Journal of
Materials in Life Sciences

Vol. 3 No. 1 (2024)

Full Paper

Magnetic-Solid Phase Extraction of 5-Fluorouracil using Newly Synthesized Surface-Imprinted Magnetic Hollow Porous Polymer

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Article history
Received
27 September 2023
Revised
21 November 2023
Accepted
27 March 2024
Published online
25 May 2024

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Abstract

A magnetic hollow porous molecularly imprinted polymer (MHPMIP) was developed using a surface imprinting technique (mesoporous MCM-48 as a core). Its potential as a magnetic-solid phase extraction (MSPE) sorbent for the extraction of 5-fluorouracil (5-FU) anticancer drug was investigated to overcome some drawbacks caused by conventional SPE sorbents. Although SPE offers advantages such as rapidity and simplicity, the issues of low selectivity and sensitivity still remain unsolved. Moreover, the current determination methods of 5-FU in water samples are seriously disadvantaged due to major matrix interferences. Thus, extensive sample preparation is required before instrumental analysis. The MHPMIP was synthesized via precipitation polymerization using a functional monomer, methacrylic acid (MAA), crosslinking agent, ethylene glycol dimethacrylate (EGDMA), an initiator 4,4'-azobis (4-cyanopentanoic acid) (ACPA), and also the template, 5-FU. The specific surface area of MHPMIP was determined to be 63.47 m^2/g with a pore volume of 0.2325 cm^3/g using Brunauer-Emmett-Teller (BET) method. The MHPMIP possessed a good adsorption capacity of 2.089 mg/g. The adsorption capacity of 5-FU was significantly higher than that of the non-imprinted one (MHPNIP) (0.02324 mg/g). Coupled with FASI-CZE, the MSPE using the MHPMIP as sorbent showed that it could extract 5-FU from wastewater samples at low ppm levels. The limit of detection (LOD) and the limit of quantitation (LOQ) of the MSPE-FASI-CZE method was found to be 3.228 and 9.782 mg/L, respectively, in the linear concentration range of 5 to 15 mg/L with a good correlation (R^2 = 0.9973). A recovery up to 105.7% (RSD% 9.428, n = 3) was obtained for 5-FU (5 mg/L) spiked in real samples.

Keywords Molecularly imprinted polymer, magnetic-solid phase extraction, 5-fluorouracil, capillary electrophoresis

1.0 INTRODUCTION

Cancer has become the deadliest disease throughout the world and continues to torture humanity on a global scale [1]. Anticancer drugs, also known as antineoplastic agents, are used in chemotherapy to treat solid malignancies, including colorectal, breast, lung, gastrointestinal, and many others. 5-Fluorouracil (5-FU) is one of the most important agents among the various therapeutic molecules used in hospitals [2]. It primarily functions as an inhibitor of thymidylate synthase, which prevents the enzyme from acting and synthesising pyrimidine thymidine, a nucleoside necessary for the replication of deoxyribonucleic

acid (DNA) [3]. The cytostatic compounds often lead to pharmaceutical pollution, specifically in hospital and municipal wastewater, via the urine or feces of medically treated patients. Additionally, hospital wastewater presents a multifaceted composition, including substantial quantities of pharmaceuticals, disinfectants, heavy metals, reagents, and microorganisms [4]. Due to these ubiquitous matrix interferences, analyzing anticancer drugs is not straightforward. Thus, research on this analyte in a variety of sample types with complex matrices requires the development of sensitive and trustworthy analytical methods for the measurement of 5-FU. The application of gas chromatography (GC) and other chromatographic separation techniques [5,6] and liquid chromatography [7-9] have been widely used in determining anticancer drugs in complex samples over many years due to their high sensitivity. The detection technique used for quantification purposes is dominated by mass spectrometry (MS/MS) [10]. However, despite their advantages, issues such as the consumption of solvent and derivatization process due to certain circumstances prior to sample injection cannot be avoided. A successful HPLC-based approach with 5-FU is quite challenging to perform due to its poor retention on reversed-phase material. In any case, the resolution has been discovered as an issue due to the interferences in biological samples. Still, they might become a crucial factor in a matrix that is constantly changing, such as wastewater samples [11]. Since capillary electrophoresis (CE) has not been extensively used in the analysis of 5-FU to date, it is important to note that CE is a highly separation analytical technique, which provides quick separation with higher resolution and exceptional efficiency (500,000-700,000 theoretical plates per meter) [12] for analytical challenges that often can only be met with difficulty by LC.

Because sample preparation consumes the most labor and time during analysis and has the potential to introduce errors and imprecision into the final outcome, obtaining satisfactory results from the analysis of real samples is imperative. Numerous techniques for preparing samples have been developed, such as dispersive liquid-liquid microextraction (DLLME), liquid-liquid extraction (LLE), solid phase extraction (SPE), liquid phase microextraction (LPME), and solid phase micro-extraction (SPME). [13]. Among sample preparation methods, SPE is simple, quick, and cost-effective. SPE provides advantages over traditional LLE due to its promising properties, such as simplicity, rapidity, and cost-effectiveness. Nevertheless, conventional SPE methods have issues with the sorbents because, most of the time, the sorbents used can retain other materials that could interfere with the detection of analytes, making it more difficult to separate analytes effectively in complex samples due to poor selectivity. The core of the SPE technique is the sorbent material because it directly influences the selectivity, as well as the sorptive capacity of the final SPE device. Molecularly imprinted polymers (MIPs) have been investigated as sorbents in magnetic solid-phase extraction (MSPE) [14-16]. Molecular imprinting is a well-established method for creating robust molecular recognition [17] elements for various target molecules. MIPs are ideal for molecular recognition due to their specific binding sites and excellent chemical, thermal, and mechanical stability. Bulk and precipitation polymerization methods are two common methods used in preparing MIPs. Despite their advantages and successful polymerization reports, these methods still display poor recognition, suffering from the presence of remnants of surfactant as well as time-consuming [18]. These problems could be alleviated by surface imprinting using mesoporous silica MCM-48 [19], with templates imprinted on or near the material's surface. The MIP modified with MCM-48 as sacrificial support could improve the limits of MIP due to its high stability, high surface area, and pore volume to easily distribute the analyte 5-FU into the MIP and produce hollow porous MIP (HPMIP).

Magnetic nanoparticles (MNPs) are gaining popularity for their high efficiency, including a large surface area-to-volume ratio and easy separation from samples without special equipment. There is significant recent interest in using MNPs for environmental contaminant separation, extraction, and cleanup [20, 21]. MNPs have a magnetic solid phase, primarily made up of iron minerals and magnetic iron oxides like magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3). Fe_3O_4 is favored for its large surface area, small particles, excellent paramagnetic properties, water dispersibility, stability, ease of synthesis and functionalization, and low toxicity [22]. Magnetic sorbents undergo physical or chemical adsorption or functionalization with various materials. These adjustments increase the capacity for extraction, improve selectivity, inhibit aggregation within the sample solution, and bolster chemical stability [23]. In this method, magnetic MIP is dispersed in a sample solution. After incubating until the target analytes adsorb, the magnetic MIP is separated using a magnetic field due to its superparamagnetic properties. Target molecules are easily desorbed with solvent. The magnetic materials redisperse in solution after removing the magnetic field, simplifying washing and desorption. This process eliminates the need for centrifugation or filtration to separate the magnetic MIP from the sample after extraction [24]. In the present work, magnetic hollow porous molecularly imprinted polymer (MHPMIP) was proposed for the magnetic SPE (MSPE) of fluorinated pyrimidines, 5-FU.

