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Molecularly imprinted polymers with ionic liquid as the functional monomer for selective solid-phase extraction of gastrodin.

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Abstract: *Gastrodia elata* is a famous traditional herb. It is a challenging task to efficiently separate the active ingredient Gastrodin (p-hydroxymethylbenzene- β -D-glucopyranoside) from *gastrodia elata* by simple procedure. According to the results of computer molecular simulation & UV-vis spectroscopy, water-soluble 1-vinyl-3-tetradecylimidazole bromide([C₁₄VIM⁺] [Br⁻]) was selected as the best ionic liquid functional monomer compared with methacrylic acid(MAA), acrylic acid(AA), 3-(2-carboxyethyl)-1-vinylimidazolium bromide ionic liquid (imidazole propionate, [COOHpvimBr]) and dehydroabiestic acid [2-(acryloyloxy)ethyl] ester (DAAEE). The force between [C₁₄VIM⁺] [Br⁻] and GAS (with multiple hydroxyl groups) is stronger than other monomers with more polar groups. The molecular imprinted polymers (MIPs) and non- molecular imprinted polymers (NIPs) with high selectivity to gastrodin(GAS) were synthesized utilizing ([C₁₄VIM⁺] [Br⁻]) as a novel

functional monomer. In addition, surface molecular imprinted polymers (SMIPs) and surface non-molecular imprinted polymers (SNIPs) supported on silica gel were also prepared. These polymers were characterized by infrared spectrum, scanning electron microscope, specific surface area tester and thermogravimetric analyser (TGA). The adsorption performance of the MIPs and NIPs was investigated by adsorption kinetics experiment and static adsorption experiment. By Molecular Imprinting-SPE extraction (HPLC), the content of gastrodin in eluent (70% ethanol) was significantly increased compared with it in plant extract, which is better than that of SMIPs-SPE. The recoveries of the spiked samples for the GAS ranged from 97.75-103.43% with relative standard deviation values lower than 2.88%. The molecular imprinted polymer was a potential separation material for gastrodin.

Keywords: molecularly imprinted polymers; GAS; ionic liquid; solid-phase extraction

1. Introduction

Gastrodia elata is a traditional herb with a variety of pharmacological activities and is widely used in Asian countries^[1,2]. As the water-soluble active ingredient of *Gastrodia elata*^[1,3], Gastrodin (p-hydroxymethylbenzene- β -D-glucopyranoside, the structural formula was shown in **Fig.2**) presents anticonvulsant, anxiolytic, and analgesic effects^[4-10]. Thus, Gastrodin has been widely used for the treatment of cardiovascular & cerebrovascular, headache & dizziness, limb numbness and pediatric convulsions^[10-14]. At present, due to the complex matrix of *Gastrodia elata* herbs and difficulties in the separation and purification of Gastrodin, the traditional techniques for the detection of GAS include HPLC, HPLC-MS/MS^[15-18], spectrophotometry and high performance capillary electrophoresis^[19]. The pre-

processing of these methods generally uses traditional solid phase extraction(SPE) small column clean-up, which lacks selectivity and specificity. Therefore, it is of great interest to establish a rapid, highly selective and reproducible technique for the targeted separation of GAS to meet the needs of quality control and pharmacological activity studies of gastrodin.

The molecular imprinting technique is based on the principle of simulating antibody-antigen or enzyme-substrate interactions so that the prepared blotted polymer has some recognition properties for the target molecule ^[20,21]. Compared with some other separation techniques previously studied, it shows high resistance to interference, high selectivity, good stability, a long life, universal utility and high recognition properties ^[22,23]. At present, molecular imprinting technique has been more researched and applied in the field of separation and purification of traditional Chinese medicine. For instance, Tian prepared the quercetin molecularly imprinted polymer which was used to purify the crude flavonoid extract of flavones. The average recoveries of myricetin and quercetin were 88.0% and 78.0%, respectively ^[24]. The artemisinin molecular imprinted polymers were synthesized by Xie. The polymers were used as the SPE packing for the extraction of artemisinin. The maximum static adsorption of MIP for artemisinin was 8.46 mg·g⁻¹^[25]. But there are still many shortcomings in its research: (1) the binding mechanism of functional monomers to template molecules is not studied deeply enough; (2) there are too few functional monomer species; (3) there are few studies on hydrophilic compounds in the study of MIPs. Although the technique still has many problems to be solved, molecularly imprinted solid-phase extraction can also separate and enrich target compounds more effectively than conventional SPE ^[4,26-30], which is a new highly selective technique for target substance pretreatment and

purification. In addition, it has been rapidly developed in the last decade and successfully applied to separate and purify the active ingredient in complex Chinese herbal extracts in several fields^[31-32].

