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RESEARCH PAPER

Synthesis of a New Magnetic Molecularly Imprinted Micro/Nano Polymer Using 2-Acetamidoacrylic acid as a Monomer for the Extraction and Determination of Amphetamine in Human Urine

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ABSTRACT

This study successfully synthesized a novel magnetic molecularly imprinted Micro/nano polymer (MMIP) for the selective extraction of amphetamine using a surface molecular imprinting approach. Functionalized Fe_3O_4 nanoparticles were employed as magnetic cores, with amphetamine serving as the template molecule. 2-Acetamidoacrylic acid was selected as the functional monomer, tetraethyl orthosilicate (TEOS) as the cross-linker, benzoyl peroxide (BPO) as the initiator, and methanol as the porogenic solvent. The resulting polymers were comprehensively characterized by Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA), and vibrating sample magnetometry (VSM). Adsorption studies revealed that the synthesized MMIPs exhibited pronounced selectivity toward amphetamine compared to the non-imprinted counterparts. The optimized polymers were effectively applied as adsorbents for the extraction and quantification of amphetamine in human urine, achieving recoveries between 90.0 % and 96.9 %. The limits of detection (LOD) and quantification (LOQ) were determined to be 0.0547 mg L^{-1} and 0.182 mg L^{-1}, respectively, based on seven replicate analyses. These findings demonstrate that the prepared MMIPs offer a reliable and efficient platform for the selective extraction and determination of amphetamine in complex biological matrices.

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INTRODUCTION

In recent years, there has been a notable rise in drug use among adolescents, emerging as a substantial issue in many societies.[1] Substances of misuse provide a significant societal danger. [2] The imperative to establish economical, swift, dependable, and sensitive analytical techniques for the identification of drug usage is currently essential for managing drug addiction. Amphetamine is a highly addictive psychostimulant associated with various significant socioeconomic problems globally. Numerous techniques, including gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography-mass

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spectrometry (HPLC-MS), electrochemical assays, and ion mobility methods, have been established and validated for the quantitative analysis of Amphetamine in diverse biological specimens, due to their specificity, sensitivity, and dependable quantification capabilities. Nevertheless, several characteristics of these methods (including bulkiness, multi-step processing, expensive consumables, and maintenance) must be resolved to transform them into a convenient, cost-effective methodology that provides swift sample-to-answer outcomes.[3]

MIPs are a category of specialized affinity materials featuring recognition cavities that match the form, size, and functional groups of template molecules [4] Molecularly imprinted polymers (MIPs) can be synthesized using traditional techniques like bulk polymerization, precipitation polymerization, and emulsion polymerization .The manufacture of MIPs involved a copolymerization reaction between template molecules and functional monomers through non-covalent or covalent interactions. Thereafter, the monomers and crosslinking agents undergo polymerization around the template to form a hyper-cross linked polymer network. Selective binding sites are formed by eliminating the template. MIPs may be chosen as an appropriate option for the separation and preconcentration of complex matrices due to their advantageous characteristics, including affordability, straightforward synthesis, superior selectivity and sensitivity, remarkable reusability, and high resilience to experimental conditions [5]. Molecularly imprinted polymers (MIPs) have recently advanced significantly; however, notable limitations persist in their practical application. In highly cross-linked polymers, the elution of target molecules is hindered by increased viscosity, leading to a marked decrease in the selectivity and specificity of MIPs, which adversely affects separation and purification efficacy [6]. Magnetic molecularly imprinted polymers (MMIPs) have garnered considerable interest from researchers, as they present a potential remedy to these challenges. MMIPs not only retain the advantages of MIPs but also exhibit enhanced dispersion stability, facilitate magnetic separation, and possess modifiable chemical surfaces [7]. MMIPs enable rapid separation through the application of an external magnetic field. MMIPs can be acquired using multiple ways; the typical synthesis procedure normally comprises four phases. The initial step involves synthesizing magnetic nanoparticles (MNPs), followed by the surface modification of the magnetic components. The third step entails polymerization using functional nanoparticles as the magnetic core, in the presence of template molecules, functional monomers, and a cross linker. The final step consists of extracting the template molecules from the polymer.in this study A novel and precise method was developed for the extraction of amphetamine from human urine using Molecularly Imprinted Magnetic Polymers (MIMPs). This technique offers a fast and highly selective approach for the efficient isolation of amphetamine, ensuring superior extraction performance. Additionally, it reduces the need for complex procedures or high costs, making it a practical and effective solution.

MATERIALS AND METHODS

Materials

Amphetamine (AMP) was provide from the medico legal institution (Baghdad ,Iraq) (2-Acetamidoacrylic acid was purchased from Themo Fisher SFcientific /India).Tetraethyl orthosilicate(TEOS) from Sigma Aldrich, benzoyl peroxide was purched from General Durg House ,Daryaganj New Delhi (INDIA) ,Methanol was purched from Chem Lab NV(Belgium) Acetic acid Glacial from Alpha Chemika (INDIA),,Fe_3O_4(Sigma –A-ldrich), Sodium Hydroxide(NaOH)Pancreac Spin .Hydrochloric Acid (HCl),CDH India.

Instruments

Monitoring of the analysis was performed using FTIR- Smart iTX-thermo scientific Nicolet iS20 wer ,UV-VIS (shimadza UV-spectroscopy 1800 pc(Japan) ,Microwave reactor,TGA(SDT Q600 V20.9 Build 20, University of Kashan) ,Field Emission Scanning electron microscope(SEM), Vsm(University of Kashan),XRD(Laboratory-University of Kashan).

Procedures

Synthesis of Fe_3O_4@SiO_2

A total of 2.16 mmol of Fe_3O_4 nanoparticles was dispersed in 80 mL of a methanol–water mixture. Subsequently, 20 mL of distilled water and 4 mL of tetraethyl orthosilicate (TEOS) were added to the suspension, which was stirred continuously for 8 hours at ambient temperature. The resulting product was isolated, washed several times with distilled water to remove any unreacted residues, and then dried under vacuum at 45 °C to yield

Fe_3O_4@SiO_2 nanoparticles [8].

Synthesis of Fe_3O_4@SiO_2@AMP-MMIP and Fe_3O_4@SiO_2@AMP-MNIP

A quantity of 0.8 g of Fe_3O_4@SiO_2 was dispersed in 100 mL of methanol, followed by the sequential addition of 4 mmol of amphetamine (template molecule), 16 mmol of 2-acetamidoacrylic acid (functional monomer), 8 mL of tetraethyl orthosilicate (TEOS) as the cross-linker, and 0.7 mmol of benzoyl peroxide (BPO) as the initiator. The resulting suspension was stirred for 1 hour at room temperature and then transferred to a 150 mL round-bottom flask, which was placed into a microwave reactor equipped with a rotor. Microwave-assisted polymerization was carried out at a power of 80 W and a stirring speed of 120 rpm for 15 minutes. Upon completion, the polymer was repeatedly washed with a 5:1 (v/v) mixture of methanol and glacial acetic acid to remove the template molecule, and then dried to yield the magnetic molecularly imprinted polymer, a non-imprinted polymer was prepared under the same condition without adding Amphetamine and recorded as Fe_3O_4@SiO_2@ MNIP [9].

