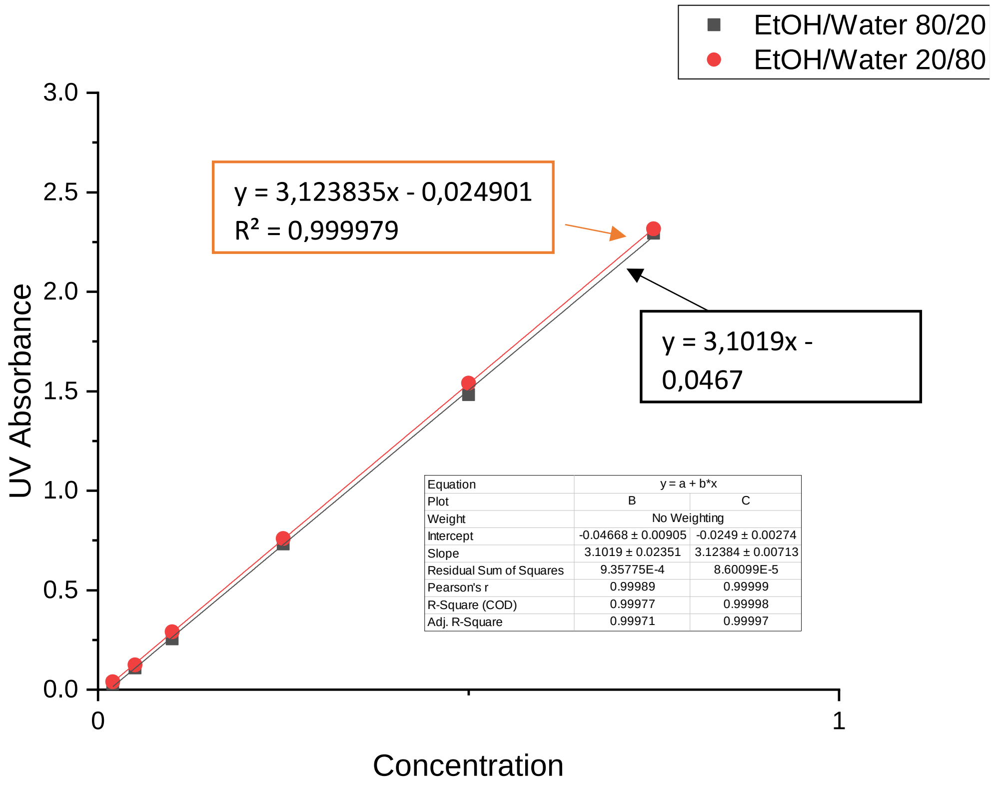


Figure 19: Structure of Catechol

4.3.1.2. Rutin

The calibration curves in the *figure 20* allowed to calculate the concentration at the equilibrium for each solvent. For Rutin, the comparison is only made between two solvents because of its solubility problem in ethyl acetate. Contrary to its less hydrophilic behavior, regardless of the materials, in the *figure 21* it is shown that the mixture of ethanol and water (20/80) gave a better retention.



Equation	y = a + b*x	
Plot	B	C
Weight	No Weighting	
Intercept	-0.04668 ± 0.00905	-0.0249 ± 0.00274
Slope	3.1019 ± 0.02351	3.12384 ± 0.00713
Residual Sum of Squares	9.35775E-4	8.60099E-5
Pearson's r	0.99989	0.99999
R-Square (COD)	0.99977	0.99998
Adj. R-Square	0.99971	0.99997

Figure 20: Calibration curves of Rutin

The rutin has more affinity with the material than with the solvent used. The *figure 22* shows that regardless of solvents, the newly synthesized adsorbent (MCC-P4VP) gave the highest amount retained. The solvent EtOH/Water 20/80 and the material MCC-P4VP, as for Catechol, seems to be best solvent and the best material to use.

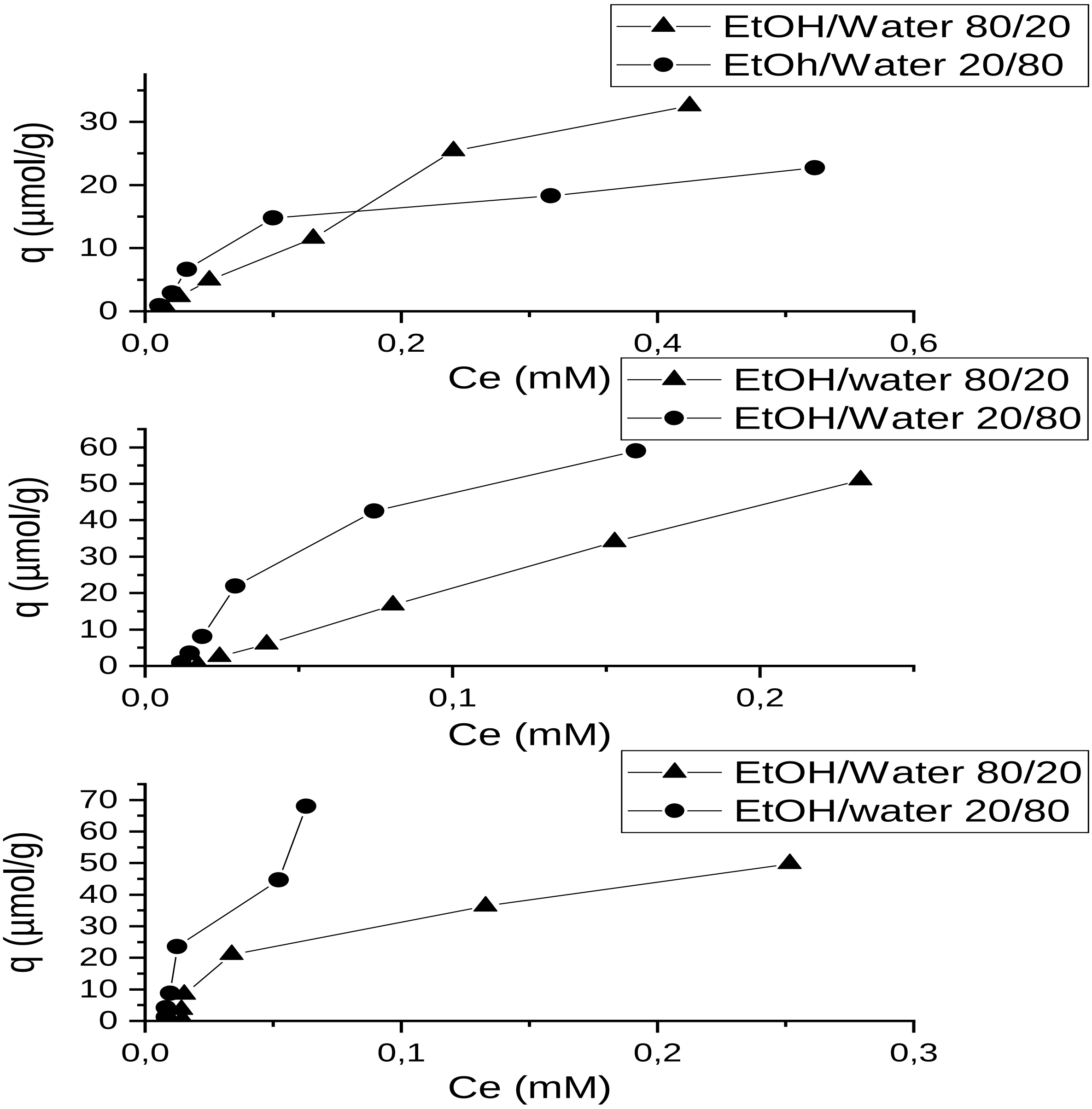


Figure 21: Rutin isotherms for each material (a) Reillex402 (b) Reillex425 (c) MCC-P4VP

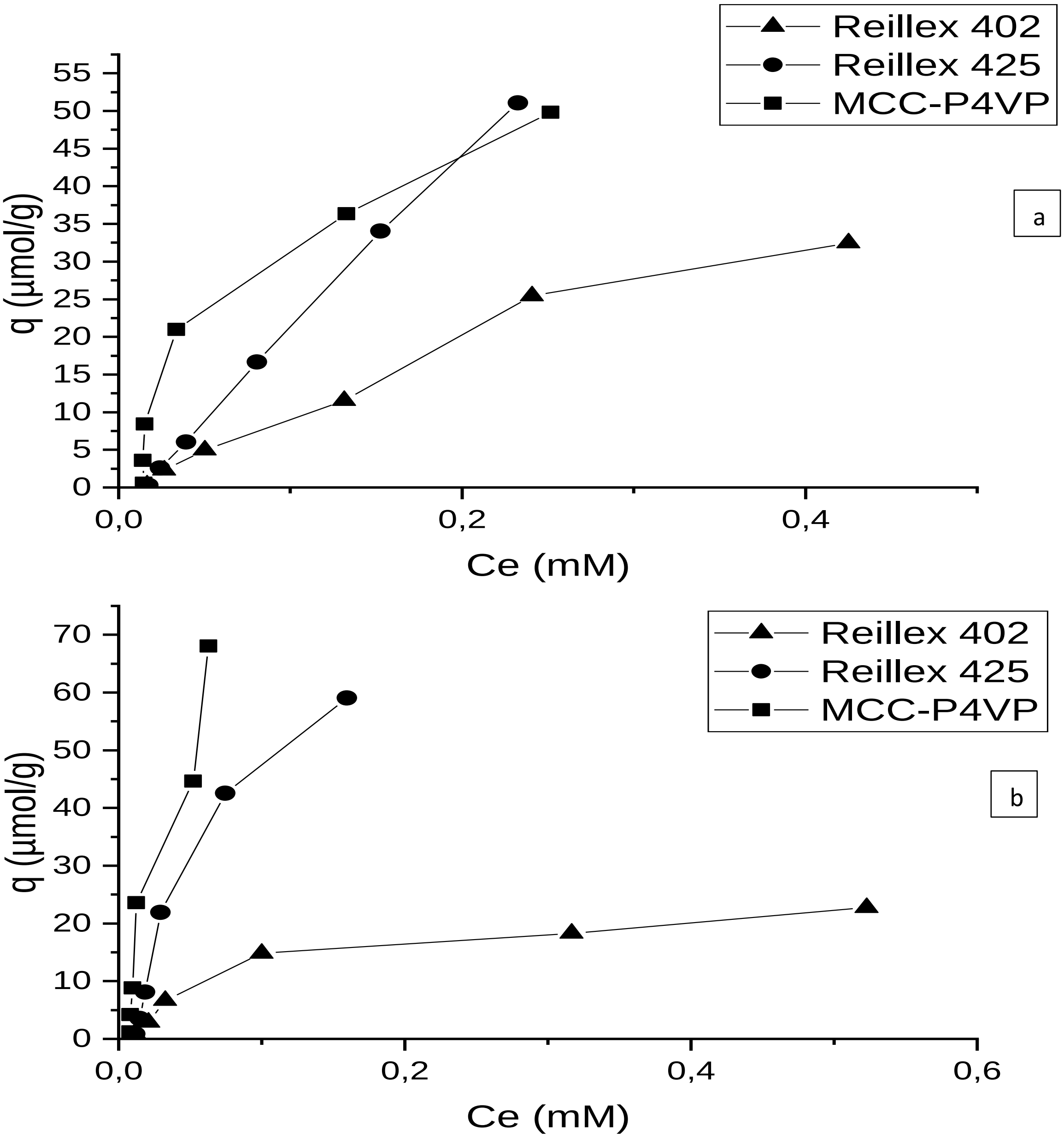


Figure 22: Rutin isotherms for each solvent (a) EtOH/Water 80/20 (b) EtOH/Water 20/80

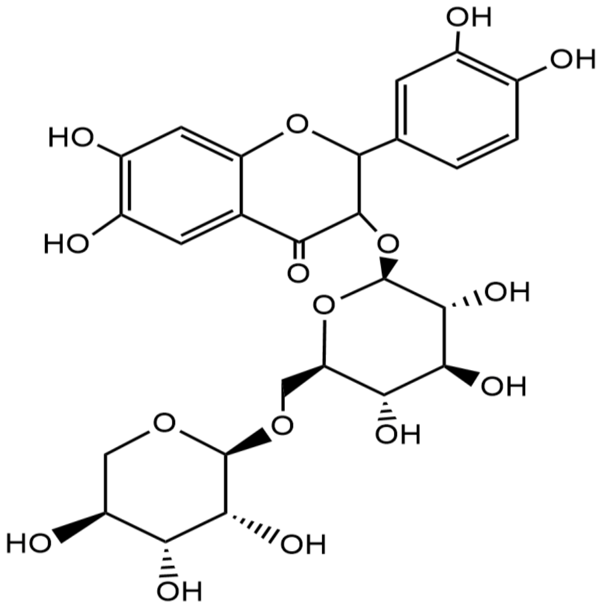
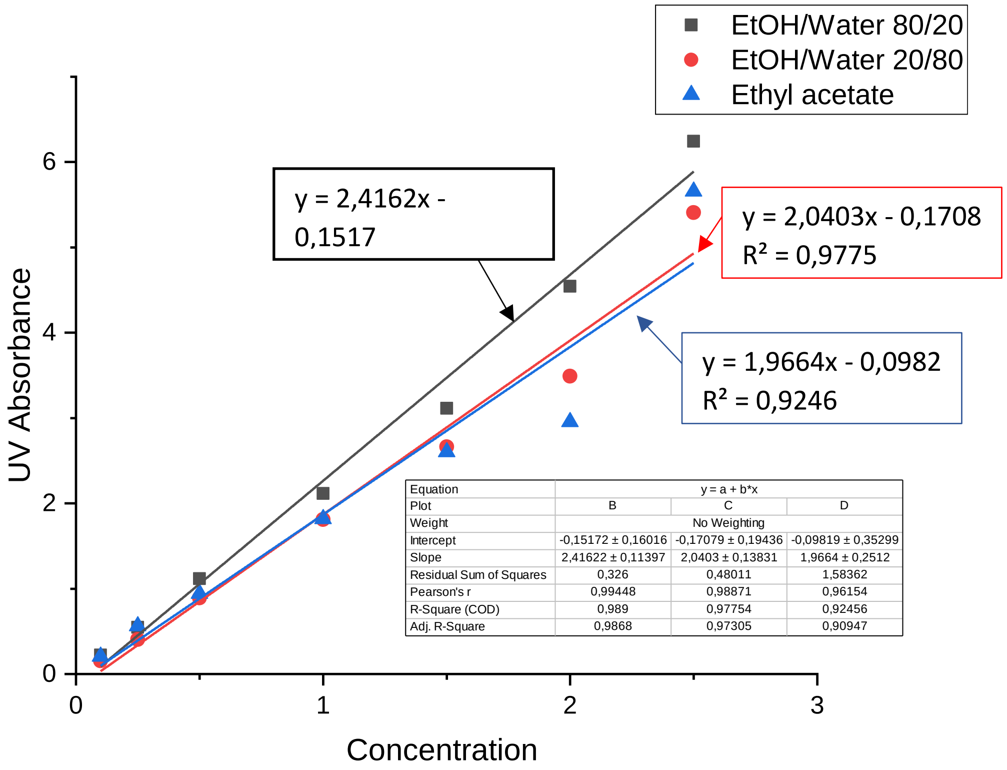


Figure 23: Structure of Rutin

4.3.1.3. Vanillic acid

The calibration curves, as for the other commercial polyphenols used, in the *figure 24* allowed to calculate the concentration at the equilibrium for each solvent. The behavior of vanillic acid is different. The comparison made in the *figure 25* shown that the EtOH/Water 20/80 gave good results with only two materials (Reillex 402 and MCC-P4VP). The other material, Reillex 425) worked better with the ethyl acetate.