2.0 EXPERIMENTAL

2.1 Chemicals and Solutions

The study exclusively used analytical-grade chemicals and reagents. Aqueous solutions were made using ultrapure water (resistivity = 18.2 MΩ) sourced from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chemicals such as methacrylic acid (MAA), 4,4'-azobis (4-cyanopentanoic acid) (ACPA), hydrofluoric acid (HF, 49%), ethylene glycol dimethacrylate (EDGMA), iron(III) chloride hexahydrate ($FeCl_3.6H_2O$), iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$), perchloric acid (HClO, 70%), pure silica mesoporous MCM-48, ammonium hydroxide (NH_4OH, 28%), boric acid (H_3BO_3) and hexadimethrine bromide (HDMB) were purchased from Sigma Aldrich (Sigma Chemical Co., St Louis, MO, USA). Ethanol, methanol (MeOH), acetonitrile (ACN), and ethyl acetate with the highest HPLC grade of purity and di-sodium tetraborate decahydrate ($Na_2[B_4O_5(OH)_4].8H_2O$) were obtained from Merck (Darmstadt, Germany). Other chemicals such as 5-fluorouracil (5-FU, ≥99% HPLC) and 5-bromouracil (5-BrU, 98%), tegafur (98%, HPLC), and uridine were all purchased by Sigma Aldrich (Sigma Chemical Co., St Louis, MO, USA). Sodium hydroxide (NaOH) pellets were purchased from QREC (ASIA) Sdn Bhd (Selangor). Prior to CE analysis, all solutions were passed through a 0.45 m nylon filter. Stock solution of 100 mM di-sodium tetraborate

decahydrate, $Na_2[B_4O_5(OH)_4].8H_2O$ and 1 M boric acid, H_3BO_3 was prepared for all field-amplified sample injection-capillary zone electrophoresis (FASI-CZE) separation. The running buffer's pH was adjusted using H_3BO_3, then diluted to 25 mM in 10 mL and the solution should be passed through a 0.45 µm nylon syringe filter prior to utilization. Stock solutions of 5-FU and 5-bromouracil (5-BrU, internal standard) at 1000 ppm were made by dissolving 0.0254 g in a 25 mL volumetric flask for each.

2.2 CE instrument conditions

CE experiments were performed with an Agilent CE system (7100, Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector (DAD). Electrophoretic separations were accomplished using a 64.5 cm polyimide-coated fused-silica capillary with an effective length of 56.0 cm. The capillary was placed at a temperature of 25°C. Two wavelengths of 234 nm and 270 nm were performed on the analyte for the detection of UV absorption using UV-DAD. Before use, the new capillary was pretreated with 1 M NaOH at 950 mbar for 60 minutes, Milli-Q water for 20 minutes, and borate buffer (pH 9.3) only as BGE for 10 minutes. At the beginning of the day, the capillary was flushed with Milli-Q water for 5 minutes to ensure the cleanliness of both the inlet and outlet electrodes. The capillary was further conditioned with NaOH (0.1M) for 10 minutes, again with water (10 minutes), with 1% w/v HDMB (20 minutes), and lastly with 20 minutes BGE (added with diluted HDMB [0.1% w/v]). Between each run, the capillary was rinsed with BGE (added with 0.1% w/v HDMB) for 5 minutes to ensure that the capillary wall was still coated with HDMB surfactant before sample injection. The electropherograms were controlled by Agilent ChemStation software. FASI was performed by introducing the sample into the capillary using electrokinetic injection (EKI) at -5 kV for 5 s, using -25 kV separation voltage and BGE (borate buffer with pH 9.3). The standard solutions were prepared in a 10x diluted borate BGE buffer solution containing 20% v/v of organic modifier used as a sample matrix to ensure the ionization of 5-FU and its internal standard of 5-bromouracil (5-BrU). The FASI-CZE method was carefully fine-tuned across three parameters (sample injection time, background electrolyte concentration in sample, and BGE concentration) to boost sensitivity and cut down analysis time. The optimized conditions were chosen based on the highest peak height ratio of the analytes.

2.3 Preparation of MHPMIP

The processes involved in the preparation of MHPMIP were: (1) synthesis of HPMIP and (2) immobilization of Fe_3O_4 on HPMIP. Initially, the HPMIP was made using a two-step process that involved the precipitation polymerization of MIP onto the interior surface of MCM-48 nanospheres, followed by the removal of the 5-FU template and MCM-48 silica matrix. The HPMIP was produced with a template, functional monomer, and crosslinker agent ratio of (1:8:10/5-FU:MAA:EGDMA). The template and functional monomer were dissolved in a porogenic solvent (ACN/MeOH) solution in a 100 mL round bottom flask. The mixture was subsequently supplemented with MCM-48, EGDMA, and ACPA. The polymerization mixture underwent 30 min of degassing, followed by 40 min of nitrogen purging. Subsequently, the flask was gently stirred in an oil bath as the temperature gradually increased from room temperature to 60°C over 2 hours, maintaining that temperature for 24 hours. After polymerization, the particles were filtered and repeatedly washed in ethanol to produce a clear supernatant. The particles were thoroughly immersed in 10% HF ethanol solution using a polytetrafluoroethylene (PTFE) beaker for 12 hours to entirely remove the MCM-48 silica matrix and the imprinted molecule 5-FU; the product was identified as HPMIP after filtration and ethanol washing (HF should never be kept in a glass container). To avoid contact with the eyes, skin, and clothing, it must be handled with extreme caution and special PPE. The HPMIP was then dried under vacuum at 50°C for 24 hours. The procedure was followed by the co-precipitation method (adopted from Adlnasab et al. [23] to produce magnetic HPMIP NPs by adding (1:2/Fe^{2+}:Fe^{3+}) on the surface of the HPMIP. In the final step, 2 g of the previously synthesised HPMIP, iron(III) chloride hexahydrate ($FeCl_3.6H_2O$), and iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$) were mixed into a 200 mL mixture of aqueous MeOH (4/1; v/v). The mixture was then homogenized using ultrasonic energy, purged under a nitrogen stream for 20 minutes and stirred at 80°C. 20 mL of NH_4OH was added dropwise while stirring continuously, and the mixtures were continuously agitated for an hour at the same temperature and under a nitrogen stream. The MHPMIP product was then cooled to room temperature and collected using an external magnet. The products were purified using ethanol and ultrapure water, then dried under vacuum at 50 ºC for 24 hours until reaching constant weights. The magnetic hollow porous non-imprinted polymer (MHPNIP), synthesized similarly without the 5-FU template, was used for comparison. Figure 1 illustrates the schematic preparation of MHPMIP.

2.4 MSPE procedure

This work involved the evaluation of the effectiveness of the prepared MHPMIP as sorbent in adsorptive extraction using MSPE and enrichment of 5-FU. The extraction was done optimally using magnetic solid phase extraction (MSPE), involving sorbent addition, vortexing, and removal with a magnet. Before MSPE, wastewater samples were filtered with 0.45 µm nylon membrane filters using a vacuum filtration system. MHPMIP sorbent (50 mg) was first added to a 10 mL spiked wastewater sample solution with 5-FU (5 mg/L) adjusted at pH 7 in a 15 mL conical tube. The samples were vortexed for 2 minutes at 1600 rpm rotational speed, and the MHPMIP sorbent was subsequently separated using an external magnet. After decanting the sample solution, the sorbent was then subjected to a liquid desorption step (ultrasonic-assisted) to desorb the adsorbed 5-FU using 350 uL of MeOH-ethylacetate (25:75, v/v) as desorption solvent using an ultrasonic bath sonicator for 5 minutes. The eluate was extracted with a magnet, dried with nitrogen, and reconstituted with 200 µL of 1.5 mM borate BGE at pH 7 to match the analyte's pH. The desorbed analyte concentration was then measured by CE, with validation including linearity, accuracy, recovery, and sensitivity checks. The method's linearity was assessed by plotting 5-FU peak area ratio against drug concentration and determining the

R^2 value from the regression curve. Repeatability was evaluated by calculating the standard deviation from three replicates for 5-FU analysis and from three consecutive injections of the same sample for instrumental repeatability. Recovery was determined using data from three replicate samples processed with the MSPE method, with analyte percentage in the final extract expressed post pre-concentration step.

