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*Original Research Article*

# Application of Molecularly Imprinted Polymers Towards Efficient Extraction and Chromatographic Detection of Natural Products

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ABSTRACT

The expanding use of phytopharmaceuticals, as a more safe and efficient remedies for different pharmacological activities, and as dietary and nutritional supplements, has created a need for having efficient, eco-friendly, and selective extraction techniques to enhance the economic and environmental greenness of the applied traditional extraction methods. Recently, the application of molecularly imprinted polymers (MIPs), has grasped the attention in the field of natural products due to their ease of design and preparation with specific recognition cavities that can selectively bind to a target analyte. The present review aims not only to discuss the application of MIPs with the past ten years in the field solid phase extraction of different classes of natural products (NPs) but also their impact on the effectiveness of chromatographic separation and detection will be elaborated. Based on their performance, MIPs represent a promising class of artificial adsorbents that can be commercialized for beneficiary extraction of natural products with much less consumption of solvents and higher selectivity when compared to the traditional types of chromatographic adsorbents besides the possibility of their 3D printing to create more optimized and uniform manifolds to match the different types of extraction and detection applications of natural products (NPs).

***Keywords*:** Molecularly imprinted polymers, Natural products (NPs), Solid phase extraction, Monoliths columns, High-performance liquid chromatography (HPLC)

## Introduction

Natural products (NPs) represent an important source for human health and have been used since ancient times as a folklore remedy for the treatment of many diseases and illnesses. The use of NPs as medicinal plants has well known all over the world for hundreds of centuries, Parmelia omphalodes (Linnaeus) *Acharius* is used on foot to prevent inflammations, *Alhagi maurorum Medik*, which belongs to a camelthorn species of legume, is used by Ayurvedic people to provide a sweet gummy substance that is composed mainly of melezitose, sucrose, and invert sugar for treatment of anorexia, constipation, dermatosis, epistaxis, fever, leprosy and obesity.

Also, in Northern Europe and Eastern America, *Ligusticum scoticum* Linnaeus, which is a perennial flowering plant in the celery family, is eaten raw as the first meal of the day to defend against daily infections, [1,2] and not to forget the thousands of Ancient Egyptian Papyrus, especially Ebers Papyrus, reporting the use of opium, cannabis, myrrh, fennel, cassia, and some naturally extracted oils from linseed and castor in many treatments to the extent that cloves of garlic are usually found in Egyptian burial sites due to the old believes in their endless health benefits.[3,4]

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Based on this ancient cultural heritage, the use of NPs was extended to their applicability as antioxidant, antibacterial, antifungal, antiviral, anticancer, and anti-inflammatory agents.[5,6–8] Accordingly, many pharmaceutical companies paid great attention to the industrial usage of herbal phytopharmaceuticals to avoid synthetic drugs' harmful side effects and to reduce the cost of production. Thus, NPs are not only used in folklore medicines, but also as dietary products, and nutritional supplements.[9,10]

Despite the huge importance of NPs applications, they represent a challenge with lots of difficulties in the research and development process due to the complex nature of active components in the herbal extract, the type of extraction process followed, and the single component isolation processes that involve the use of traditional solvent extraction methods and consume large amounts of organic solvent. These extraction processes, besides being environmentally unfriendly proved to be costly and time-consuming.

Recently, modern trends for rational waste valorization dictated the implementation of extraction methodologies within eco-friendly processes including the use of ultrasonic, microwave-assisted, and supercritical fluid approaches which can improve the extraction rate. The ultrasonic extraction method is dependent on ultrasonic waves, which cause cavitation and promote solute dissolution and diffusion as well as heat transfer. increases extraction efficiency, an additional benefit of ultrasonic extraction is its low solvent and energy consumption, as well as its use for extraction of thermally labile and unstable natural compounds.[11,12] Also, microwave-based extraction methods create heat by interacting with polar chemicals in the plant matrix, such as water and some organic components, using the ionic conduction and dipole rotation mechanisms. In the microwave extraction method, both mass and heat transfers are in the same direction, resulting in a combined effect that speeds up extraction and improves extraction yield. [13]

While using the supercritical fluid extraction method applied in recent years, the use of supercritical solvents in the extraction process that it has similar solubility to liquid and similar diffusivity to gas and can dissolve a wide variety of natural products, because of little pressure and temperature changes in their solvating characteristics altered substantially around their critical points. Because of its appealing properties such as low critical temperature (31°C), selectivity, inertness, low cost, non-toxicity, and capacity to extract thermally labile chemicals, supercritical carbon dioxide (S-$CO_2$) was frequently utilized in the extraction process. and low polarity makes it perfect for extracting non-polar natural compounds such as lipids and volatile oils.[14,15] Though operation processes, the large solvent consumption, the yield of limited capacity, and needed for some expensive equipment still represent a limitation of some of these mentioned methods. This raises the need to develop highly specific solid phase extraction sorbents that can be applied to enhance the extraction efficiency and in turn, get over other possible environmentally hazardous effects, and herein, the use of molecularly imprinting polymers is highly recommended and presents a promising approach for efficient ecofriendly sorbents.[16]

Molecularly imprinted polymers (MIPs) represent a class of porous synthetic polymers that have specific recognition and selectively developed cavities toward the target molecules. [17] They can be better described in terms of the Lock and key which are stable polymers with specific sites for a specific template molecule, and superior selectivity of targeted natural product.[18] The imprinted polymers are usually prepared through the polymerization of functional monomer(s) and a template target in the presence of crosslinker and initiator. After the polymerization, and upon washing the resulting MIP by removal solvent that dissolves and knocks out the target template from the polymeric matrix, binding sites with similar shape, size, and functionality to the target analyte are generated.[19]

In recent years molecular imprinting technique (MIT) has grasped the attention of researchers due to its various advantages, being simple, convenient in preparation, low cost, physical and chemical stability, and reasonable selectivity and specificity. [20] MIT showed expansions in many other applications, as indicated in Figure 1, and not restricted to the separation and isolation of molecules but they were extended to be modifiers in electrochemical sensors, immunoassays, and catalytic enzymatic activity. [21]

The use of molecular imprinting polymer as sorbents in SPE facilitated obtaining pure compounds with promising therapeutic effects. Recently, the use of the MISPE technique for the separation and extraction of NPs from complex extracts has turned out to be of high interest based on the increase in the number of MIP-based publications over recent years as shown in Figure 2.

The current review will shed light on the different applications of molecularly imprinted polymers as sorbents for different classes of natural products and the impact of such applications on the chromatographic separation of isolated active components from herbal extracts.

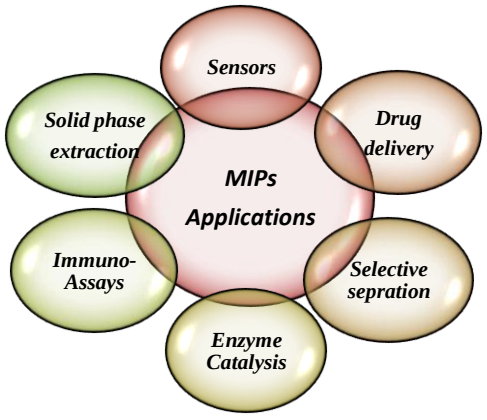



**Figure 1**: Applications of molecularly imprinted polymers.

*Conventional separation methods of natural products*

Traditional separation methods for pure components of natural products depend on the physical or chemical differences between individual compounds. Generally, chromatographic methods are commonly applied being sensitive and more accurate compared to other non-chromatographic approaches. The most common chromatographic separation methods may include adsorption (AC), partition (PC), gel filtration (GFC), ion exchange, supercritical fluid chromatography, and semi-preparative high-performance liquid chromatography[19]

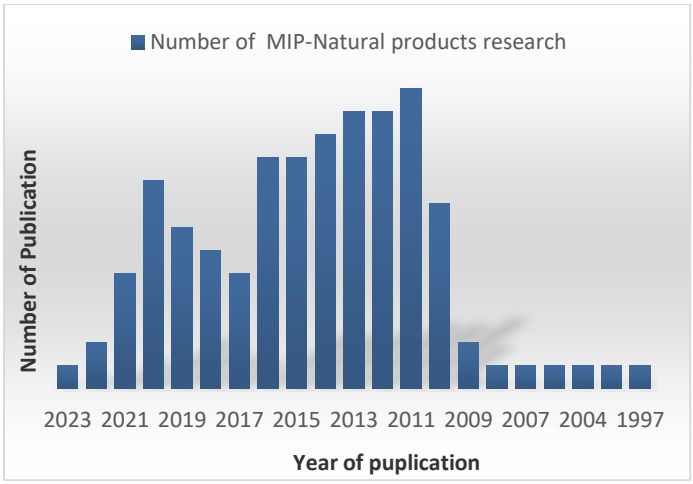



**Figure 2**: Number of publications concerning the use of molecular imprinting polymers in the isolation of natural products from 1997-2023.

*Adsorption chromatography (AC)*

It's the oldest and simplest method for separation based on the liquid-solid partition, where, the adsorbent surface such as silica gel, microporous resins, and alumina (aluminum oxide) acts as a stationary phase. The most common stationary phase used in columns is silica gel with its polar silanol groups allowing polar NPs to be retained longer on such columns compared to nonpolar ones. Due to the high tendency of hydrogen bonds and dipole-dipole interactions to take place between polar natural compounds and silica gel, AC is widely used in the initial separation stage, due to its simplicity, high capacity, and low cost of adsorbent. [22]

AC has different types, for example, thin layer chromatography (TLC) where the adsorbent is a thin layer on a solid support for the separation of NPs components depending on differential migration when the solvent moves along the powder spread on the support. Thin layer chromatography is considered an important tool in the development of separations due to being a very simple and easy technique that allows the separation process to take place by applying small spotting of the total extract on the surface of the adsorbent and using a small amount of mobile phase to elute the component(s) based on affinity to adsorbent as shown in Figure 3a.

