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SYNTHESIS AND ADSORPTION STUDY OF PENICILLIN G USING AN ACRYLAMIDE-EGDMA MOLECULARLY IMPRINTED POLYMER

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ABSTRACT

The widespread use of penicillin G demands highly selective analytical extraction methods for environmental and food residue monitoring. This study synthesized a Molecularly Imprinted Polymer (MIP) for the selective extraction of penicillin G via bulk polymerization, yielding 5.0 g of the MIP and 3.635 g of the non-imprinted polymer (NIP). Acrylamide was employed as the functional monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linker, and benzoyl peroxide (BPO) as the initiator. The template was successfully extracted using integrated Soxhlet and batch methods with a methanol-acetic acid (9:1, v/v) solvent system. FTIR spectroscopy confirmed the successful incorporation of monomeric functional groups, while Scanning Electron Microscopy (SEM) revealed a highly porous, irregular morphology in the MIP compared to the dense NIP. Equilibrium adsorption studies demonstrated that the MIP achieved an adsorption capacity of 6.40 mg/g, significantly outperforming the NIP (2.36 mg/g), yielding an imprinting factor (IF) of 2.71. Furthermore, the MIP exhibited competitive selectivity against ibuprofen as an analog molecule, achieving a selectivity coefficient (α) of 1.16. These results confirm that the synthesized acrylamide-based MIP provides high structural specificity, making it a highly robust sorbent for penicillin G analysis.

Keywords: Molecularly imprinted polymer, penicillin G, acrylamide, solid-phase extraction, selective adsorption

INTRODUCTION

Penicillin G (benzylpenicillin) is a fundamental broad spectrum beta lactam antibiotic massively utilized in medical interventions to treat gram positive bacterial infections and extensively applied as a prophylactic agent in the agro industry (Masrianto et al., 2019; Olatoye et al., 2016). However, due to its high volume of usage and the inability of organisms to completely metabolize the administered doses, substantial amounts of penicillin G residues are continuously excreted, ultimately accumulating in aquatic environments and food chain products such as milk and meat (Arsène et al., 2022; Treiber & Beranek-Knauer, 2021). Chronic exposure to penicillin G residues, even at trace levels, poses a risk of triggering anaphylactic reactions in sensitive individuals and acts as a major contributor to the escalating global crisis of antimicrobial resistance (Arsène et al., 2022; Ibrahim et al., 2026; Lee & Sun, 2017). Therefore, highly precise analytical monitoring of these residues across various sample matrices is essential to mitigate both ecological and health impacts (Chan et al., 2022).

The quantitative analysis of antibiotic residues at trace levels generally relies on advanced instrumentation, such as High-Performance Liquid Chromatography (HPLC) (Chan et al., 2023). Although this instrument offers superior detection limits, its performance is highly dependent on the sample preparation step, given the high complexity of environmental and biological matrices (Kumar & Venkatesh, 2024; Williams et al., 2023). Current conventional preparation methods still present several fundamental drawbacks. Liquid-Liquid Extraction (LLE), for instance, is considered highly time-consuming, labor-intensive, and requires large volumes of toxic organic solvents, making it increasingly unfavorable from a green analytical chemistry perspective (Armenta et al., 2022; Queiroz & Souza, 2021). Alternatively, while conventional commercial Solid-Phase Extraction (SPE) is relatively more practical and solvent-efficient, its standard adsorbent materials (such as C18 silica) rely almost entirely on non-specific hydrophobic interactions. This vulnerability allows an abundance of interfering molecules with similar polarities to be co-extracted alongside the target analyte. Consequently, this co-extraction triggers severe matrix effects, suppresses ionization during mass spectrometric detection, and significantly disrupts overall chromatogram accuracy and detection limits (Kumar & Venkatesh, 2024; Williams et al., 2023).

To achieve a more precise, selective, and eco-friendly sample preparation procedure, Molecularly Imprinted Polymers (MIPs) have emerged as highly promising hybrid adsorbent materials (Barhoum & Altintas, 2020; Jamieson et al., 2021). MIPs are smart synthetic polymers synthesized via a biomimetic approach to mimic the specific "lock-and-key" recognition mechanism analogous to antigen-antibody interactions (Khan et al., 2021). This molecular imprinting process involves three fundamental stages: (1) prepolymerization, where the target analyte forms non-covalent bonds with functional monomers; (2) cross-linking to lock the three-dimensional orientation of the polymer network; and (3) template removal to generate “memory cavities” whose size, shape, and chemical environment are precisely complementary to the target molecule (Ibrahim et al., 2026; Khan et al., 2021).