Binding Experiments of AMP-MMIP and MNIP

Static equilibrium experiments were carried out by mixing 0.01 g of AMP-MMIP with 50 mL Amphetamine solution the mixture were stirred for specified time then filtered and determined the Amphetamine content in this solution by using the uv-spectoscopy at 256 nm .And the effects of Adsorbent weight on Adsorption(0.01-0.05 g) ,different time(10-120 min),temperatures(25-45°C), and pH values(3-9) on MMIP adsorption were studied .the absorption capacity of the AMP-MMIP was calculated by the following equation[10]:

$$qe = Ci - Ce \times v / m \quad (1)$$

Where, qe ($mg.g^{-1}$) is the equilibrium adsorption, Ci ($mg.L^{-1}$) represent the initial concentration, Ce($mg.L^{-1}$) the equilibrium concentration of Amphetamine, v(L) volume of AMP-solution and m(g) the weight of AMP- MMIP.

In The adsorption Kinetic experiments , Fe_3O_4@SiO_2@AMP-MMIP(0.01g) was added in to AMP solution (5) mL with a concentration of 100 $mg.L^{-1}$ and incubated at 25 Cº for different time intervals .After incubation the AMP-MMIP was separated by a magnet and the AMP content in supernatant solution were determined by uv-vis.spectroscopy.

Selectivity of the AMP-MMIP

To assess the selectivity of the MMIP, several drugs, including Heroin (Her), Amphetamine (AMP), Artane (ART) and Pregabalin (pre), were evaluated. The experiment involved the addition of 0.01 g of the AMP-MMIP was distributed into four distinct conical flasks. Subsequently, 5 mL of Artane, Heroin, Pregabalin, and Amphetamine, each at a concentration of 35 $mg.L^{-1}$were added accordingly. The flasks were agitated at ambient temperature for contact time. After the shaking period, the solutions were separated by using magnets. The concentration of the compounds were then determined using UV-VIS spectroscopy at a maximum wavelength of Amphetamine.

Reusability and stability

The reusability of the magnetic molecularly imprinted polymers of Amphetamine was evaluated under the same experimental conditions. After each extraction cycle, the used MMIPs were regenerated by sequential washing with a mixture of methanol and acetic acid (5:1, v/v), followed by deionized water. The regenerated sorbents were then reused in subsequent extraction cycles to assess their stability and performance over repeated use.

Extraction procedure

Urine was collected from healthy volunteers(males ,around 20-year old).stock standard solution of Amphetamine were prepared in water and methanol (9:1 v/v) .standard solution were prepared by adding (2 mL, 4mL, 5mL, 6mL, 9mL) of AMP solution to a 10 mL volumetric flask and the solution was diluted to the mark with urine and vortexed for 3min.then the urine samples spiked by AMP were centrifuge for 20 min at 8000 rpm and then filtered through a cellulose acetate filter paper (0.20 µm pore size, 5 mL of the filtered supernatant was mixed with 0.01g of AMP-MMIP in a(10) mL centrifuge tube, then the mixture was shaken on a platform shaker for (120 min) to reach adsorption equilibrium ,the AMP-MMIP were collected at one side of the centrifuge tube and the evident supernatant was removed with the assist of a magnet .later 1.0 mL of eluting the adsorbed Amphetamine by vortexing for 2 min .then the AMP in the elute was filtrated for

the final analysis.

RESULTS AND DISCUSSION

Magnetic molecularly imprinted polymers (MMIPs) were successfully synthesized for the selective extraction of amphetamine through the use of 2-acetamidoacrylic acid as the functional monomer. This monomer possesses both amide and carboxylic acid groups, which facilitate the formation of hydrogen bonds and electrostatic interactions with the target analytes, thus enhancing molecular recognition and binding specificity. The synthesis process involved two main steps :

Step one: Surface Modification of Magnetic Nanoparticles: Fe3O4 nanoparticles were coated with a silica layer through the use of tetraethyl orthosilicate (TEOS), providing a stable surface and reactive sites suitable for subsequent polymerization. This coating also enhances the colloidal stability and dispersibility of the magnetic core.

Step two: Polymerization and Template Imprinting: The functional monomer (2-acetamidoacrylic acid), cross-linker (TEOS), and the template molecule (amphetamine) were dissolved in methanol. Polymerization was initiated by benzoyl peroxide (BPO) as a radical initiator, resulting in the formation of a polymer layer around the magnetic core. Following polymerization, the template molecules were removed through the use of (5:1) methanol: glacial acetic acid mixture to disrupt the non-covalent interactions and generate recognition sites that are complementary in shape and functionality to the target molecules Fig. 1 illustrates the synthesis process of AMP-MMIP.

The resulting MMIPs exhibited significant binding affinity and selectivity amphetamine, confirming the suitability of 2-acetamidoacrylic acid as an effective functional monomer for imprinting these drug molecules.

The chemical composition of MNIP (A), unwashed AMP-MMIP (B), and washed MMIP(C) was analyzed by FTIR spectroscopy .the FTIR spectrum (Fig. 2A) shows the characteristic peaks associated with chemical structure. A prominent peak at approximately 3400 cm^{-1} signifies the O-H stretching vibration, suggesting the presence of hydroxyl groups. Peaks between 1000-1100 cm^{-1} are ascribed to the Si-O-Si stretching vibrations of the silica framework. Peaks between 400-600 cm^{-1} correspond to Fe-O stretching vibrations from the magnetic Fe_3O_4 core. These

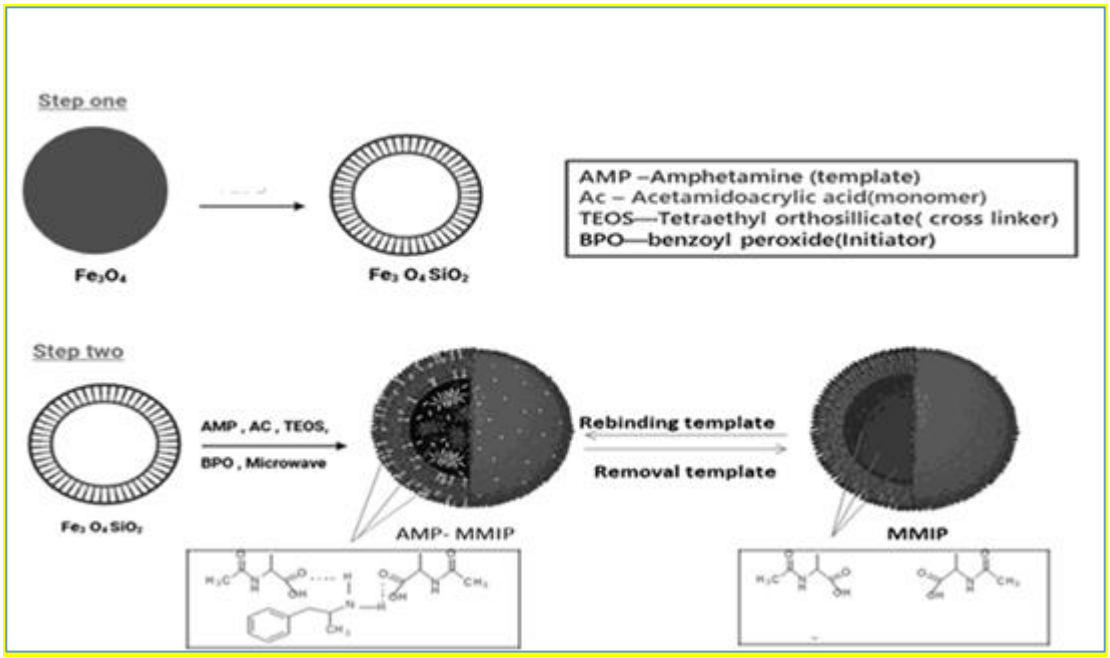

Fig. 1. Synthesis process of AMP-MMIPS.

