Preparation, Characterization and Application of Molecularly Imprinted Polymer for Ginsenoside Rg1

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Abstract. ***Background:*** Ginsenoside Rg1, a bioactive compound found in ginseng, has attracted significant attention due to its therapeutic effects. Despite its promising potential, the accurate detection and quantification of ginsenoside Rg1 in ginseng and other complex substrates have proven to be challenging. ***Objective:*** To effectively detect trace target components in complex matrices of Traditional Chinese Medicines (TCMs), the use of molecularly imprinted polymers (MIPs) has emerged as a promising approach. MIPs are synthetic materials that are designed to possess specific recognition sites for target molecules. They offer several advantages, including high efficiency, selectivity, and stability, making them ideal candidates for the extraction and analysis of target compounds in TCMs. ***Methods:*** The performance of molecularly imprinted and non-imprinted polymers (MIPs and NIPs) was evaluated including selective recognition capacity, adsorption isotherm, and adsorption kinetics. Optimization of various parameters affecting molecular imprinted solid phase extraction (MISPE), such as the binding mode of template molecule to functional monomers, the polymerization time, ratio of template molecule and functional monomer, dosage of cross-linker and initiator, stirring speed, template molecule elution was investigated. The formed MIPs were characterized in detail by Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectrometer (FT-IR) to evaluate the specific adsorption capacity. ***Results:*** Ginsenoside Rg1 exhibited a percent recovery ranging from 84% to 90%, with a maximum saturated adsorption amount of 62.22 μg/mg. Additionally, the relative standard deviations (RSDs) were found to be within the range of 3.23%. ***Conclusion:*** In conclusion, these findings present a promising approach for the effective separation and enrichment of active ingredients from ginseng.

Keywords: Ginsenoside Rg1; Molecularly Imprinted Polymer; Solid-phase Extraction; Precipitation Polymerization; Molecular Recognition; Optimization; Application.

1. Introduction

Ginseng (*Ginseng Radix Et Rhizoma*), the roots and rhizomes have been always famed as the king of herbs. It was frequently used to notify “qi” and nourish “yin” [1], and it can be available on interventions to improve the Qi Blood deficiency, insomnia, and forgetfulness symptoms [2, 3]. It has been reported to treat many diseases, including cancer [4], diabetes [5], cardiovascular disease [6] and neurodegenerative diseases etc. [7]. Ginsenoside Rg1 was also known for the crucial pharmacological effect, mainly including anti-oxidation, scavenging free radicals, and anti-apoptosis [8]. Precisely because of its unique pharmacological activity, more and more researchers set sight on targeted extraction and content determination. Ginsenoside Rg1 can be detected in single herbs, but the decrease in the proportion of ginseng in the compounded multiple-herb system makes it difficult in detection. However, current detection methods only obtained trace amounts of the target component, accompanied by impurity interferences near the trace ginsenoside Rg1. Therefore, it is critical to set a preparation method to obtain ginsenoside Rg1 with high enrichment level in complex matrices.

At present, solid phase extraction (SPE) can be widely used to extract analytes from complex matrices.[9], it can be achieved eliminating impurities and enriching compounds. However, SPE has the disadvantage of lacking selectivity, resulting in more structurally similar compounds being extracted at the same time. To overcome the problem, we concentrate on MISPE. It has been widely used as adsorbent for SPE by virtue of their specific recognition capabilities, it also has the ability to selectively extract target compounds and structural analogs. The MIPs have the synthetic material with multiple binding sites, coupled with high affinity for target molecules and their analogs [10]. It has preferentially combined the monomeric fraction to form MIPs with strong adsorption, high stability, and efficient template-monomer interactions. In comparison with other molecular recognition systems. MIPs demonstrated high stability at temperature, pressure, acids and bases, organic solvents, which was an important reason that it was used as separating material [11].

In previous studies, it has been shown that the maximum quantity of apparent adsorption is only 27.74 mg g^{-1}, which was appeared in the single-template MIPs of ginsenoside Rg1 by precipitation polymerization [12], it showed the fewer sites and adsorption. In this paper, we have successfully established and optimized the MIPs as highly selective and absorbent material for separating and enriching of ginsenoside Rg1. Now, The MIPs were synthesized using ginsenoside Rg1 as template molecule, 4-vinylphenylboronic acid (4-VPBA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as cross-linker and ethanol as pyrogen. After the evaluation of the imprinting efficiency, Rg1-MIPs was optimized and applied for the selective extraction. In addition, MISPE combines high-performance liquid chromatography (HPLC) to separate and determine trace amounts of ginsenoside Rg1.This experiment was intended to establish a bionic synthetic material with high adsorption performance, which was a valuable method for the separation and enrichment of structurally similar compounds in natural products.

2. Materials and Methods

2.1 Chemical and Regents

Ginsenoside Rg1, Ginsenoside Re, Ginsenoside Rb1, Rutin and Quercetin were provided by National Institutes for Food and Drug Control (Beijing, China), 4-vinylphenylboronic acid was obtained from Shanghai Haohong Biopharmaceutical Technology Co., Ltd. (Shanghai, China), Acrylamide (AM) and 2,2'-Azobis (AIBN) were purchased from Tianjin Damao Chemical Reagent Factory. (Tianjin, China). Ethyl glycol dimethacrylate was purchased from Shanghai Maikelin Biochemical Technology Co., Ltd. (Shanghai, China). All other analytical reagents were purchased from Tianjin Bohua Chemical Reagent Co., Ltd. (Tianjin, China).