Due to the complex matrix of *Gastrodia elata* herbs and difficulties in the separation & purification of gastrodin, many scientists have studied the molecular imprinting solid phase extraction of gastrodin. A molecularly imprinted polymer with high selectivity to gastrodin was prepared using alkenyl glycosides glucose as functional monomer by Huang^[28]. Ji^[33] synthesized the gastrodin molecular imprinted polymers by 1, 2, 3, 4, 5-pentafluoro-6-vinylbenzene & allyl 2, 3, 4, 6-tetra -O-acetyl-glucopyranoside as functional monomers. They synthesized molecularly imprinted polymers with new functional monomers which had certain selectivity for extraction and separation of gastrodin from *Gastrodia elata*. Unfortunately, these monomers have relatively complex structures and are difficult to synthesize with low yields, which makes it difficult for large-scale industrial application. Therefore, exploring new functional monomers with simple structure and strong interaction for gastrodin is the key to solve the above problems.

Based on the concept of Green Chemistry, many scientists have paid more and more attention to room temperature ionic liquids (ILs), it also known as ionic liquids, which is literally a liquid organic salt composed purely of positive and negative ions^[34] and it is a new type of green polar solvent in recent years. ILs can be used as solvents, porogenic agents, templates, functional monomer and so on. In the synthesis of MIPs, ILs can improve the solubility of the system and the binding capability between functional monomer and template molecule. In addition, it is characterized by low toxicity, high yield, simple synthesis,

excellent thermal & chemical stability, high solubility, designable structure and high conductivity^[35]. Therefore, these reagents are also known as “designable green reagent^[36]”. ILs are easy to design and connect with reactive groups such as amino, hydroxyl, cyano and carboxyl groups. ILs also have the ability to interact with organic molecules or inorganic ions through various interactions (such as π - π stacking, ionic bonding, hydrogen bonding, electrostatic, hydrophobic and hydrophilic interactions), which can promote the interaction between ionic liquids and target molecule. The potential of ILs to replace traditional volatile organic solvents for extraction has outstanding advantages, such as high ionic conductivity, large surface area, supramolecular recognition ability and so on. The application of ionic liquids in the field of molecularly imprinted polymers due to their unique physical and chemical properties has aroused wide interest among researchers. Especially, they are widely used as SPE materials for separating natural active ingredients^[37].

In this paper, molecular imprinting technology was used with gastrodin as template molecule and ethanol as porogen. Through UV-vis spectroscopy and computer molecular simulation, 1-vinyl-3-tetradecyl imidazolium bromide ionic liquid ([C₁₄VIM⁺] [Br⁻]) is the best monomer, compared with acrylic acid (AA), 3-(2-carboxyethyl)-1-vinylimidazolium bromide ionic liquid (imidazole propionate, [COOHpvimBr]), methacrylic acid (MA) and dehydroabietic acid [2-(acryloyloxy) ethyl] ester (DAAEE). The optimal ratio of template molecule to monomer is determined as 1:4. Molecularly imprinted polymer and non-imprinted polymer of GAS were successfully synthesized by precipitation polymerization and studied the adsorption mechanism of GAS. In addition, it was used as a solid phase extraction column packing to separate and purify GAS in *Gastrodia elata*, and 70% ethanol was used for

elution. MISPE was established for separating GAS, which has the characteristics of high sensitivity and good selectivity. The molecular imprinted polymer prepared by 1-vinyl-3-tetradecyl imidazolium bromide ionic liquid ($[C_{14}VIM^+][Br^-]$) is a potential separation material for gastrodin, which also provided a theoretical basis for the subsequent research and development of GAS selective adsorption materials.