Another type is column chromatography, which is a method that allows the solvent/solutes to go down a column while the stationary phase absorbs each component separately. The placements of the components on the column are determined by their affinity for the adsorbent. The component that is most strongly adsorbed is shown at the top of the column, as shown in Figure 3b.

*Partition chromatography (PC)*

This method is dependent on a liquid-liquid partition (LLP), based on the distribution of the constituent components of a mixture between two immiscible liquid phases. Of the individual components of a mixture, one as the mobile phase and the other being fixed on the solid support as the stationary phase, the polar stationary phase is usually on a silica or alumina support, and the process is referred to as the normal

phase (NP) while in the opposite case, it is called reversed phase (RP) (liquid) liquid chromatography. LLP is suitable for a great number of substances in a wide polarity range.

*Flash chromatography (FC)*

Flash chromatography is an air-pressure-driven hybrid of medium-pressure and shorter-column chromatography. FC differs from conventional techniques in two ways: firstly, slightly smaller silica gel particles are used, and secondly, due to the restricted flow of solvent caused by the small gel particles, pressurized gas is used to drive the solvent through the column of the stationary phase, this type of chromatography is very useful in the separation of NPs of crude extracts.[23,24]

FC is considered to be exceptionally simple and allows rapid preparative separations with a high-purity yield of extracted components. Automated flash chromatography systems usually consist of a gradient pump, sample injection ports, a UV detector, and a fraction collector to collect the eluent, as shown in Figure 3c. Typically, these automated systems separate samples from a few milligrams up to an industrial kg scale, columns are packed by silica gel with particle sizes from 25-200µm, for normal phase separations using pure nonpolar solvents as mobile phase. While using polar solvents as mobile phase and other types of silica gel packed with $C_8$, and $C_{18}$, a reversed-phase manifold is rendered that preferably uses FC separations to reduce the consumption of organic solvents. [25,26]

Although chromatographic separations represent the main approach applied to obtain almost pure compounds from a complex mixture, nevertheless, they suffer from being time-consuming, and cost-ineffective in terms of organic solvents consumption which implies an environmental hazard itself, the complexity of separation of enantiomers, low selectivity besides light and thermal instability of some of the separated components. [27–29]

Recently, some modern trends for rational waste valorization dictated the implementation of separation methodologies within eco-friendly processes. To achieve this strategy efficiently, the utilization of specific solid-phase extraction (SPE) sorbents is required.

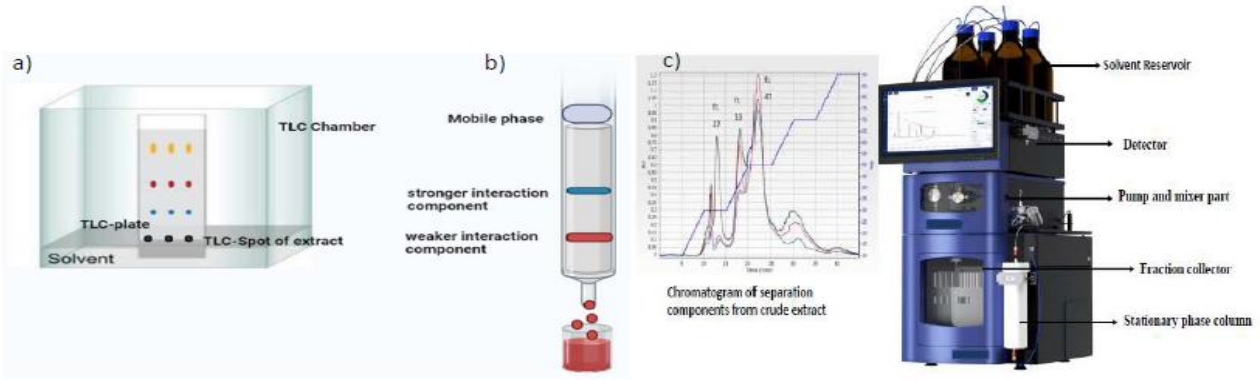



**Figure 3:** Schematic diagram of a) TLC chromatography, b) column chromatography, c) Puri flash apparatus and compartments.

*Solid-phase extraction sorbents*

Solid-phase extraction (SPE) is the most extensively utilized approach for providing cleaner components, preconcentrated trace amounts of compound samples, isolation of analytes from a liquid matrix, and purified extracts. The principle of solid-phase extraction is based on the partitioning between a liquid phase (sample matrix) and a solid phase (sorbents). The complex liquid including the desired target component is loaded onto solid sorbents, and washed to get rid of unwanted components, then the desired analytes can be recovered using another solvent (s) as indicated in Figure 4. [30–32]

The choice of SPE sorbent that is capable of binding the compounds of interest, is usually based on the physical and chemical nature of the analytes of interest, the type of sample matrix, and the possible interactions that might take place between the sorbent and analytes, which affects the extraction process in terms of visibility and time. Furthermore, the polarity of the analyte represents a critical guiding factor that influences the selection of the sorbent material.

The most common chromatographic sorbents used in SPE for NPs include silica gel, derivatized silica as $C_2$, $C_4$, $C_8$, $C_{18}$, Florisil, and ion exchange sorbents. Hydrophilic modified styrene polymer, a pyrrolidone group modified styrene polymer [31], and magnetic chitosan were also reported as sorbents for separating phenolics from *Fructus Cnidii*. [33] Also, pure zirconium silicate and bismuth citrate powder were applied for the separation of caffeoylquinic acids from different natural sources. [34]

Although many of these sorbent materials were proven to be efficient in the separation of NPs with high recovery and reusability in some cases, yet, they still suffer from being of high preparation costs, low specificity, and selectivity. This creates a need for a class of tailored sorbents that can be designed to match the properties of the target analyte(s) and the requirements of the extraction process in terms of specificity, selectivity, and polarity.

Molecularly imprinted polymers represent promising alternative artificial sorbents that can help to get over the abovementioned drawbacks, as they were shown to be powerful SPE sorbents, offer specific molecular recognition properties for a given compound or class of NPs, highly selective, reusable, of good thermal stability and require low cost, and convenient eco-friendly preparation procedures. Thus, in the upcoming sections, all details about Molecularly Imprinted Polymers-based Solid Phase Extraction (MISPE) sorbents, and their contribution to the separation of NPs from crude extracts will be discussed. [35]

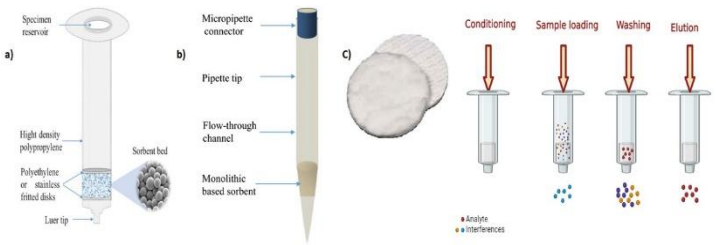



**Figure 4**: Illustration of SPE principle and common types of SPE.

*Molecularly imprinted polymers (MIPs)*

MIPs have been proven to be excellent sorbents for extracting target molecules due to the possibility of tailoring matrixes with specific shapes, sizes, and functional groups of templates. The synthesis of

molecular imprinting polymers involves complexation in a solution of target molecules (template) with functional monomer(s) through either covalent or non-covalent bonds, as schematically presented in Figure 5.

The polymerization reaction can proceed by covalent linkage when the template and chosen monomer share electron pairs in between them to form chemical bonds. MIPs based on reversible covalent bonds between target molecules and functional monomers can difficulty achieve thermodynamic equilibrium and in turn slow binding and dissociation due to their strong covalent interaction, thus, this approach is considered to be inflexible to match the requirements for SPE applications.

On the other hand, when MIPs are based on non-covalent interactions that relay on the occurrence of electrostatic attraction, metal chelation, charge transfer, hydrophobic action, and van der Waals between template and monomer where either of them is an electron donor and the other is acceptor, are more favorable. Non-covalent interactions offer more flexibility in the selection of monomers and templates, and the approach is universal due to easy binding, wide applications, and easy removal of templates,[21] through using a highly polar slovents) to knock out the template.

MIP mainly consists of a template (target molecule), suitable monomer(s), crosslinker, initiator, and a solvent that is commonly referred to as a porogenic agent due to its impact on the variation of the pores sizes of the MIP, as shown in Figure 5. The template is considered to be the main component that affects the choice of the polymerization approach to create the specific recognition sites in MIP. It should be of excellent stability under the polymerization conditions and have a functional group(s) that does not participate in or prevent the polymerization. The polymerizable functional monomer(s) with double bonds or characteristic functional groups can have specific interactions with template molecules. The presence of the cross-linking agent is very necessary to assure the stabilization of a three-dimensional network structure formed after the interaction of the template and functional monomer(s), also, the porogenic solvent selection plays a significant role in the polymerization process, as it should be capable of dissolving all components of the prepared MIP. In addition, the porogenic solvent can help the formation of the porous structure of the polymer. The polymerization is initiated generally by free radicals such as azobisisobutyronitrile or benzoyl peroxide and is usually heated at 50–70 °C or photo-initiated at room temperature.[36–38]

To optimize the polymerization reaction and select the best monomer(s) to bind with the target analyte, computational calculations have started to take part as one of the essential steps before chemical interactions, to avoid the wrong choice of monomer type or ratio and reduce the expenses of reagents used and time to run trial and error preparation experiments.