2.2 Preparation of MIPs and NIPs

The MIPs was synthesized by precipitation polymerization method. For polymer synthesis,0.05mM of ginsenoside Rg1 and 0.5mM 4-VPBA were dissolved in 10 mL of mixed anhydrous ethanol. The reaction mixture was sonicated to mix up, EGDMA(0.7mM) and AIBN (0.8mL) were adv template until the template could not be detected. The NIPs were also prepared simultaneously in identical method to MIPs, although in the absence of the template molecule.

2.3 Optimization of Extraction Parameters

To further optimize the preparation method of Rg1-MIPs, we conducted a comprehensive study on the effects of various parameters, including the polymerization reaction time (3, 6, 12, and24 hours), the ratio of template molecule to monomer (1:4, 1:6, 1:8, 1:10, 1:12, 1:15), crosslinker concentration (0.3, 0.5, 0.7, 0.9 mmol), initiator dosage (0.4, 0.8, 1.2 mL), and stirring speed (400, 1000, 1500 rpm).

2.4 Characterization of MIPs and NIPs

Prepared powder of MIPs and NIPs were examined by SEM (1530) to observe morphology and dispersion. The qualitative analysis process of Rg1-MIPs was characterized using FT-IR (8700) for scanning spectral.

2.5 Chromatograph Method.

HPLC analysis was performed by using synchronous C18 column (250mm *4.6mm, 5μm). Mobile phase was acetonitrile(A) and pure water(B). The chromatogram was assessed at 203 nm. The column temperature was maintained at 30°C, and the quantitation analysis was set into injecting 10 μL sample solution at a flow rate of 1 mL min^{-1}. The gradient elution process was as follows:0~35 min, 19%A; 35~55 min, 19~29%A; 70~100 min, 29~40%A.

2.6 Sample Preparation

The source of ginseng (RS190413) was identified by Professor Guo Baolin from Institute of Medicinal Plants, Chinese Academy of Medical Sciences. Ginseng was collected and transported into our laboratory. The sample was cleaned, cut, grounded into powder. The powder of 0.5g was accurately weighed and added into 70% ethanol (25 mL) in triangular flask. After sonicating 40 min. the solution was used for the analysis.

2.7 Binding Test

2.7.1 Adsorption Kinetics

The adsorption kinetics for MIPs and NIPs was assessed by the following method. 5 mg MIPs and NIPs were weighed and added precisely in 0.5 mg/mL (1mL) ginsenoside Rg1 solution at selective centrifugation ranging from at 2 to 24 h, The mixture adsorption was oscillated in a thermostatic incubation shaker at 20 ℃. After completion of centrifugation, the supernatant was obtained and analyzed by HPLC to measure the concentration of ginsenoside Rg1, the results were determined three times in parallel. To assess the adsorption capacity of Rg1-MIPs, experiments about isotherm, kinetics and selective adsorption were performed. The adsorption amount(Q) was evaluated according to the following equation:

Adsorption amount $Q = \frac{(C_0 - C_1)V}{M}$

Where C_0 (μg/mL) and C_1 (μg/mL) are the initial and equilibrium concentrations of Rg1, V (mL) is the measurement of the volume of the adsorption solution; M (mg) represents the mass of the polymer.

2.7.2 Adsorption Isotherms

The adsorption isotherms were confirmed by the following method. 5 mg MIPs and NIPs were mixed in 1mL ginsenoside Rg1 solution at selective concentrations of 0.5 mg mL^{-1}, 1.0 mg mL^{-1}, 1.5 mg mL^{-1}, 2.0 mg mL^{-1} and 2.5 mg mL^{-1}. and then the mixtures were put into constant temperature culture shaker at 20 ℃ for 12h, and then centrifuged to take the supernatant. The concentration changes of ginsenoside Rg1 was analyzed by HPLC. The Q was calculated according to the above formula.

2.7.3 Substrate Selectivity

The substrate selectivity was studied by the following method. 5mg MIPs and NIPs were respectively added in different mixed solution. Solution 1 was 2 mg mL^{-1}(1 mL) mixed solutions with ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, rutin and quercetin. Solution 2 was mixed solutions (1 mL) of ginsenoside Rg1 (0.50 mg mL^{-1}), ginsenoside Re (1.50 mg mL^{-1}), ginsenoside Rb1 (2 mg mL^{-1}), rutin (2.50 mg mL^{-1}), quercetin (2.50 mg mL^{-1}). The adsorbed amount Q was used to assessed according to the formula (1).

2.8 Molecularly Imprinted Solid-phase Extraction

The extraction was conducted by using SPE. The MIPs (25mg) was installed on the solid-phases extraction columns. The eluent sequential was pass through the system including methanol (1 mL), 50% methanol (1 mL), and pure water (2 mL). The influence of non-selective adsorption was removed with 30% methanol. The target component was eluted in batches (1mL×8) with 70% methanol-ice acetic acid (9:1, V/V). Finally, the collected solution was evaporated and the residues were rediscovered in 5 mL methanol for HPLC analysis.

3. Results

3.1 The Optimized of MIPs

In the field of molecular imprinting, the selection of the binding approach between the template molecule and the functional monomer is a crucial step. Our team has always considered the options of covalent and non-covalent binding. Taking into account the structural characteristics of the template molecule ginsenoside Rg1, which contains multiple glycosylation sites, we opted for an esterification reaction with 4-VPBA. This reaction can form five- or six-membered cyclic boronic esters by dehydration with 1,2-cis-o-diols or 1,3-cis-diols present in the sugar structure [13]. Hence, we focused on a combination of covalent and non-covalent preparation methods.