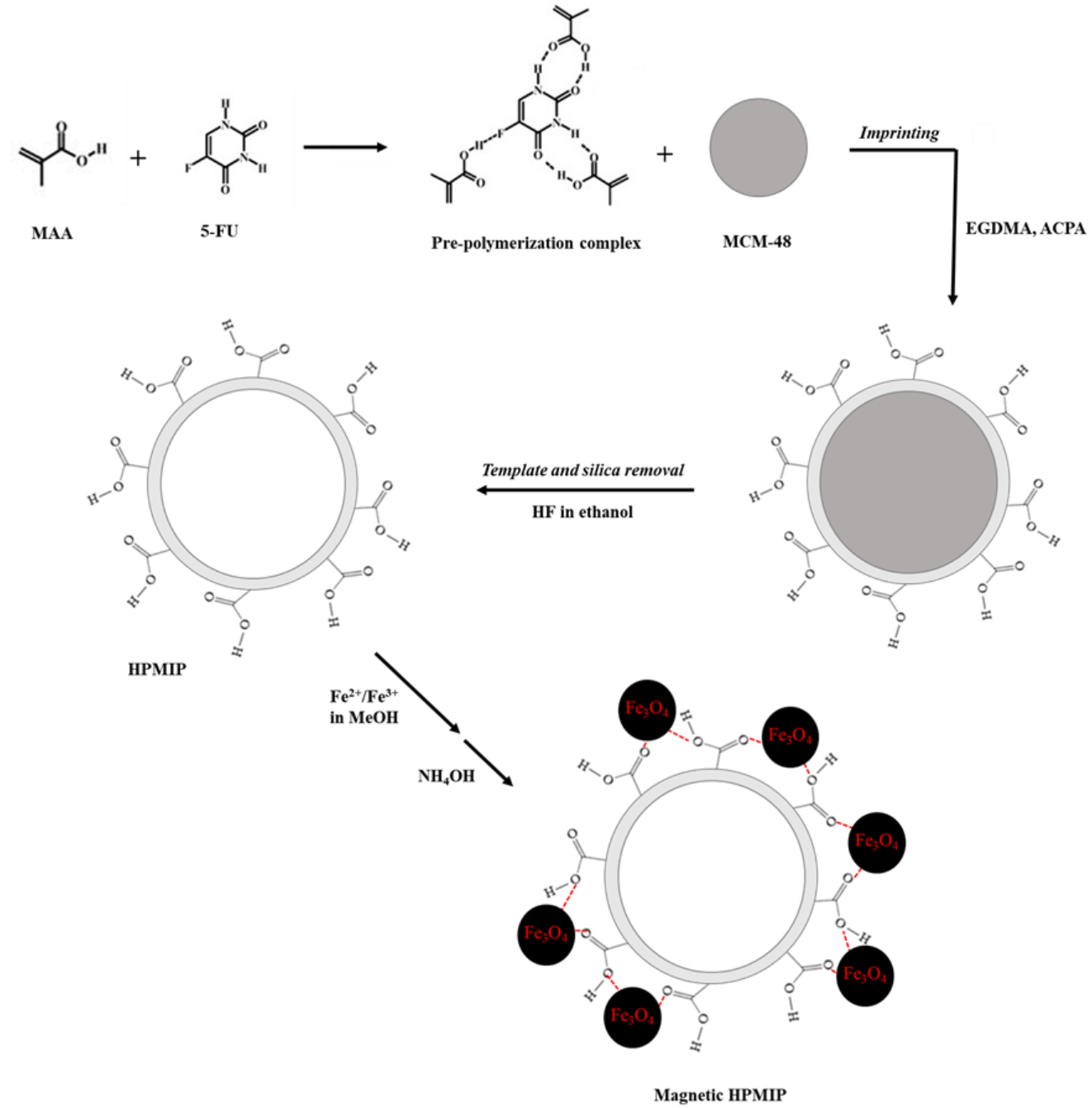

Figure 1 The flow diagram for preparing MHPMIP.

3.0 RESULTS AND DISCUSSION

3.1 Synthesis and characterization of MHPMIP sorbent

In this study, MHPMIP was synthesized via two methods of polymerization: bulk and precipitation polymerization. Initially, the MHPMIP was synthesized using a non-covalent bulk polymerization technique modified by Puoci et al. [25] in the presence of DMF solvent. This method was simple, but it practically needed the end material to be crushed or ground into usable particles (only 30-40% of polymer recovered) prior to the next step. The grinding process yielded unevenly shaped particles, eliminating

some high-affinity binding sites and substituting them with low-affinity ones. [26]. Furthermore, the produced MHPMIP took longer than expected to be removed from the solution, which was not ideal for MSPE. Furthermore, bulk polymerization yielded polymers with uneven binding site distribution, greatly limiting the use of MIPs in chromatographic adsorption. [27]. Besides, the CE results were likewise unsatisfactory since the peak of 5-FU did not exist. The occurrence of template bleeding of the synthesized material was also deduced when MSPE and CE analysis were conducted; proved by the absence of 5-FU. In contrast, MIP synthesized via precipitation polymerization did not require any crushing or grounding process. This method could directly obtain monodispersed molecularly imprinted spheres without jeopardizing the integrity and stability of recognition sites [28]. Thus, MHPMIP was prepared by precipitation polymerization to overcome all of the drawbacks associated with bulk polymerization. The ratio of the utilized cross-linker agents, MAA, and template 5-FU was key to the polymerization process. This study used ratios of 1:8 (5-FU/MAA) and 4:5 (MAA/EGDMA) as the optimum conditions. The cross-linker plays a significant role in controlling the shape of the polymer matrix, stabilizing the imprinted binding sites, and improving the mechanical stability of the polymer matrix. 10% HF acid-ethanol solution was used to remove the template and silica matrix. Both were effectively removed from the MHPMIP in a short time, a significant feature that could not be achieved when lengthy Soxhlet extraction using a mixture of MeOH-acetic acid (9:1 (v/v)) was utilized.

3.1.1 Attenuated total reflectance-Fourier transform infrared (ATR-FTIR)

The synthesized MHPMIP and its starting materials were characterized using attenuated total reflectance-Fourier transform infrared (ATR-FTIR). The overlaid ATR-FTIR spectra of (a) MHPMIP, HPMIP (non-magnetic), 5-FU, and MCM-48 are shown in Figure 2(a)-(d) respectively.