Equation	y = a + b*x		
Plot	B	C	D
Weight	No Weighting		
Intercept	-0,15172 ± 0,16016	-0,17079 ± 0,19436	-0,09819 ± 0,35299
Slope	2,41622 ± 0,11397	2,0403 ± 0,13831	1,9664 ± 0,2512
Residual Sum of Squares	0,326	0,48011	1,58362
Pearson's r	0,99448	0,98871	0,96154
R-Square (COD)	0,989	0,97754	0,92456
Adj. R-Square	0,9868	0,97305	0,90947

Figure 24: Calibration curves of Vanillic acid

For Vanillic acid, the synthesized adsorbent got better results when it was combined with solvents composed of ethanol and water. The less hydrophilic solvent (ethyl acetate) seems to get more affinity with the commercial solvent Reillex 425 as it shown in the *figure 26.*

The synthesized adsorbent gives better results only if the polyphenols are in a hydrophilic solvent. When vanillic acid is in a less hydrophilic solvent, the affinity with the material (Reillex 425) takes the top.

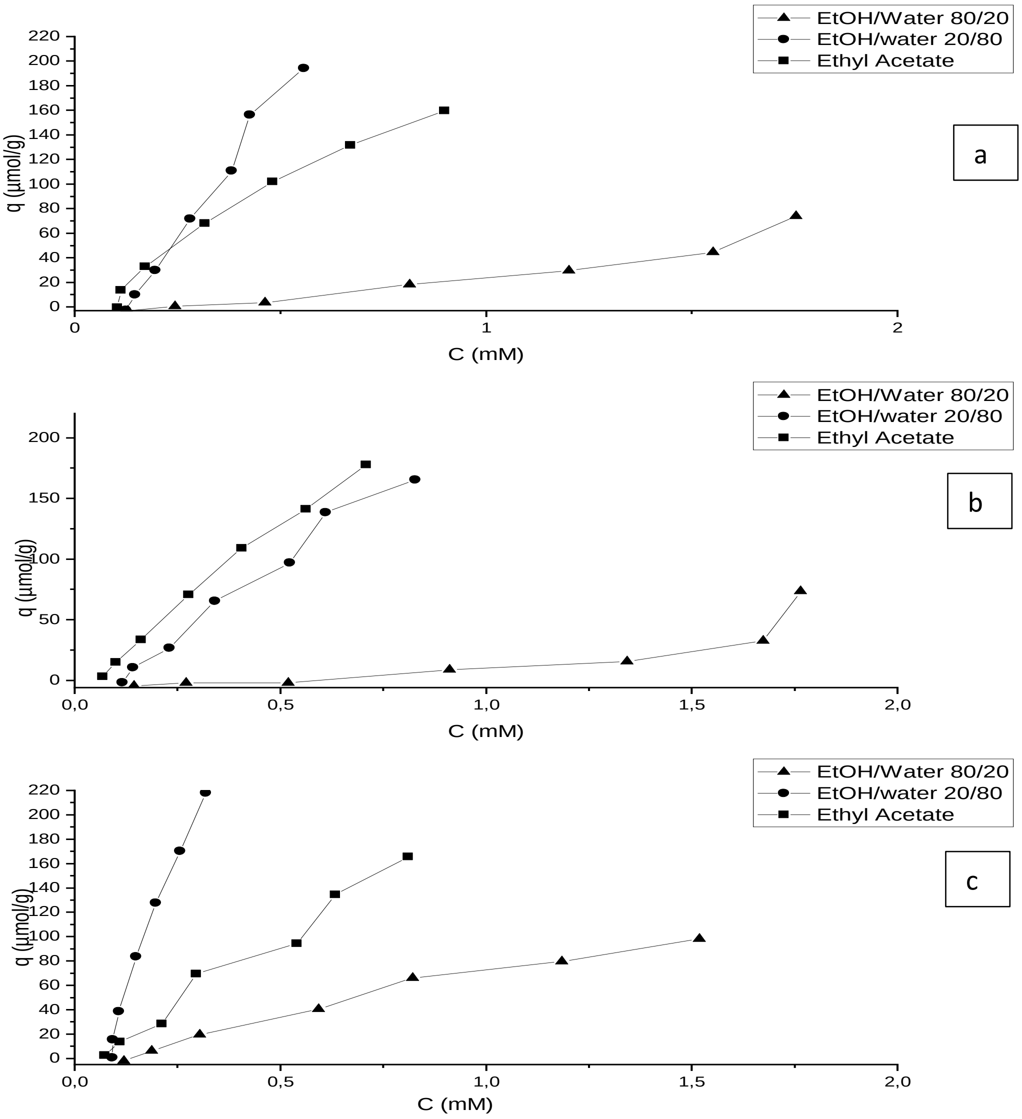


Figure 25: Vanillic acid isotherms for each material (a) Reillex402 (b) Reillex425 (c) MCC-P4VP

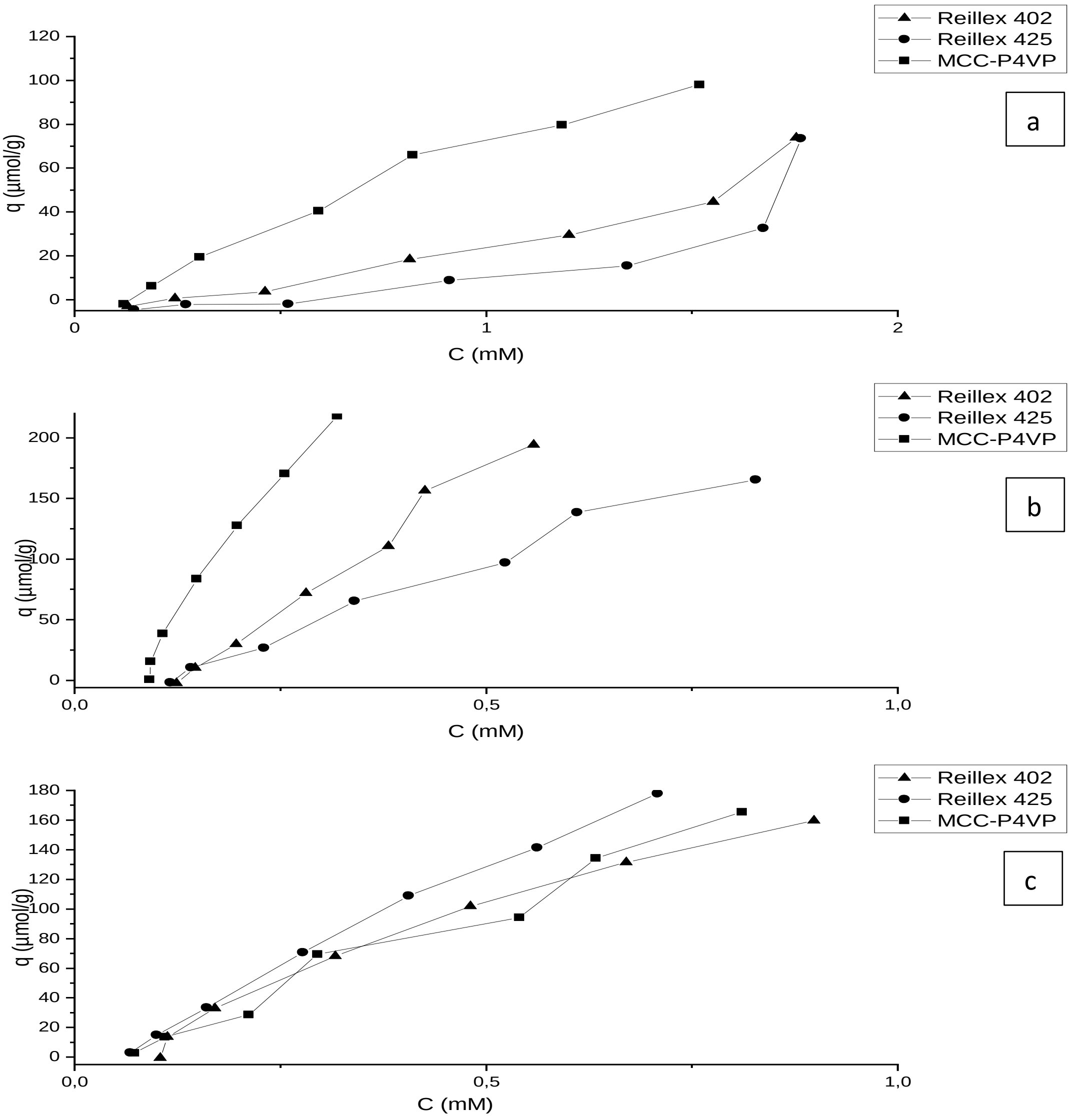


Figure 26: Vanillic acid isotherms for each solvent (a) EtOH/Water 80/20 (b) EtOH/Water 20/80 (c) Ethyl Acetate

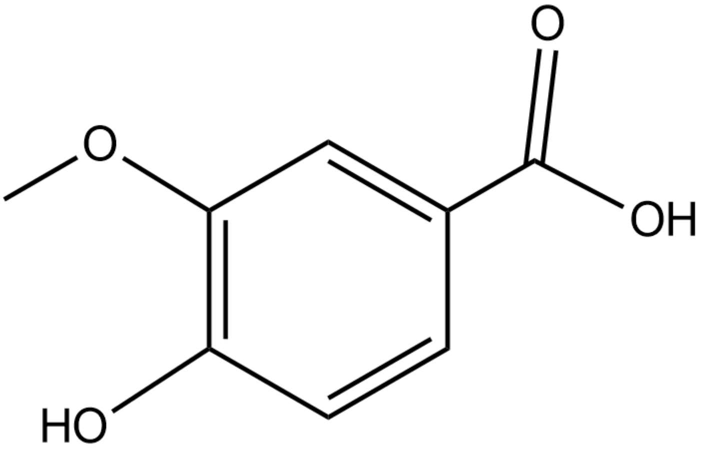


Figure 27: Structure of vanillic acid

4.3.2. Saturation experiences

The batch adsorption experience allowed to see the behavior of different adsorbent materials with polyphenols. Indeed, to perform the saturation experience, among the three adsorbent materials used in the batch experience, only two of them were chosen: Reillex 402 and the newly synthesized adsorbent (MCC-P4VP). A third material was added to perform the saturation experience: the native cellulose which has also an adsorbent capacity.

The saturation experiences lead to obtaining curves in order to have more information about the retention capacity of each adsorbent. These curves have as abscissa the eluted volumes and as ordinate the UV absorbance.

4.3.2.1. Saturation experience with the EtOH/Water olive leaves extract

This saturation experience performed with the EtOH/water 80/20 olive leaves extract shows a difference between the materials. Indeed, in the *figure 28*, the microcrystalline cellulose (MCC), although having adsorbent capacities, reaches its saturation quite quickly. From ten milliliters (10 mL) of the eluted solution, the saturation curve of MCC starts to reach its steadiness.

The Reillex 402 and MCC-P4VP, as far as they are concerned, have a little competitivity, but it is visible that Reillex 402 reaches its saturation faster than MCC-P4VP. Indeed, from thirty milliliters (30 mL) of the eluted solution, the saturation curve of Reillex 402 starts to reach its steadiness while the MCC-P4VP really reaches its saturation around fifty milliliters (50 mL) of eluted solution.

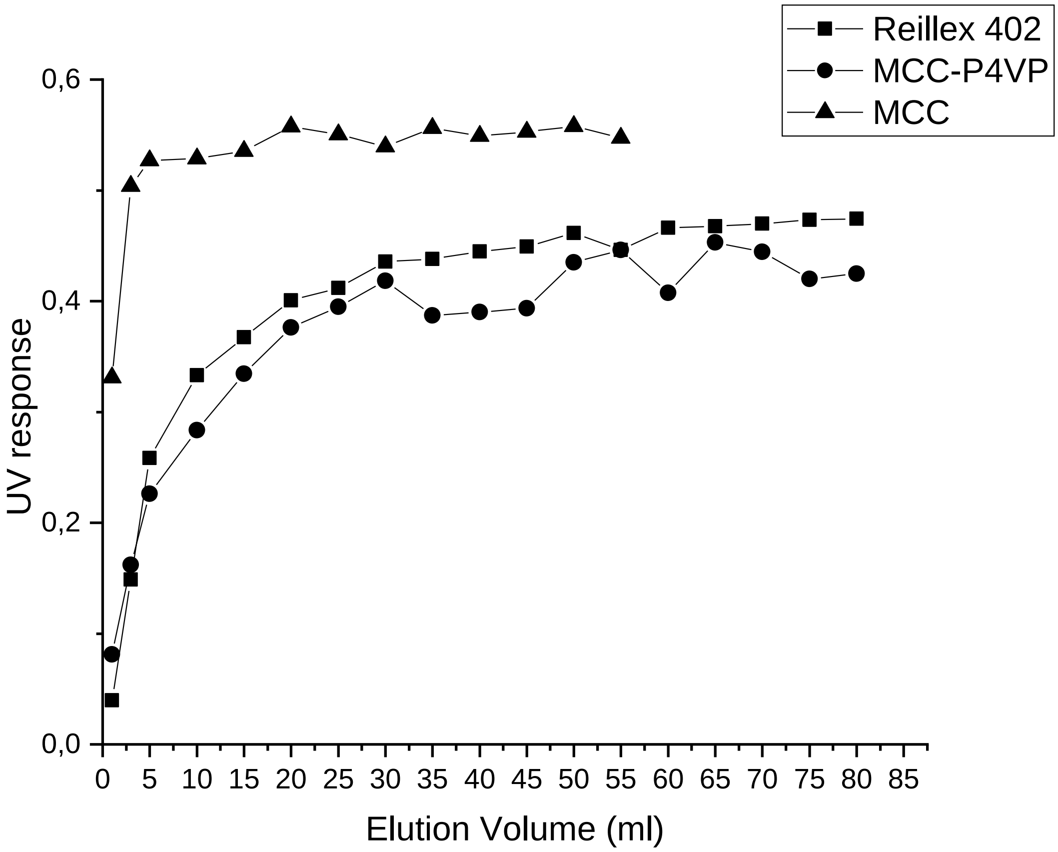


Figure 28: Saturation curves of absorbent materials with EtOH/Water olive leaves extract

To read the absorbance to draw the saturation curves, the extracts were diluted twenty times. The 3D HPLC chromatogram of the extract solutions 20 times diluted, represented in the *figure 29*, shown a little difference due to the dilution. The absorbance intensity decreases also one compound does not appear as on the 3D HPLC chromatogram obtained in the *Chapter 2*.