characteristics validate the effective synthesis of the polymer framework. Fig. 2B, the spectrum exhibits significant alterations in comparison to the (2A): New or enhanced peaks are detected in the 2800-3000 cm^{-1} range, attributed to the C-H stretching vibrations of amphetamine molecules. The peaks associated with amide groups (C=O and N-H stretching) in the range of 1600-1700 cm^{-1} exhibit minor changes or heightened intensity as a result of interactions between the polymer and amphetamine molecules. The data indicates that amphetamine molecules are effectively incorporated into the polymer matrix. Fig. 2C illustrates the FTIR spectrum of the polymer subsequent to the extraction of amphetamine. The spectrum roughly mimics the (2A), albeit with minor variations: The peaks in the 2800-3000 cm^{-1} range corresponding to amphetamine molecules are markedly diminished or nonexistent, signifying effective elimination. The original peaks associated with the polymer framework, including the hydroxyl group at 3400 cm^{-1} and the Si-O-Si and Fe-O bonds, are reinstated. Subtle variations in peak intensities may signify surface alterations resulting from the loading and unloading processes [11،12].

Fig. 3A the structure appears relatively smooth and compact, with some visible porosity. The particle sizes are measured at 39.05 nm, 45.60 nm, and 54.46 nm, indicating Nano scale dimensions. The polymer matrix is homogeneous, indicating a uniform synthesis process[13]. Fig. 3B the structure is more irregular, with larger particle sizes measured at 219.79 nm and 507.07 nm. This

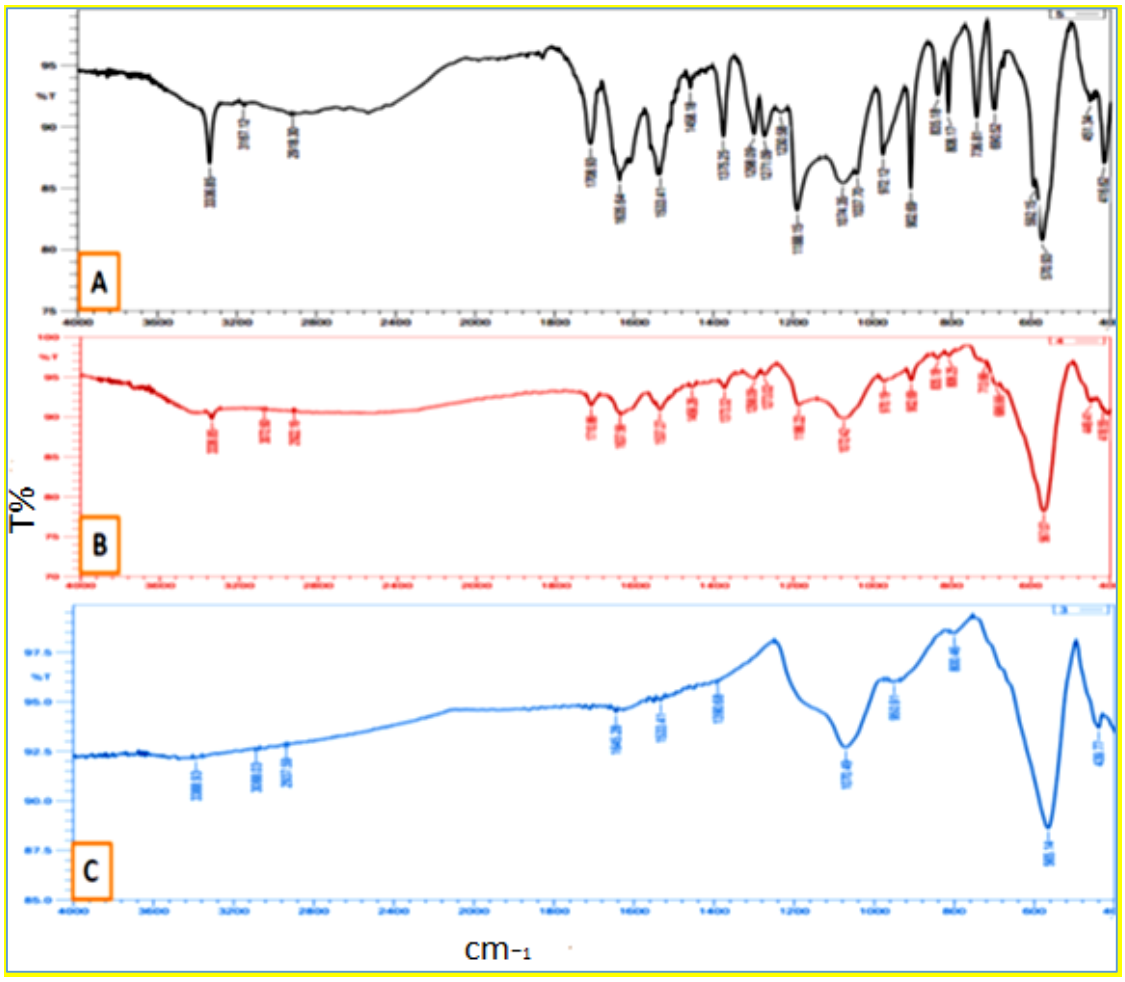

Fig. 2. (A MNIP, (B) un wash AMP-MMIP, and (C) wash AMP- MMIP.

increase in particle size is due to the attachment or incorporation of amphetamine molecules onto/within the polymer matrix. The surface appears rougher compared to Image A, indicating successful loading. Fig. 3C the structure becomes more porous and fragmented compared to the loaded polymer in Image B. The particle sizes decrease to 44.96 nm and 193.54 nm, closer to the original dimensions in Image A. The removal of amphetamine likely created voids or cavities, characteristic of molecular imprinting [14] .