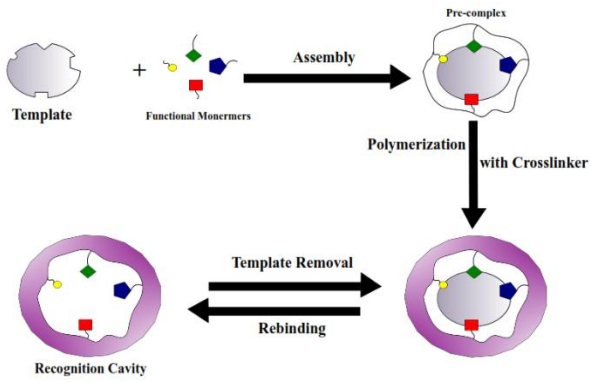



**Figure 5**: General approach for MIPs synthesis.

*Computational design of Molecularly imprinted polymers*

Recently using a computational approach to evaluate the MIP system has grasped great attention as it offers the chance to inspect and calculating the binding energy of monomer–template interaction(s) in the pre-polymerization step and indicate to choose the best monomer interaction with the target template in a suitable solvent rather than the extensive and time-consuming synthesis methods. This approach significantly reduces the amounts of solvents and chemical reagents used during the typical polymerization process and decreases the financial costs, as a result, MIP modeling is highly recommended for greener methodologies of natural product separation and extraction.[39]

Computational calculations have been used to provide a quick alternative approach for the rational design of high-affinity MIPs that are more affected by natural compounds and highly expensive and searching for an alternative compound that has the same chemical and physical properties act as a dummy template in polymerization process costs a lot of trials result to waste time and chemicals to achieve the best compound and ratio of template to monomer.[39,40] To perform the calculations, firstly, different types of graphical software are used to design optimum 3D structures and configurations of the target template and functional monomer(s) by means of the semi-empirical (SE) quantum mechanical approach as well as testing the possible solvents to be used as porogenic agents. Then, ab initio calculations can be performed on the optimized configurations to estimate the bonding energies between the reaction components, based on which the ratio of the components to be used in the experimental polymerization reaction can be depicted. Solvent interaction can also be performed in two potential reaction environments, either in vacuum or porogenic solvent.[39,41]

Of the very famous commercial software HyperChem, which relies on performing quantum mechanics and dynamics, semi-empirical, ab Initio, and Density function theory (DFT) calculations which is capable of conducting Hartree Fock, ab Initio and Density function theory (DFT) calculations based on the configurations built on using Gaussian with Gauss View Studio. On the other hand, some free and efficient software are also available with comparable efficiency to Hyperchem and Gaussian, of which, GAMESS-US which can be applied to small and larger molecules, CFOUR; Dalton; and NWChem which are a little more complicated and mainly applicable to small and medium molecules.

Screening literature indicated Gaussian software with different additions is the most popular model used to operate the calculation process, and the theoretical studies involved some methods of calculation like the PM3 method, the semi-empirical AM1 method, and the Hartree-Fock (HF) method with different basis sets like (3-21G, 6-31G) which are preferred for larger molecular systems or fast and very approximate calculations.

The use of binding energies obtained from calculations by the above-mentioned methods on complexes between the template and a set of different functional monomers has become a more frequently used strategy for choosing functional monomers and the best ratio between them. Among the parameters that affect mostly the binding interactions involve atomization energy, proton affinity, harmonic and anharmonic vibration frequencies, dipole moments, partial charges on the interaction centers of each of the templates, and the functional monomer(s) can be calculated using the following equation:

$$\Delta E = E(template - monomer\ complex) - \left[(E_{template}) - nE_{(monomer)}\right]$$

Equation 1

where: E(template-monomer complex is the total energy of template/functional monomer complex; E(template); E( monomer) is the energy of the template or monomer, respectively and n is the number of the functional monomers (defined as molar ratio).

Based on the computational calculation, the practical strategy for preparation of MIP became short-term and did not need more trials to achieve the best ratio only checking the performance of binding MIP on a calculated ratio from computational modeling So computational approach is grateful for decreasing the time of preparation of MIP and the cost of chemicals used. [42,43]

A computational design approach was applied for the optimization of the separation of rosmarinic acid (RA) from Rosmarinus officinalis employing the Gaussian 09 package. The optimized ratio between template to functional monomers (4-VP, MAA) was obtained for the 3D structures of RA, FM; MAA, and 4-VP, using the Hartree-Fock

method at [6–31 G(D)] basis set. As shown in Figure 6, RA was expected to exhibit eight active binding sites for H-bonding, yet the formation of two intra-molecular H-bonds was highly anticipated between sites (1, 2) and (5, 6), denoted by bond distances of 2.166 Å and 2.174 Å44. Site 8 highlighted in Figure 6a was not capable of forming H-bonds of moderate strength with MAA; it could be associated with the steric hindrance created by the surrounding moieties. Results also demonstrated an increment in binding energies of complexes by incorporating more FM, which reflects the formation of a more stable pre-polymerized complex. Thus, the suggested optimum RA-FM ratios that would offer the best recognition properties were 1:5 (Figure 6d) and 1:4 (Figure 6e) for 4-VP and MAA, respectively.

The computational design was also applied for designing the MIP for the separation of gallic acid from different complex matrixes, using density functional theory calculation (DFT) and optimizing the template and 18 chosen monomers at a ratio (1:1), without effect of solvent, at a basis set B3LYP/6-31G(d). Based on the optimizations, two monomers were found to show the highest binding energy, namely acrylic acid is- 21.2 kcal mol-1 and acrylamide is -19.8 kcal mol-1 45. Based on this calculation, MIPs were synthesized and their rebinding performance was determined using the Langmuir-Freundlich model. The AA and AAm imprinting factors of the prepared MIPs were depicted as 5.28 and 4.80, respectively which indicates that the experimental data is highly coherent with the computational calculations. Thus, rational computational MIP design is a safer and cost-effective way to determine the optimum functional monomer type and ratio for a specific template before MIP synthesis. [45,46]

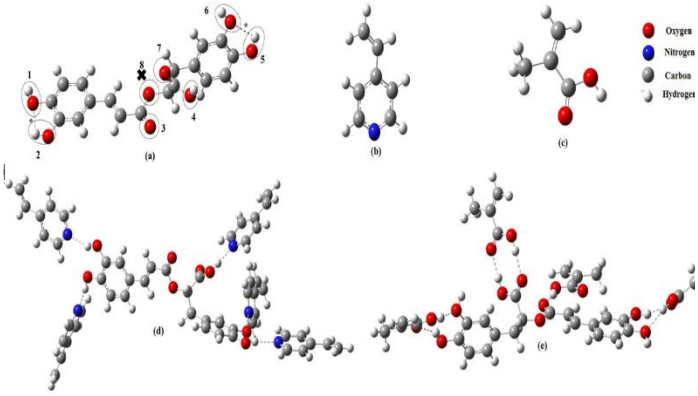



**Figure 6**: Computeroptimized structures of (a) RA, (b) 4-VP, and (c) MAA, conformations, optimum (d) RA-(4-VP)$_5$ and (e) RA-(MAA)$_4$ conformations assuming no intra-molecular H-bond formation, optimum, represents possible intra-molecular H-bonds of a distance (2.166–2.174 Å), and **x** represents the site incapable of H-bonding with MAA. [44] (*Figures are reproduced under permission from Elsevier* ).

*Molecular imprinting methods of polymerization*

Several -polymerization methods are applied in molecular imprinting leading to a variety of polymer formats including bulk, precipitation, suspension, emulsion, and surface imprinting polymerization. The choice of the polymerization method depends on the physicochemical nature of the template and the type of application the polymer will be used for. Table 1 summarizes the application of different polymerization approaches to the extraction and detection of NPs and the applied chromatographic conditions.

*Bulk polymerization*

It is regarded as the most common and general method used for the preparation of MIPs due to offering appealing properties such as rapidity and simplicity in preparation, with no requirements for specialized or expensive instruments. Preparation of MIPs by bulk polymerization involves copolymerization of the template, and a functional monomer having suitable functional groups to preferably render non-covalent interactions. Following the addition of a cross-linker and the initiator in a suitable solvent, the system should be purged with a gentle flow of $N_2$, and sealed before applying thermal or photoinitiation. After the end of the polymerization process, the synthesized MIP is usually crushed, ground, and sieved to obtain the desired average particle size according to the concerned application as shown in Figure 7. [44,47]

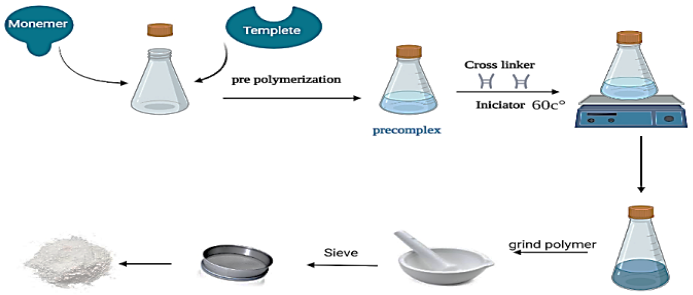



**Figure 7**: Schematic illustration of the preparation of MIP by bulk polymerization.

Most of the separation and extraction of NPs from complex whole extracts by MIP are prepared using bulk polymerization as shown in Table 1. The analysis of extracted compounds can be performed using different techniques of detection such as HPLC, UPLC, LC-MS, and or simply using a spectrophotometer. HPLC and spectrophotometric detection are widely used in monitoring the knocking out (washing) process to remove the template from the imprinted polymer, especially after applying an MIP to separate the target compound(s).