Within the framework of this study, acrylamide is specifically applied as the functional monomer. The primary amide structure in acrylamide possesses a dual electronegativity ideal for acting as both a hydrogen bond donor and acceptor (Feng et al., 2026). This mechanism can precisely interact with the carboxylate group and secondary amide bond on the beta-lactam ring structure of penicillin G (Feng et al., 2026). The practicality and effectiveness of the acrylamide monomer as an MIP building block have also been widely demonstrated in the literature for the selective extraction of other complex organic compounds, which further confirms its reliability in forming stable hydrogen bond complexes (Feng et al., 2026). This prepolymerization network is then cross-linked using ethylene glycol dimethacrylate (EGDMA) to ensure the mechanical integrity of the memory cavities is maintained post-extraction (Ismail et al., 2026).

In the context of penicillin G removal and sensing, state-of-the-art Molecularly Imprinted Polymers are predominantly based on methacrylic acid (MAA) or similar carboxylic-acid monomers cross-linked with EGDMA. Several reports describe MAA/EDGMA-based MIPs for penicillin G or its degradation products (e.g., penicilloic acid) (Ismail et al., 2026; Söylemez & Güven, 2019; Xu et al., 2024; Rahim et al., 2023; Bitas & Samanidou., 2018; Luo et al., 2015; Luo et al., 2014; Cederfur et al., 2003), demonstrating high adsorption capacities and selectivity via ionic and hydrogen-bond interactions. These conventional systems often achieve high adsorption capacities, fast binding kinetics, and good reusability. However, the majority of these studies still rely on classical carboxylic-acid monomers, which operate optimally under relatively acidic conditions and may be less compatible with neutral or mildly basic sample matrices or biological applications.

Against this background, the current work introduces an acrylamide-EGDMA MIP specifically designed for penicillin G, prepared via a straightforward bulk polymerization to yield a rigid macroporous matrix. The use of acrylamide instead of MAA represents the key novelty, as it shifts the dominant interaction mechanism from carboxylate-based to predominantly hydrogen-bonding, offering potential advantages in operating under milder pH conditions and in more aqueous-dominated environments. By thoroughly evaluating the equilibrium adsorption capacity and competitive selectivity of the acrylamide-EGDMA MIP, this study aims to clarify its originality within the current landscape of Pen-G-imprinted adsorbents and to provide a reliable fundamental sorbent material for future analytical procedures in penicillin G residue monitoring.

METHODS

Materials

The template (Penicillin G potassium salt, ≥ 98%), analog molecule (ibuprofen, ≥ 98%), functional monomer (acrylamide, 99%), crosslinker (EGDMA, ≥ 97.5%), and radical initiator (benzoyl peroxide, 70%) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade methanol (99.9%) and glacial acetic acid, used for the solvent and template extraction systems, were supplied by PT. Smart-Lab (Tangerang, Indonesia). Double-distilled water was utilized in all procedures. All chemicals were used as received without further purification.

Instrumentation

Spectroscopic characterization of the polymers' functional groups was performed using a Shimadzu Fourier Transform Infrared (FTIR) spectrophotometer (Japan), while surface morphology and porosity were observed via a JEOL IT 300 Scanning Electron Microscope (SEM, Japan). For quantitative analysis, the concentrations of Penicillin G and ibuprofen were measured at a maximum absorption wavelength (λ_{max}) of 242 nm using a Thermo Scientific™ GENESYS™ 150 UV-Vis spectrophotometer. Additionally, routine laboratory equipment included an orbital shaker for batch adsorption studies and a Quattro analytical balance for precise mass measurements.

Synthesis of Molecularly Imprinted Polymer (MIP) and Non-Imprinted Polymer (NIP)

The MIP was synthesized via bulk polymerization, a method favored for its simplicity and reliability in creating rigid adsorbent matrices (Ariani et al., 2024). Penicillin G (0.37 g) was dissolved in 20 mL of methanol in a glass reactor and homogenized in an ultrasonic bath for 2 min. Acrylamide (0.35 g) and EGDMA (3.77 mL) were added simultaneously, followed by the addition of BPO (0.15 g). The prepolymerization mixture was thoroughly homogenized and purged with high-purity nitrogen gas for 1 min to eliminate dissolved oxygen. The reactor was sealed and incubated in an oven at 70 °C for 3 h to facilitate complete polymerization. The resulting rigid polymer block was washed with methanol, air-dried, mechanically crushed, and sieved to obtain particles of uniform size (14–20 mesh). The NIP was prepared following an identical procedure and thermal conditions, but without the addition of the Penicillin G template (Bakhshpour et al., 2021; Urraca et al., 2006).