Fig. 4 illustrates an S-shaped hysteresis loop, indicating the magnetic properties of the MNIP. The material demonstrates either super paramagnetic characteristics or modest ferromagnetism. Saturation Magnetization: When the applied magnetic field reaches its peak (about ±10,000 Oe), the magnetization attains a saturation value of around +20 emu/g. This indicates the material's capacity to retain the magnetic field owing to the inclusion of Fe_3O_4 particles in its composition. Coercivity: The curve intersects near the origin with an insignificant coercivity value (Coercivity ≈ 0). This indicates that the material demonstrates super paramagnetic characteristics at a particular temperature. Remanent Magnetization: Following the removal of the magnetic field, the value of remanent magnetization approaches zero. This signifies that the material relinquishes its magnetization with the removal of the external field, a favorable characteristic for biological or environmental applications to prevent magnetic accumulation. The elevated magnetism is ascribed to the presence of Fe_3O_4, a potent magnetic substance. The SiO_2 layer diminishes interactions among magnetic particles, enhancing polymer

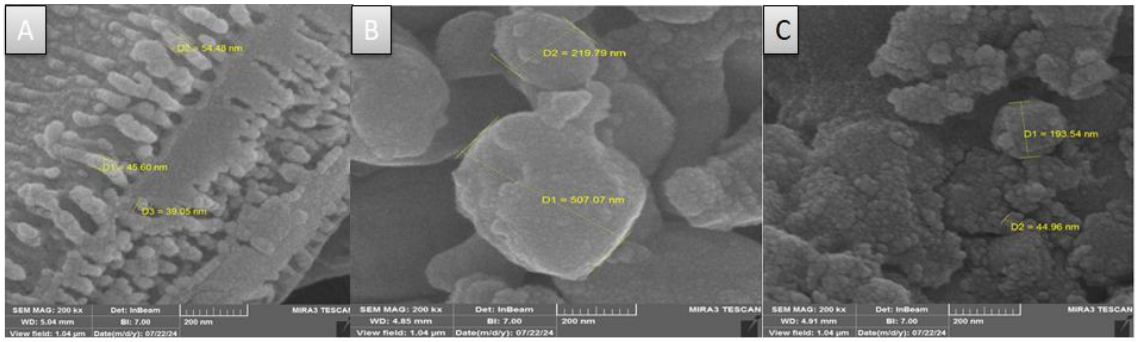

Fig. 3. FESEM images of (A) MNIP (B) AMP-MMIP (C) MMIP.

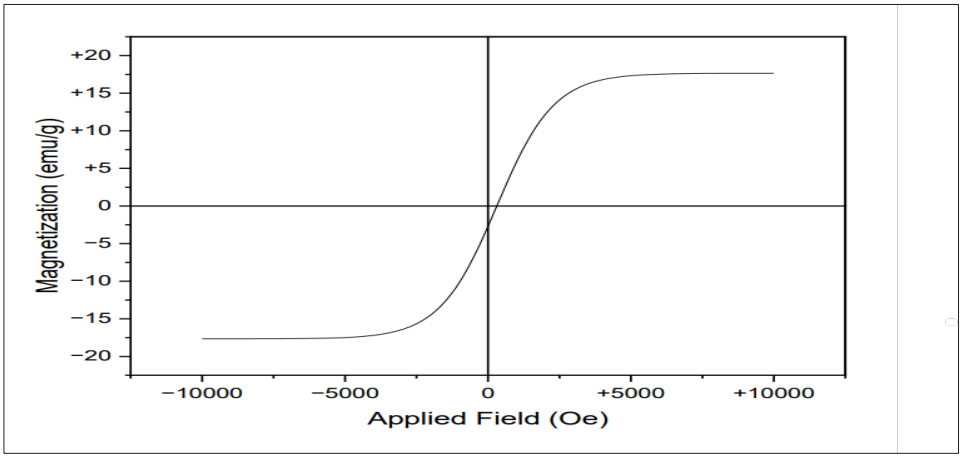

Fig. 4. VSM image of MNIP.

stability and inhibiting agglomeration. This research underscores the material's suitability for applications necessitating transitory magnetization and quick responsiveness to external magnetic fields [15,16].

Nitrogen adsorption-desorption analysis was conducted to examine the specific surface area and porous structure of AMP-MMIP, as determined by the BET equation .The specific surface area, pore volume, and average pore diameter of the MMIP were determined to be 8.9352 [$m^2.g^{-1}$], 0.095316 [$cm^3.g^{-1}$], and 42.67 [nm], respectively.(Fig. 5A) illustrates the adsorption (ADS) and desorption (DES) curves in relation to the partial pressure (P/Pº). The correlation between the two Figures indicates a homogeneous distribution of pores within the polymer substance .Fig. 5BThe linear BET plot exhibits a robust correlation between the experimental data and the linear model. The high correlation coefficient (R^2) clearly demonstrates the dependability and correctness of the analysis. These results validate the appropriateness of

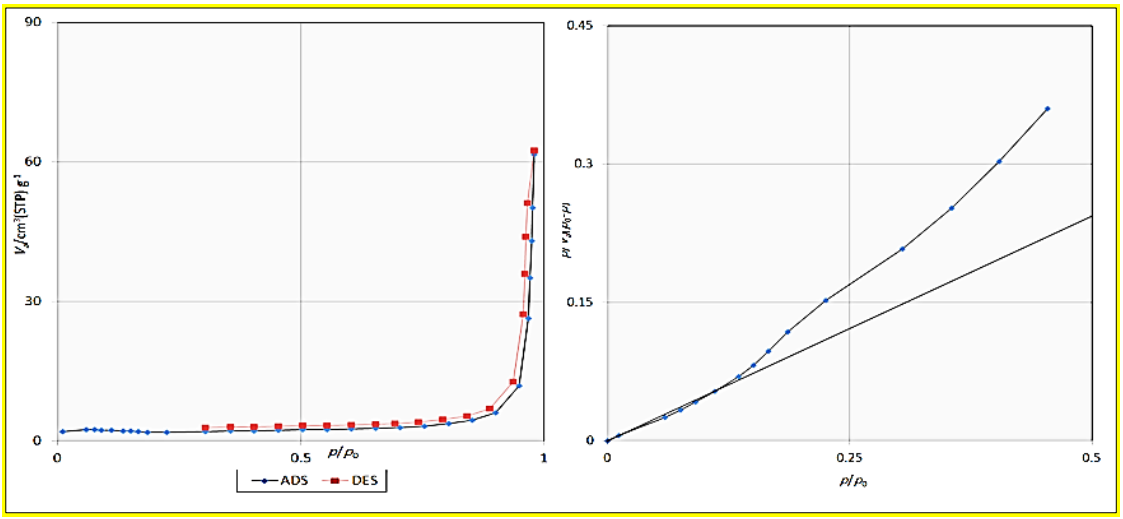

Fig. 5. N2 adsorption-desorption isotherm of the AMP- MMIP.

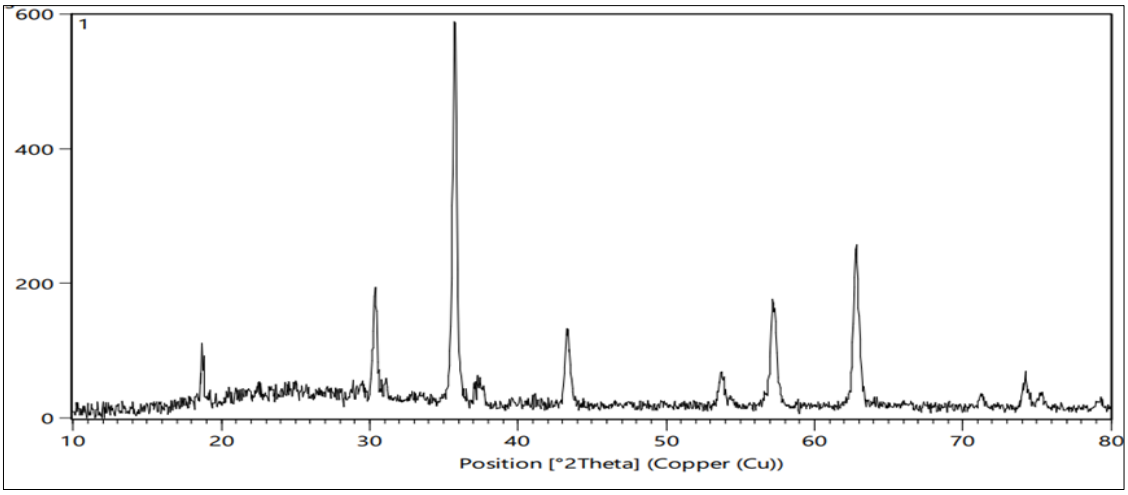

Fig. 6. XRD Spectra of MMNIP.

the BET method for assessing the surface area and porosity of the material. These results were consistent with findings published in other research[17] .

