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Magnetic Molecularly Imprinted Polymer Synthesis and Application for Selective Separation of Quercetin

Şeyda Karaman Ersoy[1*], Merve Akyüz[2], Kevser Sözgen Başkan[2]

[1]Fenerbahçe University, Faculty of Pharmacy, Department of Analytical Chemistry, İstanbul, 34758, Turkey.
[2]İstanbul University-Cerrahpaşa, Faculty of Engineering, Department of Chemistry, İstanbul, 34320, Turkey.

Abstract: Quercetin (QUE) is the most active compound in the flavone family, commonly found in the leaves, fruits, and flowers of many plants. The separation of QUE from various plant matrices has been a key research area due to its antioxidant, anti-inflammatory, antiviral, and antitumor properties. In this study, the conditions for synthesizing MMIPs and their use in QUE recovery were examined. Iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) and iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) were used to prepare magnetic nanoparticles, and Fe_3O_4 was synthesized. Tetraethyl orthosilicate (TEOS) was used to coat the resulting Fe_3O_4 surface with silica. [3-(methacryloxy)propyl] trimethoxysilane (γ-MPS) was used to functionalize the surface of the formed Fe_3O_4@TEOS structure. The synthesis was carried out using QUE as the template molecule; tetrahydrofuran (THF), ethanol (EtOH), and a solvent mixture of acetone and acetonitrile (ACN) (3:1, v/v) served as porogen solvents; acrylamide (AM), methacrylic acid (MAA), and 4-vinylpyridine (4-VP) were used as functional monomers; ethylene glycol dimethacrylate (EDMA) served as the cross-linker, and 2,2'-azobisisobutyronitrile (AIBN) was used as the initiator at different molar ratios (T:M:CrL, 1:4:20, 1:8:20, and 1:8:40). The recognition and selectivity properties of these polymers were evaluated based on absorbance values at 370 nm obtained through equilibrium assays, which used QUE solutions prepared in THF, ACN, and 50% (v/v) EtOH solvent mixtures at different ratios. It was established that the magnetic imprinted polymer prepared with a 50% (v/v) EtOH solvent mixture and molar ratios of 1:8:40 (QUE:4-VP:EDMA) exhibited the highest adsorption capacity and imprinting factor. Using the prepared QUE-MMIP, QUE was recovered with 33% efficiency from red onion peel extract.

Keywords: Quercetin, Magnetic molecularly imprinted polymer, Red onion peel.

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***Corresponding author's E-mail:** seyda.ersoy@fbu.edu.tr

1. INTRODUCTION

In recent years, there has been a growing interest in plant antioxidants that can be used in their unmodified form as natural food preservatives, replacing synthetic substances. One of these substances, Quercetin (QUE), a flavonol found in fruits and vegetables, is a food ingredient with proven beneficial effects on health and is widely used in pharmaceutical, cosmetic, and nutraceutical products. It has been reported that quercetin is abundant in onion and apple species, and it is also found in onion peels approximately 77 times more than the edible part (1-4).

However, the selective extraction of quercetin from natural sources remains a significant challenge due to the complexity of plant matrices and the presence of structurally similar phenolic compounds. Conventional extraction methods often lack the specificity required to isolate QUE, resulting in low purity and yield efficiently. Therefore, there is a pressing need for advanced materials capable of selectively recognizing and isolating target molecules such as QUE from complex mixtures.

The selective separation of QUE and other bioactive compounds from the natural matrix cannot be achieved with traditional extraction methods. Effective isolation of target analytes from complex sample matrices depends on using highly efficient extraction materials that can interact selectively with them (5-7). Many studies in the literature demonstrate that materials suitable for this purpose

can be prepared using molecular imprinting technology (6). Molecularly imprinted polymers (MIPs), produced by this technique, contain many cavities that match the shape, size, and chemical functionality of the template molecules. In other words, MIPs are materials with predefined selectivity for target molecules. This design can be explained by analogy, such as the "lock and key model" described by Emil Fischer over a century ago (8). Besides many other applications, MIPs, which are important as molecular recognition tools in analytical chemistry, are used to aid in the detection of amino acids, peptides, proteins, nucleotide derivatives, toxic substances including pesticide residues, antibiotics, artificial hormones, and food adulterants (9,10). MIPs are also valuable for identifying and extracting plant compounds (8). There are studies on using MIPs for the selective extraction, determination, and preconcentration of quercetin (11-14).

Molecularly imprinted polymers have a wide range of applications because of their high affinity and selectivity for the template molecule, their ability to retain recognition ability for a long time, high physical and chemical stability, durability, and ease of preparation. Besides the advantages of MIPs, magnetically imprinted polymers (MMIPs), which acquire magnetic properties, are also used for similar purposes. Compared to traditional solid supports, magnetic materials offer several superior features, such as a high surface-to-volume ratio, rapid and effective binding with the template molecule, and high magnetic susceptibility. Additionally, the MMIPs with the target molecule attached can be easily separated from the environment using external magnets without filtration or centrifugation (15,16). The MMIP consists of a magnetic component (such as nickel, -Fe_2O_3, Fe_3O_4, NiO, and their alloys) and an MIP component. Among magnetic materials, Fe_3O_4 is the most commonly used because it is easy to produce, has low toxicity, and has abundant hydroxyl groups that enable surface modifications (17). The synthesis of MMIPs mainly involves three steps: first, the production of magnetic nanoparticles; second, the modification of the magnetic core-shell surface; and third, the synthesis of the MIP with the target template molecule and coating of the core with this polymer. To make the MMIP ready for use, the template molecule must be removed.

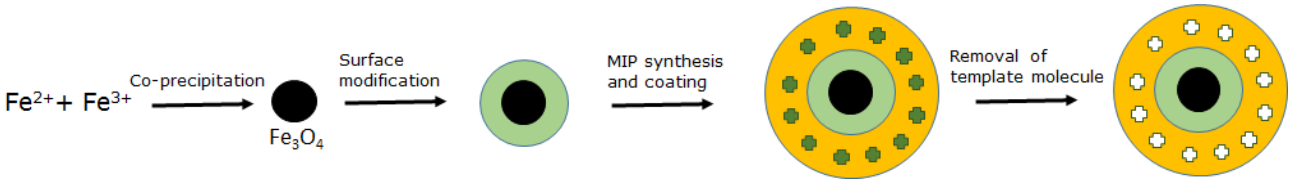

Figure 1: Schematic diagram of magnetic molecularly imprinted polymer (MMIP) synthesis.