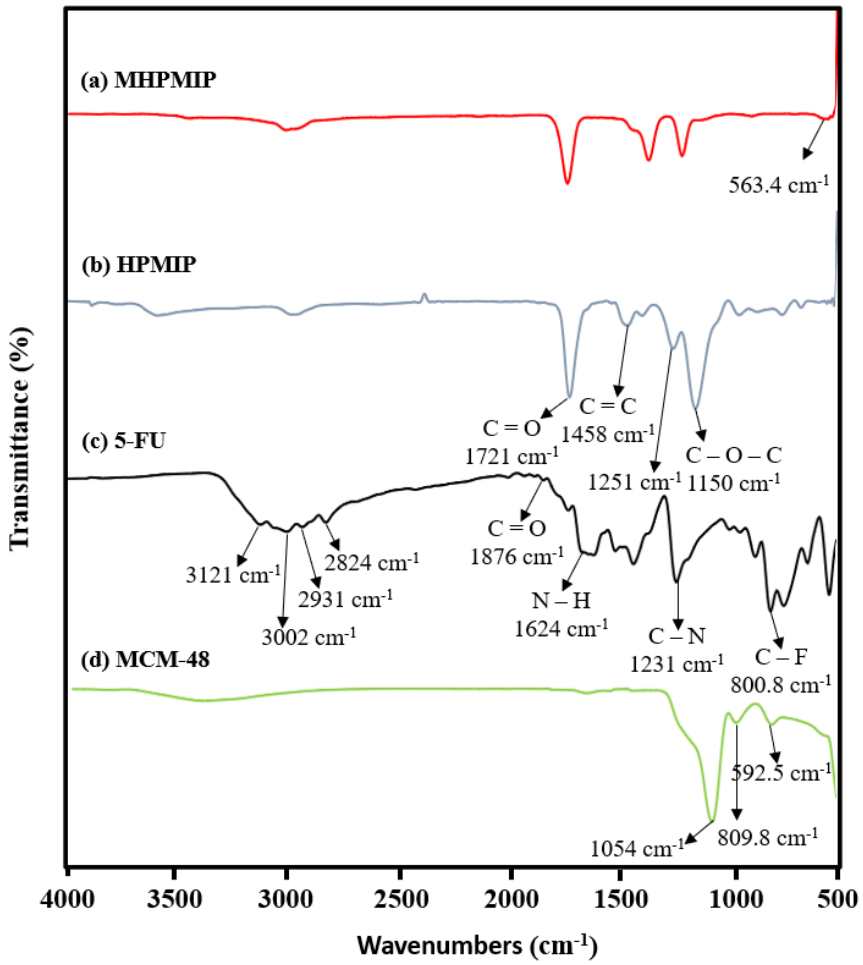

Figure 2 The overlaid ATR-FTIR spectra of (a) MHPMIP, (b) HPMIP (non-magnetic), (c) 5-FU, and (d) MCM-48.

As indicated by the ATR-FTIR spectrum of MCM-48, the typical bands around 1054 and 809.8 cm^{-1} [30] resulted from the Si-O asymmetric and symmetric stretching vibrations. The adsorption peak of Si-O bending vibration was observed at 592.5 cm^{-1}. From the ATR-FTIR spectrum of HPMIP (Figure 2(b)), it could be observed that all those functional groups come from the monomer, MAA, and crosslinker agent, EGDMA. The peaks at 1251 cm^{-1} and 1150 cm^{-1} for C–O–C stretching, along with the

peak at 1731 cm^{-1} for C=O stretching, confirm the successful preparation of HPMIP. This involved using 5-FU as a template, MAA as a functional monomer, and EDGMA as a cross-linker. The disappearance of SiO_2 bands in HPMIP demonstrated the successful removal of the silica matrix of MCM-48 from the polymer. Besides, the absence of characteristic peaks at approximately 3002 cm^{-1}, 1624 cm^{-1}, and 3121 cm^{-1}, which were attributed to the functional groups of amides (N-H stretching and bending bands) and alkene (C-H stretching band) in 5-FU could be seen in the overlaid spectra of 5-FU and HPMIP (Figure 2(b) and (c)). The amine functional group (C-N stretching band) was only observed in the 5-FU spectrum at 1231 cm^{-1}. This finding demonstrated that the silica matrix MCM-48 and 5-FU template were successfully removed, and the HPMIP was formed. The presence of a characteristic band at 563.4 cm^{-1} was observed in the MHPMIP spectrum. This peak was the first sign of the immobilization of magnetic Fe_3O_4 NPs onto the HPMIP, indicating an interaction between the Fe_3O_4 and HPMIP. The VSM test was subsequently conducted to confirm the magnetism of the synthesized material.

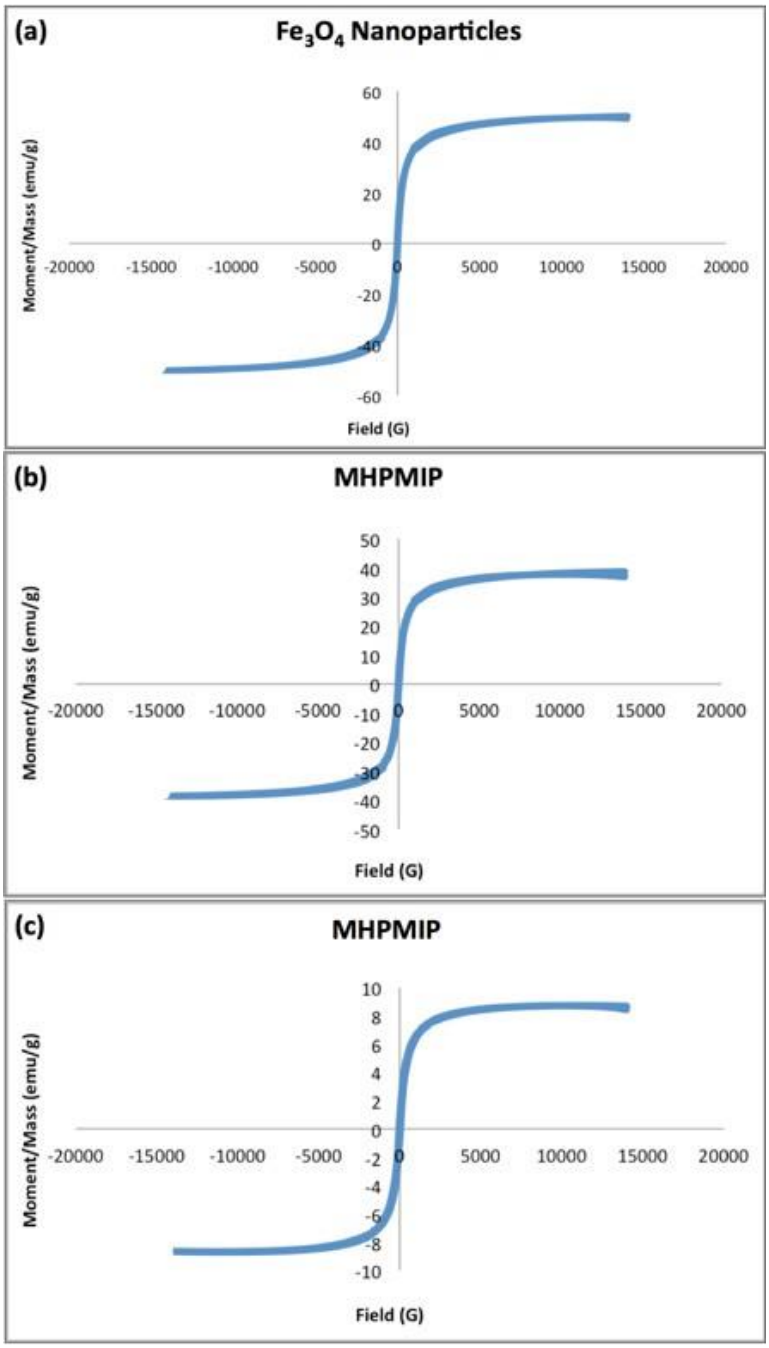

Figure 3 The hysteresis loop of (a) Fe_3O_4 NPs, synthesized MHPMIP (b) by precipitation polymerization, (c) by bulk polymerization.

3.1.2 Vibrating sample magnetometry (VSM)

Both Fe_3O_4 NPs and MHPMIP (prepared by precipitation polymerization) displayed the typical characteristic of superparamagnetic behaviour (Figure 3(a) and (b), with almost immeasurable coercivity and remanence [24]. Individual NPs with significant constant magnetic moments act akin to oversized paramagnetic atoms, swiftly reacting to applied magnetic fields with minimal remanence (residual magnetism) and coercivity (the necessary field to nullify magnetization).[29]. Magnetic Fe_3O_4 NPs had a high saturation magnetization of 50.21 emu/g, indicating a pure Fe_3O_4 crystal structure. The value of saturation magnetization for MHPMIP was found to be 38.57 emu/g. Since the polymeric shell exhibits diamagnetism, having a polymeric coating on the surface of magnetite NPs could potentially diminish the magnetic moment.