The crystal structure of MNIP was further investigated using XRD (Fig. 6). Clear and pronounced diffraction peaks were observed at 35.5°, 43°, 57°, and 62°, respectively, indicating the presence of a robust and prominent crystal plane, which typically reflects that the crystal structure of the material is in substantial agreement with the standard PDF card for the crystal planes of Fe_3O_4. JCPD Card No. 88-0866[18].

Thermo gravimetric analysis TGA was conducted to assess the thermal stability of the MNIP .Fig. 7 illustrates the weight loss trend of the non-imprinted polymer sample as the temperature rises to 800°C. A minor weight reduction is noted from (0–200°C), attributable to the evaporation of volatile constituents, including absorbed moisture or residual solvents. A notable weight reduction transpires at temperatures between 200 and 500°C,

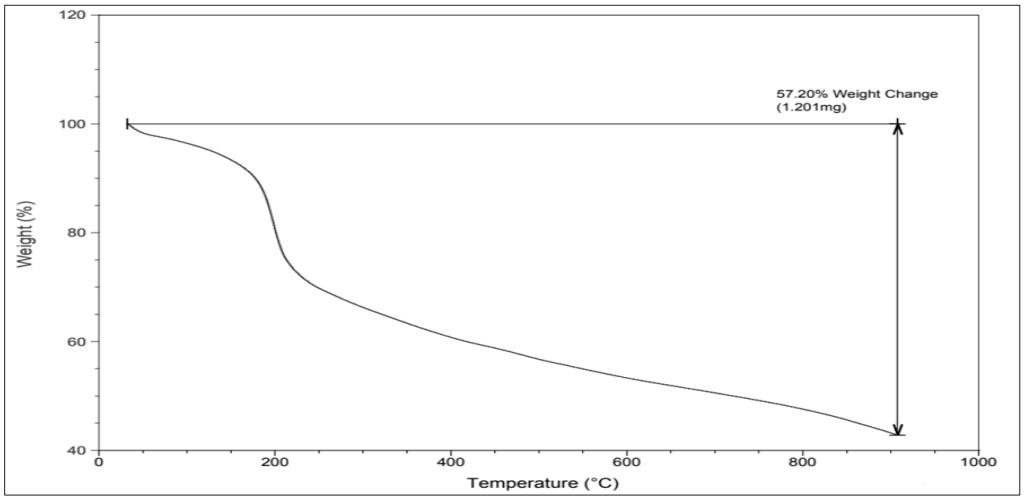

Fig. 7. TGA curve of MNIP.

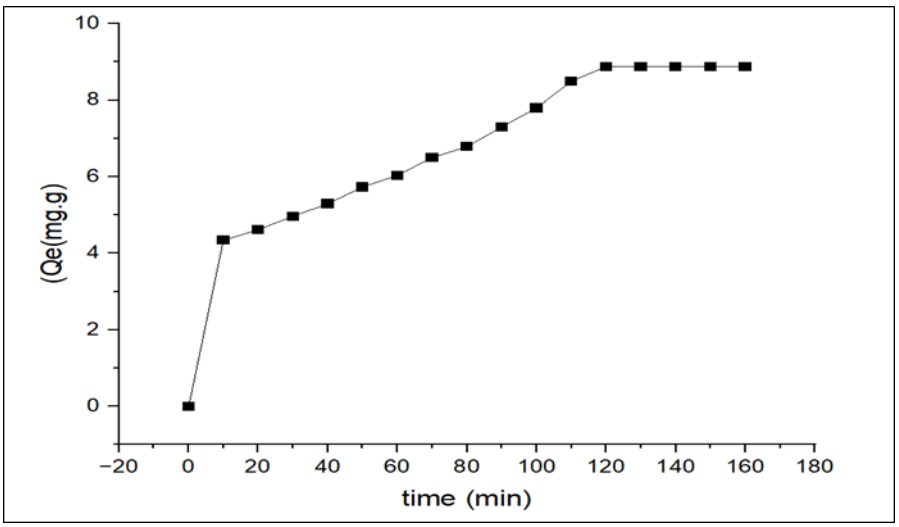

Fig. 8. Effect contact time at 25Cº and 15 $mg.L^{-1}$.

signifying the principal thermal degradation of the polymer chains. The rate of weight loss diminishes at temperatures between 500 and 900°C, indicating the production of thermally persistent carbonaceous residues (char) or the lack of additional decomposable components. The overall weight reduction is 57.20%, as demonstrated in the curve. This indicates that the polymer comprises a substantial quantity of decomposable organic matter, while the remaining 42.80% constitutes non-decomposable inorganic substances or char residues. The primary decomposition transpires between 200–500°C, indicating a moderate thermal stability of the polymer. The residual material indicates the existence of inorganic or thermally stable constituents. This behavior offers insights regarding the material's thermal degradation characteristics and composition [19].

The equilibrium time for the synthesized magnetic molecularly imprinted polymer (MMIP) was examined as an efficient adsorbent for the Amphetamine solution. The experiments were conducted under optimal settings. (Fig. 8) depicts the effect of contact duration (10–180 min) on the adsorption process. The adsorption effectiveness improved over time, reaching equilibrium at (120 min) facilitated by the presence of active sites on the composite's surface [20]. The adsorption process initiated swiftly, as the active sites were readily available for interaction. Upon reaching equilibrium, the adsorption rate decreased due to the saturation of the adsorbent surface with Amphetamine molecules [21].