Template Extraction (Leaching)

To generate specific recognition cavities, the Penicillin G template was removed from the MIP matrix through a combination of thermal and dynamic leaching processes. The polymer particles were subjected to Soxhlet extraction at ± 80 °C for 6–8 h using a solvent mixture of methanol and acetic acid (9:1, v/v) (Ariani et al., 2024). This was subsequently followed by a dynamic batch extraction using the same solvent system for 24 h under orbital shaking (100 rpm). The extraction process was considered complete when the UV-Vis absorbance of the filtrate at 242 nm approached zero, confirming the total quantitative removal of Penicillin G (Bakhshpour et al., 2021; Urraca et al., 2006).

Physicochemical Characterization

The surface morphology of the synthesized polymers (MIP and NIP) was evaluated using Scanning Electron Microscopy (SEM). Polymer powders were dispersed on double-sided carbon tape attached to a specimen holder prior to observation. Additionally, Fourier Transform Infrared (FTIR) spectroscopy was performed to identify the chemical functional groups. Polymer samples ((NIP, unleached MIP, and leached MIP) were ground with anhydrous potassium bromide (KBr) at a 1:9 ratio, compressed into translucent pellets at 20 psi using a hydraulic press, and analyzed across the mid-infrared spectral range.

Adsorption Capacity and Selectivity Studies

Static batch adsorption experiments were performed by suspending 10 mg of the polymer (MIP or NIP) in 10 mL of a 20 ppm Penicillin G standard solution. The suspensions were shaken at 100 rpm for 24 h at room temperature to achieve thermodynamic equilibrium. The final equilibrium concentration of Penicillin G (C_t) was determined via UV-Vis spectrophotometry at 242 nm, and the adsorption capacity (Q_e, mg/g) was calculated using the standard mass balance equation.

The imprinting factor (IF) was calculated as the ratio of the MIP adsorption capacity to that of the NIP ($IF = Q_{MIP}/ Q_{NIP}$). To evaluate competitive selectivity (cross-reactivity), 10 mg of MIP was incubated with 10 mL of a 30 ppm ibuprofen solution under identical conditions. The relative selectivity coefficient (α) was calculated as the ratio of the MIP's adsorption capacity for Penicillin G to its capacity for ibuprofen ($\alpha = Q_{Penicillin}/ Q_{Ibuprofen}$). All batch binding experiments were carried out in triplicate to ensure data reproducibility, and the results are presented as mean ± standard deviation.

RESULT & DISCUSSION

Synthesis and Template Extraction (Leaching)

The molecularly imprinted polymer (MIP) matrix was synthesized via bulk polymerization, driven by the formation of a prepolymerization complex based on non-covalent hydrogen bonding between the carboxylate/amide groups of penicillin G and the functional monomer acrylamide. The decomposition of the BPO initiator at a constant temperature of 70 °C triggered the crosslinking agent EGDMA to lock the three-dimensional conformation of the polymer network (Wang et al., 2019). The schematic representation of these fundamental synthesis stages, illustrating the prepolymerization interaction, the formation of the cross-linked network, and the generation of specific imprinted cavities after template removal is visually summarized in Figure 1.

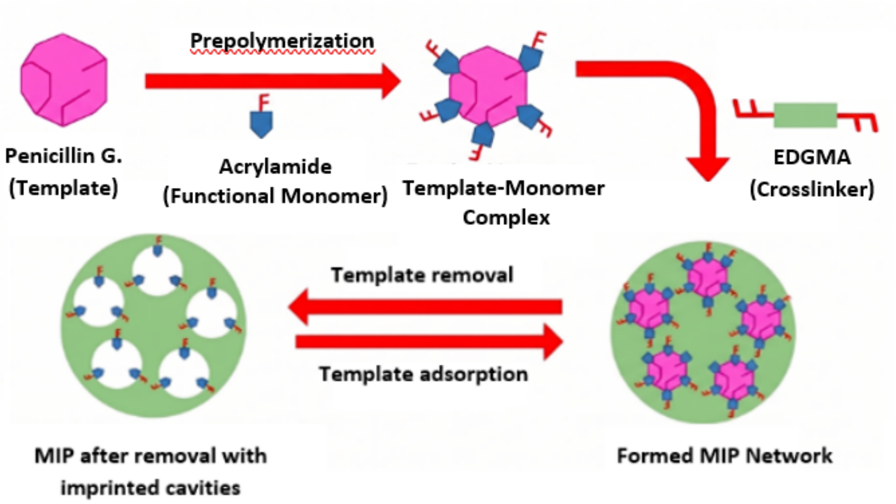

Figure 1. Schematic Representation of The Penicillin G Imprinting Process Using Acrylamide and EGDMA