After passing through volumes of extract solutions in the columns, the non-adsorbed solutes which were collected and analyzed by HPLC-DAD. These 3D HPLC chromatograms obtained allowed to have an idea of the polyphenols retained in the adsorbents.

Regarding the saturation curve, which was obtained, only the samples of two materials (MCC-P4VP and Reillex 402) were analyzed. For each material, two samples were chosen. To understand better and to interpret these chromatograms, they will be compared with the chromatogram of the initial extract solution (20 times diluted).

- **HPLC-DAD of the collected samples after adsorption with MCC-P4VP:**

As it said above, in the 3D HPLC chromatogram in the *figure 8* four compounds were identified, after dilution only three compounds were identified. The *figure 29* shows the 3D HPLC chromatogram of the initial extract solution diluted 20 times. In this chromatogram, the peaks of Verbascoside (1), Luteolin-7-glucoside (2), and Oleuropein (3) were identified. The peak of Hydroxybenzoic acid disappeared due to the dilution.

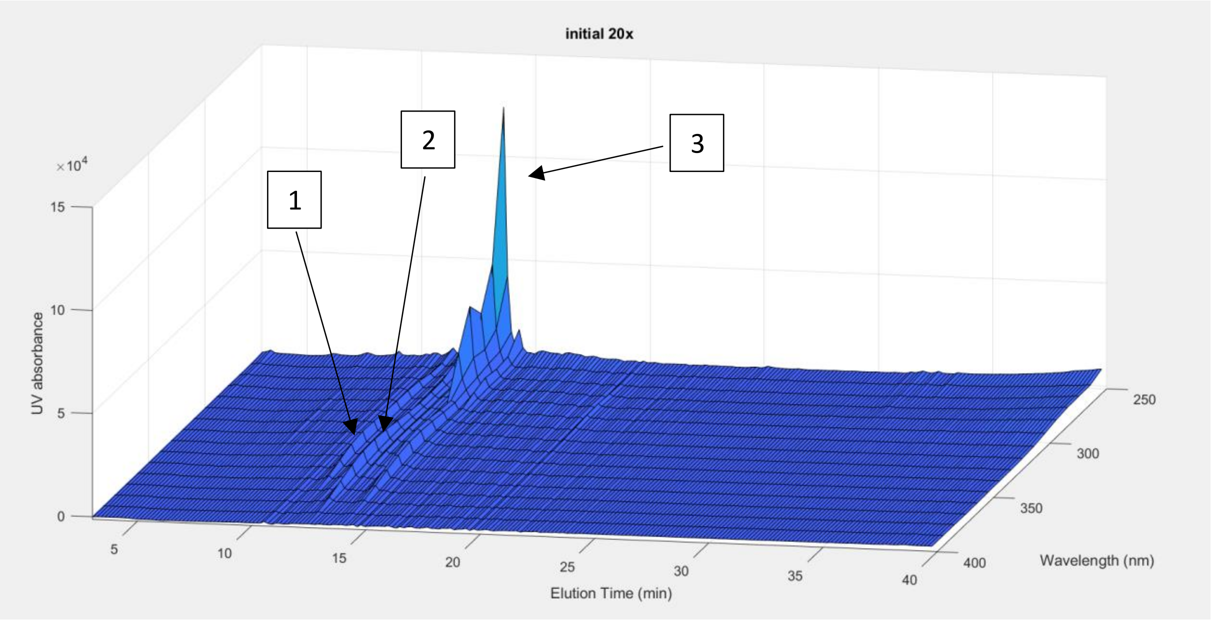


Figure 29: HPLC chromatogram of EtOH/Water 80/20 Olive leaves extract 20x diluted (1)Hydroxybenzoic acid (2)Luteolin-7-glucoside (3)Oleuropein

After passing through the column 3 mL diluted extract solution, the 3D HPLC chromatogram, represented in *figure 30(a)*, of the non-adsorbed solute shows a lot of peaks, but the most visible and distinguishable peaks are the peaks of Oleuropein (2) and Hydroxybenzoic acid (1) with lower UV absorbance than in the initial solution. There are also the peaks of non-identified compounds.

After passing through 55 mL of diluted solution, the 3D HPLC chromatogram represented in the figure 30(b), also shown two peaks. The Oleuropein peak appears again. Instead of the Hydroxybenzoic acid, the other identified compound is Luteolin-7-glucoside.

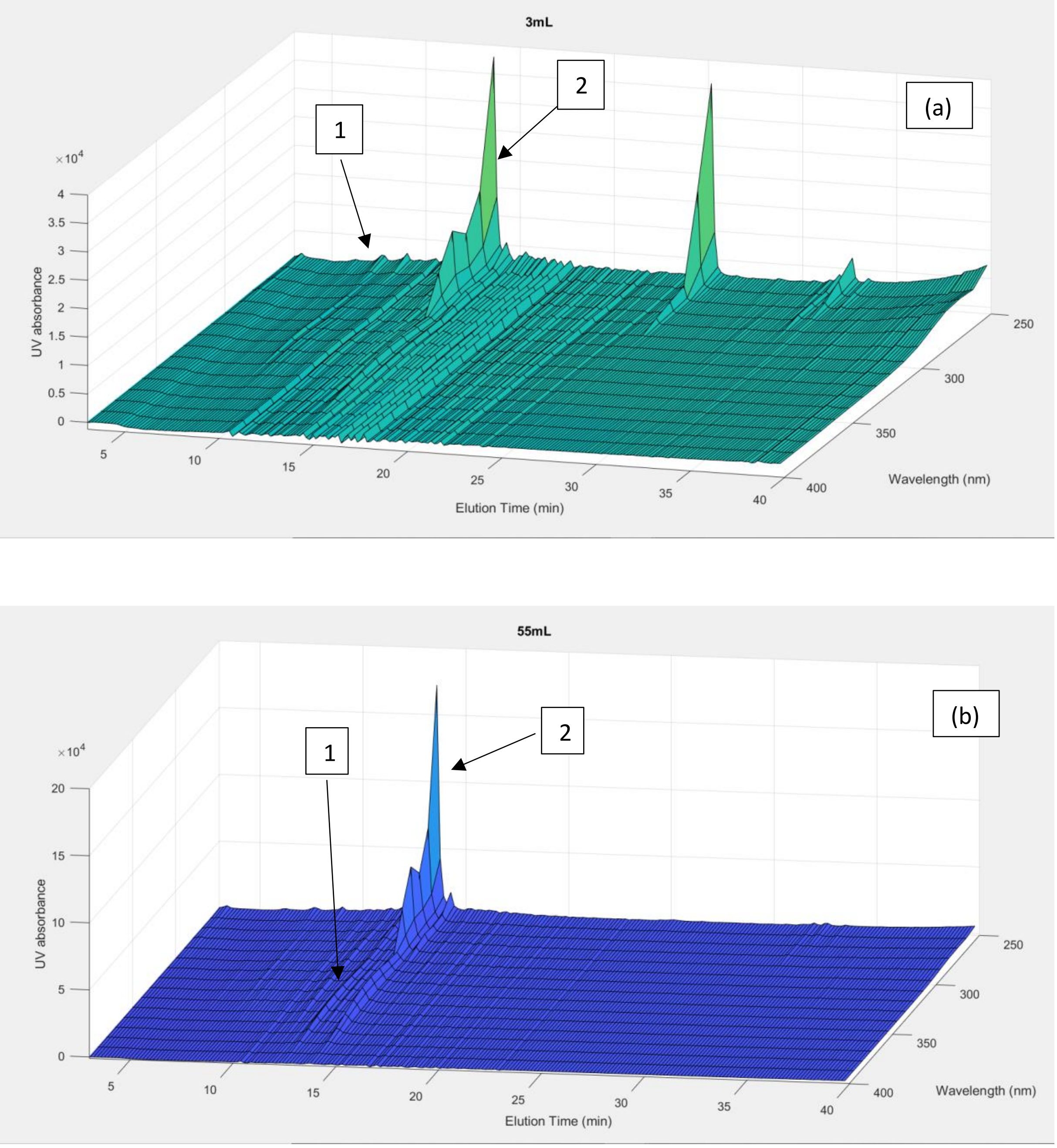


Figure 30: HPLC chromatogram of non-adsorbed solute by MCC-P4VP after passing through 3mL and 55mL of diluted extract solution

- **HPLC-DAD of the collected samples after adsorption with Reillex 402:**

For Reillex 402, the samples chosen to analyze them by HPLC are the non-adsorbed solutes after passing through 3 and 15 mL of diluted extract solution. The obtained chromatograms represented in *figure 31* for this adsorbent material do not show a lot of peaks as with MCC-P4VP, which allows a better identification of compounds.

Among the visible peaks on these chromatograms of the non-adsorbed solute only one peak can be identified. After passing through 3 mL, figure 31(a), and after passing through 15 mL, figure 31(b) of passed extract solution, one compound can be clearly identified. It is the Oleuropein. There are also the peaks of non-identified compounds after 20 minutes of elution.

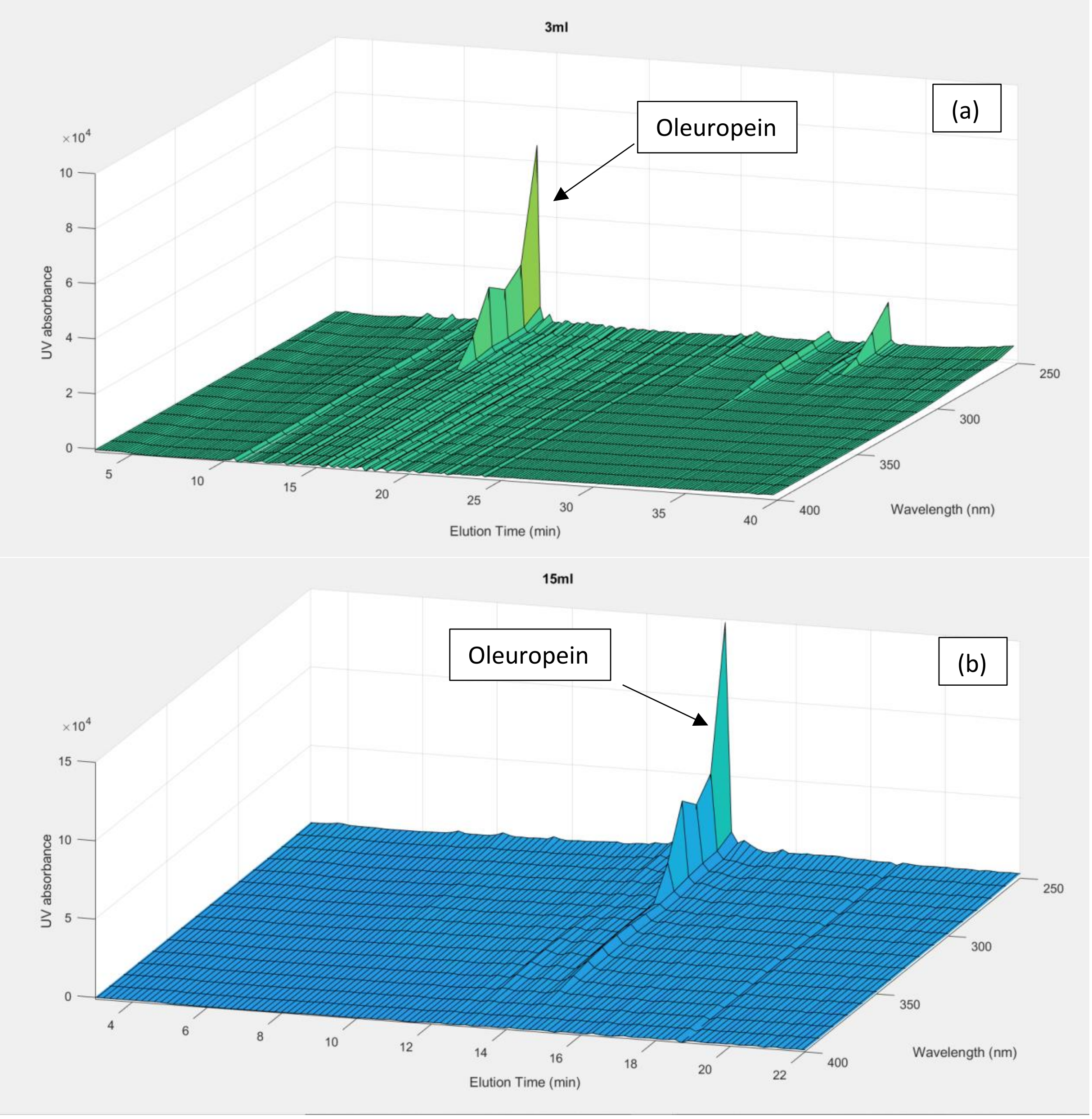


Figure 31: HPLC chromatogram of non-adsorbed solute by Reillex 402 after passing through 3mL and 15mL of diluted extract solution

4.3.2.2. Saturation experience with Ethyl acetate olive leaves extract

The obtained saturation curves with ethyl acetate olive leaves extract showed a behavior completely different than the previous one. Indeed, the behavior of microcrystalline cellulose is special. In *figure 32*, the native cellulose MCC reaches its saturation much slower than the other adsorbent materials. Without achieving steadiness, MCC starts to reach its saturation around sixty milliliters of eluted solution.