MMIPs have been used for the selective separation of various plant-derived bioactive compounds, including QUE (7,15,16,18,19). The synthesis of magnetic MIPs for recovering bioactive compounds from natural samples is a relatively new research area in our country, with limited studies available. This study presents the synthesis of a novel QUE-imprinted MMIP and its application for QUE recovery from a natural matrix. QUE, a prominent flavonol, was chosen as the template molecule for imprinting. Bulk polymerization was selected for QUE-MMIP synthesis because of its ability to produce uniform structures. The optimal functional monomer, porogen solvent, and T:M:CrL molar ratio were determined for MMIP synthesis. A magnetic non-imprinted polymer (MNIP) was also synthesized under the same conditions as MMIP. Fourier Transform Infrared-Attenuated Total Reflectance (FTIR-ATR) spectroscopy was used to analyze structural differences between QUE-MMIP and MNIP. The static and theoretical adsorption capacities of QUE-MMIP were evaluated using Freundlich and Langmuir isotherms. Selectivity tests were conducted with rutin (RT), chlorogenic acid (CLA), vanillic acid, and gallic acid (GA) standards. For QUE-MMIP applications, red onion peel extract—known for its high QUE content—was used as a natural sample. Adsorption studies were performed with batch tests, and the results were analyzed by HPLC-PDA. This work reports the development of a novel magnetic molecularly imprinted polymer (MMIP) for selective quercetin extraction, offering improved adsorption capacity, high selectivity, and rapid magnetic separation, contributing to advancements in natural product isolation techniques.

2. EXPERIMENTAL SECTION

2.1. Chemicals

The following chemicals and solvents of analytical reagent grade were supplied from the indicated sources: Vanillic acid (VA; ≥99 % purity), gallic acid (GA; ≥98 % purity), chlorogenic acid (CLA; 98 % purity), acrylamide (AA; ≥99.9% purity) (Fluka, Steinheim, Germany); methanol (MeOH; gradient grade, ≥99.9 % purity), acetonitrile (ACN; gradient grade, ≥99.9 % purity), iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$;≥99%), iron(III) chloride hexahydrate ($FeCl_3.6H_2O$; ≥99% purity), 3-(methacryloxy)propyl trimethoxysilane (γ-MPS; ≥98% purity), tetraethyl orthosilicate (TEOS; reagent grade, ≥99.0% purity), ammonia (NH_3; anhydrous, ≥99.95% purity) (Sigma-Aldrich, Steinheim, Germany); rutin hydrate (RT; ≥94 purity) (Sigma, Steinheim, Germany); quercetin (QUE; ≥95 purity), 4-vinylpyridine (4-VP; ≥95 purity), methacrylic acid (MAA; ≥99% purity), ethylene glycoldimethacrylate (EDMA; ≥98% purity; cross-linking reagent polymerization reactions), 2,2-azobisisobutyronitrile (AIBN; ≥98%; free radical initiator) (Aldrich, Steinheim, Germany); *o*-phosphoric acid (≥85% purity);ethanol (EtOH; suitable for HPLC; ≥99.99%), tetrahydrofuran (THF; suitable for HPLC; (≥99.9% purity), acetone (ideal for HPLC, ≥99.9% purity)(Merck, Darmstadt, Germany). All the chemicals were of analytical grade.

2.2. Instruments

An HPLC system (Waters Breeze 2, Milford, MA, USA) equipped with a PDA detector (Waters 2998), a binary gradient pump (Waters 1525), and a Zorbax Eclipse XDB C18 column (4.6 mm x 250 mm x 5 µm) was used for chromatographic analyses. Data acquisition was performed using Empower PRO (Waters Associates, Milford, MA). Absorbances were measured with a Varian Cary 1E UV–Vis spectrophotometer (Sydney, Australia) using matched quartz semi-micro cuvettes of 1.4 mL volume and a Daihan HS-30D mechanical mixer (South Korea). Multi Bio RS-24 shaker (Latvia) for batch testing. A Witeg water bath was used for incubation. The evaporation of the extracting solvent was performed with a Büchi R210/215 rotary evaporator (Flawil, Switzerland). Additional equipment included an Isolab vacuum pump (İstanbul, Turkey), an Agilent 12-port SPE system (Waldbronn, Germany), and a Daihan vacuum oven (South Korea). Pure water for all solutions was obtained from a Millipore Simpak1 Synergy185 ultrapure water system (France).

2.3. Development of HPLC Method for The Analysis of Phenolic Compounds

In this study, to evaluate the usability of the synthesized MMIP for the selective separation of QUE from phenolic compounds, the analyses were carried out by HPLC method. A new method based on gradient elution was developed using bidistilled water (B) containing 0.2% *o*-H_3PO_4 with MeOH (A) as the mobile phase (Table 1). Flow rate is 1 ml min^{-1}, detection wavelength is 370 nm.

Table 1: HPLC method for the analysis of compounds in the studied samples.

Time	A%	B%	Curve
0-3 min	35	65	6
3-5 min	50	50	6
5-10 min	80	20	6
10-15 min	100	0	6

2.4. Synthesis of Magnetic Molecularly Imprinted Polymers (MMIPs)

In this study, magnetic molecularly imprinted polymers with QUE were synthesized to enable selective separation of QUE and its derivatives within the flavonol group of phenolic compounds. The synthesis of the polymer specific to QUE involved non-covalent interactions conducted in suitable solvent media (such as THF, ACN, or ACN:DMSO), using various molar ratios of template molecule, monomer, and crosslinker (1:4:20, 1:8:20, 1:8:40) under optimal polymerization conditions (in a water bath at 60 °C for 24 hours under N_2). These procedures were also applied to the non-imprinted polymer (without the template molecule, MNIP). The synthesis of magnetic molecularly imprinted polymers involves two main steps: first, the synthesis of magnetic material, and second, the polymerization process.

2.4.1. Synthesis of magnetic material

4.30 g of $FeCl_2.4H_2O$ (iron(II) chloride tetrahydrate) and 11.68 g of $FeCl_3.6H_2O$ (iron(III) chloride hexahydrate) were weighed, and 200 mL of ultrapure water was added. The mixture was stirred mechanically at 80 °C under nitrogen gas at 800 rpm. Fe_3O_4, which is unstable in the presence of O_2 and/or H+ ions, can easily oxidize to Fe_2O_3. To prevent this, precipitation was carried out by adding excess ammonium hydroxide (20). After adding 22 mL of NH_3:H_2O (28%, v/v), stirring continued for 4 hours. The Fe_3O_4 particles were separated using an external magnet, and the upper aqueous phase was discarded, following the method of Chai et al. (21).

2.4.2. Modification of the surface of the magnetic cores

After obtaining Fe_3O_4 magnetite, its surface was coated with a SiO_2 film. The procedure is as follows: First, the magnetic nanoparticles were evenly dispersed in 80% (v/v) MeOH, then 4 mL of NH_3:H_2O (28%, v/v) solution and 3 mL of TEOS were added slowly. The silica-coated magnetic nanoparticles were then separated using an external magnet and washed several times with ultrapure water. The purpose of coating the magnetite cores with SiO_2 is to improve their dispersion in water and prevent agglomeration. Additionally, the SiO_2 shell allows for further modification of the magnetite nanoparticles. In the next step, the surface modification was completed by coating the SiO_2-coated magnetic nanoparticles (Fe_3O_4@SiO_2) with γ-MPS {[3-(methacryloxy) propyl]trimethoxysilane}. For this, the silica-coated nanoparticles were mixed in 40% (v/v) EtOH under a nitrogen gas flow, then 3 mL of γ-MPS was added to the mixture, and stirring continued for 6 hours. After this, it was stirred for an additional hour at 70 °C. The final product, Fe_3O_4@SiO_2@MPS (Figure 2), was washed with EtOH and ultrapure water until the rinse water reached pH 7.0-7.5 and dried for 24 hours in a vacuum oven at 60°C. The use of γ-MPS is because it provides an attachment site for the polymer via its vinyl group (-CH=CH_2) (7).