Following the completion of the polymerization process, the synthesis successfully yielded 5.0 g of the MIP and 3.635 g of the non-imprinted polymer (NIP). The macroscopic visual appearance of both synthesized polymer particles is presented in Figure 2.

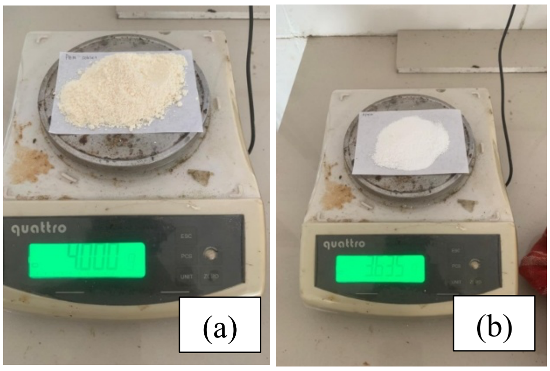

Figure 2. The Visual Appearance of The Synthesized: (a) MIP; and (b) NIP Particles

Physicochemical Characterization

The successful synthesis of the MIP and NIP was confirmed through FTIR spectroscopy, as shown in Figure 3. The successful synthesis of the molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP) was confirmed through Fourier Transform Infrared (FTIR) spectroscopy. Both polymers exhibited characteristic absorption bands consistent with the acrylamide-EGDMA copolymer framework, while notable spectral differences between the two materials provided evidence of template-monomer interactions during the imprinting process.

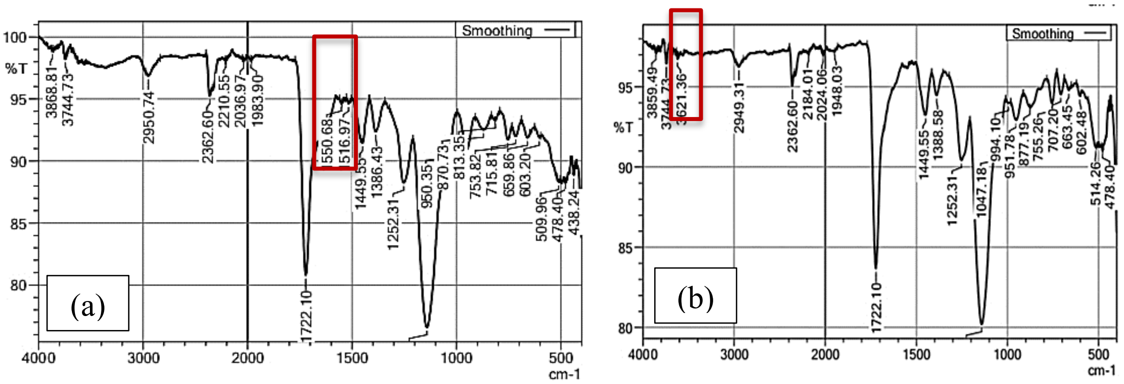

Figure 3. FTIR Spectra of The Synthesized: (a) MIP; and (b) NIP Matrices

Looking at the high-frequency region, both the MIP and NIP exhibited absorption bands near 3868 and 3744 cm⁻¹, which indicate free O–H stretching vibrations. Additionally, a broad envelope spanning 3300–3500 cm⁻¹ points to the N–H stretching of the acrylamide monomers (Al Faysal et al., 2024). Interestingly, the NIP spectrum showed an isolated band at 3621 cm⁻¹ that did not appear in the MIP. This absence suggests that the hydroxyl groups within the imprinted polymer are heavily engaged in hydrogen bonding with the Penicillin G template, thereby depleting the population of free O–H groups (Fauziah et al., 2021). Both spectra also shared a weak signal around 2950 cm⁻¹, confirming the aliphatic C–H stretching from the polymer backbone.