The Reillex 402 and MCC-P4VP showed the same behavior as the previous saturation experience. The shape of the curve seems to be the same in the two saturation experiences, even if it reaches its saturation faster. The saturation of Reillex 402 is reached around twenty milliliters of eluted solution. And MCC-P4VP, as far as its concerned, reaches its saturation faster than the other adsorbent materials; from fifty milliliters of the eluted solution, it started to reach its saturation.

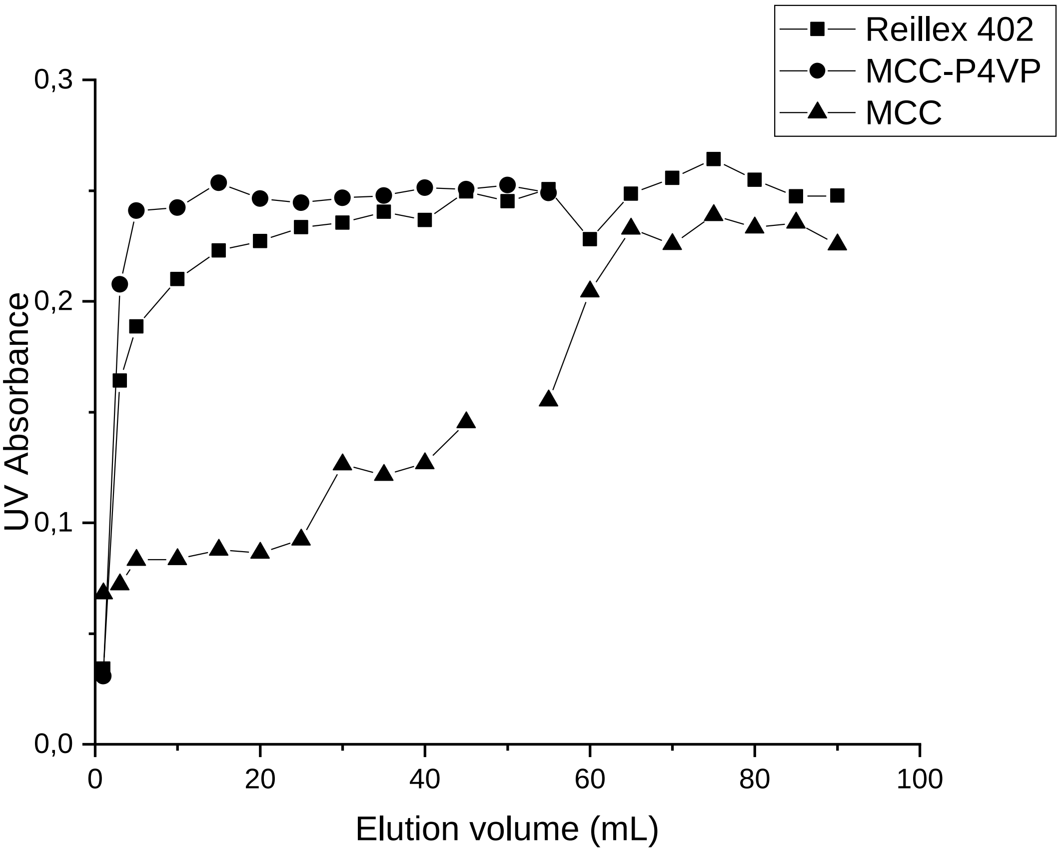


Figure 32: Saturation curves of different materials with ethyl acetate olive leaves extract

- **HPLC-DAD of the collected samples after adsorption:**

To read the absorbance to draw the saturation curves, the extracts were diluted twenty times. The 3D HPLC chromatograms of the initial extract solution and the diluted solution are the same, only the absorbance intensity decreases because of the dilution.

After passing through the column a certain volumes of extract solutions, the non-adsorbed solutes which were collected and analyzed by HPLC-DAD. These chromatograms thus obtained allowed to have an idea of the polyphenols retained in the adsorbents.

Regarding the interesting results obtained with the saturation curve, only the samples from native cellulose (MCC) were analyzed. Among all the collected samples, only two of them were chosen to be analyzed by HPLC: 55 and 90 mL. The obtained chromatograms of the sample were compared with the chromatogram of the initial diluted extract solution.

The 3D HPLC of the diluted extract solution represented in *figure 32* shows the same compounds peaks than in the *figure 9* form *Chapter 2*. The identified compounds are Verbascoside (1), Luteolin-7-Glucoside (2), and Oleuropein (3) are observable.

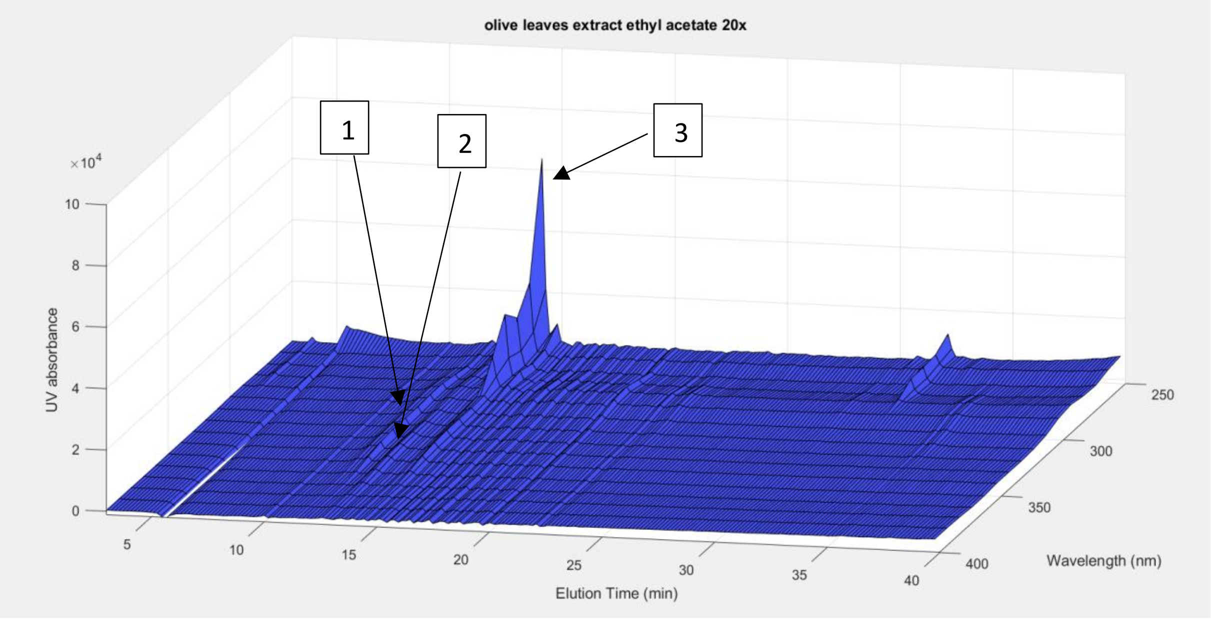


Figure 33: HPLC chromatogram of initial ethyl acetate extract solution 20x diluted

After passing through 3 and 20 mL of diluted solution, the obtained chromatograms show a lot of non-distinguishable peaks with lower intensity. This is the reason why 55 mL, and 90 mL were chosen. After passing through 55 mL of diluted extract solution in the column, the 3D HPLC chromatogram, represented in *figure 34(a)*, only one peak could be identified: Oleuropein.

After passing through 90 mL of diluted extract solution in the column, the obtained 3D HPLC chromatogram represented in the *figure 34(b)* showed the same peak. The only identified peak is oleuropein.

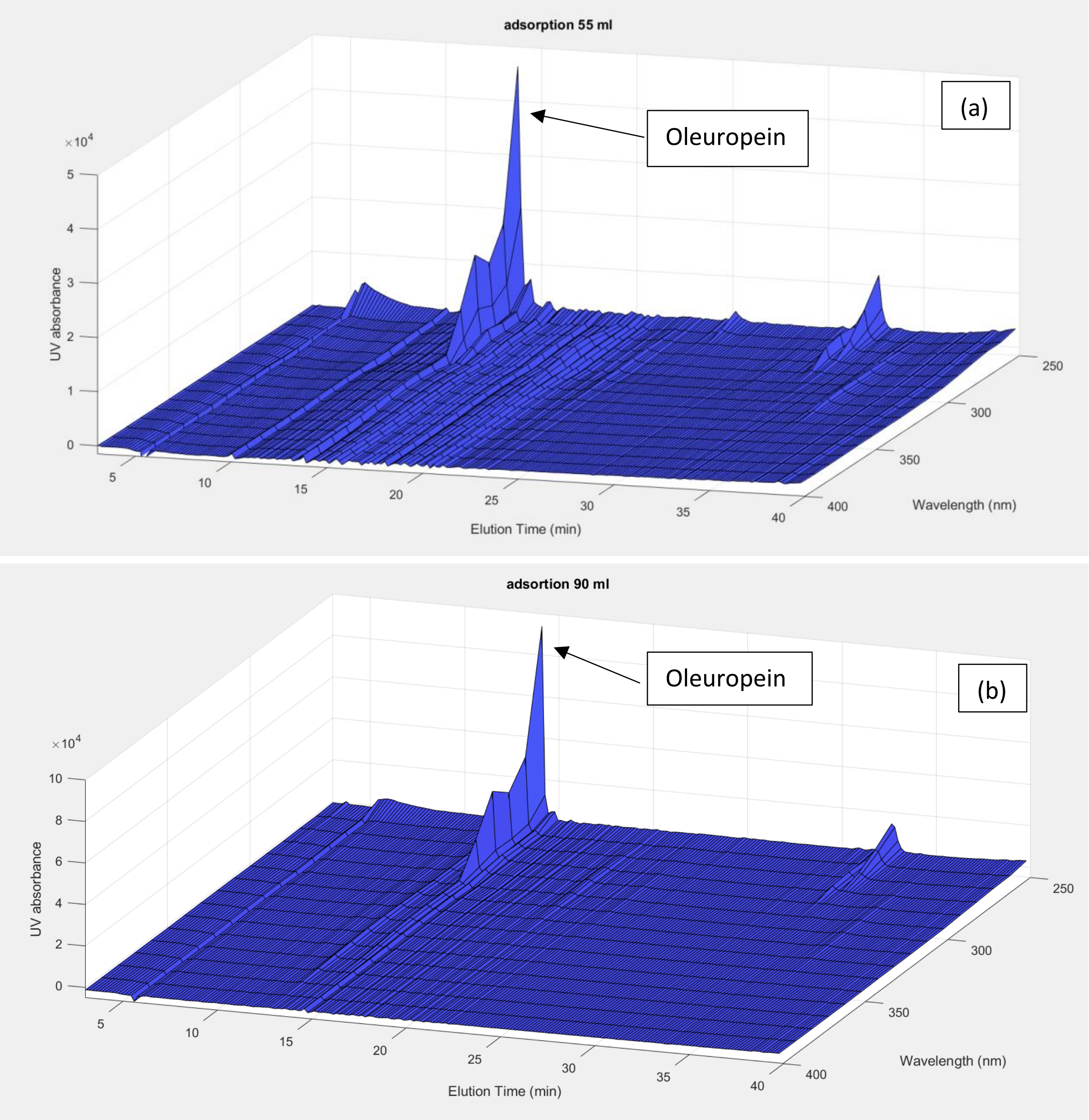


Figure 34: HPLC chromatogram of non-adsorbed solute by MCC after passing through 55 and 90mL of diluted solution

4.4. Conclusion

The batch experience was made to observe the behavior of polyphenols in contact with the synthesized adsorbent but also to compare the commercial adsorbents containing vinyl pyridine to the synthesized adsorbent. Whether with catechol, rutin, or even vanillic acid, the synthesized adsorbent manages to have better results than the other adsorbents. Moreover, to find the best solvent to use with the new adsorbent, three solvents were used in this batch experience. Among these solvents, it is the most hydrophilic solvent that gives better results regardless of the material used. It is, therefore, solvent-based on ethanol and water with a ratio of 20/80, which will suit best for the use of the new adsorbent.

The curves obtained in the batch experience allowed to choose the materials of interest in order to perform the saturation experience. Indeed, among the three materials used, only two materials were chosen to be compared to the native cellulose, which also has adsorbent capacities. The saturation experiences were performed with the extracts of olive leaves, which were prepared using two different solvents. The results obtained allowed to understand that the capacity of the retention of the materials depends on the solvent used. Also, as in the batch experience, ethyl acetate seems to be the least suitable solvent for the newly synthesized adsorbent.

In addition to the retention capacity gauge of the adsorbents, the saturation experience allowed the retention of some phenolic compounds. In order to recover these polyphenols, desorption will be performed.

Chapter 5: Polyphenol's recovery

5.1. Introduction

Desorption is the complementary experience of adsorption. While adsorption allows the retention of adsorbate, desorption allows the recovery of these adsorbed compounds. To recover the polyphenols, desorption must therefore be done.

After the saturation experiences were done for the two principals extracts, desorption experiences were performed. To do these experiences, five solvents were chosen. The elutions were performed, beginning by the most hydrophilic and ending by the least hydrophilic solvent.

In the end, analysis was performed to identify the polyphenols eluted by each solvent.

5.2. Experimental section

5.2.1. Chemicals

Methanol (MetOH), acetic acid bought from Fischer Scientific, and deionized water were used as solvents for the elution. Acetonitrile was purchased from Fischer Scientific, and deionized water was used to prepare the mobile phase for the HPLC-DAD.

5.2.2. Elution experiences

After the saturation of materials, elution was performed to recover the polyphenols. This experience was performed by washing the column with five types of solvents, starting with the most hydrophilic and ending with the most hydrophobic. The solvent used were deionized water, MetOH/Water 50/50 v/v, MetOH/water 80/20 v/v, MetOH/Water 95/5 v/v, and MetOH/Acetic Acid 90/10 v/v. Each solvent was added by 5mL and eluted before adding the next solvent, as it mentioned in the *table 9*.

These five fractions were used only with the extract made with the mixture of ethanol and water. For the other extract (ethyl acetate), other solvents were used: deionized water and ethanol.