Bulk polymerization was applied for selective extraction of quinic acid (QA) from coffee bean (*C. arabica L.*) extract. [48] Computational modeling was performed to select from three different functional monomers (allylamine, methacrylic acid (MAA), and 4-vinylpyridine (4-VP)) based on which a ratio of 1:5 using 4-VP was chosen as a functional monomer showing better binding performance. All the tested polymers were found to show low binding in an aqueous medium as a result of the high solubility of QA in water. A binding capacity of 4.88 ± 0.32 and 2.28 ± 0.38 µmol/g, was attained by using the optimum MIP and its corresponding NIP with an imprinting factor of 2.14. Although the aqueous medium is known to disrupt hydrogen bonding interactions between template and monomer, in the rebinding results of QA a pronounced difference between the binding of QA to the MIPs and the corresponding NIPs was observed. This suggests that hydrogen bonding is not the only factor behind the MIP selectivity towards QA. It can be concluded that, during the imprinting process, different interactions took place based on the size, shape, and functionality of the template.

Many other NPs were successfully extracted using bulk polymerization some of which are capsaicin and dihydrocapsaicin extraction from chili peppers, [49] chlorogenic acid, crypto chlorogenic acid, and neo chlorogenic acid from *Lonicera japonica* and *Lianhua qingwenby*, [50] sinapic acid,[51] rosmarinic acid,[44] chicoric acid,[52] quercitin,[53] and rutin[54] are extracted from their natural source by bulk polymerization protocol, and obtained results were better in specificity, selectivity, and higher binding capacity than traditional protocols. [44,51]

Despite the MIPs synthesized via the bulk polymerization method can be easily prepared, yet, they have some drawbacks due to the production of polymeric materials of irregular shape and size especially after the grinding which may lead to the development of heterogeneous binding sites and destruction of the specific recognition cavities.[55]

*Surface imprinting polymerization*

Surface imprinting polymerization commonly involves the grafting of MIPs on the surface of a solid substrate or around nano/micro-sized solid particles such as (silica, chitosan, magnetite, graphene, gold nanomaterial, etc.). The significant advantage of this approach is that it has easily accessible binding sites because the target molecules are

not required to migrate through the complex structure of MIPs formed by bulk imprinting. As a result, target binding is not diffusion-restricted but also the polymeric matrix allows simple and quick rebinding and release of the template, consequently, this technique is widely used for the imprinting of large molecules and proteins using either soft lithography or core-shell surface imprinting.

Soft lithography generates nano/micro-sized patterned MIP films on solid substrates using a soft polymeric stamp., a pre- polymerized layer coated on a transducer surface and the template including (proteins, and microorganisms) then applying pressure for a certain time and UV light for initiation of the reaction as a result of which, a thin patterned polymeric film will be formed. after drying, the template can be removed using a suitable solvent which renders a highly selective and specific surface imprinted film.[56] This method is widely used in the recognition and separation of large macromolecules as proteins and has limited use in the medical field because of constraints related to biocompatibility requirements and the structural and mechanical properties of the resulting polymeric particles.[57]

Unlike the case of soft lithography, core-shell surface imprinting, depends on grafting MIP(s) on the surface of nano/micro-sized particles rather than a flat solid surface which increases the surface-to-volume ratio of binding sites and offers excellent dispersion that results in fast binding kinetics, and the templates within the thin shells can be entirely removed to generate efficient recognition sites. These features of the core-shell surface imprinting method can overcome many drawbacks of bulk polymerization and become an efficient method of extracting natural products from many plants' extracts.

On comparing the performance of bulk polymerization of quercetin using acrylamide with that made using silica magnetic nanoparticles, as a solid surface substrate for grafting the MIP, and EGDMA as a crosslinker with a ratio of 1:4:20, the results indicated that the surface imprinted MIP showed a relatively high surface area and higher adsorption capacity of 4.84mg/g that was almost two times that of bulk polymerized MIP which gave an adsorption capacity of 2.7mg/g. [58]

The core-shell surface sorbent was also applied for the extraction of di hydro quercetin from *Larix griffithiana* using amino-functionalized core-shell magnetic nanoparticles ($NH_2$-$SiO_{2@}Fe_3O_4$) as a surface substrate, di hydro quercetin as a template, 4-VP as a monomer, EGDMA as a crosslinker. After polymerization, $MIP_{@}SiO_2@Fe_3O_4$ was obtained by magnetic separation and drying under vacuum. Di hydro quercetin was removed by Soxhlet extraction using a mixture of methanol–acetic acid. After removing the template, the resulting MIP was applied to separate di hydro quercetin from the extract solution as shown in Figure 8, and HPLC results indicated a binding capacity of 4.02µg/g (Dihydroquercitin/MIP). [59]

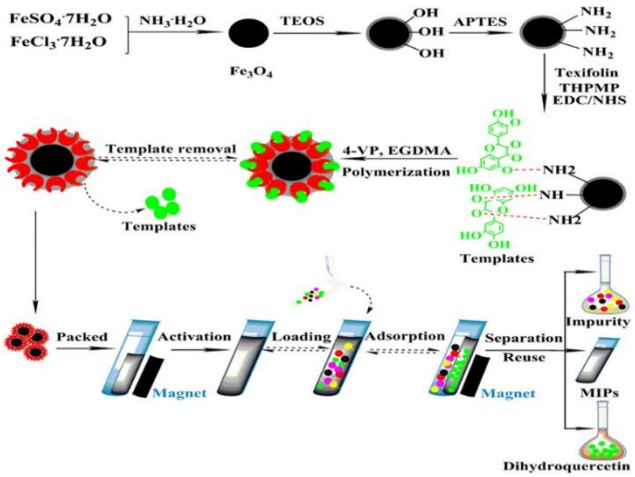



**Figure 8**: Preparation, washing, and rebinding process of separation dihydro quercetin from *Larix griffithiana* using amino-functionalized core-shell magnetic nanoparticles ($NH_2$-$SiO_2@Fe_3O_4$) as a surface substrate [59] (*Figures are reproduced under permission from John Wiley and Sons*).

Chlorogenic acid, which is considered a challenging compound due to its thermal and light instability, is another example of an important natural product extracted using surface imprinted MIP, mesoporous silica nanoparticles, and magnetic porous nanoparticles ($Fe_3O_{4@}mSiO_2$) as a surface support. The MIP was prepared by mixing ($Fe_3O_{4@}mSiO_2$), 4VP as the monomer, chlorogenic acid as a template, and EGDMA as a crosslinker. Methanol was used to knock out chlorogenic acid, and the rebinding process was tracked using an HPLC resulting in a highly selective and high adsorption capacity of 42.22mg/g. [60]

Accordingly, this approach represents a possible alternative for bulk polymerization and can be efficiently used for extraction as most of the binding sites are superficially exposed to the template which makes the rebinding more easier and enhances the extraction efficiency.

*Precipitation polymerization*

This type of polymerization has proven to be a highly flexible one-step method for creating MIP micro-/sub-microspheres with high yields and uniform particles. Precipitation is more sufficient than traditional polymerization approaches as it does not require the use of a surfactant, and needs no wasteful and time-consuming grinding procedures. The process involves the polymerization of monomers in a dilute solvent, particle growth is primarily generated by the entropic precipitation of nano gel particles and then the continuous capture of oligomers from the solution, and once the particles exceed a certain size, they start to precipitate in a form of spheres of almost identical size depending on the stirring rate and volume of solvent used. [61,62]

Extraction of natural compounds using MIP prepared using precipitation polymerization was proven efficient in obtaining pure stable and economic natural products. Proanthocyanidin extraction from *Camellia oleifera Abel* was found to overcome the struggles of traditional techniques that require high consumption of organic solvents, complex extraction steps, and low stability of proanthocyanidin. The polymerization was performed by dissolving proanthocyanidin in methanol followed by adding chitosan and acrylamide as a monomer(s) in acetonitrile as a porogen and Co-trimethylolpropane tri methacrylate was added as a crosslinker. After 24 hrs of polymerization reaction, the resulting MIP gave a rebinding capacity of 6.82mg/g without any oxidative degradation of proanthocyanidin, which is considered one of the drawbacks of traditional extraction procedures, the experimental setup of the polymeric reaction is shown in Figure 9. [63]

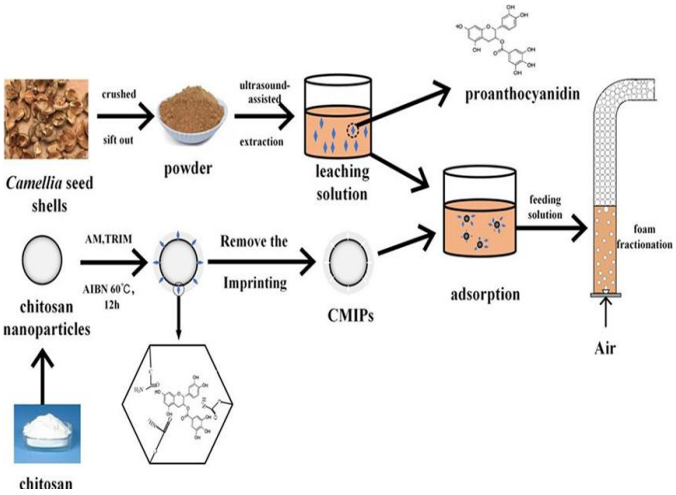



**Figure 9**: Extraction of proanthocyanidin from *Camellia oleifera Abel* using precipitation polymerization synthesis of the MIP [63] (*Figures are reproduced under permission from Elsevier*).