While both matrices displayed signals between 1948 and 2210 cm⁻¹, the MIP bands shifted toward higher wavenumbers (2210, 2036, and 1983 cm⁻¹) compared to those of the control NIP (2184, 2024, and 1948 cm⁻¹). This blue shift points to an altered local chemical environment, driven by the successful formation of a prepolymerization complex between the functional monomer and the template molecule (Sullivan et al., 2021; Xie et al., 2020).

Successful crosslinking of the polymer network was confirmed by a dominant C=O stretching band at 1722 cm⁻¹ in both materials, which originates from the EGDMA ester groups (Zhang, et al., 2017; Zhang, et al., 2018). The most distinct structural evidence of imprinting, however, emerged between 1500 and 1600 cm⁻¹. Two sharply defined bands at 1550 and 1516 cm⁻¹ appeared solely in the MIP spectrum. These peaks represent conformationally restricted Amide II vibrations (a combination of N–H bending and C–N stretching) from acrylamide. Their exclusive presence provides direct spectroscopic proof of hydrogen bonding interactions between the acrylamide amides and the functional groups of Penicillin G during complexation (Kaur et al., 2024). Without the template, the NIP's amide groups remained conformationally free, resulting in just a broad, unresolved signal in the 1449–1388 cm⁻¹ range (Kuodis et al., 2020). Both polymers also showed an asymmetric C–O–C stretch around 1252 cm⁻¹, typical of the EGDMA linkages.

In the fingerprint region, the control NIP yielded relatively sharp peaks near 1047 and 994 cm⁻¹. In contrast, the MIP displayed broader and weaker counterparts in this area. This broadening reflects restricted local chain mobility, a physical consequence of the rigid, specific binding cavities formed within the imprinted network (Zhang et al., 2017; Zhang et al., 2018). Slight wavenumber shifts in the lower frequencies (e.g., ~509 cm⁻¹ in the MIP versus ~514 cm⁻¹ in the NIP) further highlight these underlying structural variations.

Overall, the FTIR data clearly validates the copolymerization of the acrylamide-EGDMA system. More importantly, the distinct spectral differences, specifically the missing 3621 cm⁻¹ band, the blue-shifted peaks, and the unique Amide II restrictions at 1550 and 1516 cm⁻¹, serve as solid evidence that a molecularly imprinted architecture with selective recognition cavities for Penicillin G was successfully generated.

Morphological Characterization

The surface morphology and topographic features of the synthesized polymers were investigated using Scanning Electron Microscopy (SEM). As depicted in the SEM micrograph (Figure 4 (a)), the MIP matrix, following the complete removal of the Penicillin G template, exhibited a highly irregular, rough, a lot of pores and granular surface structure (Amin et al., 2018; Herlina et al., 2025). This pronounced porosity provides direct structural evidence of the micro-cavities generated within the poly(acrylamide-co-EGDMA) framework post-extraction (Qronfla et al., 2023). Such a highly permeable architecture is fundamental to the molecular recognition mechanism, as it exposes specific binding sites that are complementary in size, shape, and chemical functionality to the target antibiotic.

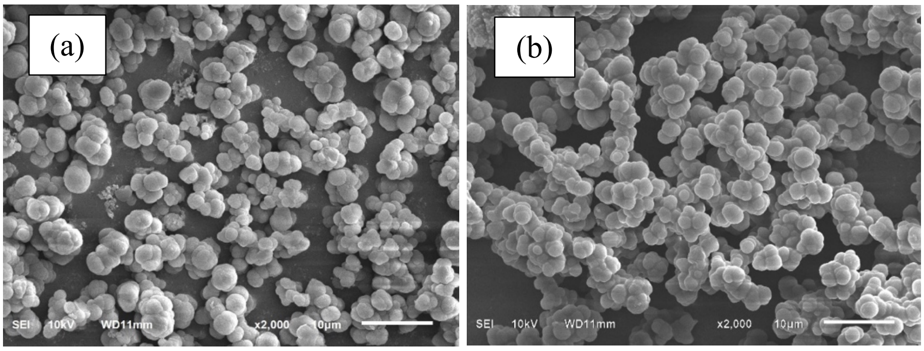

Figure 4. Scanning Electron Microscopy (SEM) Micrographs of: (a) MIP After Leaching; and (b) NIP at 2000x Magnification

By contrast, the corresponding micrograph of the non-imprinted control polymer (NIP) (Figure 4 (b)) revealed a dense, solid, and relatively smooth morphological profile (Herlina et al., 2025). In the absence of a template molecule during the copolymerization process, the resulting network remained tightly packed, lacking the formation of systematically distributed macropores (Amin et al., 2018; Bakhtiar et al., 2019; Meléndez-Marmolejo et al., 2022). This dramatic morphological divergence between the highly porous MIP and the compact NIP serves as robust qualitative validation of the successful molecular imprinting effect.