In previous experiments, we evaluated the affinity and selectivity of the functional monomers by measuring the adsorbed amount (Q) and polymer yield (polymer production/feeding amount). We used AM and 4-VPBA as the functional monomers. The analysis of these indicators revealed that4-VPBA, as a combined functional monomer, exhibited favorable adsorption properties. Interestingly, when the non-covalent monomer AM was absent, both the adsorbed amount and polymer yield were maximized. The results of the adsorption and polymer yield at different molar ratios of 4-VPBA and AM are presented in Table S1. These findings indicate that the combination of covalent and non-covalent functional monomers, specifically 4-VPBA and AM, yielded optimal adsorption properties and polymer synthesis. The selection of appropriate molar ratios is crucial in achieving the desired outcomes.

By investigating the polymerization reaction time, we determined that the optimal duration for preparing the MIPs was 12 hours (Table S2). Interestingly, we observed that extending the reaction time beyond this point resulted in an increased polymer yield. This phenomenon can be attributed to the prolonged high-speed stirring, which potentially disrupted the complex formed between the template molecule and the monomers. As a result, more monomers cross-linked with the cross-linker, leading to a higher polymer yield. The findings suggest that the duration of the polymerization reaction plays a crucial role in achieving the desired MIPs. The optimal reaction time of 12 hours strikes a balance between polymer yield and the preservation of the template-monomer complex. Prolonging the reaction time beyond this point may compromise the stability of the complex, but it can enhance the overall polymer yield through increased cross-linking. These insights highlight the importance of carefully controlling the polymerization reaction time to obtain MIPs with optimal properties. Future studies could explore the impact of reaction time on the binding affinity and selectivity of the MIPs to further enhance their performance.

Through careful examination of the ratio of template molecules to functional monomers, we have discovered that the polymer yield reaches its maximum when the ratio is 1:10 (Table S3). This finding indicates that the proper balance between the template molecules and functional monomers is crucial for the successful formation of molecularly imprinted polymers (MIPs). Furthermore, the dosage of crosslinker and initiator also plays a significant role in the formation of MIPs. Our results have shown that the adsorption amount and polymer yield are maximized when the crosslinker is present at a concentration of 0.7 mM and the initiator AIBN is added at a dosage of 0.8 mL (15 mg/mL) (Table S4 and S5).

In addition to the ratio of components, the stirring speed during the polymerization process can greatly influence the morphology, particle size, and dispersion of the synthesized MIPs. To assess the impact of agitation velocity, we characterized the synthesized polymer using scanning electron microscopy. The results revealed that the maximum adsorption was achieved when the agitation rate was set at 1500 r/min (Figure 1) (Table S6).

In summary, we have successfully optimized several key parameters for the preparation of MIPs. Under the optimized conditions, which include the addition of 0.05 mmol of template molecule Rg1, 0.50 mmol of 4-VPBA,0.70 mmol of crosslinker EGDMA, and 0.80 mL of initiator AIBN (15 mg/mL), at a stirring speed of 1500 r/min for a duration of 12 hours at 60°C, we can obtain MIPs with excellent properties. These findings provide valuable insights into the efficient synthesis of MIPs and contribute to the advancement of molecular imprinting technology.

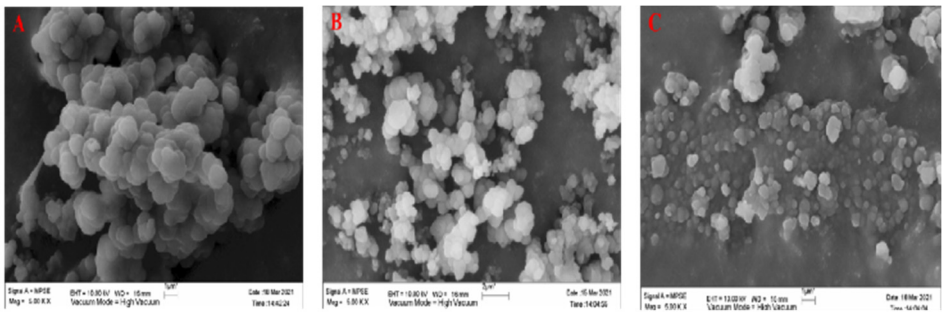

Fig 1. The SEM image of MIPs: (A)400 r/min(B)1000 r/min(C)1500 r/min

3.2 The Characterization of MIPs

In this research, FT-IR and SEM were used to characterize the synthesized polymer, The SEM can be seen that the morphology and structure of the polymer in Figure 2. Under the optimized condition, the image clearly showed that the polymerized products have a highly homogeneous size and shape. Furthermore, they reveal the presence of micro-, meso-, and macro-pores, as well as flow-through channels. These structural features contribute to excellent mass transfer properties and lower back pressure. The uniform and open network skeleton provides a large surface area and minimal flow resistance. As a result, this structure is highly beneficial for the extraction of target molecules from the sample.