MIP based on precipitation polymerization is considered an efficient way to separate ellagic acid from pomegranate peel. Ellagic acid is a polyphenolic flavonoid compound, with potent nutraceutical efficiency in the treatment of cancer, inflammation, and degenerative diseases. The polymerization reaction proceeded by dissolving ellagic acid, 4-vinyl pyridine (monomer), and divinylbenzene (crosslinker) in a mixture of tetrahydrofuran and acetonitrile. The mixture was stored overnight to develop the pre-polymerization complex, before adding the initiator and heating. The resulting imprinted polymer showed a relatively uniform size of about 1 µm with a binding capacity of 37.07mg/g towards ellagic acid. [64]

Another example involves the extraction of farrerol, reported to be potent for cardiovascular diseases and Alzheimer's disease treatment, from *Rhododendron aganniphum* using quercetin as a dummy template and 4-vinyl pyridine as the monomer.[65] The polymer was found to show a maximum binding capacity at low-affinity sites on the surface of MIP of 20.67mg/g, while at high-affinity recognition sites within the polymeric matrix, an efficiency of 10.04mg/g was attained. The performance of d-MISPE cartridges packed with the prepared polymers was performed using HPLC detection of *Rhododendron aganniphum* (1 g) samples spiked with farrerol at four levels (1, 10, 50, and100 µg/g) and the method showed recoveries between 85.7% and 104.1%, indicating the efficiency of the prepared sorbents for farrerol extraction from real samples.

All of the mentioned examples, and others given in table 1, show that precipitation polymerization yields imprinted polymers with spherical uniform shape that increases the binding capacity of natural compounds from extracts. However, the main disadvantage of such an approach is the use of a significant amount of solvent, which reduces the method's greenness. Also, it requires using a large concentration of templates which are sometimes economically expensive, especially on using rare templates. [61,62]

*Suspension polymerization*

Suspension polymerization is considered a suitable polymerization approach for water-soluble templates, amino acids, or metal ions as water is commonly used as a dispersion medium. Synthesis of MIP by suspension polymerization involves complexion between functional monomer/ template and a cross-linker in the dispersion medium, followed by continuous stirring until all solid phases dissolve before adding the initiator and starting the thermal initiation. The obtained polymer is shown to commonly have uniform spherical particles, characterized in the range from micrometers to millimeters. Suspension polymerization is considered a perfect method of polymerization to form molecular imprinting polymer microspheres used as stationary phases for separation and purification.

Camptothecin (CPT) from *Camptotheca acuminata* fruit suspension polymerized MIP was prepared using 2% wt PVA/water as the dispersion medium, methacrylic acid as a monomer, and ethylene glycol maleic rosinate acrylate (EGMRA) as a crosslinker. After polymerization, the product was centrifuged, and the precipitated MIPs and NIPs were packed into commercial stainless-steel HPLC empty columns (250 mm × 4.6 mm i.d.). [66] The separation ability of the columns towards CPT was greater than two, indicating its stronger interactions with both the MIPs and NIPs, as a result of the presence of carbonyl and nitrogen atoms and carboxyl groups. However, the IF values were between 1 and 1.1, indicating that there was a weak imprinting effect on the MIP column. The magnitude of IF is mainly dependent on the magnitude of k on the blank polymer; in other words, only the blank polymer shows that the molecular imprinting process can improve the nonspecific effect of template molecules and functional monomers when synthesizing MIP. Although CPT has multiple polar groups, it is still possible to form intermolecular hydrogen bonds with functional monomers.

The main drawback of suspension polymerization is that the dispersion medium may interfere with template recognition, so it has limited application in noncovalent imprinting approaches unless an appropriate dispersion medium is chosen. [61]

*Emulsion polymerization*

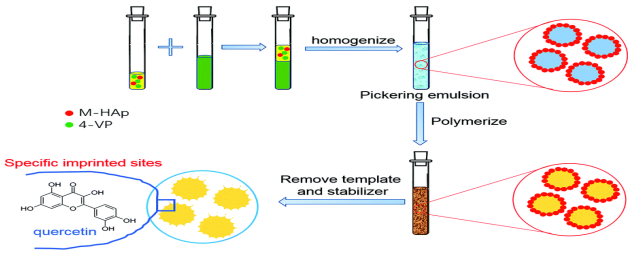
Emulsion polymerization is a process providing high-yield mono-dispersed polymer particles. In this type of polymerization, water is typically used as the continuous phase, while emulsion surfactants or cyclodextrins are used to form a water-in-oil or oil-in-water emulsion for the polymerization mixture. The monomer, crosslinker, and initiator are dissolved in one phase, mostly the organic phase, while the template and surfactant are dissolved in the opposite phase. The two solutions are then vigorously mixed, and the resulting emulsion contains fine mono-dispersed polymer particles with particle sizes ranging from tens to hundreds of nanometers. De-emulsification occurs after polymerization by adding an organic solvent such as acetone.

This approach was successfully applied for the isolation of quercetin from *Gleditsia sinensis(G.sinensis*)[67]. The preparation steps were performed using quercetin as a template, 4-VP (monomer); hydroxyapatite, a bioactive calcium phosphate ceramic, was used as a Pickering stabilizer due to its good bioactivity, high biocompatibility, good mechanical property, and rich surface chemistry (stabilizer) and, then the pre-polymerization mixture was crosslinked using divinyl benzene in tetrahydrofuran as the organic porogenic solvent as shown in Figure 10. After ultrasonication, water was added to the mixture. A homogenizer at 15000 rpm was used to vigorously mix and shake these two phases for 5 minutes, resulting in a stable emulsion. The mixture was then incubated at 70ºC after adding AIBN (initiator) for 12 hrs, followed by multiple washing steps in different methanolic and acetic acid mixtures to remove quercetin. The obtained MIP with specific recognition sites showed a binding capacity of 769µg/g.

An obvious disadvantage of this approach; is related to the use of surfactants that may isolate the template in their core and reduce the efficiency of the knocking-out process which results in a decrease in the number of active recognition cavities. [68,69]

*Isolation of Natural products by Molecularly imprinted polymers-based solid phase extraction (MISPE)*

Isolation of natural products from plant extracts by traditional solid-phase extraction sorbents suffers from several drawbacks, the most significant including limited separation efficiency of compounds from complex mixtures, due to broad selectivity towards several functional groups, and dependence on physiochemical properties like polarity commonly shared in many natural compounds. In contrast, molecularly imprinted polymer application offers multiple unique features for being of high affinity and selectivity, low cost, and ease of preparation in a tailored design to match the nature and physical properties of the target analytes, which made them superior adsorbents in solid-phase extraction (SPE). For this reason, the use of molecularly imprinting polymer solid-phase extraction (MISPE) is shown to be a promising technique in the isolation of natural products with high purity and sufficient amounts.[70,71]



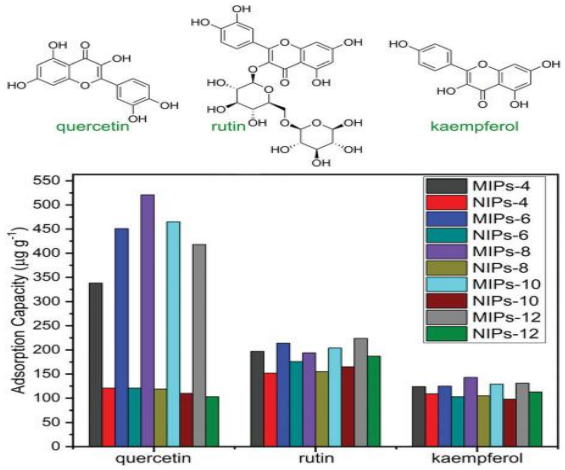



**Figure 10**: Application of emulsion polymerization for Quercetin extraction from *Gleditsia sinensis(G. Sinensis)* and comparison of its adsorption capacity towards rutin and kaempferol. [69] (*Figures are reproduced from Royal Society of Chemistry open access source*).

The most common NPs separated by the MISPE technique belong to alkaloids, phenolic, flavonoids, and terpenoids classes which have large therapeutic and biological activities. For example, rosmarinic acid, a phenolic compound, has wide therapeutic effects in Alzheimer's disease, it can be used to improve cognitive function as well as to reduce the severity of renal diseases, besides its antioxidant and anti-inflammatory activities that especially protect epidermal inflammations. Using MISPE it was isolated from *Rosmarinus officinalis L.*, and *Salvia hypoleuca* extracts efficiently avoiding multi-step processes and with excellent selectivity. [44, 47,72]

MISPE was also applied for the extraction of six phenolic compounds namely: ellagic acid, kaempferol3-rutinoside, salidroside, gallic acid, vanillic acid, and ferulic acid in one step from raspberry fruits (*Rubus chingii Hu*), a significant Chinese herbal medicine serving as a food and medicine with excellent nutritional and therapeutic value.[73,74] These compounds have shown a massive influence on the treatment of Alzheimer's disease, kidney deficiency, frequent urination, and other several pharmacological effects.