Table 9: Summary of solvents used during the desorption

Fractions	Solvents	Volume (mL)
1	Deionized water	5
2	MetOH/Water 50/50	5
3	MetOH/Water 80/20	5
4	MetOH/Water 95/5	5
5	MetOH/Acetic acid 90/10	5

5.2.3. Identification of polyphenols

To identify the polyphenol eluted by each solvent, the characterization by HPLC-DAD was performed. The analysis was made at room temperature with a flow rate of 1mL/min. Two solvents were used as mobile phase: (A) acetonitrile/water 10/90 v/v and (B) acetonitrile/water 90/10 v/v. The two solvents were at pH=3; the pH was adjusted by using acetic acid. The linear gradient method was used, starting with 100% of (A) and ending with 100% of (B).

The Jasco MD-4010 photodiode array (PDA) detector was used to perform the analysis with a Nucleosil® C18 column L × I.D. = 15 cm × 4.6 mm and 5µm particle size.

5.3. Results and discussion

As for the saturation experience, the samples of the elution were collected and analyzed by HPLC to identify the polyphenol eluted. The identification of phenolic compounds was made using the commercial polyphenols. The obtained 3D HPLC chromatograms were compared to the chromatogram of the initial extract solution shown in the previous chapters.

5.3.1. Recovery from EtOH/ Water 80/20 olive leaves extract

For each material, five samples were collected. From the first solvent to the last, the collected samples are named Fraction1 to Fraction5.

- <u>**Material: MCC-P4VP**</u>

After the elution of all the solvent, all the samples were analyzed by HPLC. The *figure 35*, the 3D HPLC chromatograms of Fraction 1, Fraction 3, Fraction 4 and Fraction 5 were represented. The peaks of Hydroxybenzoic and Oleuropein are identified in the chromatogram of Fraction 1 represented in the *figure 35(a)*. Fractions 2 and 3 gave the same chromatogram even if the intensities of UV absorbance of the identified peak are different. The only identified peak in the *figure 35(b)* is Hydroxybenzoic acid.

Among the peaks on the obtained 3D HPLC chromatogram of the Fraction 4 represented in *figure 35(c)*, the identified peaks are Hydroxybenzoic acid and Luteolin-7-glucoside. And the 3D HPLC chromatogram of Fraction 5 gave a lot of peaks, but in the *figure 35(d)* only one peak was identified: Luteolin-7-glucoside peak.

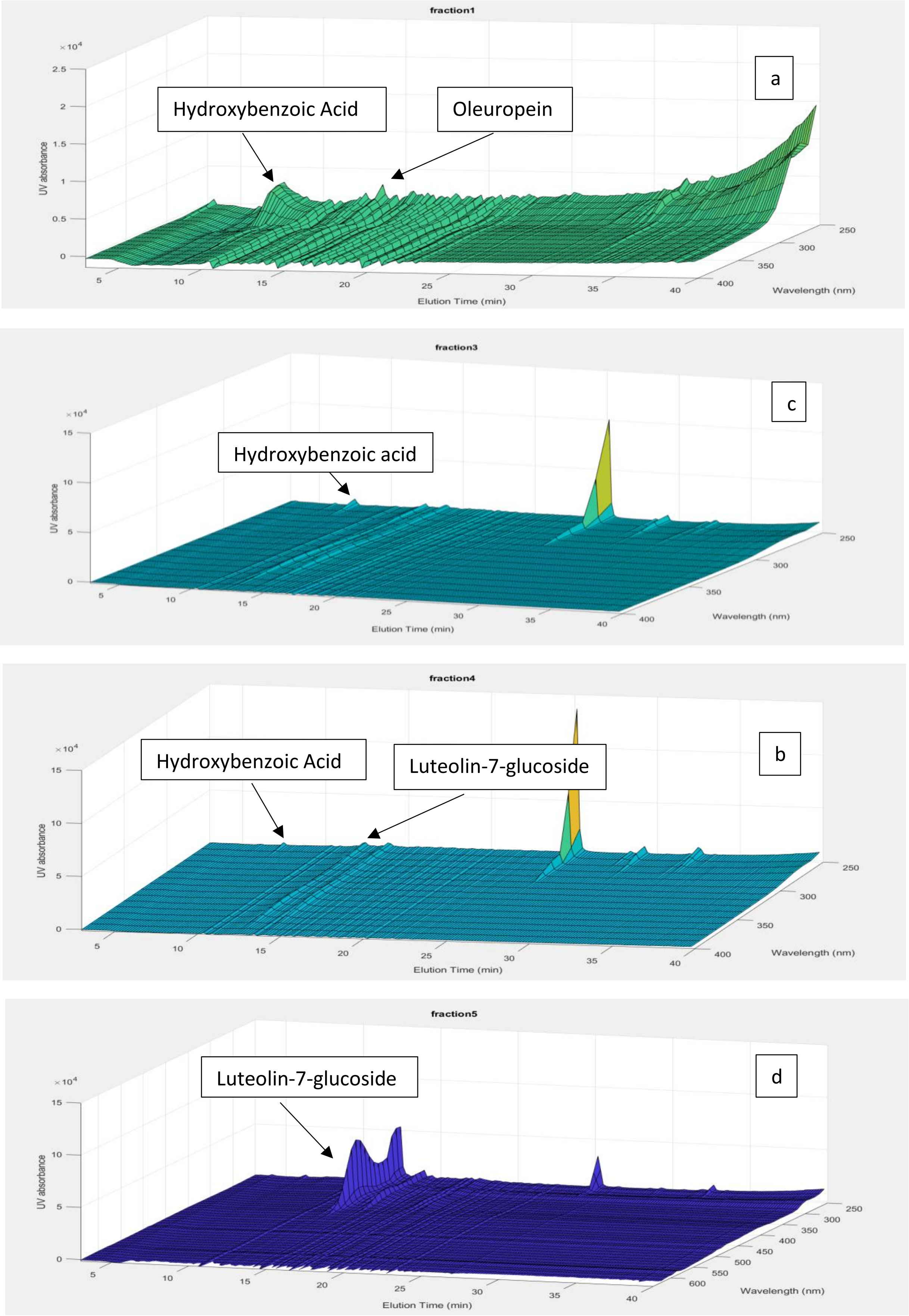


Figure 35: Chromatograms of elution with MCC-P4VP (a) fraction1 (b) fraction3 (c) fraction4 (d) fraction5

In the previous chapter, the 3D HPLC chromatogram of the extract solution diluted 20 times gave 3 identified peaks: Verbascoside, Luteolin-7-glucoside, and Oleuropein. The desorption experience performed by washing the column with solvents allowed to recover polyphenols. These polyphenols were identified by HPLC. The comparison between the diluted initial extract and the different samples from the desorption was made. The *table 10* gives the list of phenolic compounds contained in the diluted extract solution and the recovered polyphenols. Moreover, another phenolic compound was found. It can be explained by the fact that this phenolic compound is present in the extract at low concentration.

Table 10: Comparison of phenolic compounds before and after sorption experience with MCC-P4VP

Phenolic compounds in initial solution extract (20x diluted)	Phenolic compounds after elution of the five fractions
Verbascoside	Hydroxybenzoic acid
Luteolin-7-glucoside	Luteolin-7-glucoside
Oleuropein	Oleuropein

- **Material: Reillex 402**

After the elution of all the solvent, all the samples were analyzed by HPLC. The *figure 36*, the 3D HPLC chromatograms of Fraction 1, Fraction 2, Fraction 3, and Fraction 5 were represented. Oleuropein was the only identified peak on the 3D HPLC chromatogram of Fraction1 represented in the *figure 36(a).* On the chromatogram of Fraction 2 represented in *figure 36(b),* the peaks of Verbascoside and Oleuropein were identified. Three peaks were identified in *figure 36(c)* which represents the chromatogram of Fraction 3. The identified peaks are Verbascoside, Oleuropein and Luteolin-7-glucoside.

The Fraction 4 and 5 have the same identified phenolic compounds even if the intensities of UV absorbance are different. The figure 36(d) represents the 3D HPLC chromatogram of Fraction 5 and Verbascoside, Luteolin-7-glucoside, and Apigenin-7-glucoside were identified on it.

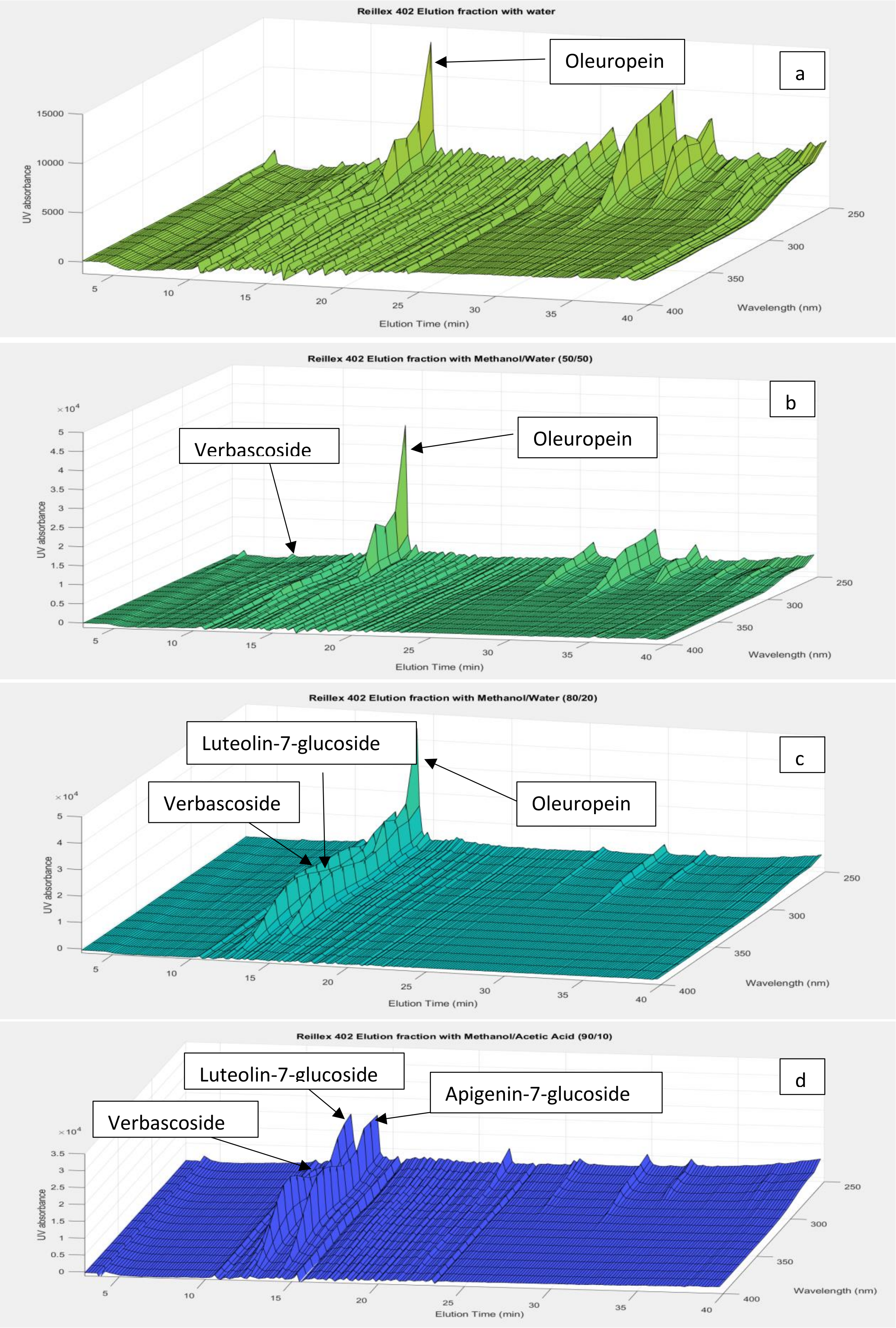


Figure 36: Elution chromatograms (a) fraction1 (b) fraction2 (c) fraction3 (d) fraction5

In the previous chapter, the 3D HPLC chromatogram of the extract solution diluted 20 times gave 3 identified peaks: Verbascoside, Luteolin-7-glucoside, and Oleuropein. The desorption experience performed by washing the column with solvents allowed to recover polyphenols. These polyphenols were identified by HPLC. The comparison between the diluted initial extract and the different samples from the desorption was made. The *table 11* gives the list of phenolic compounds contained in the diluted extract solution and the recovered polyphenols. Moreover, another phenolic compound appeared in fraction 4 and 5. It can be explained by the fact that this phenolic compound is present in the extract at low concentration.

Table 11: Comparison of polyphenols before and after sorption experience with Reillex402

Phenolic compounds in initial extract solution (20x diluted)	Phenolic compounds after elution of the five fractions
Verbascoside	Verbascoside
Luteolin-7-glucoside	Luteolin-7-glucoside
Oleuropein	Oleuropein
	Apigenin-7-glucoside

5.3.2. Recovery from Ethyl acetate olive leaves extract

After the saturation experience for the extract made with ethyl acetate as the solvent to recover phenolic compounds, the fractions were not the same as the previous one. With this extract, the fractions of elution are different. For the elution experience with MCC-P4VP, only two fractions were used: deionized water and ethanol. And with MCC, three fractions were used: deionized water, ethanol, and ethanol at 50 ˚.

- **Material: MCC-P4VP**

After the elution of the two solvents, the samples were analyzed by HPLC. These chromatograms represented in the *figure 37* shown the same phenolic compounds. In *figure 37(a)* and *figure 37(b)* only one peak was identified on the chromatogram: Oleuropein.