The characteristic peak of 4-VPBA was observed at approximately 1608, 1553, and 1511 cm^{-1}, corresponding to the B-O and O-H stretching vibrations of 4-VPBA. Additionally, the characteristic peaks of C=O were observed at 1261 and 1150 cm^{-1}, indicating the successful synthesis of MIPs through the polymerization of ethylene glycol dimethacrylate and 4-VPBA.Comparing the peak intensities of the stretching vibration of O-H in the MIPs before and after removing the template, it was found that the intensity was lower in the MIPs without template removal (Figure 3). This observation suggests that the template molecule Rg1 and the functional monomer 4-VPBA were simply mixed in a certain ratio during the synthesis process. Moreover, this finding further confirms the successful synthesis of Rg1-MIPs in the current experiment. The characteristic peaks obtained from the infrared spectroscopy analysis provide strong evidence for the successful formation of MIPs and the specific interaction between the template molecule Rg1 and the functional monomer 4-VPBA. This synthesis method holds promise for the development of molecularly imprinted polymers with high selectivity and affinity for Rg1, enabling their potential use in various applications such as selective extraction, separation, and purification of Rg1 from complex mixtures.

3.3 Specificity Evaluation of MIPs and NIPs

To assess the substrate selectivity, a mixed solution containing ginsenoside Rg1, Re, and Rb1 was examined. The results revealed that the MIPs exhibited a significant binding capacity for ginsenoside Rg1, Re, and Rb1.This finding suggests a structural similarity between ginsenoside Rg1, Re, and Rb1,

as they all share the same parent nucleus as ginsenoside Rg1. However, there is a distinction in the number of glycoconjugate groups and their connection positions, which ultimately led to a lower adsorption capacity of Rg1-MIPs compared to the template molecule at the same concentration level. Furthermore, Figure 4 illustrates a positive correlation between substrate concentration and adsorption within a certain range. This indicates that as the concentration of the substrate increases, the adsorption capacity of the MIPs also increases. In conclusion, the study demonstrates the favorable binding capacity of MIPs for ginsenoside Rg1, Re, and Rb1. The structural similarities between these compounds, along with their shared parent nucleus, contribute to their successful adsorption onto the MIPs. However, the variation in glycoconjugate groups and connection positions influences the adsorption capacity of Rg1-MIPs compared to the template molecule. Additionally, the positive correlation between substrate concentration and adsorption highlights the importance of optimizing the concentration levels for efficient adsorption.

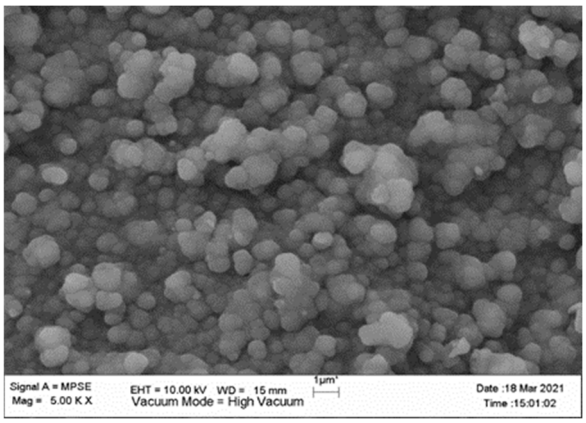

Fig 2. The SEM image of MIPs

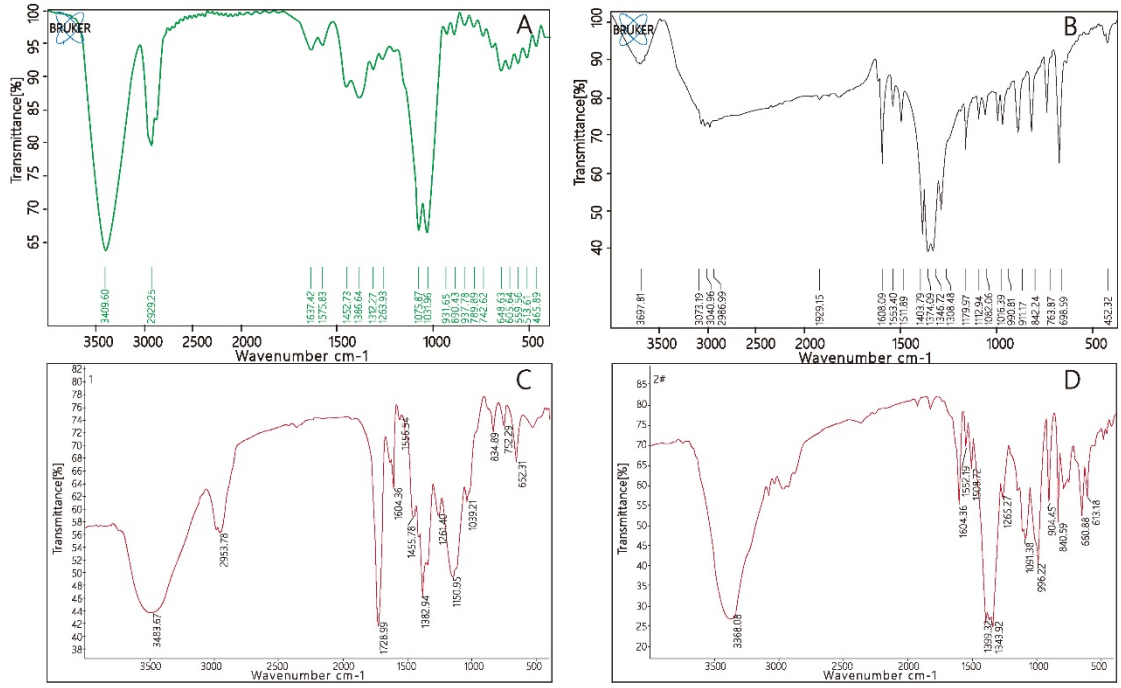

Fig 3. The FT-IR spectra (3-A, Ginsenoside Rg1; 3-B, 4-VPBA; 3-C, Untemplated Rg1-MIPs; 3-D. Mixture of Rg1 and 4-VPBA)