The isolation of camptothecin, a known potent drug in the treatment of gastrointestinal tumors, bladder tumors, liver cancer, and leukemia, from *Camptotheca acuminate* fruit applying traditional methods such as alkaline water percolation is a long process with low efficiency. Also, other methods including reflux or Soxhlet extraction are time-consuming and may give poor-quality camptothecin. On the other hand, using MISPE for the separation and purification of camptothecin resulted in a good yield and purity of more than 90%. [66]

Resveratrol, a polyphenol non-flavonoid compound present in strongly pigmented vegetables and fresh fruits as well as dried nuts such as peanuts, demonstrated several pharmacological activities of anticancer, antioxidant, and immune system regulator activities. Isolation of resveratrol yielded almost 11.5 mg/g by MISPE technique and increased the content level of resveratrol extract solution from *Polygonum cuspidatum* from 4.23 % to 23.74 %, which indicates the excellent efficiency and selectivity of MISPE compared to traditional methods. [75,76]

Ailanthone is a quassinoid obtained from the leaves of *Ailanthus altissima* known for its cytostatic, herbicidal, antituberculosis, anti-malarial, and anti-viral activities. MISPE application has proven to be a method of choice for obtaining purified ailanthone with low cost and high yields almost 0.77mg/g from the methanolic extract and 0.73mg/g from water extract which is more than another extraction method. [77] Similarly, galegine from *Galega officinalis*, a compound with anti-diabetic activity, can be obtained cost-effectively using MISPE with 330μg/g from a released fraction of extract which is considerably a higher yield compared to that of the traditional approach.[78]

Furthermore, molecular imprinting solid phase extraction can be used as a sorbent with excellent recognition ability that allows separating more than one natural product from a complex mixture, especially on applying magnetic MIPs with graphene oxide as sorbent. An example is the isolation of four flavonoids i.e. Ferrerol, taxifolin, kaempferol, and hyperin from *Rhododendron* species where farrerol is considered a famous flavonoid compound exhibiting various pharmacological effects, including phlegm removal, relieving asthma cough, anti-inflammatory, anti-allergic, anti-platelet aggregation, antioxidant, and anticancer.[28] MISPE was combined with liquid chromatography-tandem quadrupole mass spectrometry (LC-MS/MS) and was shown to exhibit limits of detection (LOD) of 0.06-0.08 g/L indicating the efficiency of the sorbents for extraction.

Similarly, the isolation of five iridoid glycosides (IGs) (garden-side, genipin, genipin-1-O-gentiobioside, teniposide acid, and geniposide,) from *Gardenia jasminoides Eills*, via MIP which acts as a highly selective adsorption surface for iridoid glycosides and flavonoids obtaining a more rich fraction from complex extracts [79]. Compared to the previously reported extraction column, The presented MISPE columns have shown to have 50 adsorption-elution cycles without obvious loss of recovery of IGs compared to eight adsorption-elution cycles. The low reusability of these earlier SPE columns might be caused by excessive hydrolysis of ester groups. In sharp contrast, the reusability of the MISPE columns.

Other flavonoid compounds like chrysin and morin were also separated using MIP from *Oroxylum indicum* using a one-step selective method of isolation with high adsorption capacity of 127.5mg/g and with lower cost, and time-consumption compared to traditional methods. [80–83] Using MISPE, morin was isolated in a cost-effective way attaining a high adsorption capacity of 3.24 mg/g polymer.

Generally, many types of MISPE manifolds were efficiently applied for the separation of NPs such as direct incubation, syringe-barrel, cartridges, variable disks, pipette Tips, 96-Well SPE plates, and monolithic columns. The most commonly used formats in MISPE are direct incubation, cartridge types, and monolithic columns as will be described in detail in the upcoming sections. [84]

*MISPE -direct incubation*

It is considered the simplest technique for extraction and isolation of the target molecule from a complex mixture. It is commonly used for the separation of bulk NPs from crude extracts that are left in contact for an enough duration of time with MIP powder designed to have selective recognition sites for a targeted component in the solution of crude extract. After this incubation step, the target component will be adsorbed in the specific cavities in the polymeric matrix, that can be filtered and washed using a suitable solvent to isolate the attached template.

This approach was successfully applied for the extraction of chicoric acid from *Chicorium intybus L.plant* in which the synthesized MIP was incubated with extract solution for 2hrs, then the amount of chicoric acid bound was determined using HPLC indicating that the MIP was bound selectively to 70% of chicoric acid while in case of other structures analogs, the binding efficiency did not exceeds 30%. Also, each gram of MIP powder has effectively bound to 12.41μmol of chicoric acid and 1.5, 1.4, and 1.017μmol in the case of other structural analogs like Caffiec acid, Caftaric acid, and chlorogenic acid, respectively which indicates that the developed MIP is highly selective and specific to chicoric acid. [44]

Another example of direct incubation isolation using MIP as sorbents was applied for sinapic acid extraction from *Botrytis italica, L.* (broccoli) among its closely related structural analogs including ferulic acid and caffeic acid in ethyl acetate fraction of the total extract in which the optimized MIP powder was incubated with *Botrytis italica, L.* (broccoli) extract solution for 30 min to achieve complete separation of the targeted molecule [85]. The samples were analyzed by HPLC before and after incubation and it was found that the ethyl acetate fraction of the total herbal extract showed no peak for sinapic acid after interaction with MIP which indicated full binding to the polymer but for ferulic acid, the peak area decreased by 60%, while for caffeic acid, a decrease of only 11% was noticed. On the other

hand, incubation with the nonimprinted polymer (NIP) has resulted in a decrease of 5%, 3%, and 2% of the peak area for sinapic acid, ferulic acid, and caffeic acid, respectively which indicated that the non-specific binding sites contributed minimally in the extraction and that the MIP has specific binding sites capable of binding to sinapic acid in the presence of other structural analogs.

*Cartridge MISPE*

This is the most popular manifold for SPE application in which a plastic or glass container with an open end, is filled with MIP powder as the adsorptive phase, instead of silica, as in the common commercially available types of chromatographic extraction cartridges. The MIP used is designed to have specific recognition cavities synthesized for the target component, in between silica or asbestos frits.

To assure the imprinting efficiency, the non-imprinted polymer (NIP), prepared in a similar procedure to that applied for its equivalent MIP, but in absence of the target analyte is used as control to assure that the extraction procedure is mainly a result of the specific recognition interactions and not only due to superficial interactions between the functional groups on the surface of either MIP or NIP and should be packed in a cartridge with the same dimensions and amount like that of MIP. [86]

The herbal extract comprising the target analyte is usually passed through MIP, and NIP cartridges then, a suitable solvent is used for eluting the target component entrapped within the MIP cavities and retained than other components that can be eluted using a different eluting solvent that is not capable to dissolving the target template and finally, the eluted fraction can be analyzed using a suitable detection method such as HPLC, LC/MS, UPLC, etc... It very crucial in such setup in this approach to optimize several parameters including the amount of polymer used to fill the cartridge, the type of eluent, the loading solvent, the cartridge capacity and the washing solvent, thus it is considered to be more complicated with many variables when compared to the simple direct incubation manifold besides applying to limited amounts of extracts that is loaded according to the volume of the cartridge used.

This approach was applied for the extraction of capsaicin and dihydrocapsaicin from chili peppers extract. The MIP loaded in the cartridge was synthesized via bulk polymerization of vanillyl amine as a dummy template, 4-VP as functional monomer, and EDMA crosslinker. 500mg from either prepared MIP and NIP were loaded in a 5ml empty SPE then the extract solution was passed through the MIP/ or NIP -SPE columns using a suction pump at a flow rate 1ml/min. Finally, 4.0 mL of ethanol was used as elution solvent, the resulting solution was analyzed by HPLC. The results indicated that capsaicin, and dihydrocapsaicin were adsorbed with maximum capacity of 219 mg/g (template /MIP) at 20 min interaction time. This indicates that the use of DMISPE can overcome the limitations of other separation methods which produce capsaicinoids is energy intensive and time-consuming procedures, especially on an industrial scale, furthermore, capsaicin, and dihydrocapsaicin are thermally labile under reflux conditions. [49]

Quercetin from red onion peels was successfully extracted using the MISPE technique. The MIP was prepared through bulk polymerization using Quercetin as a template, 4-VP as a functional monomer, and EDMA crosslinker in a ratio of 1:4:20, respectively in can as a porogenic solvent. The washed polymer was packed in a 3ml empty polytetrafluoroethylene (PTFE) cartridge with a disc frit fitted on the bottom and the top of the MIP bed at Flow rates optimized at 0.3, 1, and 0.5-ml/min for loading, washing, and eluting operations, respectively. The washing included six steps: first, 5 mL ACN, then two times 4 mL H2O (total 8 mL), and three times ACN: $H_2O$ (50:50, v/v) (6, 4 and finally 2 mL, total 8 mL). While the elution was performed twice with MeOH: ACN (4:1, v/v) for volumes of 6 and 3 mL, respectively. The eluted solution was analyzed by HPLC and an attained recovery of 58% with methanolic extract and 86% from hydrolyzed extract.[16]

Another example of extraction of some flavonoids components directly from gingko leaves using the MISPE technique, involved using a 2.5ml cartridge packed with MIP prepared via bulk polymerization method for quercetin as a template, 4-VP as functional monomer, and EDMA crosslinker. The packed MIP cartridge was conditioned by methanol solvent with a flow rate of 0.2ml/min, then 2ml from the extract solution was added followed by 12 ml acetone to rinse the cartridge, and finally 3ml of methanol: acetic acid mixture (9:1) mixture was added as an eluent solution for 3times to separate quercetin, kaempferol and isorhamnetin. HPLC analysis of the eluent solution indicated recovery of 101.19% for quercetin 96.76% kaempferol and 95.68% isorhamnetin.