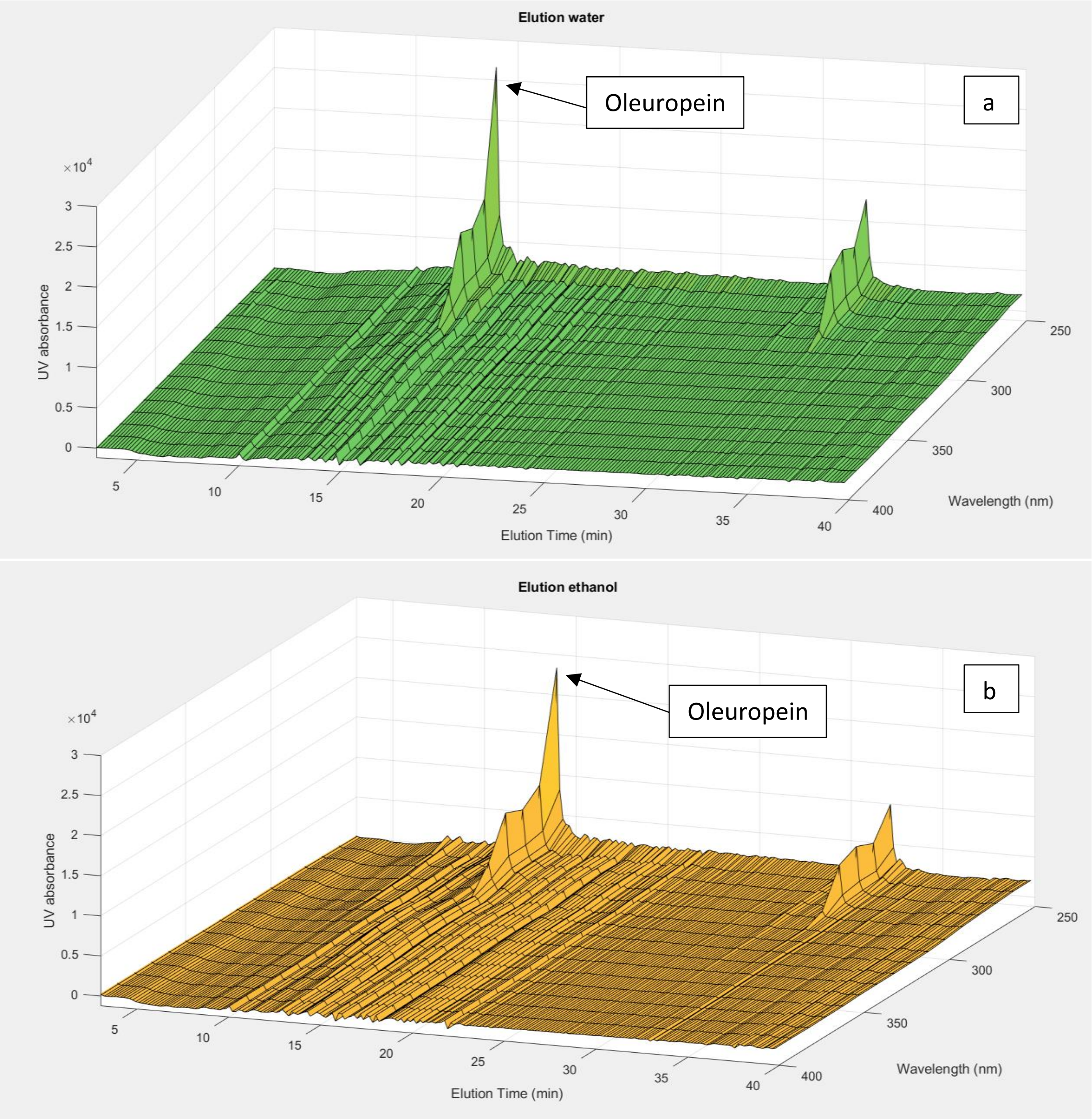


Figure 37: HPLC chromatogram with MCC-P4VP (a) fraction1 (b) fraction2

In the previous chapter, the 3D HPLC chromatogram of the extract solution diluted 20 times gave 3 identified peaks: Verbascoside, Luteolin-7-glucoside, and Oleuropein. The desorption experience performed by washing the column with solvents allowed to recover polyphenols. These polyphenols were identified by HPLC. The comparison between the diluted initial extract and the different samples from the desorption was made. The *table 12* gives the list of phenolic compounds contained in the diluted extract solution and the recovered polyphenols. Regardless on the solvent used, only one phenolic compound was eluted.

Table 12: Comparison of polyphenols before and after the sorption experience

Phenolic compounds in initial extract solution (20x diluted)	**Phenolic compounds after the elution of the two fractions**
Verbascoside Luteolin-7-glucoside Oleuropein	Oleuropein

- **Material: MCC**

For this material, three samples were analyzed by HPLC. The three obtained 3D HPLC chromatograms, represented in *figure 38*, gave some peaks, and among these peaks, three compounds were identified. On the chromatogram of Fraction 1, in *figured 38(a),* the peaks of Verbascoside and Oleuropein were identified. In *figure 38(b),* only one peak was identified on the chromatogram of Fraction 2: Oleuropein. And the Fraction 3 allowed to also recover one identified polyphenol: Luteolin-7-glucoside which is represented in *figure 38(c).*

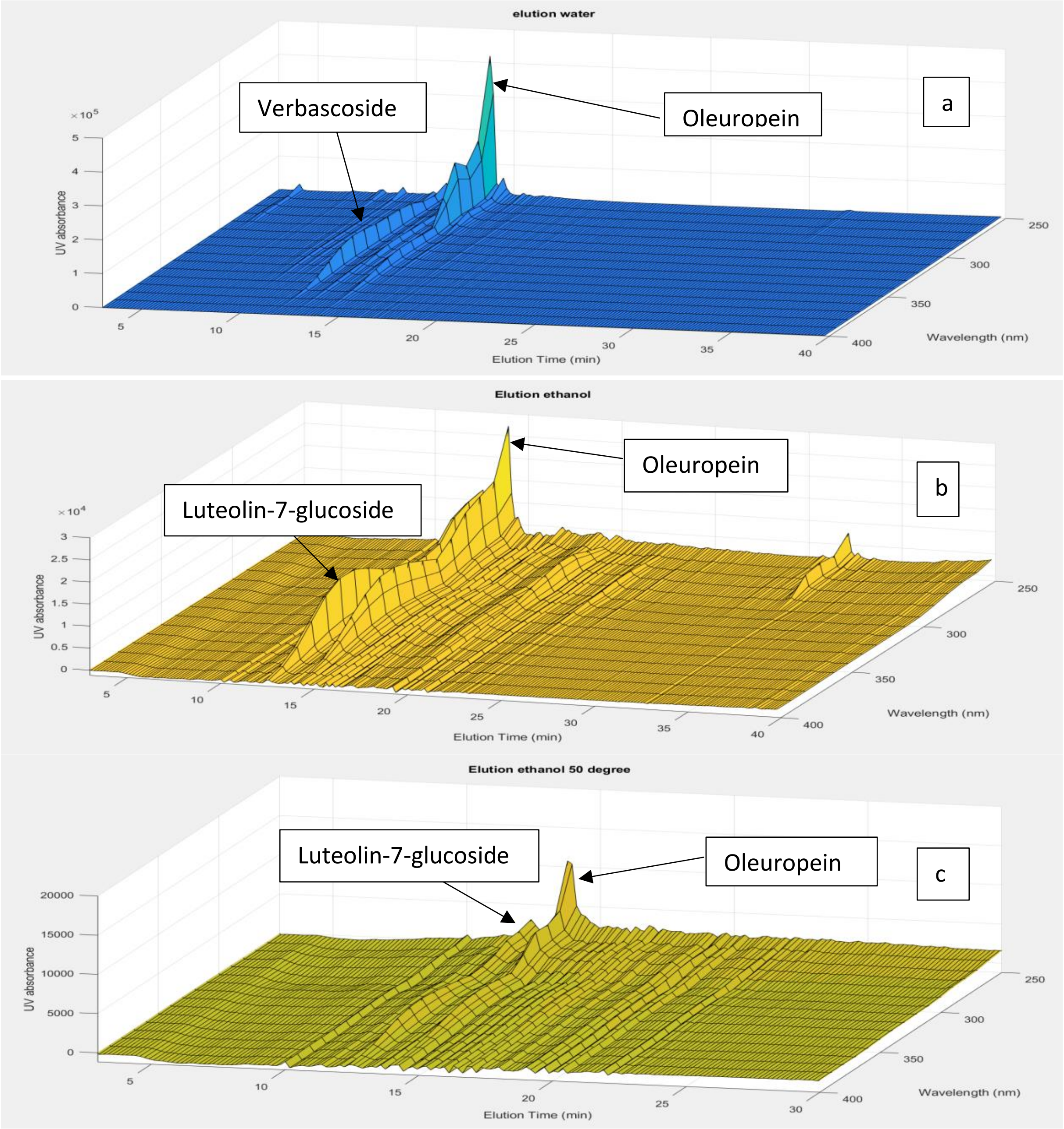


Figure 38: Chromatograms of elution with MCC (a) fraction1 (b) fraction2 (c) fraction3

In the previous chapter, the 3D HPLC chromatogram of the extract solution diluted 20 times gave 3 identified peaks: Verbascoside, Luteolin-7-glucoside, and Oleuropein. The desorption experience performed by washing the column with solvents allowed to recover polyphenols. These polyphenols were identified by HPLC. The comparison between the diluted initial extract and the different samples from the desorption was made. The *table 13* gives the list of phenolic compounds contained in the diluted extract solution and the recovered polyphenols. With the native cellulose MCC, all the phenolic compounds identified on the chromatogram of the initial extract solution were recovered after the sorption elution.

Table 13: Comparison of polyphenols before and after the sorption experience

Phenolic compounds in initial extract solution (20x diluted)	Phenolic compounds after the elution of the two fractions
Verbascoside	Verbascoside
Luteolin-7-glucoside	Luteolin-7-glucoside
Oleuropein	Oleuropein

5.4. Conclusion

Desorption is the complementary process of adsorption. The adsorbed solute (adsorbate) is recovered. The desorption was made by washing the column with different solvents. It was started by the most hydrophilic and was ended by the less hydrophilic. These solvents were eluted by this order to have the same order for the elution of the polyphenols.

The recovery of phenolic compounds depends on the solvent used and its behavior with the polyphenol and the adsorption material used. The first phenolic compounds eluted were those eluted by the hydrophilic solvent: Hydroxybenzoic acid and Oleuropein. The less hydrophilic solvents were those that eluted the compounds like Luteolin-7-glucoside and Apigenin-7-glucoside.

Reillex 402 and native cellulose MCC are the materials that recovered 100% of phenolic compounds identified in the olive leaves extract solutions. Le new synthesized adsorbent material for its part only eluted between 33 and 66% of phenolic compounds depending on the solvent used to perform the extraction of the olive leaves extracts.

Chapter 6 : Adsorption isotherms

6.1. Introduction

An adsorption isotherm is a representation of the amount of solute adsorbed by the adsorbent. It is shaped as a plot of the amount adsorbed q versus the equilibrium concentration C_e.

Adsorption isotherms are used to predict adsorption results by finding certain parameters, characterizing the properties of the adsorbed phase, and determining the adsorbent texture.

There are many isotherm models as the Linear model, Langmuir model, Freundlich model, etc. For this work, Langmuir and Freundlich were used to treating adsorption data. Due to its simplicity and its good agreement with experimental data, the Langmuir model is the most popular isotherm model [41]. At the origin, the Freundlich was an empirical equation [42].

The curves obtained with the Batch experience were used to use the isotherms model.

6.2. Adsorption isotherm

During the Batch experience, three commercial polyphenols were used with three different solvents and three different materials. But for the adsorption isotherms, only the results of two materials (Reillex 402 and MCC-P4VP) were used. The results of the Batch experience in Chapter 4 were taken into consideration. Among the three solvents, the solvent which gave better results was the EtOH/Water 20/80; therefore, only this solvent was used to perform the isotherm models with the three reference polyphenols.

6.2.1. Langmuir isotherm model

The Langmuir model was developed based on the thermodynamic equilibrium theory, which supposed that:

- The surface of the sorbent is uniform
- Adsorbed molecules do not interact
- All adsorption occurs through the same mechanism

- Only a monolayer is formed at the maximum adsorption [41]

The Langmuir isotherm model can be represented by the following equation :

$$q_e = q_{max} \frac{k_L c_e}{1+k_L c_e} \qquad (8)$$

In equation 8, q_e is the amount adsorbed at the equilibrium, q_{max} is the maximum adsorption capacity, C_e is the concentration at the equilibrium, and K_L is Langmuir adsorption constant related to the free energy adsorption [42].

This equation allows fitting the experimental data for each reference polyphenol.

- For the Catechol, the Langmuir isotherm model applied to experimental data represented in *figure 39* shown that a better fitting is got with Reillex 402 than with MCC-P4VP. Indeed, the coefficient of determination of Langmuir-Reillex is higher (R^2=1) than the coefficient of determination of Langmuir-MCCP4VP. The maximum which can be adsorbed by Reillex 402 is 9554.3762 µmol/g with a K_L of 0.014. For MCC-P4VP the maximum adsorbed is 289.0645 µmol/g with a K_L of 1.2701.