The adsorption kinetics of ginsenoside Rg1 on the MIPs were investigated, as depicted in Figure 5. It was observed that equilibrium was reached after 12 hours for both MIPs and NIPs. At this equilibrium point, the adsorption capacity of MIPs and NIPs was determined to be 31.90 μg/mg and 14.58 μg/mg, respectively. This observed phenomenon can be attributed to the presence of binding

cavities located on the surface of the microsphere particles in the MIPs. These cavities facilitate the attachment of the target analyte, ginsenoside Rg1, leading to a reduction in transport resistance and an enhancement in the rate of adsorption. The equilibrium time of 12 hours indicates that sufficient time is required for the binding sites within the MIPs to interact with ginsenoside Rg1 and reach a point of saturation. This finding highlights the importance of allowing an appropriate duration for the adsorption process to achieve optimal results in terms of adsorption capacity. Overall, the adsorption kinetics study demonstrates the effectiveness of the MIPs in selectively capturing ginsenoside Rg1, with a higher adsorption capacity compared to the NIPs. This can be attributed to the presence of specific binding sites within the MIPs, which facilitate the efficient and selective adsorption of the target analyte.

Figure 6 illustrates the relationship between the adsorption capacity and the concentration of ginsenoside Rg1. As the initial concentration of Rg1 in the adsorption solution increased to 2 mg/mL, both MIPs and NIPs reached saturation, with their respective saturation adsorption amounts being 62.22 μg/mg and 29.70 μg/mg. Notably, the adsorption capacity of MIPs for ginsenoside Rg1 significantly increased with higher initial concentrations. Additionally, the curves demonstrate that the adsorption capacity of MIPs was notably higher than that of NIPs at the same concentration. This can be attributed to the fact that MIPs possess a larger number of specific binding sites, which are a result of the combination of geometric selectivity of the MIPs' imprinted pores and reversible covalent bonding between the functional monomer and the template molecule.

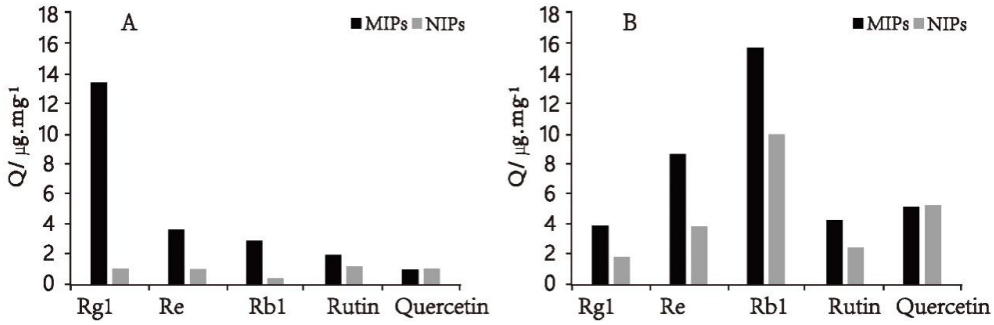

Fig 4. Binding capacities of MIPs and NIPs for Ginsenoside Rg1, Re, Rb1, Rutin and Quercetin
4-A: substrate solutions at the same concentration level;4-B: substrate solutions at different concentration levels

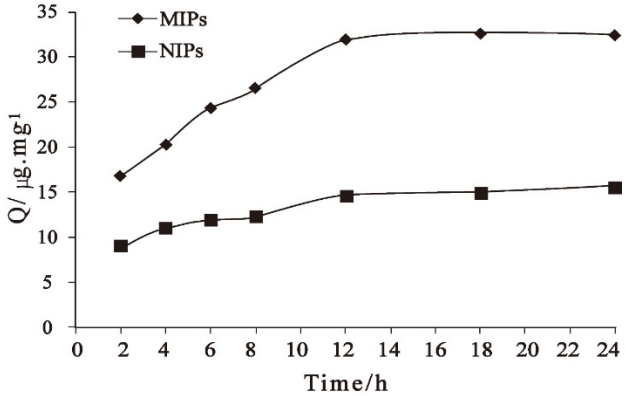

Fig 5. Adsorption kinetic curve of MIPs and NIPs for the template

3.4 Optimization of MISPE Conditions

The washing step is a critical procedure in the MISPE process as it aims to minimize non-specific interactions between the polymeric matrices and the target analytes. To optimize the MISPE procedure, factors such as the elution mode and solvent selection for the template molecule need to be considered. In order to determine the most effective elution method, three different elution

methods were compared. It was found that constant temperature agitation yielded the best elution effect, with a remarkable elution rate of template molecules reaching as high as 70.58% within a short duration of only 2 hours (Table S7). The choice of solvent also plays a crucial role in the MISPE process. Therefore, various proportions of methanol and acetic acid were tested as eluents. The results demonstrated that the elution rate of ginsenoside Rg1 from the MISPE column was 79.35% using the optimized solvent composition. Based on these findings, a compromise was made, and a 70% methanol/glacial acetic acid solution with a ratio of 9:1 (V/V) was selected as the optimum eluent. Furthermore, it was observed that strong polar solvents had the ability to reduce specific interactions. Therefore, a strong polar solvent was utilized to effectively remove ginsenoside Rg1 from the SPE column, resulting in promising results (Table S8). These findings underscore the significance of optimizing the washing step in the MISPE procedure to enhance elution efficiency and minimize non-specific interactions. The selection of the appropriate elution mode and solvent composition is crucial for achieving successful extraction of the target analytes, such as ginsenoside Rg1, from the polymeric matrices.