2. Results and Discussion

2.1. Computer simulation

MD technique was used to study the interaction between GAS and monomers. The binding energies of the compounds between GAS and five monomers ($[C_{14}VIM^+][Br^-]$, DAAEE, imidazo ipropionate, MAA and acrylic acid) were counted. Meanwhile, the binding energies of GAS & $[C_{14}VIM^+][Br^-]$ with different proportions were also counted. **Fig.7a** showed that binding energies were -195.151 Kcal/mol ($[C_{14}VIM^+][Br^-]$), -92.9591 Kcal/mol (AA), -115.986 Kcal/mol (DAAEE) and -95.9767 Kcal/mol (MAA), -179.687 Kcal/mol (Imidazole propionate), respectively. Therefore, $[C_{14}VIM^+][Br^-]$ was chosen as the optimal functional monomer. Significantly, the force between $[C_{14}VIM^+][Br^-]$ and GAS (with multiple hydroxyl groups) is stronger than other monomers with more polar groups. It is speculated that the polymer synthesized with $[C_{14}VIM^+][Br^-]$ as the functional monomer may also have stronger recognition ability for GAS than other monomers. The results in **Figure 7b** showed that $[C_{14}VIM^+][Br^-]$ to GAS (1:4) is the best molar ratio. In addition, the functional monomers were further screened by analyzing of Ultraviolet and visible spectrophotometry, which was coincided with that of computer simulation.

2.2. Ultraviolet and visible spectrophotometry analysis

The mixture of GAS and 6 monomers was respectively scanned by UV-Vis. Moreover, The mixture of GAS & $[C_{14}VIM^+][Br^-]$ with different proportions was also researched. The results (**Fig. 8**) showed that comparing the absorption peaks, $[C_{14}VIM^+][Br^-]$ is the best monomer and there is little difference between the molar ratios of 1:6 and 1:4. Therefore, in order to save raw materials, 1:4 was chosen as the good molar ratio for preparing GAS-MIPs. The experimental results were showed no obvious difference with the MD calculation. The interaction diagram of GAS and $[C_{14}VIM^+][Br^-]$ is shown in **Fig. 9**. It showed that van der Waals force may play a major role between $[C_{14}VIM^+][Br^-]$ and GAS. The reason may be that $[C_{14}VIM^+][Br^-]$ contains 14 alkyl groups, which prevents the formation of hydrogen bonds with GAS. Compared with $[C_{14}VIM^+][Br^-]$, $[COOHpvimBr]$ can form hydrogen bond with Chikusetsu saponin IVa^[38].

2.3. Characterization

2.3.1. ¹H NMR spectrophotometry analysis

The ¹H NMR results of DAAEE, $[COOHpvimBr]$ and $[C_{14}VIM^+][Br^-]$ was shown in **Fig.S1, S2 and S3** respectively. The analysis results could be found in the supporting information.

2.3.2. Fourier transform infrared spectroscopy characterization

The FT-IR spectrum of $[C_{14}VIM^+][Br^-]$ ionic liquid was shown in the **Fig.10(b)**. The absorption at 1553.48 cm⁻¹ is mostly corresponded to the C=N in C₆N₇. The peak at 3135.57 cm⁻¹ was due to the C-H stretching vibration peak of the alkene and 1650.49 cm⁻¹ is the C=C stretching vibration peak. In addition, the bending vibrations peak of long-chain alkyl-CH₂ is

at 725.95 cm^{-1} . Above characteristic absorption peaks indicate that the target product $[\text{C}_{14}\text{VIM}^+][\text{Br}^-]$ was successfully obtained. In GAS spectrum (10 d), 3351.16 cm^{-1} belonged to the vibration peak of O-H in GAS, the peak of C-O-C in GAS appeared at 1240.88 cm^{-1} and 830.43 cm^{-1} to the characteristic peak of para-double substitution in the benzene ring. In the MIP spectrum (10a), the double bond absorption peak at 1655 cm^{-1} disappeared significantly, indicating that $[\text{C}_{14}\text{VIM}^+][\text{Br}^-]$ ionic liquid is involved in the polymerization. The stretching vibration absorption peak of C=O of EGDMA at 1731 cm^{-1} can be seen in MIP spectrum (10a). The characteristic peak in GAS can also be reflected in MIP spectrum (10a). These results showed that polymerization was synthesized successfully. Figure(10c) showed the IR spectrum of MIP after the adsorption of GAS, from which it could be seen that the absorption peak intensity of GAS is slightly higher than that in MIP.