The impact of the adsorbent surface weight (AMP-MMIP) on the adsorption process from an aqueous Amphetamine solution was investigated using different weights (0.01-0.05 g) and a solution concentration of (15 $mg.L^{-1}$) at a temperature of 25 Cº. Fig. 9 illustrates that when the weight of the adsorbent (w) grows from 0.01 g to 0.05 g, the adsorption capacity (Qe) progressively rises. This suggests that augmenting the weight increases the active surface area of the adsorbent, hence improving its capacity to collect adsorbate molecules. Impact on Adsorption at lower weights (0.01-0.02 g), the augmentation of Qe is very gradual. As the weight escalates to 0.03 g and beyond, the enhancement becomes more pronounced due to augmented contact between the adsorbent and the adsorbate molecules. Maximum Increase in Qe at elevated weights (about 0.05 g), Qe nears saturation, and the rate of rise diminishes. This signifies that the majority of the accessible adsorption sites are occupied

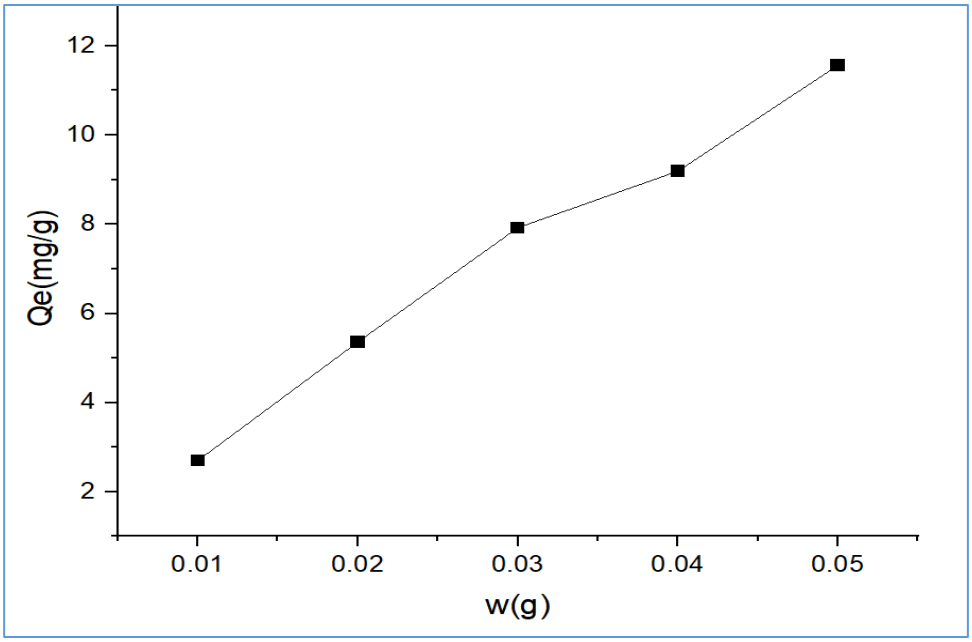

Fig. 9. Effect of weight of AMP-MMIP on the adsorption capacity of Amphetamine solution.

[22, 23].

The sample pH significantly influences the analytical conditions and the acidic or basic functional groups of MMIP. The pH value of the sample was modified from (2.0 to 9.0) to evaluate its impact, as depicted in Fig. 10. Under very acidic conditions (PH 2-4), the carboxyl groups (-COOH) of the MMIP are likely to be protonated, hence diminishing their negative charge. This restricts the electrostatic interactions between the polymer and the amphetamine, resulting in a diminished adsorption capacity (Qe). Furthermore, surplus H^+ ions contend with amphetamine molecules for binding sites. At pH 7 the carboxyl groups of the MMIP are deprotonated ($-COO^-$), augmenting the negative charge of the polymer surface. This amplifies electrostatic interactions with the positively charged amphetamine molecules, attributable to their amine groups. The molecularly imprinted spots on the polymer

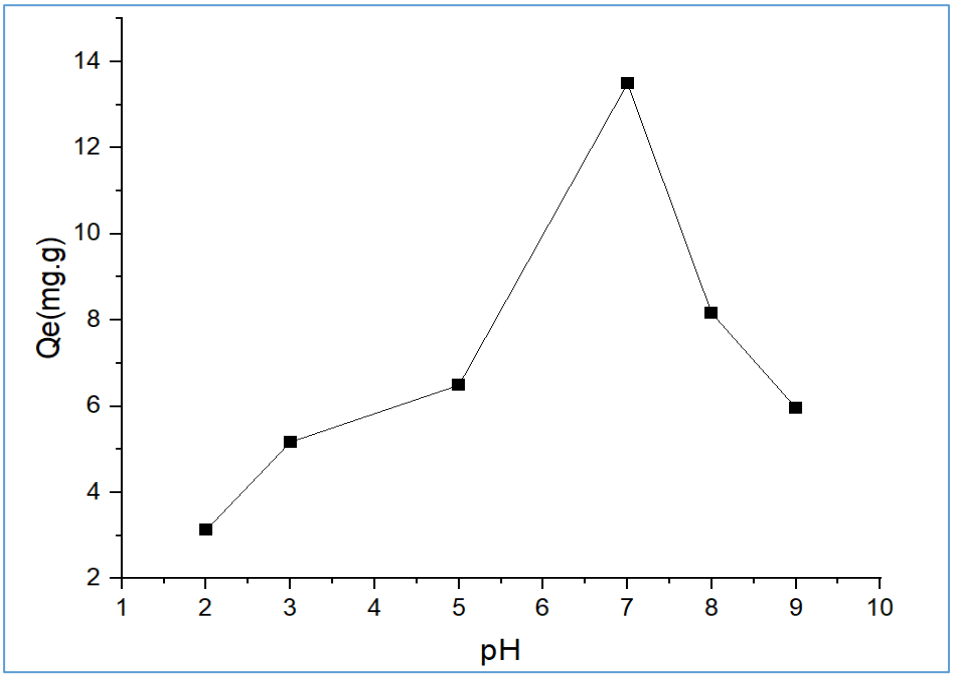

Fig. 10. Effect of pH Solution.

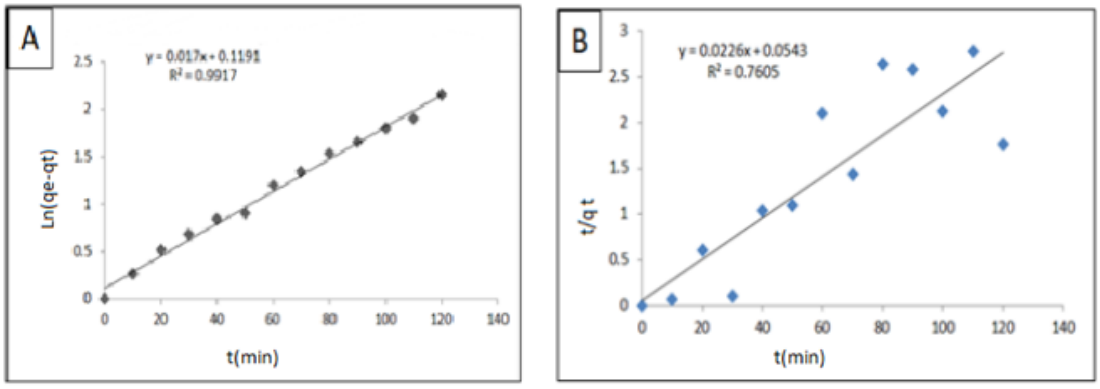

Fig. 11. (A) pseudo-first-order kinetics (B) pseudo-Second-order kinetics.

surface are tailored for the particular recognition and binding of amphetamine molecules, yielding the maximum adsorption capacity at this pH. Under alkaline conditions, (pH 8-9) Amphetamine molecules may undergo deprotonation of their amine groups, resulting in the loss of their positive charge and diminishing their interaction with the negatively charged polymer surface. Moreover, an overabundance of OH^- ions in the solution may disrupt the binding process, hence diminishing adsorption capability[24].

Fig. 11A demonstrates that the linear correlation coefficient (R^2) for the pseudo-first-order kinetics is 0.9917.The value is rather insignificant, but the correlation coefficient for the pseudo-second-order kinetics surpasses 0.7605, as demonstrated in Fig. 11B.This illustrates that pseudo-first order kinetics efficiently clarify the adsorption process of The adsorption mechanism of AMP on Fe_3O_4@SiO_2@MMIP[26].