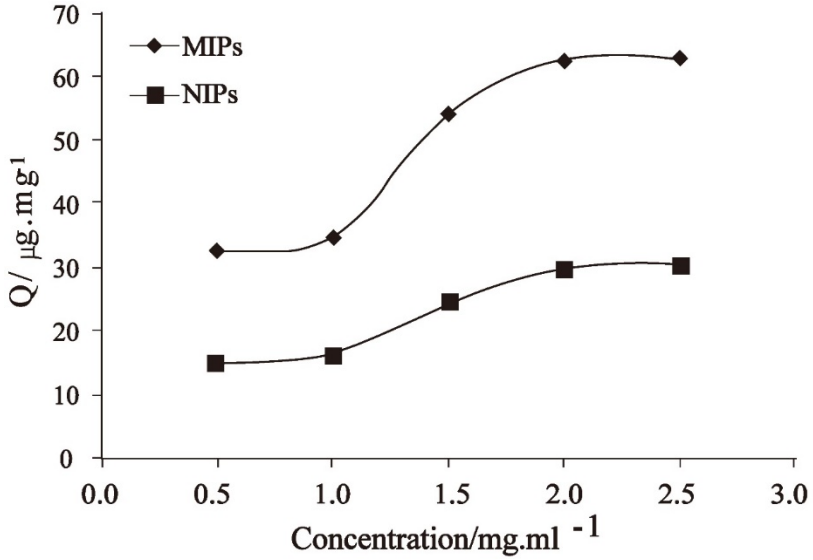

Fig 6. Adsorption isotherms curve of MIPs and NIPs for the template

3.5 Application of Ginsenoside Rg1-MIPs to TCM Sample

To evaluate the applicability of the established MISPE method in real matrices, it was employed for the extraction of ginsenoside Rg1 from ginseng extract. Figure 7-B illustrates the chromatogram of the eluent obtained after passing the ginseng extract through the MISPE column. Remarkably, the peak areas corresponding to ginsenoside Rg1 were significantly reduced in the effluent after MISPE treatment. By quantifying the proportion of Rg1-MIPs (25 mg), it was determined that approximately 58% of the ginsenosides Rg1 were adsorbed onto the MIPs, indicating a high affinity and efficient adsorption capacity of the MIPs for the target compound.

Figure 7-D depicts the chromatogram of the eluent obtained using 30% methanol, revealing smaller peak areas for the target components. To address this issue, a lyophilization process can be employed to eliminate non-specifically adsorbed impurities and non-covalently bound ginsenosides. This additional step aims to enhance the specificity and purity of the target component. Subsequently, the MIPs was eluted using a 70% methanol-glacial acetic acid (9:1, V/V) solution, and the content of the target component was determined based on the peak area of the eluate in the chromatogram. According to the peak area observed in Figure 7-C of the eluent chromatogram, it was estimated that approximately 84% to 90% of the target components were successfully eluted. These results demonstrate the effectiveness of the MISPE method in selectively extracting ginsenoside Rg1 from

ginseng extract, highlighting its potential for practical applications in the analysis and purification of ginsenosides.

By comparing Figure 8-A and B, it was observed that the peak areas of ginsenoside Rg1 in the extracts remained almost unchanged after adsorption by NIPs. This indicates that NIPs lacked specific binding sites for the target component. In contrast, the majority of the ginsenoside Rg1 was retained in the 30% methanolic fraction, with a recovery rate ranging from 7% to 12%. These findings confirm the absence of specific affinity in NIPs for ginsenoside Rg1.On the other hand, the synthesized Rg1-MIPs exhibited a significantly higher affinity and selectivity towards the template molecule ginsenoside Rg1, as demonstrated by the quantitative analysis of the target components using HPLC. The adsorption number of MIPs on ginsenoside Rg1 was determined to be 87.5%, further validating the effectiveness of the MISPE method for extracting ginsenosides. These results are summarized in Table-1.

Overall, the stark contrast in the adsorption behavior between NIPs and Rg1-MIPs confirms the successful imprinting of ginsenoside Rg1 in the synthesized MIPs, highlighting their high affinity and selectivity for the target compound. This further emphasizes the potential of MISPE as an efficient method for the extraction and purification of ginsenosides.