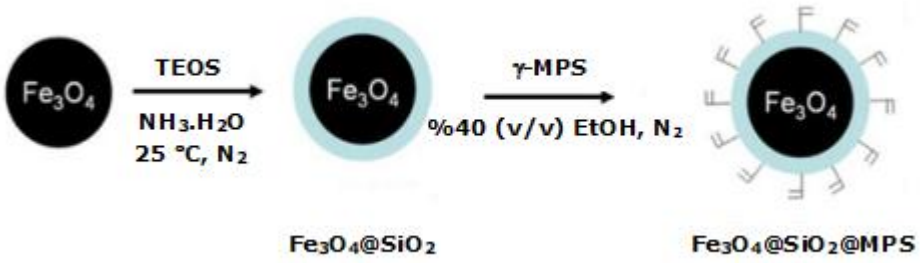

Figure 2: Synthesis of γ-MPS-SiO_2@Fe_3O_4.

2.4.3. Synthesis of QUE-MMIP and MNIP

2.4.3.1. Selection of the appropriate functional monomer

MMIP synthesis was performed using suspension, ultrasonic, and bulk polymerization methods, with batch test results showing that bulk polymerization was the most effective. Functional monomers 4-VP, AA, and MAA were employed to synthesize both QUE-imprinted and non-imprinted magnetic polymers. To identify the optimal monomer, imprinted polymers were prepared with a template:monomer:cross-linker (T:M:CrL) molar ratio of 1:8:40, while non-imprinted polymers were synthesized without a template molecule, using THF as the solvent.

For QUE-imprinted magnetic polymer synthesis, a solution containing 0.1 mmol QUE and 0.8 mmol monomer in 20 mL of THF was prepared in a sealed tube. Pre-polymerization was started by bubbling N_2 gas through the solution for 10 minutes, followed by a 30-minute incubation in darkness. Next, 100 mg of γ-MPS-SiO_2@Fe_3O_4 was added and mixed for 2 hours. After adding 4 mmol EDMA and 20 mg AIBN, the mixture was sonicated for 15 minutes and then purged with N_2 gas for 10 minutes. Polymerization was completed by sealing the tube and incubating it at 60 °C in a water bath for 24 hours. The polymers were then isolated and dried in a vacuum oven at 60 °C. Template removal was carried out via Soxhlet extraction using MeOH:HAc (8:2, v/v) as the solvent, with spectrophotometric monitoring until absorbance reached zero. Afterward, washes with MeOH and ultrapure water were performed until the pH stabilized between 6.5 and 7.5, followed by vacuum drying at 60 °C. The synthesis of non-imprinted magnetic polymers (MNIPs) followed the same procedure, minus the template molecule. A schematic diagram of the QUE-MMIP synthesis process is shown in Figure 3.

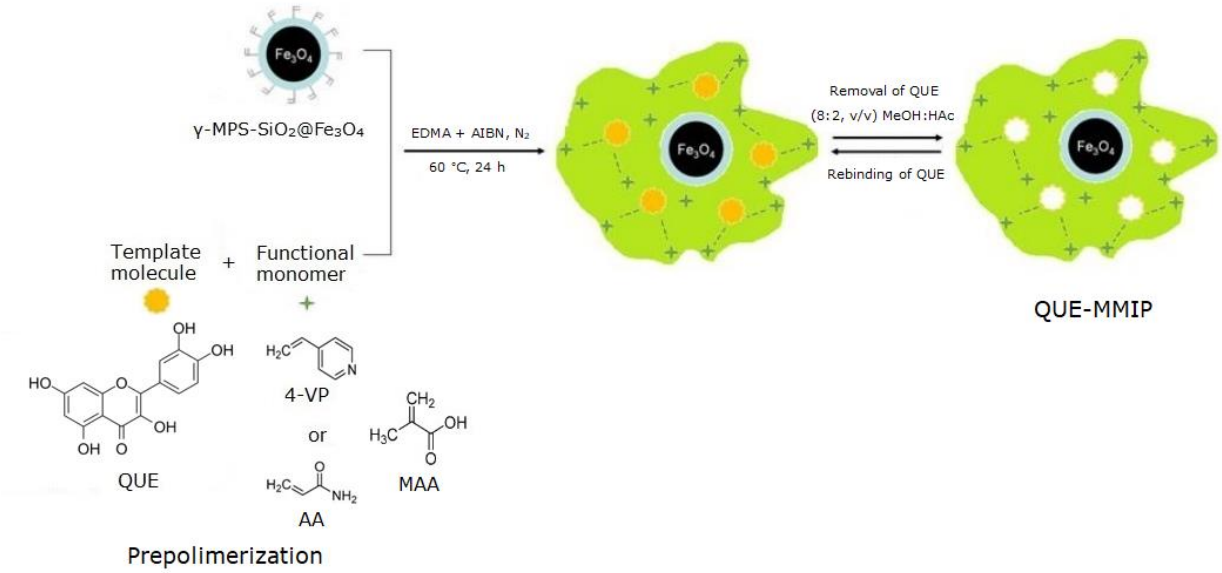

Figure 3: Schematic diagram of QUE-MMIP synthesis.

2.4.3.2. Selection of the appropriate porogen

After identifying 4-VP as the most suitable monomer for QUE-MMIP in the previous section, polymer synthesis was performed using this monomer, but with different solvents or solvent mixtures each time, following the procedure described above. The solvents and solvent mixtures used include acetone:ACN (3:1, v/v), EtOH, acetone, THF:MeOH:H_2O (6:3:1, v/v/v), and THF. To determine the most appropriate porogen, QUE was loaded into the prepared QUE-MMIP using the batch test, and the adsorption efficiency was measured spectrophotometrically.

2.4.3.3. Selection of the appropriate template/monomer/cross-linker ratio

QUE-MMIP syntheses were performed using 4-VP monomer and ethanol as the solvent at molar ratios of 1:8:20, 1:5:40, and 1:8:40 (T:M:CrL). Additionally, polymers were prepared at molar ratios

of 1:8:20, 1:4:20, and 1:8:40 using the same monomer and THF as the solvent. QUE loading was conducted on all prepared polymers with a batch test, and the most suitable molar ratios for the preparation of QUE-MMIP were determined based on the adsorption efficiency values obtained spectrophotometrically.

2.5. Examination of QUE-MMIP Rebinding Parameters

2.5.1. Effect of time on rebinding

To evaluate the effect of time on QUE rebinding, 20 mg of MMIP and MNIP were placed in six separate tubes, each containing 4 mL of a 60 µM QUE solution prepared in 50% (v/v) EtOH. Samples were shaken in a water bath at room temperature for 2, 4, 6, 18, 24, and 48 hours. MMIP was separated using an external magnet, and the remaining QUE concentration was determined by measuring absorbance at 370 nm after filtration through microfilters. The optimal adsorption time was identified based on the adsorption-time graph.