Despite the multiple optimization steps for the conditioning, loading, and washing solvents, the extractions recoveries are considerably high as indicated by the results of HPLC detection, especially in the presence of complicated extracts with less chemical hazards in terms of the amounts of used solvents, and no possibility of thermal degradation as in case of reflux extraction especially for temperature sensitive NPS.

*Monolith column MISPE*

This format of MISPE was successfully applied as a working or preparatory column in high-performance liquid chromatography to separate enantiomers and analogs, racemic mixtures, and target molecules from complex extracts. Monolith columns have diverse properties like high porosity, high robustness good permeability, fritless, and large surface-to-volume ratio. In the case of packed SPE cartridges, the obtained polymer from the traditional preparation of molecularly imprinted polymer is first ground and sieved to obtain the appropriate size of packing material. This process may involve the production of non-regular sized or shaped MIPs, which seriously affects the application and may cause back pressure to be used as a working HPLC, thus, the application of monolith column has emerged to avoid many of such serious drawbacks. [87]

In general, monolith means “column consisting of a single large block of stone”. Monolithic columns usually consist of one piece of a continuous porous material that is synthesized in situ to the wall of a tube and is characterized by having a high permeability due to uniform distribution of the MIP particles throughout the length of the column which facilitates the efficient separation of target analytes. [88]

It is processed in one-step polymerization in the chromatographic column as applied in the case of extraction of caffeine and theophylline from black tea using a monolith column packed with MIP prepared by bulk polymerization type using methacrylic acid as a monomer in toluene (Porogen solvent) packed into stain steel 150 ×3.9mm chromatographic column after adding the crosslinker and the initiator to start the polymerization process. The column was left in a water bath with the temperature maintained at 55°C for 24 h. After polymerization the column was connected to HPLC and washed with tetrahydrofuran and methanol/acetic acid (80:20 v/v), respectively, to remove the porogenic solvents and the template molecule. Then a mixture of caffeine and theophylline was efficiently separated using the monolith MIP column. Although caffeine and theophylline are close in structure where the group attached to the nitrogen atom is a single hydrogen atom but in caffeine this group is a $CH_3$ group. Therefore, the analysis of such compounds in the presence of others represents a real challenge, yet, monolith MIP columns made the separation process between two structures easier. [38]

Another approach for MIP columns is that the polymerization and washing step are finished first then the washed MIP with justified specific recognition of a target molecule is packed under high pressure in the chromatographic column. This approach was applied for the separation of Camptothecin from *Camptotheca acuminata* fruit, in which the MIP was prepared by suspension polymerization using Camptothecin, methacrylic acid, ethylene glycol maleic rosinateacrylate (EGMRA) as template, monomer, and crosslinker, respectively in chloroform then 2%wt polyvinyl alcohol /water was added and the mixture emulsified and extruded through a micro-porous membrane (8.0 μm) by $N_2$ pressure at 0.1 M Pa to achieve uniform-sized- droplets. Then, the mixture was stirred at 80 °C for 24 h under $N_2$, after this step the polymer was centrifuged, collected, and washed using hot distilled water to remove PVA. This was followed by Soxhlet extraction with acid/MeOH (10/90), Then the MIP was

packed to stain steel 250×4.6mm empty column, using MIP as stationary phase for separation Camptothecin. [66]

## Conclusion

Molecular imprinting polymers are considered powerful and excellent analytical tools which is capable of solving many problems occurring mainly in the separation and extraction of natural compounds from complex extracts, not only on a research scale but also on commercial and industrial scales, some of the international companies increase their attention for producing molecular imprinted solid phase extraction cartridges have imprinted polymers as sorbent materials to separate the targeted compound from complexes extracts as some of the companies produced MISPE Cartridges in the market for extract natural products from extracts such as patulin from apple extract and some phenolic compounds.

In recent years, we have noticed a huge increase in the rate of production of MISPE for more targeted molecules due to special features of this cartridge as low cost-sensitive and selective to the target molecule, reusable cartridge and it will be facilitated more extraction and isolation a lot of purely natural products with a large amount, stable with low consumables and short time. However, there are some considerations to be kept in mind while applying MIPs, mainly due to the use of organic solvents during the synthesis and the washing steps of the polymers, which constitute an environmental hazard, also most of the chemical reagents used in the synthesis such as the monomer(s), crosslinker might are toxic, other problems as template leakage, irregular particle shapes in case of bulk polymerized MIPs, heterogenous recognition sites and low affinity binding sites,

Thus, the development of more effective MIPs is crucial to get over the above mentioned concerns, this can be achieved through combination of MIP with other porous or nanostructured materials or using 3D printed technology to prepare more homogenously designed polymeric layers or membranes, especially most of the used monomers in MIPs synthesis are acrylic derivatives that were found to have high potential in 3D printing and applications.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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**Table 1:** Application of different MIP synthesis approaches for the extraction and detection of NPs and the chromatographic analysis parameters.

| Natural compound/s | Source | PY-M | F.M | C.L | Modifier | Porogen Solvent | IF | T/M/CL | B.C (mg/g) | HPLC parameters (Column dimension) F.R(ml/min) R.T(min) Temp (C°) | M.P (v/v) | Ratio | Dec | Ref |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Ailanthone** | *Ailanthus altissima* | Bulk | 4-VP | TRIM | - | CAN | 3.8 | 1:9:45 | N/A | $C_{18}$ (125×4.6mm,5µm) 0.6ml/min 2.41 min Temp:25 C° | Water: MeOH | (78:22) | UV 254nm | [77] |
| **Anthocyanin** | *Garciniamangostana L (Mangosteen)* | Bulk | 4-VP | EGDMA | - | EtOH | 3.14 | 1:4:25 | 0.87 | UV spectrophotometer | N/A | | UV | [89] |
| **Capsaicin, dihydrocapsaicin** | Chili peppers | Bulk | 4-VP | EGDMA | - | TCM | 2.54 2.7 | 1:4:10 | 18.11 19.86 | $C_{18}$ (250×4.6mm,5µm) 1ml/min 10min Temp:25 C° | Water: MeOH | (70:30) | UV 280nm | [49] |
| **Chlorogenic acid Cryptochlorogenic acid Neochlorogenic acid** | *Lonicera japonica and Lianhua qingwen* | Bulk | 4-VP | DVB | - | MeOH | 2.24 2.42 2.90 | 1:4:8 | 112 109 119 | $C_{18}$ (250×4.6mm,5µm) 1ml/min - Temp:30 C° | 0.2%FA: ACN | (90:10) | UV 280nm | [50] |
| **Galegine** | *Galega officinalis L* | Bulk | MAA | EGDMA | - | ACN | - | 1:5:30 | 0.33 | $C_{18}$ (250×4.6mm,5µm) 1ml/min 3min Temp:30 C° | Water pH 3: MeOH | (80:20% ) | UV 290nm | [78] |
| **Glycyrrhizic acid** | *Glycirrhizaglabra (liquorice)* | Bulk | NVP | NMBA | - | - | | 1:4:20 | 15 | C18 (250×4.6mm,5µm) 1ml/min Temp:25 C° | Water: MeOH | (60:40%) | UV 254nm | [90] |
| **Hesperidin** | *Citrus Latifolia var. persa (lime )* | Bulk | AA | EGDMA | - | nBU/MePh/ MeOH | 2.1 | 1:2:40 | - | $C_{18}$ (150×4.6mm,5µm) 0.5ml/min Temp:35C | 0.5% GAA in water: MeOH | (80-20%) | UV 285nm | [71] |
| **`7-hydroxy coumarin Galbanic acid** | *Asafoetida plant* | Bulk | MAA | EGDMA | - | EtOH | 2.4 | 1:6-20 | - | $C_{18}$ (150×4.6mm,5µm) 1ml/min Temp:30 C° | Water: MeOH | 15:85% | UV 320nm | [91] |
| **Matrine** | *Sophora moorcroftiana* | Bulk | MAA | EGDMA | - | CFM | - | 1:3:25 | 27.3 | $C_{18}$ (100×2.1mm,3µm) 0.5ml/min Temp 25 C° | 0.5%FA MeOH | | HPLC–MS/MS | [92] |