Additionally, the sharp and angular microparticle fragments observed in both samples are characteristic of polymers synthesized via the bulk polymerization technique, which requires the rigid polymer monolith to be mechanically crushed and sieved prior to application (Amin et al., 2018). Analytically, the enhanced surface roughness and porosity of the MIP are highly advantageous; they substantially expand the interfacial specific surface area and minimize mass transfer resistance during the selective rebinding of Penicillin G (Qronfla et al,m

2023, Tan & Tong, 2007). Furthermore, the high crosslinking density provided by EGDMA imparts sufficient mechanical rigidity to the particles, ensuring the imprinted cavities retain their structural memory and remain functional across potential adsorption-desorption cycles.

Equilibrium Adsorption Capacity Evaluation

The adsorption affinity of the recognition sites was evaluated through static batch adsorption equilibrium experiments (100 rpm, 24 h). The equilibrium adsorption capacity (Q_e) of the MIP matrix reached 6.40 ± 0.15 mg/g, which was drastically enhanced compared to the adsorption performance of the NIP, which remained at only 2.36 ± 0.08 mg/g. The significance of this capacity enhancement suggests that the adsorption mechanism in the MIP is primarily driven by the steric spatial matching and the orientation of hydrogen bonding sites within the specific imprinted cavities, rather than solely by nonspecific surface physisorption (Kuru et al., 2020; Wang et al., 2019).

Selectivity and Molecular Recognition

To quantitatively assess the structural specificity of the synthesized materials, both the Imprinting Factor (IF) and cross-reactivity profiles were evaluated. The ratio of the MIP's equilibrium adsorption capacity to that of the NIP generated an IF value of 2.71. This prominent value underscores the matrix's robust and highly specific recognition affinity toward the Penicillin G template (Urraca et al., 2006; Meléndez-Marmolejo et al., 2022). To further probe the binding selectivity, cross-reactivity assays were performed using ibuprofen as a competitive analog. Although ibuprofen possesses a complementary carboxylate functional group, its overall steric volume and molecular geometry deviate significantly from the bulky beta-lactam structure of Penicillin G (Benito-Peña et al., 2006; Pupin et al., 2020). Under equivalent concentration parameters, the MIP's uptake of ibuprofen was restricted to only 2.84 mg/g, which is distinctly lower than its adsorption of Penicillin G (3.30 mg/g). This binding disparity translates to a selectivity coefficient (α) of 1.16. These results provide compelling evidence that the imprinted memory cavities effectively employ a steric and functional exclusion mechanism, enabling the MIP framework to selectively discriminate against non-target interferents while accommodating the original analyte. While this study successfully demonstrates the competitive exclusion of ibuprofen, further cross-reactivity studies utilizing structurally closer beta-lactam analogs (such as amoxicillin or ampicillin) will be considered in future work to comprehensively map the specific recognition profile of the imprinted cavities

CONCLUSION

In conclusion, an acrylamide-based molecularly imprinted polymer (MIP) was successfully synthesized via bulk polymerization, serving as a highly selective sorbent for the extraction of Penicillin G. Physicochemical and morphological characterizations confirmed the effective formation of specific memory cavities within the polymer network. This was evidenced by distinct hydrogen-bonding interactions in the FTIR spectra and a highly porous, granular surface topography observed in the SEM micrographs, which starkly contrasted with the dense structure of the non-imprinted polymer (NIP).

Thermodynamically, the MIP exhibited a superior equilibrium adsorption capacity of 6.40 mg/g, significantly outperforming the NIP (2.36 mg/g) and generating a notable imprinting factor (IF) of 2.71. Furthermore, the synthesized matrix demonstrated excellent structural recognition and selectivity against the competitive analog ibuprofen, yielding a selectivity coefficient (α) of 1.16. These findings collectively validate that the proposed MIP framework possesses robust steric and functional exclusion mechanisms. Consequently, this synthesized material stands out as a highly promising, reliable, and efficient solid-phase extraction (SPE) sorbent for the accurate trace analysis of Penicillin G residues in complex environmental and biological matrices.

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