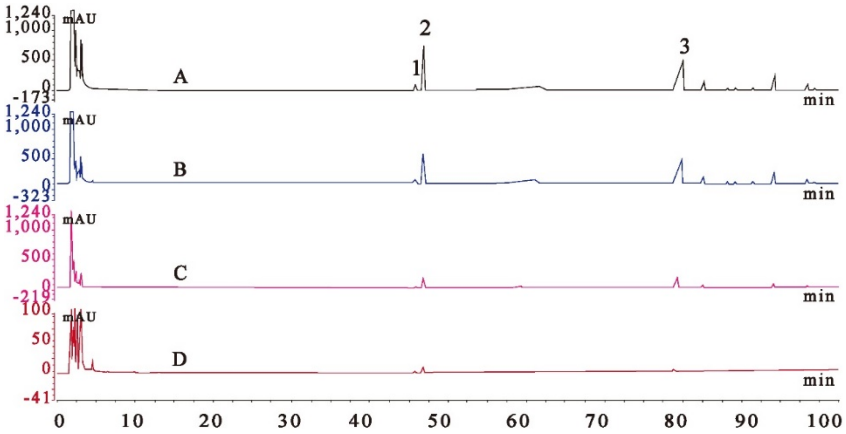

Fig 7. Chromatograms of the Ginsenoside Rg1 from the SPE protocols(MIPs)A. Ginseng extract; B. Upper sample effluent; C. Elution solution; D. Leaching solution;1. Ginsenoside Rg1; 2. Ginsenoside Re; 3. Ginsenoside Rb1.

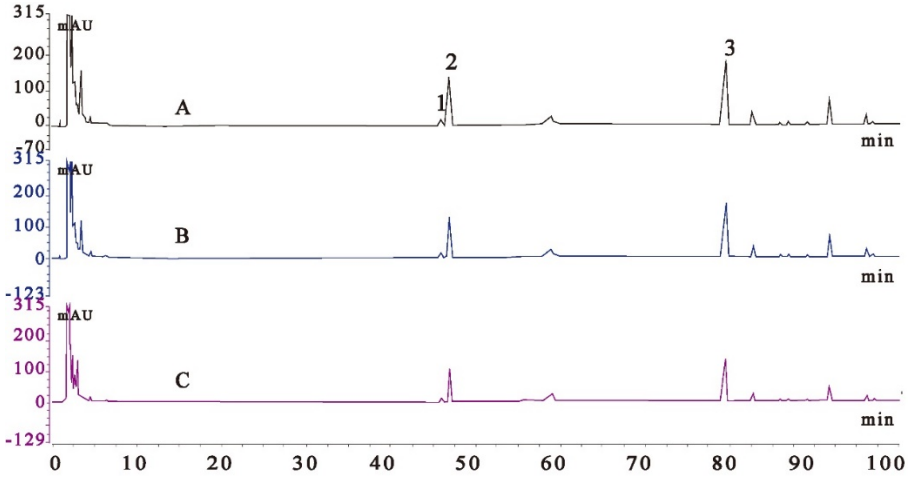

Fig 8. Chromatograms of the Ginsenoside Rg1 from the SPE protocols(NIPs)A. Ginseng extract; B. Upper sample effluent; C. Elution solution; D. Leaching solution; 1. Ginsenoside Rg1; 2. Ginsenoside Re; 3. Ginsenoside Rb1.

Table 1. Determination of Rg_1, Re and Rb_1 in ginseng extract after clean-up with MIP-SPE protocol(n=3)

SPE column	component	loading/μg	leaching/μg	elution/μg	recovery rate /%	RSD/%
MIPs	Rg_1	59.25	5.09	49.90	84.22	3.23
	Re	457.12	34.10	411.00	89.91	2.74
	Rb_1	907.33	19.96	777.28	85.67	2.34
NIPs	Rg_1	16.73	13.37	1.86	11.12	6.78
	Re	34.45	30.53	2.40	6.97	3.01
	Rb_1	86.67	74.59	9.97	11.50	3.44

4. Discussion

The molecularly imprinted polymers (MIPs) in this study were designed to specifically bind to the surface and internal binding sites of the template molecules through molecular forces. During the polymerization reaction, reversible covalent bonds were cleaved, resulting in the removal of the molecular templates. As a result, the cavities with complementary shape, size, and function [14] to the target monomers were retained.

It is worth emphasizing the significance of ginseng's diverse range of active substances, which often exhibit similar structures or share common structural units. This inherent complexity poses considerable challenges in terms of separating, extracting, and purifying these compounds. However, MIPs provide an effective solution to this problem by offering precise customization to meet specific requirements. MIPs possess several advantageous characteristics, including straightforward preparation processes, cost-effectiveness, reusability, and, most notably, their ability to demonstrate specific molecular recognition and high efficiency in enrichment and separation. These unique properties make MIPs particularly well-suited for the separation of structurally similar substances present in ginseng. By utilizing MIPs, researchers can overcome the difficulties associated with isolating and purifying these complex mixtures, thereby facilitating further exploration of ginseng's bioactive components.

Previous literature studies have predominantly focused on the preparation of molecularly imprinted polymers for flavonoids and phenols, with fewer studies on triterpenoids [15,16]. Therefore, our experiments are significant in addressing this research gap. Our experimental results demonstrate that the prepared MIPs exhibit higher selectivity and adsorption capacity. Through optimization, the MIPs for ginsenoside Rg1 achieved a percent recovery ranging from 84% to 90%, with a maximum saturated adsorption amount of 62.22 μg/mg. This MIPs can serve as an efficient adsorbent for the selective isolation and enrichment of ginsenoside Rg1 from complex mixtures. This finding holds great importance for the preparation of high-purity ginsenoside Rg1 drugs and their derivatives.