2.5.2. Effect of solvent on rebinding

To investigate the effect of solvent on the rebinding of QUE with MMIPs, loading experiments were conducted using different solvents after the complete removal of QUE from the MMIPs. In these studies, ACN, THF, and 50% (v/v) EtOH solvents were utilized. Solutions of 60 µM QUE prepared in different solvents were added to 20 mg of MMIP and MNIP in each case, and the mixtures were shaken for 2 hours. After separating the polymer with an external magnet, the solutions were filtered, and the optimal loading solvent was determined based on the absorbance measured at a wavelength of 370 nm.

2.5.3. Eff*ect of QUE concentration on rebinding*

To investigate the effect of QUE concentration on the rebinding to QUE-MMIPs, 20 mg of the prepared polymer was weighed and 4 mL of QUE solutions in the concentration range of 20-100 µM prepared in the specified solvent were added. After shaking in a water bath at room temperature for the specified time, MMIPs were separated with an external magnet. The amounts of QUE in the solutions filtered through 1.0/0.45 µm GF/PET micro filters were determined using absorbances at 370 nm wavelength.

2.6. Determination of the Adsorption Properties of QUE-MMIPs

2.6.1. Batch adsorption test

Batch adsorption test was applied with QUE standards prepared at different concentrations for QUE-MMIP and MNIP. 20 mg of the prepared QUE-MMIP and MNIP were weighed and 4 mL of QUE solutions in the 20-80 µM concentration range were added and shaken in a water bath at room temperature for a specific period. After the polymers were separated with the help of an external magnet, the solutions were filtered through 1.0/0.45 µm micro filters. The QUE amounts in the filtrates were determined by using the absorbances at 370 nm.

By analyzing Freundlich and Langmuir isotherms using batch adsorption data, QUE adsorption isotherms were applied to MMIP and MNIP.

For Freundlich Isotherm, MMIP and MNIP adsorption characteristics were assessed. The following equation, {Eq. (1)}, expresses the Freundlich isotherm, the first known relationship explaining the sorption equation. (2) Q_e: Adsorbed QUE amount on polymer (µg/g) C_e: Equilibrium concentration, or the amount of adsorbate in solution at equilibrium (in millimeters), by taking the logarithm of both sides, the equation can be made more linear.

The Freundlich adsorption isotherm is represented as follows in mathematics:

$$Q_e = K_f C_e^{1/n} \qquad \text{(Eqn. 1)}$$

By taking the logarithm of both sides, the equation can be made more linear.

$$\log Q_e = \log K_f + 1/n \log C_e$$

The second most used adsorption isotherm, the Langmuir model, is written as:

$$Q_e = Q_{max}\, b\, C_e/(1 + bC_e) \qquad \text{(Eqn. 2)}$$

$$C_e/Q_e = 1/(Q_{max}\, b) + C_e/Q_{max}$$

Where Q_e is the amount of adsorption (on adsorbent) per unit weight of MMIP at equilibrium (µg/g), b is the Langmuir adsorption equilibrium constant (L/mol), which represents the adsorption energy, and Q_{max} is the theoretical maximum adsorption capacity (µg/g). C_e is the equilibrium or final concentration (µM) of solution after adsorption.

2.6.2. Selectivity experiments

Selectivity tests of QUE-MMIP and MNIP prepared in appropriate molar ratios for some phenolic compounds were performed using rutin (RT) from the flavonol group of flavonoids such as QUE, chlorogenic acid (CLA) from the hydroxycinnamic acid class, gallic acid (GA) and vanillic acid (VA) from the hydroxybenzoic acid class of phenolic acids.

For this purpose, 20 mg of MMIP and MNIP were weighed into separate tubes, and 4 mL of 60 µM phenolic compound solutions were added and shaken in a water bath at room temperature for a certain period. Polymers were separated with a magnet and the spectra of the solutions passed through the microfilter were taken between 200-400 nm. The amount of each compound remaining in the solution was determined by measuring at the wavelength of maximum absorption (λ_{max}). Then, to determine the adsorption status of the above-mentioned compounds in the presence of QUE, a mixture solution containing 60 µM RT, CLA, VA, GA, and QUE in 50% (v/v) EtOH was prepared. The initial and post-polymer treatment concentrations of the mixtures were determined by HPLC method.

2.7. Investigation of the Structures of MMIPs

FTIR-ATR (Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance) spectra were obtained for QUE-MMIPs and MNIPs prepared under optimal conditions, and their structures were compared.

2.8. Application of QUE-MMIP in Natural Sample

Red onion peels were used as a natural sample containing QUE.

2.8.1. Preparation of onion peel extracts

0.9 g of air-dried onion peel sample was weighed and extracted in three steps with 25 mL of 70% (v/v) MeOH for 60 minutes, 15 mL for 45 minutes, and 10 mL for 15 minutes in the ultrasonic bath. These three extracts were combined, and the final volume was completed to 50 mL. Then, this extract was evaporated in a rotary evaporator under vacuum at 45 °C until it reached dryness. The dry residue was then dissolved in a specific volume of the loading solvent.

2.8.2. Application of QUE-MMIP for analysis of red onion peel extract

The extract, which was evaporated to dryness and redissolved in the loading solvent, was first analyzed by HPLC, and QUE content was determined. 4 mL of this extract was taken separately and shaken in a water bath for 2 hours with 20 mg of QUE-MMIP and MNIP. After the polymers were separated with an external magnet, the remaining solutions were passed through the micro filters, and HPLC analysis was performed. The solid QUE-MMIP remaining after filtration was washed with water, 50% (v/v) EtOH and MeOH:HAc (8:2, v/v) were analyzed by HPLC.

3. RESULTS AND DISCUSSION

3.1. Optimization Studies for the Synthesis of QUE-MMIP

3.1.1. Suitable monomer determination

To identify the most suitable monomer for the synthesis of QUE-MMIPs, three different monomers (AAm, MAA, and 4-VP) were used in a 1:8:40 molar ratio T:M:CrL in THF. Polymers were synthesized with and without imprinting. No polymerization occurred in the MAA-based synthesis. Polymers synthesized with AAm and 4-VP were loaded with 60 µM QUE, and it was observed that the polymer synthesized with 4-VP exhibited the best adsorption.

3.1.2. Suitable porogen determination

After determining 4-VP as the most suitable monomer for the QUE-MMIP synthesis, various solvents as porogen, including EtOH, THF, THF:MeOH (6:3:1), acetone, and acetone:ACN (3:1, v/v), were used to synthesize polymers in a 1:8:40 ratio. Polymers without the template molecule were also prepared using the same solvents. Adsorption tests were conducted in batch mode (60 µM QUE), and the imprinting factors (IF) were determined. The results are shown in Table 2.

Table 2: IF values of polymers synthesized in a 1:8:40 ratio using different porogens.