3.1.3 Field emission scanning electron microscopy-energy dispersive X-ray (FESEM-EDX)

The materials' structure and elemental composition were analyzed using field emission scanning electron microscopy paired with energy dispersive X-ray (EDX). In this study, the morphological structures of three (3) different materials, i.e., MHPMIP (imprinted and magnetic), MHPNIP (non-imprinted and magnetic), HPMIP (imprinted and non-magnetic) and pure MCM-48, were investigated (as shown in Figure 4(a)-(d)). The cloudy agglomeration of magnetite NPs on the surface of both MHPMIP and MHPNIP was clearly visible (red arrow in Figure 4(a) and (b)), confirming the surface morphology of MHPMIP and MHPNIP. In contrast to the structure of HPMIP, the FESEM image showed clusters of near-sphere MIP shapes (Figure 4(c)), but the structure differed significantly from MHPMIP and MHPNIP due to the absence of magnetite.

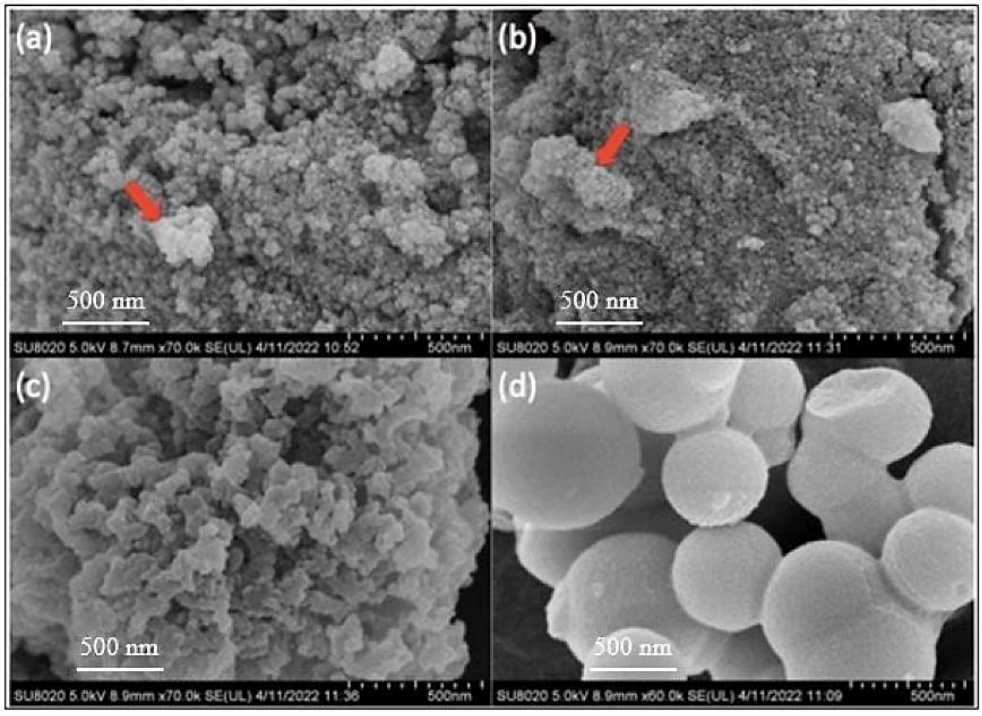

Figure 4 FESEM images of (a) MHPMIP, (b) MHPNIP, (c) HPMIP (non-magnetic), and (d) Pure MCM-48. (The red arrow indicates the cloudy agglomeration of Fe_3O_4 NPs on the surface of the MIP). Magnification: 70K.

FESEM combined with EDX was utilized to confirm the purity and elemental composition of both MHPMIP and HPMIP. (Figure 5(a) and (b)). The EDX results (Figure 5(a)) with the use of platinum coating revealed only the presence of 85.8% and 14.2% of C and O in the HPMIP, respectively. These compounds might be attributed to the functional group from MAA (functional monomer) and EDGMA (crosslinking agent) in the HPMIP. Besides, the presence of Fe, O, C, Cl, and N in the MHPMIP was found to be 34.7%, 33.0%, 20.8%, 8.5%, and 3.0%, respectively (as shown in Figure 5(b)). The highest Fe and O compositions were observed, demonstrating the presence of Fe_3O_4 NPs coating the surface of HPMIP. Furthermore, no peaks of Si or F appeared in either HPMIP or MHPMIP EDX results because they might have been completely removed when treated with an HF-ethanol solution, which was consistent with the reported literature [30]Consequently, the FESEM and EDX results confirmed the formation of hybrid MHPMIP NPs. Hence, the FESEM-EDX result observed here is corroborated by the previous ATR-FTIR

results, indicating that the method used for removing the silica matrix and template was highly effective.

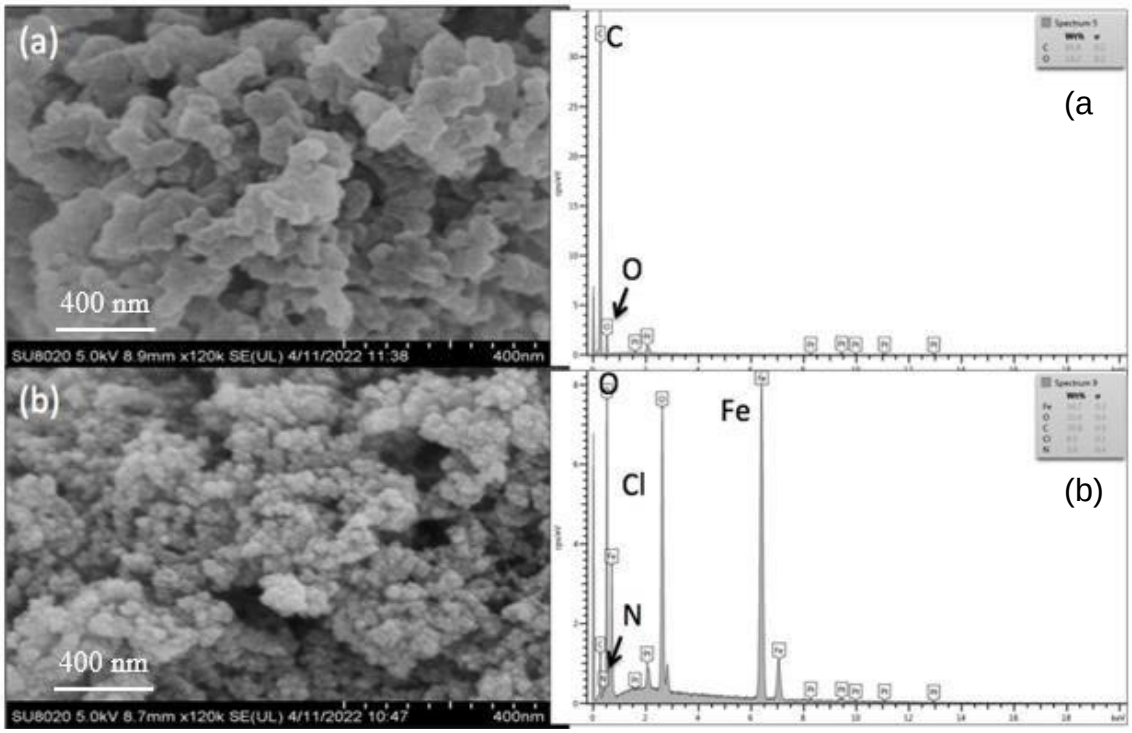

Figure 5 FESEM images and EDX results of (a) HPMIP and (b) MHPMIP (platinum coating was used for EDX analyses). Magnification: 120K

3.1.4 Brunauer-Emmett-Teller (BET) and nitrogen adsorption

The pure MCM-48 had a considerably higher BET surface area outcome with a value of 1293 m^2/g. The spherical MCM-48 exhibits a cubic mesoporous arrangement, boasting both a significant surface area and a finely tuned distribution of pore sizes. The resulting MHPMIP has a BET surface area of 63.47 m^2/g and a pore volume of 0.2325 cm^3/g. The volume of pores for adsorption of MHPMIP and MCM-48 by BJH were 0.2357cm^3/g and 0.9181 cm^3/g, respectively. The outcomes corroborated the findings of a study by Zhang and co-workers [30]. The lower BET surface area of 63.47 m^2/g for MHPMIP was expected due to the existence of Fe_3O_4 attached to the polymer surface, which eventually covers some of the surface area (also as shown in FESEM results Figure 4(a). The isotherm of MHPMIP shows the characteristics of an IV curve. In the IUPAC adsorption isotherm classification, the typical BET range is denoted within the shaded regions of Types II and IV. Type IV denotes mesoporous solids with pore diameters ranging from 2 nm to 50 nm, which confirmed the MCM-48 used in the preparation of MIP. The mean pore diameter measured 2.9 nm, aligning with the thickness of the MCM-48 walls, suggesting successful replication of the MCM-48 template structure by the created HPMIP.