5. Conclusion

In this study, MIPs was developed and utilized for the analysis of ginsenoside Rg1. The MIPs were synthesized using a precipitation polymerization technique, resulting in a highly selective detection method that was easy, quick, selective, and sensitive. The recovery rate further confirmed the MIPs' ability to effectively separate and analyze complex samples. The prepared polymers were characterized using SEM and FT-IR, and their performance was evaluated using HPLC. The optimized MISPE method demonstrated its potential for purifying the chemical composition in TCMs, offering reduced interference and improved enrichment. As shown in Figure 9, this study provides a comprehensive description of the preparation, optimization, and application of ginsenoside Rg1-MIPs. The MISPE technique proves to be a powerful tool for the selective extraction of target components, effectively isolating and enriching ginsenoside Rg1 in TCMs.

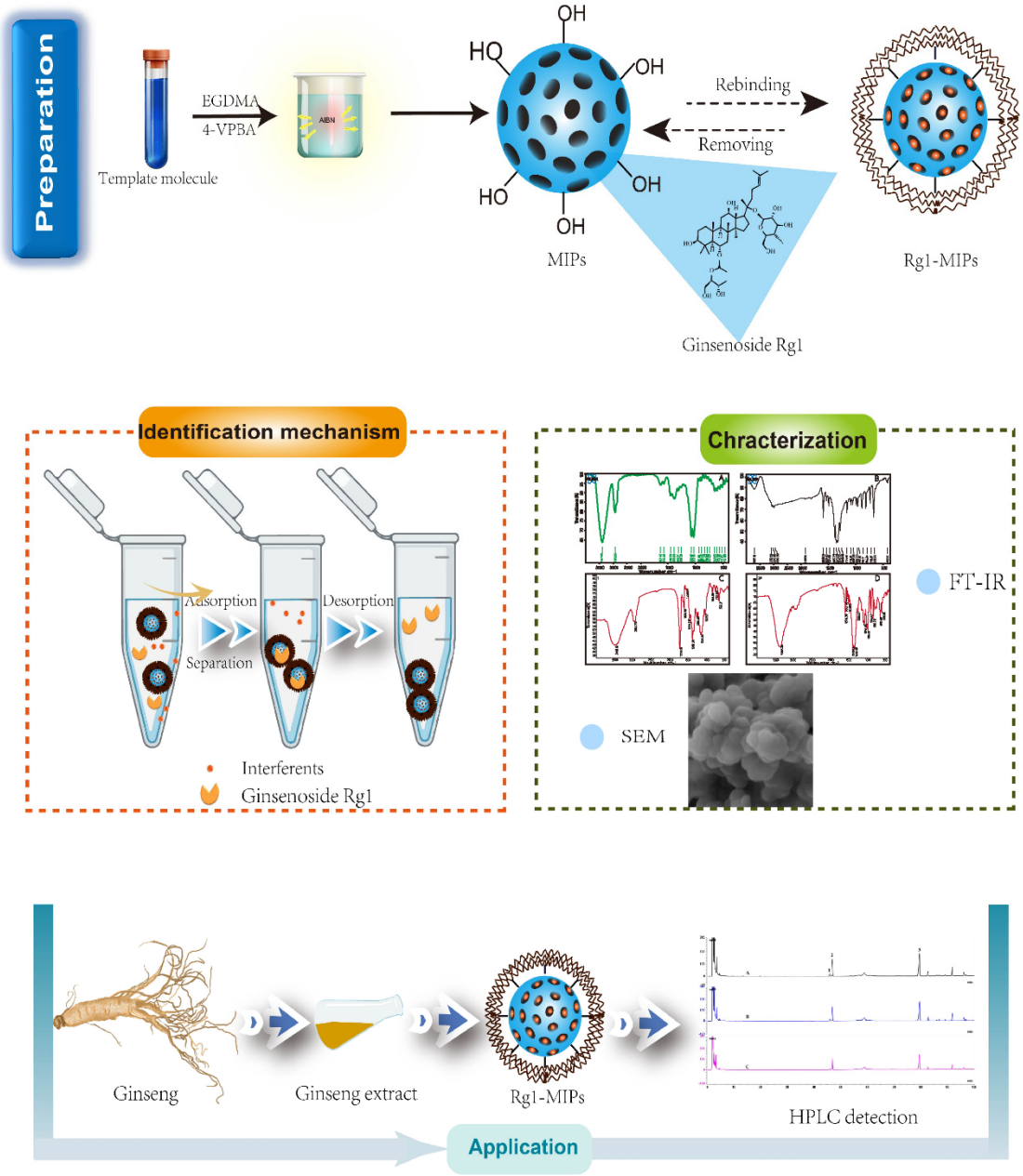

Fig 9. Rg1-MIPs:Preparation,chracterization and application.

ABBREVIATIONS

TCMs traditional Chinese medicines
MIPs molecularly imprinted polymers
NIPs molecularly non-imprinted polymers
MISPE molecular imprinted solid phase extraction
SEM scanning electron microscope
FT-IR flourier transform infrared spectrometer
RSDs relative standard deviations
SPE solid phase extraction
4-VPBA 4-vinylphenylboronic acid
EGDMA ethylene glycol dimethacrylate
HPLC high-performance liquid chromatography

AM Acrylamide
AIBN 2,2’-azobisisobutyronitrile

Ethical Approval

This article does not contain any research involving humans or animals.

Human and Animal Guidelines

Not applicable.

Consent of Publication

Written informed consent has been obtained from all persons involved in the experiment and a copy of the written consent is available for review by the Editor-in-Chief of the journal.

Conflict of Interest

We declare that Our products are independently developed, we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

Suppelementary Material

We can provide all supplementary materials regarding the experiments conducted in the article in an additional file.

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