Porogen	Polymer	Adsorption amount of QUE (µg/g)	Imprinting Factor (IF)
THF	1:8:40 0:8:40	1496 906	1.65
EtOH	1:8:40 0:8:40	1632 1269	1.28
THF:MeOH:H_2O (6:3:1, v/v/v)	1:8:40 0:8:40	Polymer formation occurred; however, the adsorption capacity of the MNIP was found to be higher than that of the MMIP	-
Acetone	1:8:40 0:8:40	No polymer formation	-
Acetone:ACN (3:1, v/v)	1:8:40 0:8:40	No polymer formation	-

3.1.3. Suitable monomer:Cross-linker determination

The ratios used in the synthesis of quercetin-printed magnetic polymers were determined based on similar studies as references. The ratios tested in the quercetin-printed magnetic polymer synthesis were 1:4:20, 1:8:20, and 1:8:40. THF was used as a pore-forming solvent to synthesize both printed and non-printed polymers, and the imprinting factors were determined (Table 3).

Table 3: Comparison of QUE-MMIP and MNIP at different molar composition ratios.

Molar ratio	Adsorption Amount of QUE (µg/g)	Imprinting Factor (IF)
1:8:40 0:8:40	1496 906	1.65
1:8:20 0:8:20	423 287	1.47
1:4:20 0:4:20	695 453	1.53

Based on the imprinting factor values, the most optimal molar ratio for QUE-MMIP was determined to be 1:8:40.

3.2. Investigation of QUE-MMIP Rebinding Parameters

3.2.1. Effect of time on rebinding

20 mg of 1:8:40 QUE-MMIP and MNIP polymers were weighed into five separate tubes, to which 4 mL of a 60 µM QUE solution prepared in a 50% EtOH (v/v) solvent mixture was added. The samples were incubated at room temperature, with a shaking speed of 250 rpm, in a water bath for 2, 4, 6, 18, and 24 hours. The amount of adsorbed QUE was calculated from the absorbance measurements at a wavelength of 370 nm. The measured value at 2 hours was considered sufficient, as the difference in retention between MMIP and MNIP stabilized at that point.

3.2.2. Effect of solvent on rebinding

20 mg of 1:8:40 QUE-MMIP and MNIP polymers were weighed, and 4 mL of 60 µM QUE solutions prepared in THF, ACN, and 50% EtOH (v/v) solvents were added to each. The samples were incubated at room temperature for 2 hours, with a shaking speed of 250 rpm, in a water bath. The amount of adsorbed QUE was calculated based on absorbance measurements at a wavelength of 370 nm. According to the obtained data, the highest adsorption value was determined with 50% (v/v) EtOH.

3.2.3. Effect of T:M:CrL ratio

To investigate the effect of QUE concentration on the polymer's binding capacity, solutions prepared at different concentrations (20-140 µM) in 50% EtOH (v/v) were added to 20 mg of QUE-MMIP and MNIP polymers, and batch testing was performed. The amounts of QUE retained by QUE-MMIP and MNIP, as well as the binding factor (BF) values determined based on concentration, are presented in Table 4.

Table 4: The IF values determined as a result of the adsorption of QUE solutions at different concentrations by MMIP and MNIP.

QUE concentration (µM)	Adsorption amount of QUE on MMIP (µg/g)	Adsorption amount of QUE on MNIP (µg/g)	IF values
20	352	223	1.57
40	1097	789	1.39
60	1496	906	1.65
80	1890	1453	1.30
100	2183	1666	1.31
120	2200	1734	1.26
140	2434	1987	1.22

3.3. Results of the Adsorption Characteristics of QUE-MMIPs

3.3.1. Batch adsorption test

The Freundlich and Langmuir adsorption isotherms were used to evaluate the adsorption properties of QUE-MMIP and MNIP. For both QUE-MMIP and MNIP, the linear Freundlich adsorption isotherm was plotted by examining the relationship between log Ce and log Qe, as shown in equation 1. The slope of the line and its intercept were used to determine the values of 1/n and log K_f. Figure 4 shows the Freundlich adsorption isotherm.

Table 5 represents the Freundlich adsorption isotherm values for QUE-MMIP and MNIP.

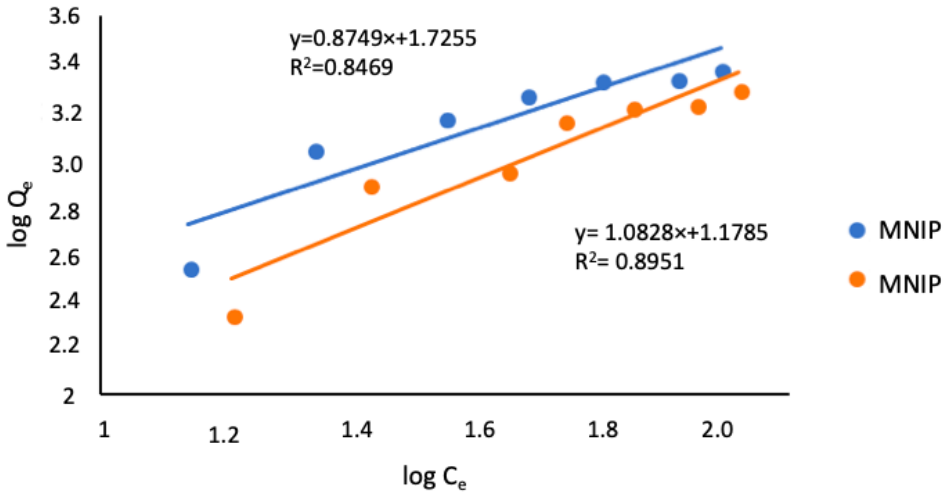

Figure 4: Freundlich Adsorption Isotherm for QUE-MMIP and MNIP (Q_e: the amount of adsorption (on adsorbent) per unit weight of polymer at equilibrium (µg/g), C_e: the equilibrium or final concentration (µM) of solution after adsorption.)

Table 5: Freundlich and Langmuir Adsorption Isotherm values of QUE-MMIP and MNIP (Kf: Freundlich constant; n: Adsorption intensity constant; Q_{max}: Theoretical maximum adsorption capacity; b: Langmuir adsorption equilibrium constant)

Polymer	Freundlich Constants			Langmuir Constants		
	Kf	n	R^2	Qmax	b	R^2
MMIP	53	1.14	0.8469	769	$3.22x10^{-3}$	0.9737
MNIP	15	0.92	0.8951	1000	$2.09x10^{-3}$	0.9000

The adsorption intensity constant (n) of QUE-MMIP was 1.14 (>1), indicating compatibility with the Freundlich isotherm. Langmuir isotherms for QUE-MMIP and MNIP were plotted using C_e and C_e/Q_e values, with Q_{max} and b calculated from the linear plot's slope and intercept. Figure 5 shows the Langmuir isotherm, while Table 5 summarizes adsorption capabilities. The RL value (0.83, RL<1) confirms the suitability of the Langmuir isotherm for QUE-MMIP. Higher correlation coefficients indicate the Langmuir model fits QUE-MMIP adsorption better than the Freundlich model (22,23).