The study utilized Freundlich isotherm Fig. 12A where the linear correlation coefficient are 0.9971for the adsorption of Amphetamine on Fe_3O_4@SiO_2@MMIP while the linear correlation coefficient calculated by the Langmuir isotherm equation and Timken isotherm are relatively small(0.8799),(0.8766) Fig. 12B and C respectively therefore ,the adsorption performance of AMP. On Fe_3O_4@SiO_2@MMIP can be well described by the Freundlich isotherm model[27] .

The thermodynamic parameters of the adsorption process were calculated to determine the Gibbs free energy. The enthalpy change (ΔH) was determined using the Van't Hoff equation (eq 3), and the reaction enthalpy slope was obtained from the graph of ln Xm vs 1/T in Fig. 13. The slope yielded a negative enthalpy value (-8.79671), indicating that the adsorption process

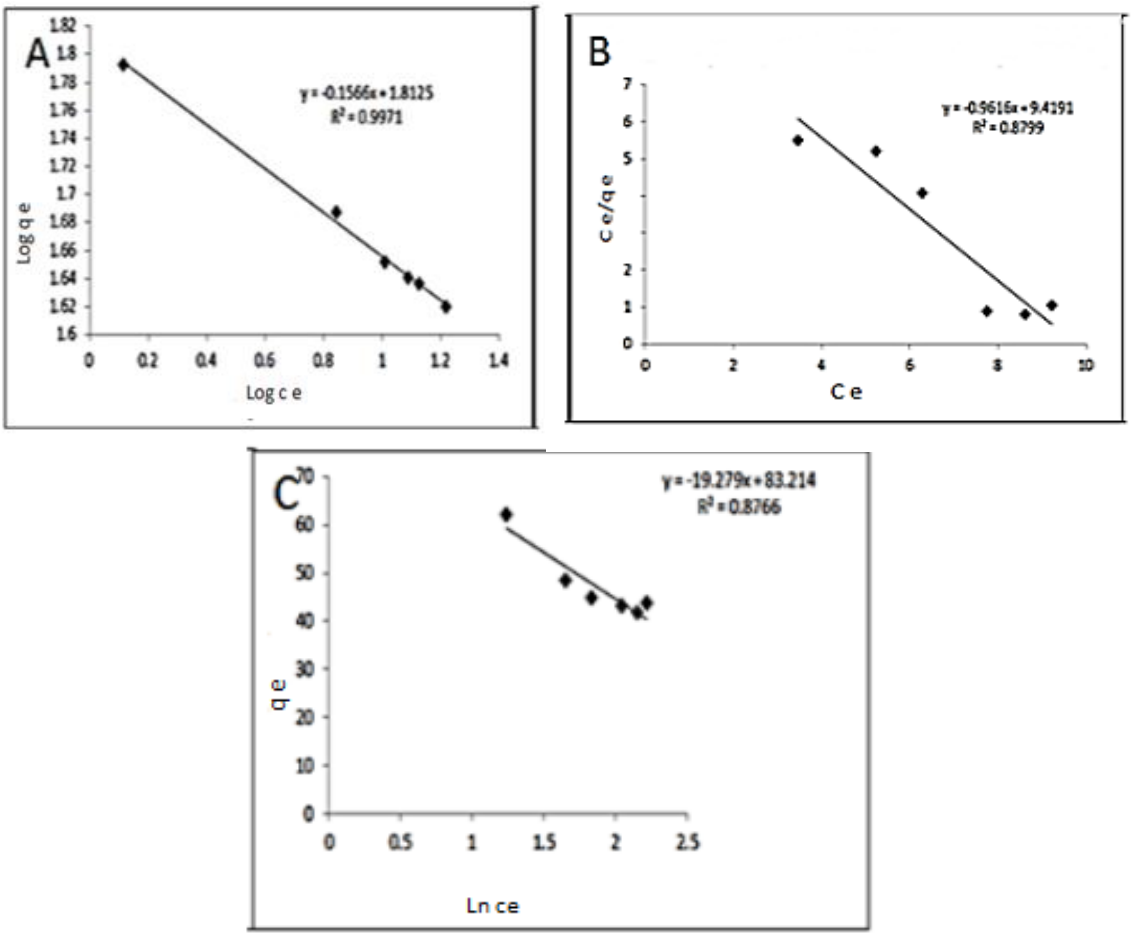

Fig. 12. adsorption isotherm (A)Freundlich isotherm(B) Langmuir isotherms(C) Temkin isotherm.

is exothermic. The negative entropy value ΔS (-1.18564) suggests a highly structured adsorption system, but the negative Gibbs free energy value ΔG (-26.1045) shows that the process is spontaneous[28].

$$\ln K = -\Delta H^{\circ}/R + \Delta S^{\circ}/R * 1/T \quad (2)$$

Where: k represents the adsorption coefficient, ΔHº is the standard enthalpy change, R is the gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$), T represents the absolute temperature in kelvins, ΔSº denotes the standard entropy change.

Following Fig. 14 the adsorption of AMP into the AMP-MMIPs under optimum circumstances, the

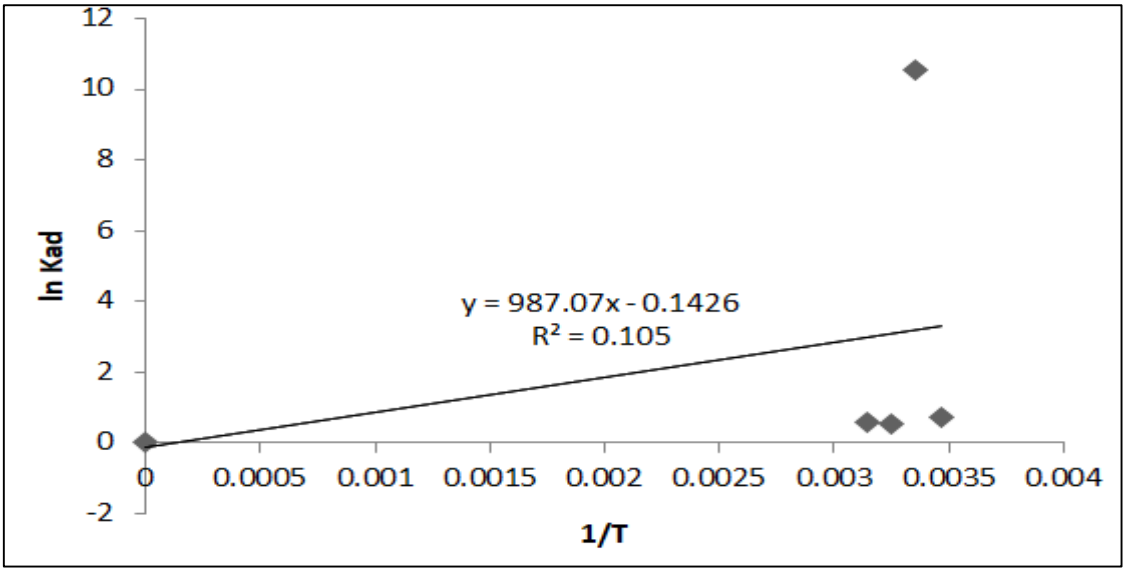

Fig. 13. Adsorption kinetic.