3.2 Optimization of magnetic-solid phase extraction (MSPE) using the synthesized MHPMIP

MSPE optimization was carried out to achieve high selectivity and recovery as well as to obtain the optimal conditions for extracting 5 mg/L of 5-FU. Peak area was chosen as the signal as it allows for a more precise comparison of different optimization parameters conditions, especially when dealing with low concentrations of analytes. Various parameters in the MSPE process were examined to determine their impact on the extraction of 5-FU: sample pH, adsorbent mass, desorption solvent ratio, desorption solvent volume, extraction duration, and desorption duration. Each parameter was studied in triplicates with three times injections for each replicate. These chosen parameters exhibited significant impacts on the response factor.

3.2.1 Effect of pH

The sample solution needs the right basic pH for efficient extraction of the weak acid analyte, 5-FU (pKa 7.3), ensuring it remains uncharged. This boosts the likelihood of transferring 5-FU to the extraction phase by reducing its solubility in the sample solution [31]. Moreover, the carboxylic acid present in the cavities of the MHPMIP has an extremely high hydrophilic affinity for 5-FU

[32]. Hence, the extraction of 5-FU is dependent on its interaction with carboxylic groups of MHPMIP. Changes in pH impact the overall charge of amine groups within the polymer matrix and the active sites of 5-FU. This influences the capacity for template molecule bonding with sorbent amine groups. Testing a sample solution within pH 7 to 12 range showed a slight decrease in peak area from pH 8 to 12 (Figure 6(a)). In acidic conditions, 5-FU's hydroxyl groups can become protonated, reducing template-monomer hydrogen bonding and increasing 5-FU solubility in water. Above pH 10.0, 5-FU deprotonation leads to decreased sorption effectiveness. Consequently, pH 7 was selected for subsequent analyses.

3.2.2 Effect of MHPMIP amount

Figure 6(b) illustrates the impact of varying the adsorbent mass from 50 to 150 mg on the peak area, revealing interesting trends in the performance of the MHPMIP. As depicted in the figure, the peak area exhibits a non-linear response to changes in the adsorbent mass. Specifically, using 100 mg of sorbent results in a noticeable decrease in peak area, while an increase to 150 mg leads to a significant enhancement in the peak area. This could be explained by the possibility that, up to 100 mg, the adsorbent has reached its saturation uptake. Up to 100 mg, the adsorbent appears to effectively capture the analyte, and further mass additions may exceed the optimal loading capacity. This could lead to a performance plateau or decline, possibly due to the analyte desorbing from the extra adsorbent mass. Therefore, in the pursuit of optimal extraction efficiency, a careful balance must be chosen to avoid overloading the adsorbent. Thus, 50 mg sorbent of MHPMIP was used as the optimum mass to extract the 5-FU from the sample solution. At this mass, the adsorbent demonstrates a favorable equilibrium between saturation uptake and efficient desorption, maximizing the extraction efficiency and resulting in an optimal peak area.

3.2.3 Effect on the ratio of desorption solvent

To evaluate the effect of ratio, working solutions with different ratios of 25:75, 50:50, and 75:25 (MeOH-ethyl acetate) were investigated. As shown in Figure 6(c), the highest peak area of 5-FU was observed at a ratio of 25:75 (MeOH-ethyl acetate). Since ethyl acetate is a polar diprotic solvent, it is highly strong to back-extract the analyte from the MIP. Therefore, the optimized desorption solvent for subsequent analyses was chosen to be a 25:75 ratio of MeOH to ethyl acetate.

3.2.4 Effect on the volume of desorption solvent

The impact of varying desorption solvent volumes (ranging from 200 to 500 µL) on the performance of MSPE was investigated (Figure 6(d)). It was noted that increasing the volume of desorption solvent from 200 to 350 µL led to an augmentation in peak area. However, extending the volume beyond 350 to 500 µL resulted in a decline in peak area, likely due to an escalating dilution effect. Consequently, 350 µL of desorption solvent was selected to ensure complete desorption of the retained analyte on the sorbent, as it yielded the highest peak area response for the specific analyte, 5-FU. This volume was subsequently evaporated using a nitrogen stream, and the residue was reconstituted with 200 µL of diluted BGE borate buffer.

3.2.5 Effect of ultrasonication on extraction time

The influence of extraction duration on the efficiency of 5-FU (5 mg/L) extraction using 50 mg of MHPMIP sorbent in a 10 mL sample solution was investigated over a period of 2 to 10 minutes. Figure 6(e) illustrates a notable decrease in peak areas with prolonged extraction time, indicating that the signal diminishes as the MHPMIP surface attains equilibrium. This decline is likely due to agitation-induced desorption of analytes from the adsorbent. Once the absorbent reaches saturation, further extending the extraction time does not yield higher yields. Consequently, 2 minutes emerged as the optimal extraction duration.

3.2.6 Effect of ultrasonication on desorption time

The study investigated how different desorption durations, spanning from 2 to 15 minutes, affected the outcome (see Figure 6(f)). Noteworthy is the significant increase in peak area from 2 to 5 minutes. However, after the 5-minute mark, there was a gradual decrease in peak area, likely due to solvent evaporation and analyte depletion [33]. Consequently, the optimal desorption time for achieving the highest peak area, signifying complete desorption of 5-FU from the adsorbent (ultrasonic-assisted), was determined to be 5 min.