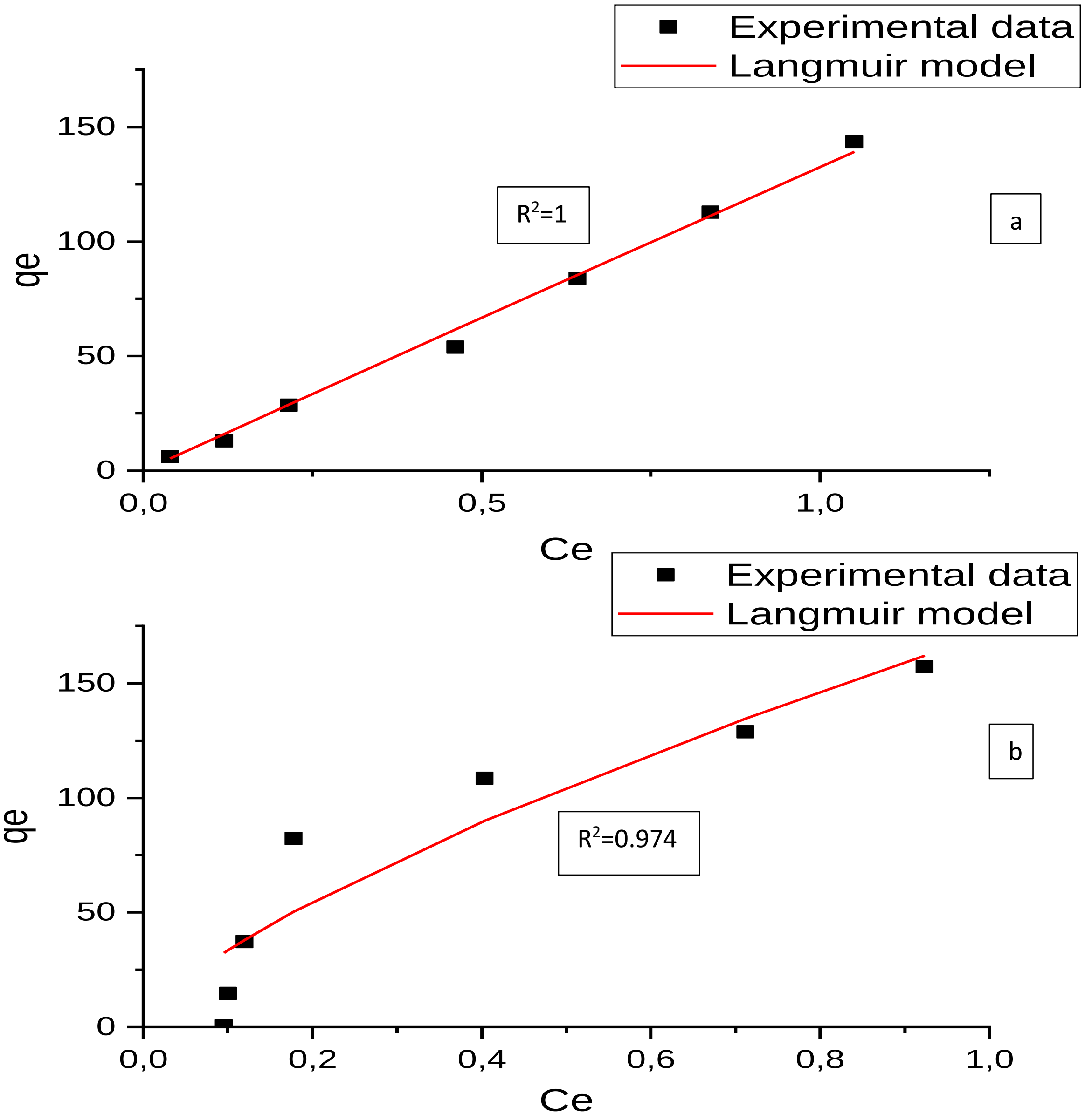


Figure 39: Langmuir isotherm adsorption of Catechol (a) Reillex402 (b) MCC-P4VP

- For Rutin, the Langmuir isotherm model applied to experimental data represented in *figure 40* shown that a better fitting is got with Reillex 402 than with MCC-P4VP even if the coefficient of determination of Langmuir-MCCP4VP is higher (R^2=1) than the coefficient of determination of Langmuir-Reillex. The maximum which can be adsorbed by Reillex 402 is 26.4998 µmol/g with a K_L of 9.4482. For MCC-P4VP the maximum adsorbed is 403.4929 µmol/g with a K_L of 2.8520.

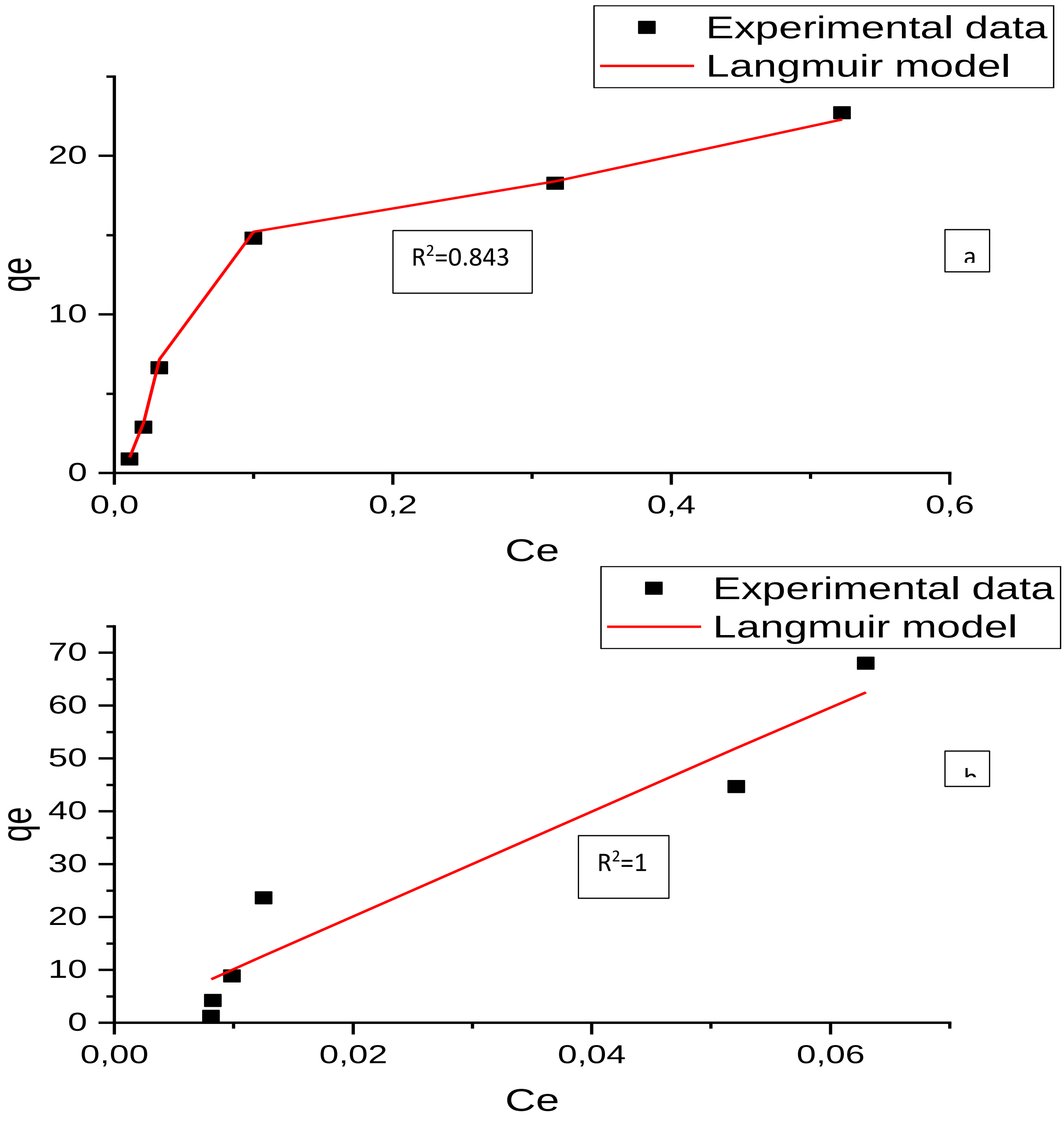


Figure 40: Langmuir isotherm of Rutin (a) Reillex402 (b) MCC-P4VP

- For Vanillic acid, the Langmuir isotherm model applied to experimental data represented in *figure 41* shown that a better fitting is got with MCC-P4VP than with Reillex 402 even if the coefficients of determination of Langmuir-MCCP4VP and Langmuir-Reillex are equals. The maximum which can be adsorbed by Reillex 402 is 1218.0650 µmol/g with a K_L of 0.2818. For MCC-P4VP the maximum adsorbed is 14392.6514 µmol/g with a K_L of 0.0139.

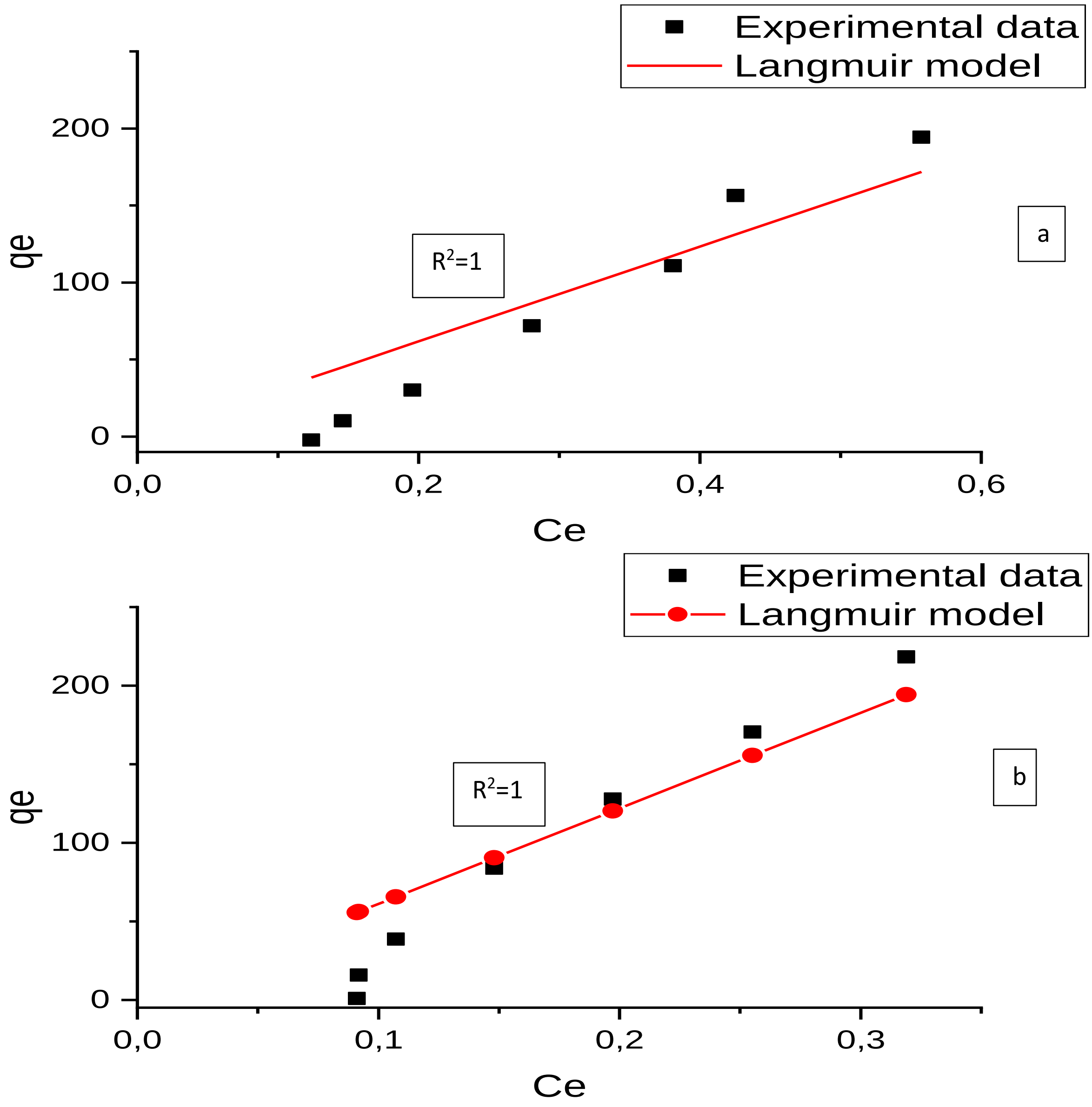


Figure 41: Langmuir isotherm of Vanillic acid (a) Reillex402 (b) MCCC-P4VP

6.2.2. Freundlich isotherm model

Freundlich equation could be thermodynamically derived based on some assumptions as:

- the adsorption sites of the adsorbent are of different energies
- the adsorption of adsorbate on the sites having the same energy level obeys the Langmuir equation.

The Freundlich isotherm model can be represented by the following equation:

$$q_e = K_F C_e^{1/n} \qquad (9)$$

In this equation, K_F and n are Freundlich constants and $0 < n \leq 5$ [2].

This equation was used to obtain the plots below.

- For Catechol, the Freundlich isotherm model applied to experimental data represented in *figure 42* shown that a better fitting is got with Reillex 402 than with MCC-P4VP as with Langmuir model. Indeed, the coefficient of determination of Freundlich-Reillex is higher (R^2=0.999) than the coefficient of determination Freundlich-MCCP4VP. The parameters of the Freundlich model, for the Reillex 402, K_F and n are respectively 135.8584 and 0.9094. For MCC-P4VP, the parameters K_F and n are 171.3453 and 1.4106.

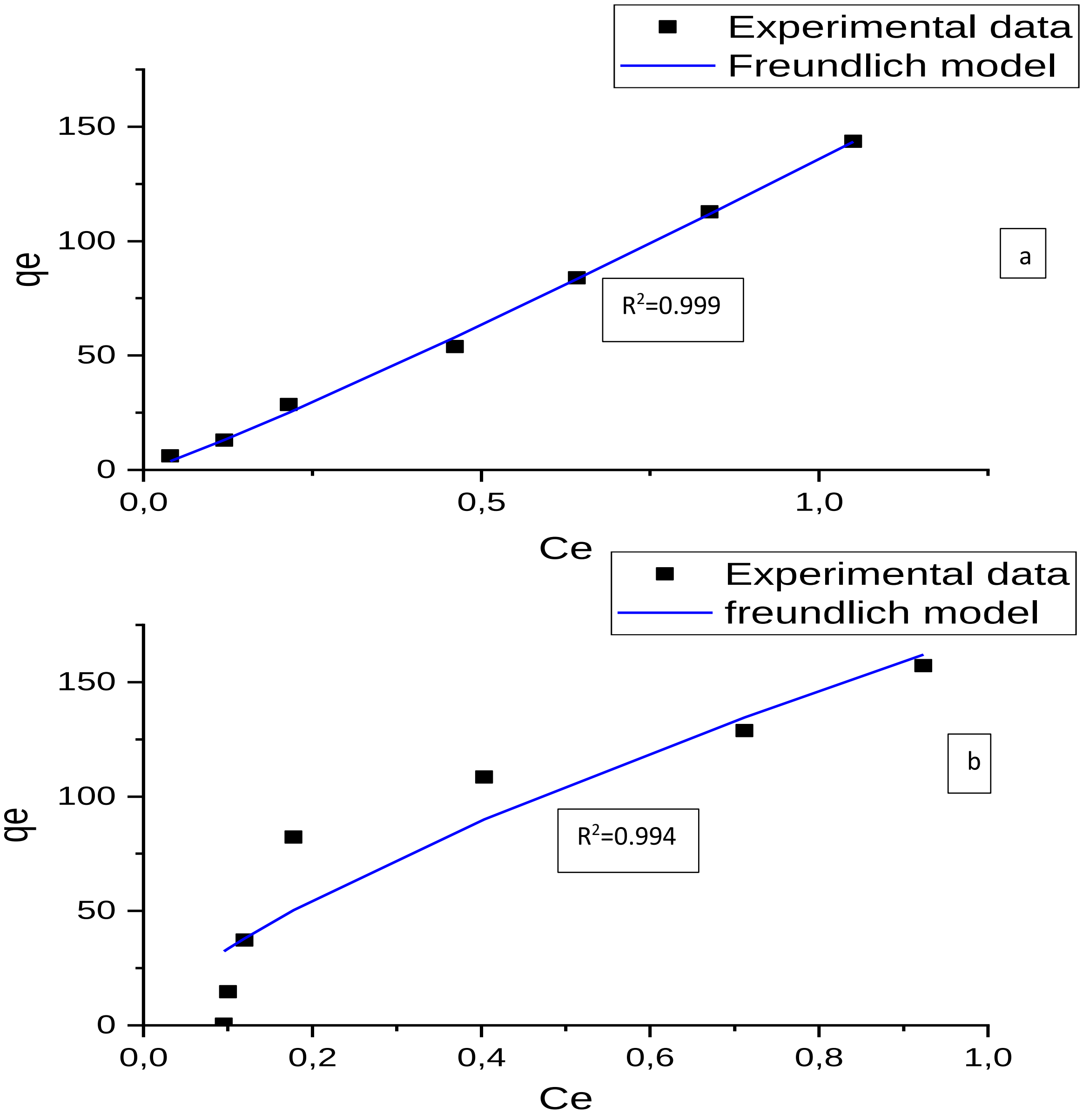


Figure 42: Freundlich model of Catechol (a) Reillex (b) MCC-P4VP

- For Rutin, the Freundlich isotherm model applied to experimental data represented in *figure 43* shows the same behavior than Langmuir isotherm model. The best data fitting seems to be obtained with the Reillex 402 even if the coefficient of determination of Freundlich-MCCP4VP is higher (R^2=1) than the coefficient of determination of Freundlich-Reillex. The parameters of the Freundlich model, for the Reillex 402, K_F and n are respectively 32.1144 and 2.0923. For MCC-P4VP, the parameters K_F and n are 1126.1362 and 0.9596.