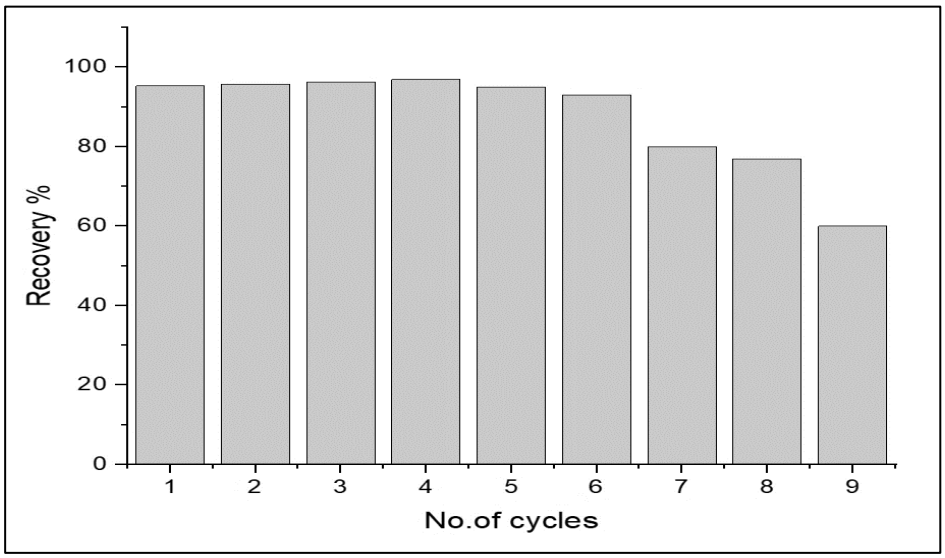

Fig. 14. Reusability of the AMP-MMIP.

AMP-MMIPs were eluted using a combination of acetic acid and methanol (V/V = 1:5) in a constant temperature shaker for 120 minutes at 25 °C. The adsorbent was subsequently retrieved under an applied magnetic field and rinsed with ultrapure water two to five times. Following complete elution, the dried AMP-MMIPs underwent an additional adsorption process, and their residual adsorption capability was evaluated. The regeneration process was conducted an additional nine times [29, 30].

Fig. 15 illustrates the selectivity of the synthesized polymeric sorbent toward amphetamine in comparison with other structurally different drugs (Artane, heroin, and pregabalin). As shown, amphetamine exhibited a remarkably high recovery exceeding 90% at a concentration of 1 mg·L^{-1}, which clearly demonstrates the strong affinity and high specificity of the imprinted recognition sites toward this analyte. In contrast, the recovery values for Artane, heroin, and pregabalin were significantly lower, ranging between 10–20%, indicating that their molecular structures and functional groups did not match effectively with the binding cavities of the polymer. These findings confirm the successful creation of selective recognition sites within the imprinted polymer that are geometrically and chemically complementary to amphetamine. The pronounced selectivity factor highlights the potential of the developed sorbent as an efficient material for the targeted extraction and detection of amphetamine from complex biological matrices.

To evaluate the analytical efficacy of the proposed methodology, the quantification of Amphetamine in human urine was performed at concentrations of 15, 20,25, 30, 35 and 40 mg.L^{-1}. Amphetamine was introduced to the sample and analyzed via UV spectroscopy. The findings for the quantification of AMP in the actual sample. The necessary data was acquired for the assessment of the target compound. Therefore, MMIP can function as a reliable sample preparation technique for the trace analysis of AMP in human urine.

The Accuracy of the method for sample solution spiked at different concentration (15, 25,35) mg.L^{-1} yielded a limit of detection LOD (0.00547 mg.L^{-1}) and limit of quantification LOQ (0.1824 mg.$L^{-1}$for the method for the Amphetamine , respectively .The recoveries ranged from 92.33% to 99% .The relative standard deviation RSDs were observed to range from 3.92% to 1.17% (n=3) . which calculated by the following equations. demonstrating that the developed method exhibited satisfactory precision and accuracy.

$$LOD = 3\delta / S \quad (3)$$

Where: δ is standard deviation, and S is slope

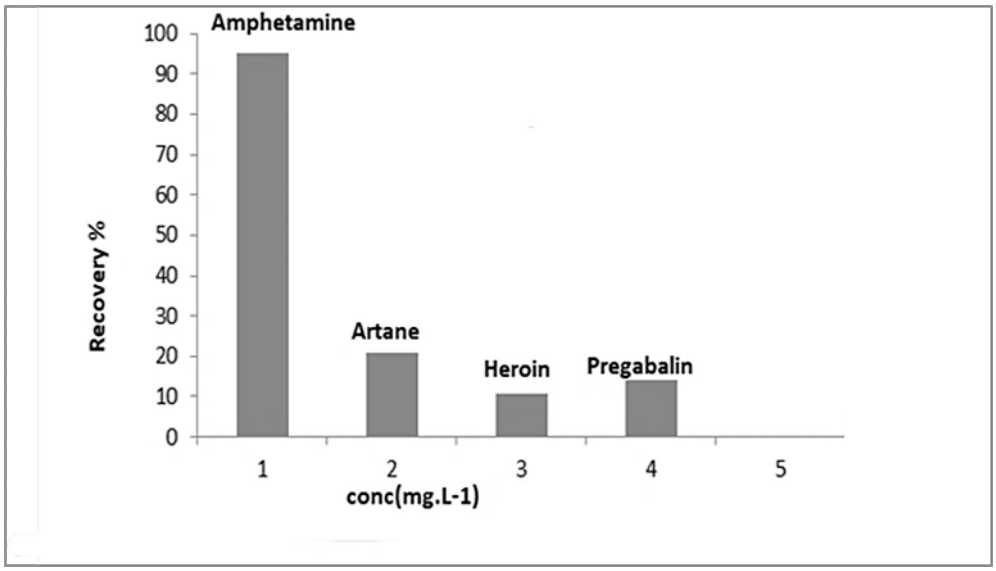

Fig. 15. Selectivity of AMP-MMIP toward Amphetamine and three drugs.

$$LOQ = 10\delta / S \quad (4)$$

Where: δ is standard deviation, and S is slope.

$$\text{Recovery \%} = (\text{Measured Amount} / \text{Added Amount}) \times 100\ \% \quad (5)$$

$$\text{RSD \%} = (\text{SD}/\text{Mean}) \times 100\% \quad (6)$$

Where, SD: standard deviation, Mean: arithmetic Average.

CONCLUSION

This study successfully synthesized MMIPs for AMP for the first time. The produced MMIPs were characterized using SEM, FT-IR, VSM, TGA, BET and XRD techniques. The presence of Fe_3O_4 nanoparticles in the MMIPs ensures efficient and rapid separation from solutions under an external magnetic field, eliminating the need for filtration or centrifugation. Isothermal adsorption experiments confirmed that MMIPs exhibit excellent selective adsorption and specific recognition abilities. Additionally, the high recovery rates observed in spiked recovery experiments validate the effectiveness of this approach. Therefore, this method can be applied to detect and quantitate Amphetamine residues in human urine.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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