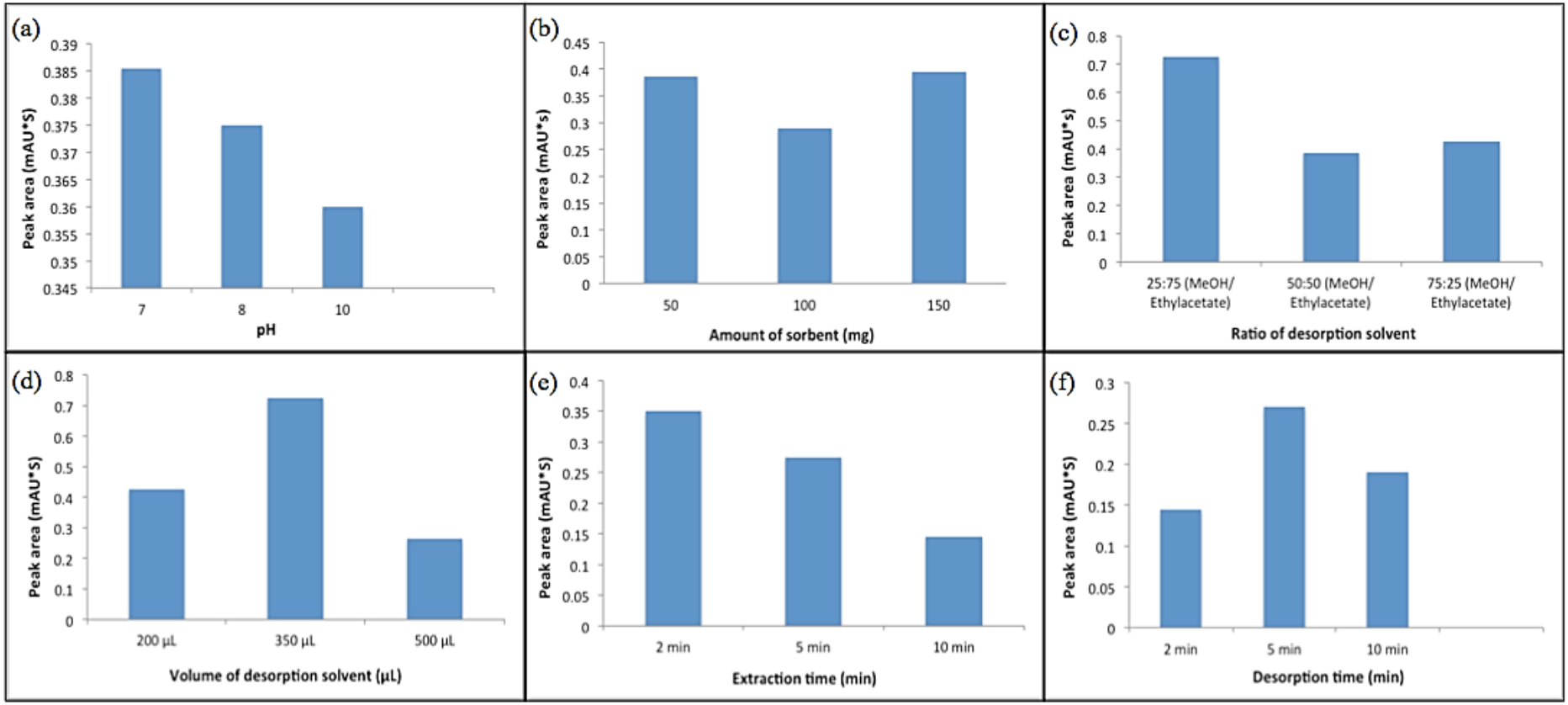

Figure 6 Optimization of the MSPE parameters (a) pH, (b) amount of adsorbent, (c) ratio of desorption solvent, (d) volume of desorption solvent, (e) extraction time, and (f) desorption time. (All the optimizations were carried out in ultrapure water spiked with 5 mg/L of 5-FU). CE conditions: polyimide coated fused-silica capillary (i.d. = 50 µm, *l* = 56 cm, L = 64.5 cm); 15 mM borate BGE (pH 9.3) (added with 0.1% HDMB); separation at 25 ℃ and -25 kV.

3.3 MSPE method validation

Utilizing a multi-point calibration approach necessitates the inclusion of a minimum of three standards to establish a comprehensive calibration curve. In this study, the MSPE method calibration plot was constructed by examining three distinct concentrations of 5-FU solutions: 5 mg/L, 10 mg/L, and 15 mg/L. This range represents potential concentrations of 5-FU in environmental matrices, ensuring the method's applicability to practical situations. The linearity, LODs, and LOQs were calculated using a linear regression curve with a good R^2 value of 0.9973. The performance of the developed MHPMIP-MSPE method for extraction of 5-FU in wastewater samples was evaluated at optimized conditions. The peak for 5-FU appeared at a migration time of 3.48 minutes, which separated within 5 minutes of analysis time. The percent recovery (%) was obtained with triplicate assays at a 5 mg/L concentration of 5-FU spiked in real samples. The highest recovery was obtained at 105.7% with an accuracy error of 0.6897 at a lower concentration level of 5-FU. The LOD and LOQ for the concentration of 5-FU at 5 mg/L (based on S/N = 3, n = 3) obtained for MSPE was 0.60 mg/L and 1.125 mg/L respectively. While the achieved detection limit may be considered suboptimal, the method remains practical and applicable, particularly in scenarios where the concentration of 5-FU in real samples is anticipated to be elevated.

4.0 CONCLUSION

A molecularly imprinted magnetic SPE (MSPE) technique using MHPMIP was developed using CE coupled with FASI-CZE protocol. The efficacy of the MHPMIP synthesized through precipitation polymerization along with surface imprinting was assessed for extracting the anticancer medication, 5-FU, from wastewater samples. The molar ratio of template/monomers played an important role in the pre-polymerization stage for the formation of binding cavities in MIP. The performance of the developed MHPMIP-MSPE method for extraction of 5-FU in wastewater samples was evaluated under optimum conditions. Coupled with FASI-CZE, the MSPE technique developed using the MHPMIP as sorbent showed that it could extract 5-FU from wastewater samples at low ppm levels. This is beneficial for samples having low to mid-ppm levels of 5-FU with severe matrix interferences. Additionally, the new FASI-CZE-UV would be useful for quantitative screening, complementing the more advanced but comparably more expensive HPLC or GC with MS/MS. This research aimed to analyze 5-FU in wastewater samples using CE due to limited literature on the topic.

Acknowledgment

A.S.A.K is grateful for the financial support of the work by the Ministry of Higher Education Malaysia (MOHE) and Universiti Teknologi Malaysia via Fundamental Research Grant Scheme (registration number: FRGS/1/2022/STG04/UTM/02/1, cost centre number: R.J130000.7854.5F528, reference number: PY/2022/03336).

References

[1] Wormington, A.M., De María, M., Kurita, H.G., Bisesi Jr, J.H., Denslow, N.D., Martyniuk, C.J., 2020. Antineoplastic Agents: Environmental Prevalence and Adverse Outcomes in Aquatic Organisms, Environmental Toxicology and Chemistry, 39(5), 967-985.

[2] Mullot, J.-U., Karolak, S., Fontova, A., Huart, B., Levi, Y., 2009. Development and Validation of a Sensitive and Selective Method Using Gc/Ms-Ms for Quantification of 5-Fluorouracil in Hospital Wastewater, Analytical and Bioanalytical Chemistry, 394(8), 2203-2212.

[3] Białk-Bielińska, A., Mulkiewicz, E., Stokowski, M., Stolte, S., Stepnowski, P., 2017. Acute Aquatic Toxicity Assessment of Six Anti-Cancer Drugs and One Metabolite Using Biotest Battery – Biological Effects and Stability under Test Conditions, Chemosphere, 189, 689-698.

[4] Serna-Galvis, E.A., Silva-Agredo, J., Botero-Coy, A.M., Moncayo-Lasso, A., Hernández, F., Torres-Palma, R.A., 2019. Effective Elimination of Fifteen Relevant Pharmaceuticals in Hospital Wastewater from Colombia by Combination of a Biological System with a Sonochemical Process, Science of The Total Environment, 670, 623-632.

[5] Zounr, R.A., Khuhawar, M.Y., Khuhawar, T.M.J., Lanjwani, M.F., Khuhawar, M.Y., 2022. GC Determination of Fluorouracil in Serum by Using Hexafluroroacetylacetone as Derivatizing Reagent, Journal of Chromatographic Science, 60(5), 409-413.

[6] Ebratkhahan, M., Zarei, M., Babaei, T., Hosseini, M.G., Hosseini, M.M., Fathipour, Z., 2022. Efficient Electrochemical Removal of 5-Fluorouracil Pharmaceutical from Wastewater by Mixed Metal Oxides Via Anodic Oxidation Process, Chemosphere, 296, 134007.

[7] Youssef, S.H., Afinjuomo, F., Song, Y., Garg, S., 2021. Development of a Novel Chromatographic Method for Concurrent Determination of 5-Fluorouracil and Cisplatin: Validation, Greenness Evaluation, and Application on Drug-Eluting Film, Microchemical Journal, 168, 106510.

[8] Gopinath, P., Veluswami, S., Thangarajan, R., Gopisetty, G., 2018. Rp-Hplc-Uv Method for Estimation of Fluorouracil–Epirubicin–Cyclophosphamide and Their Metabolite Mixtures in Human Plasma (Matrix), Journal of Chromatographic Science, 56(6), 488-497.