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| **Myricetin** | *safflower and the flowers of A. manihot (Linn.) Medicus* | Bulk | 4-VP GMA | EGDMA | - | MeOH: ACN (1:2) | | 1:6:6:20 | 10.58 | $C_{18}$ (250×4.6mm,5µm) 0.8ml/min 7min Temp 45 C° | 0.1GAA in water: MeOH (45:55%) | UV 375nm | [17] |
| **Patulin** | Apple Fruit | Bulk | MAA | EGDMA | - | MePh-MeOH (9:1) | - | 1:4:20 | - | $C_{18}$ (150×2mm,3µm) 1ml/min 3.9 Temp 25 C° | 0.1% FA: MeOH (70:30 %) | HPLC–MS/MS | [93] |
| **Punicalagin** | Pomegranate husk | Bulk | AA | EGDMA | - | THF | 3.1 | - | 7.16 | Monolith Column 1ml/min 5 Temp 35 C° | 0.2M NaH2PO4: ACN (8:92%) | UV 258nm | [94] |
| **Quercetin** | Red Onion Peels | Bulk | 4-VP | EGDMA | - | ACN | - | 1:4:20 | 2.47 | $C_{18}$ (250×4.6mm,5µm) 1ml/min | 0.2% OPA: MeOH (95:5%) | UV 258nm | [53] |
| **Quercetin and Rutin** | *Herba Artemisiae Scopariae* | Bulk | FA: Chal: Mtpp | EGDMA | - | EtOH | - | | 3.2 4.8 | $C_{18}$(250×4.6mm,5µm) 0.8ml/min 4.5, 10 min Temp 25 C° | 0.1% GAA in Water: MeOH (40-60%) | UV 325nm | [95] |
| **Rosmarinic acid** | *Rosmarinus officinalis L.* | Bulk | 4-VP | EGDMA | - | DMSO | 2.92 | - | 49.11 | $C_{18}$(250×4.6mm,5µm) 1ml/min 10min Temp 25C | 0.01%FA in Water: ACN (40-60%) | UV 330nm | [44] |
| **Rosmarinic acid** | *Salvia hypoleuca extract* | Bulk | MAA: IA: VD (5:1:1) | TRIM | - | MeOH | 4.4 | 1:(5:1:11) :35 | 41.9 | $C_{18}$(250×4.6mm,5µm) 1ml/min 7min Temp 25 C° | 0.1%G AA in Water: MeOH (40-60%) | UV 326nm | [47] |
| **Salidroside** | *Rhodiola crenulata* | Bulk | AA | EGDMA | - | DMF | - | 1:4: N/A | 1.4 | $C_{18}$ (250×2.1mm,5µm) 1ml/min 3.43min Temp 25 C° | 1% GAA in water: MeOH (55-45%) | UV 278nm | [96] |

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| **Sinapic acid** | *Botrytis italica, L. (broccoli)* | Bulk | 4-VP | EGDMA | - | DMSO | 2 | 1:4:20 | 4.78 µmol/g | $C_{18}$ (250×4.6mm,5µm) 1 ml/min 29min Temp 30 C° | 0.5% GAA in Water: MeOH: ACN (90:5:5%) | UV 320nm | [51] |
| **Theophylline** | Green tea | Bulk | APTES-AM | EGDMA | - | CFM | - | 1:4:30 | 2.09 | $C_{18}$ (150×4.6mm,5µm) 0.8 ml/min 12.5min Temp 25 C° | 2% GAA in Water: MeOH (70:30%) | UV 270nm | [97] |
| **Ellagic acid** | Pomegranate peel | Distilled precipitation | 4-VP | DVB | - | THF/ACN | | - | 37.07 | $C_{18}$ (250×4.6mm,5µm) 0.8ml/min Temp:30C° | $KH_2PO_4$, pH6.4: ACN (23:77%) | UV 254nm | [64] |
| **Iridoid glycosides (LOG/LOGA/ MOR/EMOR/ MMOR)** | *Cornus officinalis fructus* | Precipitation | AGG | DVB MBA(4-1%) | - | MeOH | - | 1:5:(6 DVB, 4 MBA) | 11.4 | $C_{18}$ (250×4.6mm,5µm) 1ml/min Temp 25 C° | Water: ACN 90:10% | UV 238nm | [79] |
| **Anthocyanins** | *Lonicera caerulea* | Surface | 4-VP | EGDMA | $Fe_3O_4$@ $SiO_2$ | ACN | | 1:8:40 | 16.57 | $C_{18}$ (250×4.6mm,5µm) 0.5ml/min Temp:30C° | 0.4%FAin Water: MeOH (20:80%) | UV 254nm | [98] |
| **Catechins** | - | Surface | 4VP/MA | EGDMA | $Fe_3O_4$@M $oS_2$ | MePh | | | | $C_{18}$ (250×4.6mm,5µm) 0.8ml/min Temp 30 C° | 0.5%GA in water: MeOH (70-30%) | UV 278nm | [99] |
| **Chlorogenic acid** | *Duzhong brick tea* | Surface | 4-VP | EGDMA | $Fe_3O_4$@m $SiO_2$ | ACN | - | 1:5:20 | 42.22 | $C_{18}$ (150×3.9mm,5µm) 0.8ml/min Temp:25 C° | 0.1%FA: MeOH (75:25) | UV 254nm | [60] |
| **Cinnamic acid** | Apple juice | Surface | 4-VP | EGDMA | $Fe_3O_4$@m $SiO_2$ | ACN | - | 1:4:20 | 4.35 | $C_{18}$ (150×4.6mm,5µm) 1ml/min Temp:25C° | 0.4% GAA in water: ACN (30:70) | UV 270nm | [100] |
| **Di hydro quercetin** | *Larix griffithiana* | Surface | 4-VP | EGDMA | $Fe_3O_4$@ $SiO_2$ | MeOH | - | 1:4:25 | 7.56 | $C_{18}$ (150×4.6mm,5µm) 0.5ml/min 2.3 Temp:25C° | 0.5%$KH_2PO_4$, pH5: ACN (60:40%) | UV 288nm | [59] |
| **Ellagic acid** | *Rubus chingii Hu* | Surface | 4-VP | EGDMA | silanized silica gel | DMSO | - | 1:6:10 | 19.10µmol/g | $C_{18}$ (150×4.6mm,5µm) 0.2ml/min 14.1 Temp:30 C° | 0.1%FA ACN (5:95%) | UPLCQ-TOF-MS | [73] |

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| **Epicatechin**<br>**Epigallocatechin gallate**<br>**Epicatechin gallate** | Green tea | Surface | MAA | EGDMA | $Fe_3O_4$@ $SiO_2$ | ACN | - | 1:8:"30 | 2.712<br>2.065<br>3.628 | N/A | N/A | N/A | [101] |
| **Gallic acid** | Pomegranate rind | Surface | DOA | DOA | $Fe_3O_4$-COOH | 10mM tris HCLbuffer | 17.5 | - | 107 | $C_{18}$ (150×4.6mm,5µm)<br>1ml/min<br>8<br>Temp:25 C° | 0.1OPA : ACN (90:10%) | UV 273nm | [102] |
| **Glycitein**<br>**Genistein**<br>**Daidzein** | *Glycine max (Linn.) Merr* | Surface | MAA | EGDMA | $Fe_3O_4$@ $SiO_2$ | ACN | - | 1:8:"30 | 1.512<br>2.426<br>3.265 | N/A | N/A | N/A | [101] |
| **Hesperidin** | *Citrus reticulata Blanco* | Surface | NIAA | EGDMA | $Fe_3O_4$@ $SiO_2$ | ACN :MeOH (3:1) | 1 | 1:3:30 | 16.48 | $C_{18}$ (250×4.6mm,5µm)<br>1ml/min<br>13min<br>Temp:30 C° | 0.1%FA ACN (70:30%) | UV 288nm | [103] |
| **Kaempferol** | Apple fruit | Surface | AM | EGDMA | vinyl-$Fe_3O_4$ @$SiO_2$ | ACN | - | 1:5:10 | 3.84 | $C_{18}$ (250×4.6mm,5µm)<br>1ml/min<br>Temp 25 C | 0.4% OPA: MeOH 50:50% | UV 370nm | [104] |
| **Luteolin** | Peanut shell | Surface | APBA | DOA | $SiO_{2@}ZrO_2$ | Buffer pH8.5: MeOH | 2.89 | - | 12.42 | $C_{18}$ (150×4.6mm,5µm)<br>1ml/min<br>Temp 30 C | .5% OPA: MeOH | UV 375nm | [105] |
| **Osthole** | *Libanotis Buchtomensis* | Surface | 4-VP | EGDMA | $Fe_3O_4$ (Nano particles) | ACN | 4.36 | - | 17.65 | $C_{18}$ (150×4.6mm,5µm)<br>1 ml/min<br>6.59<br>Temp 25 C | Water: MeOH (25:75 %) | UV 322nm | [106] |
| **Resveratrol** | *Polygonum cuspidatum* | Surface | 4-VP | EGDMA | $pCM_{@}MPS$ | ACN | - | 1:5:30 | 11.56 | $C_{18}$ (250×4.6mm,5µm)<br>1ml/min<br>Temp 25C | Water: ACN (30:70 %) | UV 303nm | [107] |
| **Sibiskoside** | *Sibiraea angustata* | Surface | VBZA | EGDMA | $Fe_3O_4$ (Nano particles) | ACN | - | 1:4:20 | 13.75 | $C_{18}$ (250×4.6mm,5µm)<br>0.5ml/min<br>Temp 30C | water: MeOH (80-20%) | UV 254nm | [108] |
| **Tanshinone I**<br>**tanshinoneIIA**<br>**cryptotanshinone** | *Salvia miltiorrhiza bunge* | Surface | MAA | EGDMA | $Fe_{3O_4@}SiO_2$ | ACN | - | 1:8:30 | 3.865<br>1.61<br>5.42 | N/A | N/A | N/A | [101] |

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