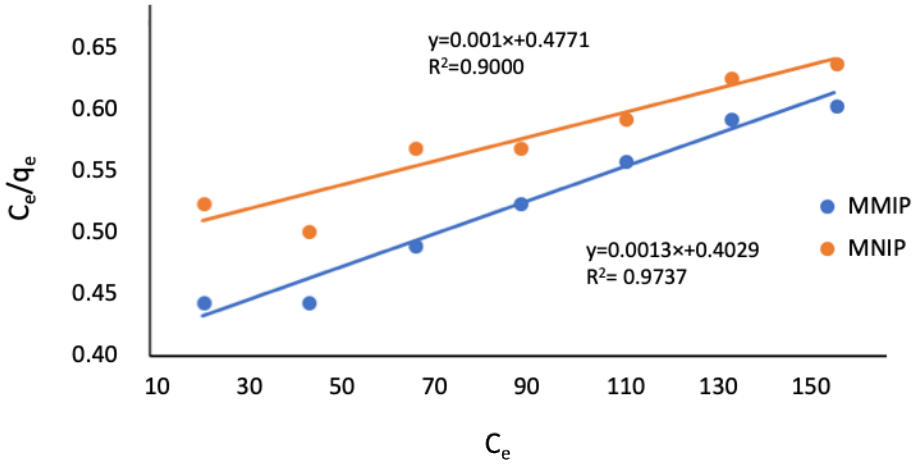

Figure 5: Langmuir Adsorption Isotherms for QUE-MMIP and MNIP (Q_e: the amount of adsorption (on adsorbent) per unit weight of polymer at equilibrium (µg/g), C_e: the equilibrium or final concentration (µM) of solution after adsorption.)

3.4. Investigation of QUE-MMIP and MNIP Structures

The FTIR-ATR spectra of QUE-MMIP and MNIP are shown in Figure 6. For QUE-MMIP, the adsorption bands observed at ~3420 cm^{-1} and ~2950 cm^{-1} correspond to -OH and C-H stretching vibrations, respectively. The vibration observed at 1719 cm^{-1} corresponds to the C=O stretching vibration. The bands observed at ~1450 cm^{-1} and ~1385 cm^{-1} are attributed to $-CH_3$ and $-CH_2$ bending vibrations, respectively. The bands observed at ~1246 cm^{-1} and ~1132 cm^{-1} are interpreted as C-O and Si-O stretching vibrations, respectively (24).

The FTIR-ATR spectra of MMIP and MNIP are nearly identical because, after the removal of the template molecule, the chemical composition of MMIP, is like that of MNIP (25).

3.5. Synthetic Mixture Application to Determine the Selectivity of QUE-MMIP for QUE

4 mL aliquots of each GA, RT, CLA, VA, and QUE were taken from the mixture prepared in 50% (v/v) EtOH at a concentration of 6×10^{-5} M and added to 20 mg of QUE-MMIP and MNIP. The samples were shaken in a water bath for 2 hours at room temperature. The mixture was analyzed by HPLC before and after this process. The resulting chromatograms are shown in Figure 7.

As shown in Figure 7, in the synthetic mixture containing 60 µM QUE along with phenolic compounds at the same concentrations, the amount of QUE adsorbed by QUE-MMIP is higher. It was hypothesized that the other components, aside from QUE and RT, bound non-specifically to the polymer without fitting into the template, since they lack a flavonol structure. The low adsorption rate of RT by QUE-MMIP was attributed to its structural difference from QUE, specifically the presence of a rutinoside group, which prevents it from fitting into the template. As depicted in Figure 7, the adsorption of QUE by MNIP was believed to result from non-specific interactions between the -OH groups in its structure and groups on the polymer surface.

3.6. Red Onion Peel Application to Determine the Selectivity of QUE-MMIP for QUE

After evaporation, 1 mL of red onion peel extract in 50% ethanol was diluted to 10 mL, then further diluted at 1:1 and 1:10 ratios. Next, 4 mL of the solution was mixed with 100 mg of MMIP and incubated at 250 rpm for 2 hours. The chromatogram at 370 nm (Figure 8) confirms the presence of quercetin, quercetin glycosides, and kaempferol. The first and third peaks were identified using PDA spectra and literature data (26).

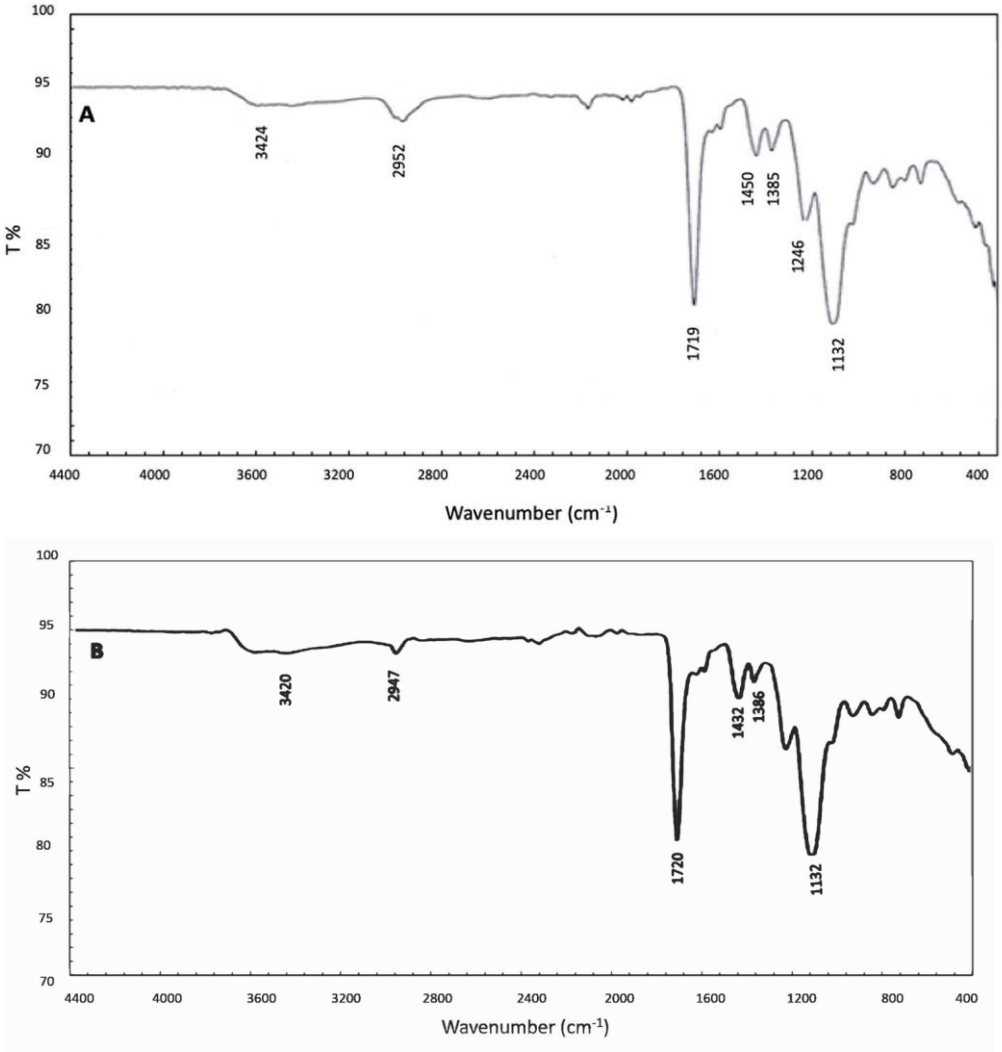

Figure 6: FTIR-ATR spectrum of MMIP (A) and MNIP (B).