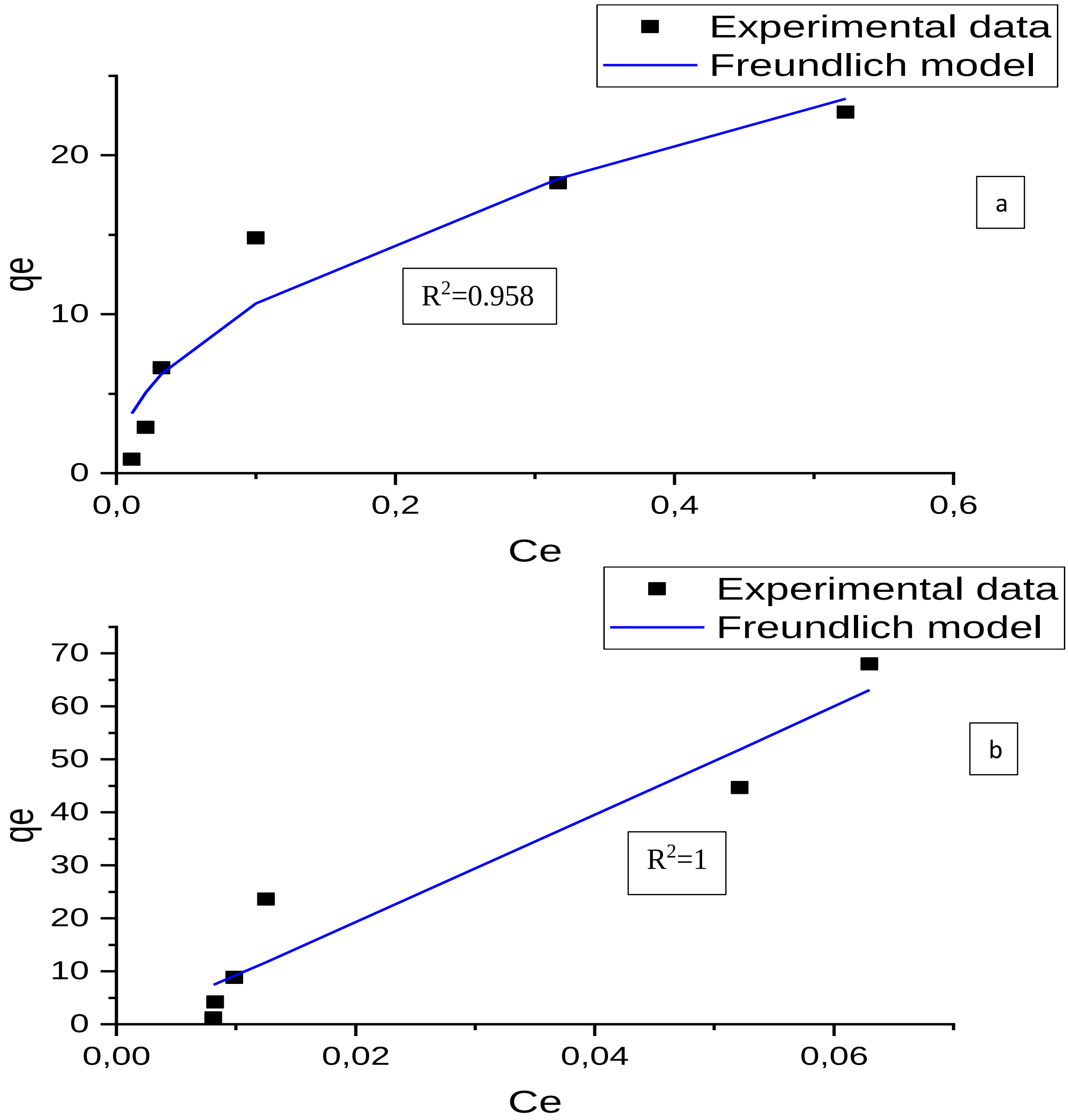


Figure 43: Freundlich isotherm with Rutin (a) Reillex402 (b) MCC-P4VP

- For the Vanillic acid, the Langmuir isotherm model applied to experimental data represented in *figure 44* shown that both materials fit the Freundlich with some deviations even if the coefficient of determination of Freundlich-MCCP4VP is higher (R^2=0.99) than the coefficient of determination of Freundlich-Reillex. For the Reillex 402, the K_F is 546.6134, and the n is 0,6031. The parameters K_F and n for the MCC-P4VP are respectively 1382.748 and 0.6373.

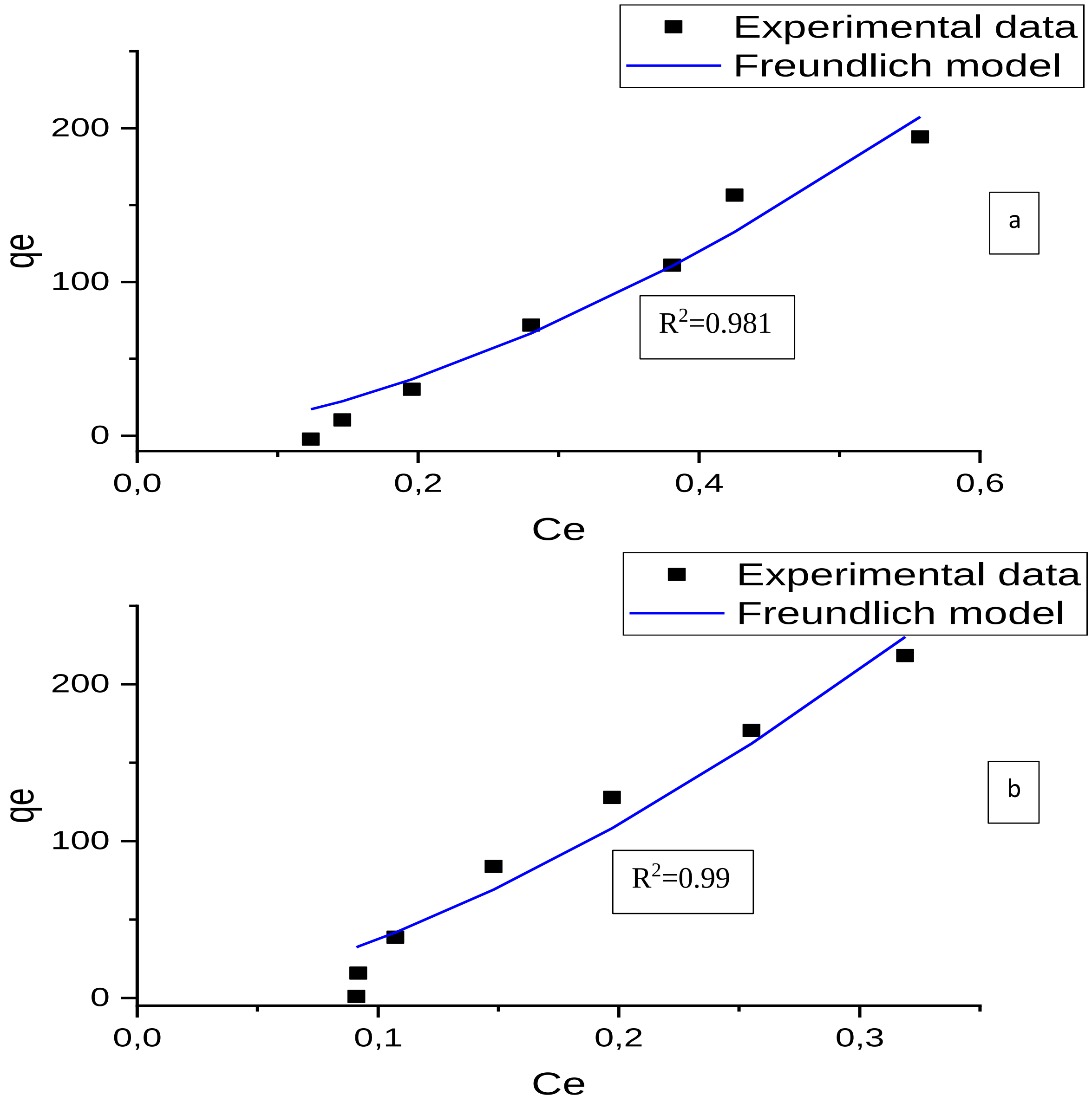


Figure 44: Freundlich isotherm of Vanillic acid (a) Reillex402 (b) MCC-P4VP

While the different parameters of each isotherm model have been calculated with the Microsoft Excel solver, the coefficient of determination of the two models was calculated by using the function *fitlm* of MATLAB in order to compare the two models between them. The coefficient of determination R^2 allows seeing which one of the two models has the best fitting. This calculation was made only for the hybrid material MCC-P4VP.

The comparison of the R^2 values, made in the *table 14*, shown that the Langmuir isotherm model is the model which fits the better the experimental data obtained during the

Batch experience performed with the new adsorbent MCC-P4VP and EtOH/Water 20/80 as a solvent; Indeed, the highest values of R^2 were obtained with the Langmuir model. And this model can be used to predict other adsorption with this material.

Table 14: Resume of Isotherm models on commercial polyphenol for MCC-P4VP

Model	q_{max}(µmol/g)	K_L	K_F(µmol/mM*g)	n	R^2
			Catechol		
Langmuir	289.064	1.270	-	-	0.88
Freundlich	-	-	171.345	1.410	0.848
			Rutin		
Langmuir	2255.389	0.453	-	-	0.904
Freundlich	-	-	1126.136	0.959	0.514
			Vanillic acid		
Langmuir	98539.517	0.006	-	-	0.978
Freundlich	-	-	1382.748	0.637	0.944

6.3. Conclusion

The Freundlich isotherm model allows to know more about the new adsorbent. Indeed, the *n* parameter provides information on the homogeneity of the material. More *1/n* tends towards *0*, more the heterogeneity of the material increase.

The Langmuir isotherm model allowed to have more information about the adsorption. Indeed, Langmuir isotherm model is used to predict homogeneous and monolayer adsorption. It can be used to predict the adsorption with the newly synthesized adsorbent.

Although the correlation coefficient shows that the model fits the data at its maximum, another model with three parameters can be used to have better fitting as the SIPS isotherm model as an example.

General conclusion

A hybrid material based on cellulose and vinyl pyridine was synthesized in order to be used as an adsorbent. It was made to absorb particular molecules through its functionalized surface. The adsorption capacity of the adsorbent was evaluated by comparing it with commercial adsorbents, Reillex 402 and Reillex 425.

The batch adsorption experience was the first experience performed to compare the adsorbents. Indeed, commercial polyphenols (Catechol, Rutin, and Vanillic acid) were chosen to perform the Batch adsorption experiences. The obtained curves by plotting the calculated amount adsorbed depending on the concentration at the equilibrium show that Reillex 425 did not give conclusive results with the reference polyphenol except with Rutin: its adsorption capacity competes with that MCC-P4VP. Reillex 402, meanwhile, competes with MCC-P4VP regarding all commercial polyphenols. Nevertheless, the synthesized adsorbent MCC-P4VP showed a better adsorbent capacity than the other adsorbent regardless of the reference polyphenols.

The synthesis of this adsorbent was achieved to perform the separation of polyphenols contained in olive leaves extracts. Extractions were achieved by using different solvents and different methods: Ultrasound and Soxhlet. The extracts used to perform the SPE were those performed by ultrasound with ethyl acetate and EtOH/Water 80/20 as solvents.

The SPE was the second experience made to compare the adsorbent between them. The adsorbent capacity of the materials was evaluated by achieving the saturation experience. The saturation experiences were performed with three adsorbents (MCC, MCC-P4VP, and Reillex 402) and for two extracts. The saturation curve obtained with the extract obtained with EtOH/Water 80/20 showed that MCC-P4VP is the one that reached its saturation slower than the other materials. But it is the opposite with the other extract. Indeed, with the extract made with ethyl acetate, MCC-P4VP reached its saturation faster than the other material, and the one that reached its saturation slower than the other is the native cellulose MCC.

After the SPE, the recovery of the adsorbates was made by washing the columns with different solvents. This experience (the desorption) allowed to know which polyphenol was adsorbed and eluted. For that, initial olive leaves extracts were analyzed by HPLC-DAD as well as non-adsorbed solutes after the adsorption and adsorbates after desorption. The identification of the phenolic compounds was made using the reference polyphenols. The comparison of chromatograms (of initial extracts solutions and after desorption) allowed to know the eluted compounds by each adsorbent and each solvent used during the washing of the columns. MCC-P4VP allowed to recover between 33 and 66% of phenolic compounds depending on the solvent used to achieve the olive leaves extracts.

Apart from the performed analysis, adsorption isotherms were made to predict futures adsorption with the adsorbent material synthesized in this work. Two simple models were used to find which match the best with the obtained data with the Batch adsorption experience. The statistical results obtained showed that the Langmuir isotherm model would be able to predict future adsorptions with a coefficient of determination close to 1.

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