[9] Shiokawa, R., Lee, X.-P., Yamada, M., Fujishiro, M., Sakamaki, H., Hasegawa, C., Ishida, H., Ikeda, K., Fujita, K.-i., Iwabuchi, S., Onda, H., Kumazawa, T., Sasaki, Y., Sato, K., Matsuyama, T., 2019. High-Throughput Method to Analyze Tegafur and 5-Fluorouracil in Human Tears and Plasma Using Hydrophilic Interaction Liquid Chromatography/Tandem Mass Spectrometry, Rapid Communications in Mass Spectrometry, 33(24), 1906-1914.

[10] Kosjek, T., Heath, E., 2011. Occurrence, Fate and Determination of Cytostatic Pharmaceuticals in the Environment, TrAC Trends in Analytical Chemistry, 30(7), 1065-1087.

[11] Nussbaumer, S., Bonnabry, P., Veuthey, J.-L., Fleury-Souverain, S., 2011. Analysis of Anticancer Drugs: A Review, Talanta, 85(5), 2265-2289.

[12] Kartsova, L.A., Bessonova, E.A., 2009. Preconcentration Techniques in Capillary Electrophoresis, Journal of Analytical Chemistry, 64(4), 326-337.

[13] Tang, S., Zhang, H., Lee, H.K., 2016. Advances in Sample Extraction, Analytical Chemistry, 88(1), 228-249.

[14] Madikizela, L.M., Tavengwa, N.T., Chimuka, L., 2018. Applications of Molecularly Imprinted Polymers for Solid-Phase Extraction of Non-Steroidal Anti-Inflammatory Drugs and Analgesics from Environmental Waters and Biological Samples, J Pharm Biomed Anal, 147, 624-633.

[15] Pietrogrande, M.C., Demaria, G., Russo, M., 2023. Determination of Particulate Polycyclic Aromatic Hydrocarbons in Ambient Air by Gas Chromatography-Mass Spectrometry after Molecularly Imprinted Polymer Extraction, Journal of Environmental Sciences, 124, 644-654

[16] Fu, Y., Pessagno, F., Manesiotis, P., Borrull, F., Fontanals, N., Maria Marcé, R., 2022. Preparation and Evaluation of Molecularly Imprinted Polymers as Selective Spe Sorbents for the Determination of Cathinones in River Water, Microchemical Journal, 175, 107100.

[17] Marć, M., Panuszko, A., Namieśnik, J., Wieczorek, P.P., 2018. Preparation and Characterization of Dummy-Template Molecularly Imprinted Polymers as Potential Sorbents for the Recognition of Selected Polybrominated Diphenyl Ethers, Analytica Chimica Acta, 1030, 77-95.

[18] [18] Zhao, Y., Bi, C., He, X., Chen, L., Zhang, Y., 2015. Preparation of Molecularly Imprinted Polymers Based on Magnetic Carbon Nanotubes for Determination of Sulfamethoxazole in Food Samples, RSC Advances, 5(86), 70309-70318.

[19] Dai, X., Wang, D., Li, H., Chen, Y., Gong, Z., Xiang, H., Shi, S., Chen, X., 2017. Hollow Porous Ionic Liquids Composite Polymers Based Solid Phase Extraction Coupled Online with High Performance Liquid Chromatography for Selective Analysis of Hydrophilic Hydroxybenzoic Acids from Complex Samples, Journal of Chromatography A, 1484, 7-13.

[20] Attallah, O.A., Al-Ghobashy, M.A., Ayoub, A.T., Nebsen, M., 2018. Magnetic Molecularly Imprinted Polymer Nanoparticles for Simultaneous Extraction and Determination of 6-Mercaptopurine and Its Active Metabolite Thioguanine in Human Plasma, Journal of Chromatography A, 1561, 28-38.

[21] Shan, S., Ma, Y., Sun, C., Guo, X., Zheng, H., Xu, X., Qin, L., Hu, J., 2021. A Novel Magnetic Solid-Phase Extraction Method for Detection of 14 Heterocyclic Aromatic Amines by Uplc-Ms/Ms in Meat Products, Food Chemistry, 337, 127630.

[22] Rocío-Bautista, P., Pino, V., Extraction Methods Facilitated by the Use of Magnetic Nanoparticles, Analytical Separation Science, pp. 1681-1724. https://doi.org/https://doi.org/10.1002/9783527678129.assep061.

[23] Adlnasab, L., Ezoddin, M., Shojaei, R.A., Aryanasab, F., 2018. Ultrasonic-Assisted Dispersive Micro Solid-Phase Extraction Based on Melamine-Phytate Supermolecular Aggregate as a Novel Bio-Inspired Magnetic Sorbent for Preconcentration of Anticancer Drugs in Biological Samples Prior to Hplc-Uv Analysis, Journal of Chromatography B, 1095, 226-234.

[24] Kazemi, S., Sarabi, A.A., Abdouss, M., 2016. Synthesis and Characterization of Magnetic Molecularly Imprinted Polymer Nanoparticles for Controlled Release of Letrozole, Korean Journal of Chemical Engineering, 33(11), 3289-3297.

[25] Puoci, F., Iemma, F., Cirillo, G., Picci, N., Matricardi, P., Alhaiqu, F., 2007. Molecularly Imprinted Polymers for 5-Fluorouracil Release in Biological Fluids, Molecules, 12(4), 805-14.

[26] Chen, L., Xu, S., Li, J., 2011. Recent Advances in Molecular Imprinting Technology: Current Status, Challenges and Highlighted Applications, Chemical Society Reviews, 40(5), 2922-2942.

[27] Rezaei, B., Rahmanian, O., 2011. Nanolayer Treatment to Realize Suitable Configuration for Electrochemical Allopurinol Sensor Based on Molecular Imprinting Recognition Sites on Multiwall Carbon Nanotube Surface, Sensors and Actuators B: Chemical, 160(1), 99-104.

[28] Wei, S., Molinelli, A., Mizaikoff, B., 2006. Molecularly Imprinted Micro and Nanospheres for the Selective Recognition of 17β-Estradiol, Biosensors and Bioelectronics, 21(10), 1943-1951.

[29] Lu, A.-H., Salabas, E.L., Schüth, F., 2007. Magnetic Nanoparticles: Synthesis, Protection, Functionalization, and Application, Angewandte Chemie International Edition, 46(8), 1222-1244.

[30] Zhang, X.-B., Li, J., You, B., Yong, G.-P., Tong, H.-W., Liu, S.-M., 2012. Hollow Porous Molecularly Imprinted Polymer Nanosphere for Fast and Efficient Recognition of Bisphenol A, RSC Advances, 2(26), 9778-9780.

[31] Forough, M., Farhadi, K., Molaei, R., Khalili, H., Shakeri, R., Zamani, A., Matin, A.A., 2017. Capillary Electrophoresis with Online Stacking in Combination with Agnps@Mcm-41 Reinforced Hollow Fiber Solid-Liquid Phase Microextraction for Quantitative Analysis of Capecitabine and Its Main Metabolite 5-Fluorouracil in Plasma Samples Isolated from Cancer Patients, Journal of Chromatography B, 1040, 22-37.

[32] Abdouss, M., Azodi-Deilami, S., Asadi, E., Shariatinia, Z., 2012. Synthesis of Molecularly Imprinted Polymer as a Sorbent for Solid Phase Extraction of Citalopram from Human Serum and Urine, Journal of Materials Science: Materials in Medicine, 23(6), 1543-1552.

[33] Musa, M., Wan Ibrahim, W.A., Mohd Marsin, F., Abdul Keyon, A.S., Rashidi Nodeh, H., 2018. Graphene-Magnetite as Adsorbent for Magnetic Solid Phase Extraction of 4-Hydroxybenzoic Acid and 3,4-Dihydroxybenzoic Acid in Stingless Bee Honey, Food Chemistry, 265, 165-172.