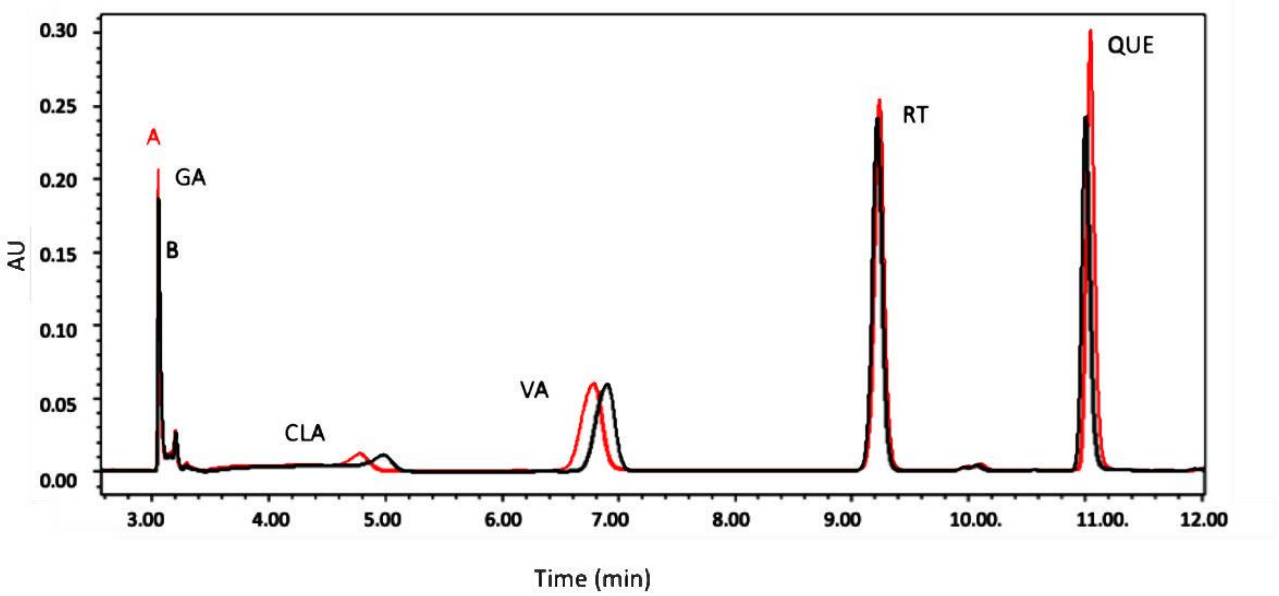

Figure 7: Chromatograms of the synthetic mixture solution before (A) and after (B) treatment with QUE-MMIP (λ = 260 nm).

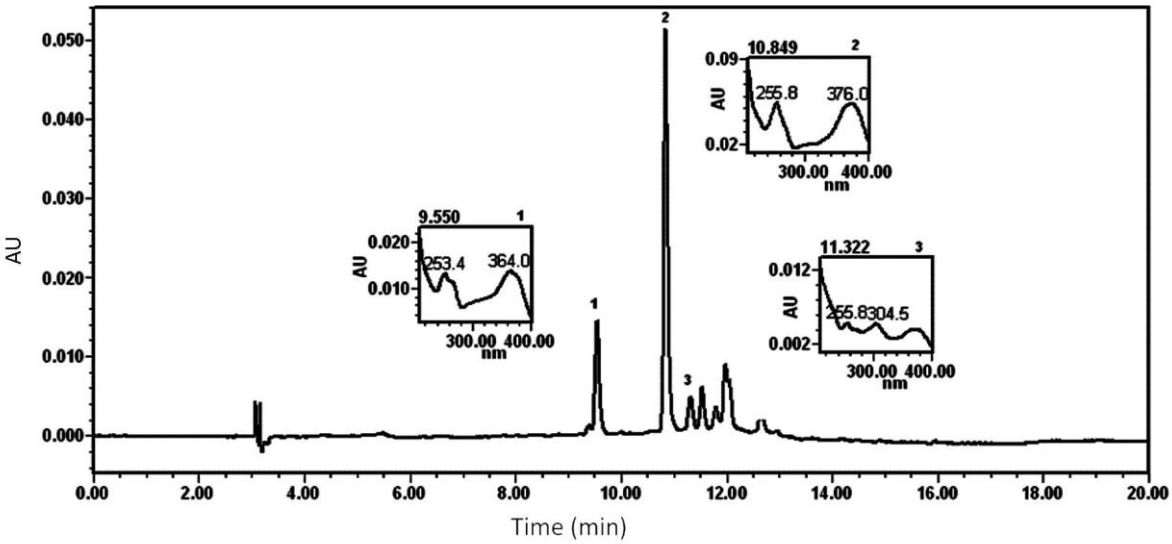

Figure 8: Chromatogram of the red onion peel extract (λ = 370 nm). (1: Quercetin glycoside, 2: Quercetin, 3: Kaempferol).

Figure 8 presents the chromatograms of QUE and its derivatives, which absorb at 370 nm, alongside other phenolic compounds absorbing at 280 nm in red onion peel extract. QUE recovery calculations were based on measurements at 370 nm. Following a 2-hour incubation of the extract with QUE-MMIP in a water bath, some non-target compounds were also retained (Figure 8). Given their phenolic structures and –OH groups, these compounds likely interacted with the polymer through hydrogen bonding or non-specific interactions within its heterogeneous regions. To eliminate retained QUE and other substances, a six-step washing procedure was implemented after QUE-MMIP treatment (Figures 8,9). Finally, to recover the QUE retained in the polymer, a two-step washing procedure was applied using 8 mL of a MeOH (8:2, v/v) mixture (Figure 9). As a result of the calculations made from the chromatograms shown in Figure 9, it was determined that 33% of QUE was recovered during the elution steps. During the washing process, quercetin glycosides and other components were removed from the medium. Table 6 shows the amounts of QUE at each stage of the process for the red onion peel extract treated with QUE-MMIP.

Table 6: QUE amounts at each processing stage of the red onion peel extract treated with QUE-MMIP.

Steps	Amount of QUE (mmol)
Loaded	2.68×10^{-5}
Adsorbed	1.68×10^{-5}
Elution 1 + Elution 2	$2.78 \times 10^{-6} + 2.72 \times 10^{-6}$
Recovery %	33 %

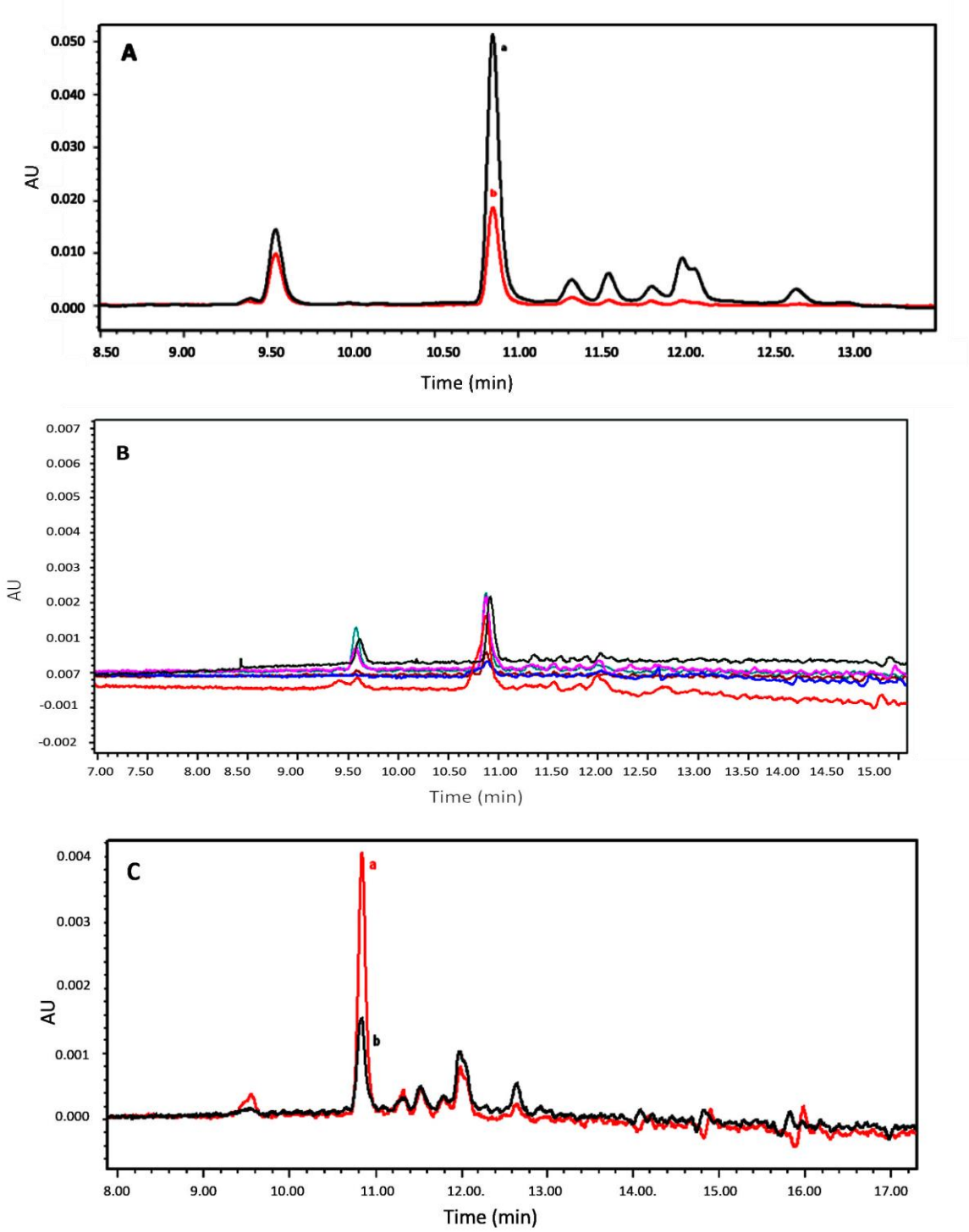

Figure 9: (A) Chromatograms of the red onion peel extract before (a) and after (b) loading (λ = 370 nm), (B)Washing chromatograms of QUE-MMIP loaded with red onion peel extract (Washing 1: 4mL H_2O, Washing 2,3,4: 4 mL 50% (v/v) EtOH), Washing 5,6: EtOH), (C) Chromatograms of the MeOH (8:2, v/v) elution following the washing steps of QUE-MMIP (λ = 370 nm). (a: elution 1, b: elution 2).

4. CONCLUSION

The synthesis of MMIPs for quercetin (QUE) is an emerging field with limited research in our country. Based on global literature, this study investigates the synthesis of QUE-imprinted polymers with magnetic properties, utilizing available resources and optimizing conditions for the best outcomes.

The targeted separation of QUE from natural matrices remains a scientific challenge, mainly due to interference from similar compounds and the limitations of traditional extraction methods. Our results show that the developed MMIP system not only overcomes these issues with improved selectivity and adsorption capacity but also allows easy magnetic separation, removing the need for filtration or centrifugation.

For the magnetic component, the method of Cai et al. (21) was adapted, with key parameters optimized, particularly the mixing speed during TEOS addition to Fe_3O_4, which was set at 800 rpm to achieve a homogeneous SiO_2 coating. Surface

modification of Fe_3O_4 was further achieved using γ-MPS to facilitate QUE binding, followed by washing to a pH range of 6.5–7.5.

QUE, a bioactive flavonoid with antioxidant, anticancer, and anti-inflammatory properties, was selected as the template molecule. Various polymerization methods (suspension, bulk, and ultrasonic) were tested, with bulk polymerization chosen for its ability to produce homogeneous polymers. Optimization studies identified 4-VP as the functional monomer, THF as the solvent, and a molar ratio of QUE: 4-VP: EDMA (1:8:40) as optimal for QUE-MMIP synthesis. AIBN was used as the initiator (20 mg). A non-imprinted polymer (MNIP) was also synthesized for comparison.

Batch adsorption tests were performed to examine how time, solvent, and concentration affect QUE uptake. The best adsorption solvent, based on the imprinting factor (BF), was 50% (v/v) ethanol, which had a BF of 1.65. The ideal rebinding time was 2 hours, after which the amount of QUE retained by both MMIP and MNIP remained steady. The highest BF values were seen at a QUE concentration of 60 µM. Static and theoretical adsorption capacities were determined, with QUE-MMIP showing a static capacity of 1496 µg/g. Freundlich and Langmuir adsorption isotherms were applied to the data, with Langmuir fitting better (R^2 > Freundlich), indicating monolayer adsorption on a uniform surface. The theoretical maximum adsorption (Qmax) for QUE-MMIP was 796 µg/g.

FTIR-ATR spectra confirmed the successful synthesis of magnetic components (SiO_2@Fe_3O_4 and γ-MPS-SiO_2@Fe_3O_4). No major differences were seen between the IR spectra of QUE-MMIP and MNIP, except for a lower peak intensity in MMIP, indicating the presence of the template molecule.

Finally, QUE-MMIP was tested for its ability to recover QUE from red onion peel extract. HPLC analysis showed that QUE was selectively adsorbed, with a recovery yield of 33%. These results, when compared to synthetic mixtures, confirmed the potential of QUE-MMIP for selective recovery of QUE from natural sources. Efficient isolation and enrichment of this bioactive flavonoid from complex matrices can facilitate its use in nutraceuticals, pharmaceuticals, and functional foods, ultimately contributing to public health by enhancing access to natural compounds with preventive and therapeutic potential.

5. CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

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