The FT-IR spectra of the silica gel, modified silica gel and SNIP were shown in **Fig.11**. As shown in curves a and b, the peaks at about 1095.83 cm^{-1} and 803.35 cm^{-1} correspond to the Si-O-Si stretch of the silica gels and modified silica gels respectively. The absorption band at 3441.23 cm^{-1} , 1640.91 cm^{-1} corresponded to the O-H stretch. Compared to curve a. The peaks at about 1720.26 cm^{-1} (curve b) corresponded to the stretching vibration of C=O in KH570. The results indicated that KH570 had been grafted on the surface of silica gel particles successfully. The adsorption peak of the C=O stretching band at 1730.54 cm^{-1} (curve c) in SNIPs for EGDMA. The absorption band at 1459.44 cm^{-1} (curve c) corresponded to the C=C and the C=N in imidazole. The broad absorption band at 3580.71 cm^{-1} (curve c) indicated the presence of unsubstituted hydroxyl group on the surface of silica gel. The results showed that

the non-imprinted polymer layer had been grafted on modified silica gel particles, which demonstrated that polymerization was synthesized successfully.

2.3.3. SEM analysis

The polymers were researched by SEM to characterizing the size and shape. **Fig.12** showed that SEM pictures of MIPs and NIPs were studied under different amplification ratios. The MIPs and NIPs showed significant differences in shape. The NIPs cross-linked closer, nevertheless the MIPs exhibited rougher and porous structures than NIPs, which indicated the existence of recognition sites in MIPs could be attributed to the removal of GAS. These results also explained that compared with NIPs, MIPs might have larger specific areas and more recognition sites. **Fig.13** showed that SEM pictures of SMIPs and SNIPs were studied under different amplification ratios. The surface morphology of SMIPs were rough and porous. The imprinted recognition sites within cavities could serve as predetermined high-affinity sites for the template. Compared to the SNIPs, the morphology of SMIPs is more regular.

2.3.4. Energy Dispersive Spectrometer analysis

EDS is commonly used in combination with SEM for micro-zone and surface analysis, mainly for qualitative and semi-quantitative analysis of elements. The successful preparation of MIPs and SMIPs was further confirmed by the EDS analysis of the elements contained in the MIPs and SMIPs. The EDS of the MIPs and SMIPs is shown in **Fig.14**, from which it can be seen that the MIPs contains the elements C, N, O and Br, indicating the MIPs with [C14VIM⁺] [Br⁻] as monomer was synthesized successfully. SMIPs contains the elements C,

N, O, Si and Br, indicating the SMIPs was also synthesized successfully.

2.3.5. Thermo-gravimetric analysis

The TGA curve of MIPs (**Fig.15a**) showed that the weight loss is small about 1.60% in the range of 25°C-300°C, which might be the decreases of water and residual organic solvent. In the range of 300°C-450°C, it showed the weight loss rate of MIPs increased sharply, reaching about 89.60%. These weight losses might be the loss of MIPs' organic skeleton, which showed that polymers are relatively stable at 300°C.

Fig. 15b shows the TGA curves of SMIP and SNIP from 25°C to 700°C under a nitrogen atmosphere. SMIPs (SNIPs) exhibited good thermal stability at 300°C, with weight loss remaining at about 5.26%. The weight loss at this stage was due to water loss. However, the weight loss of the two polymers increased sharply when the temperature reached 450°C, which can be related to the loss of organic parts of SMIPs. At this time, the degradation also included CO₂ and H₂O. At 500°C, the weight loss tended to be stable.

2.3.6. Nitrogen adsorption/desorption isotherm

The nitrogen adsorption and desorption curves in **Fig.16** are type IV adsorption isotherms. In the low pressure section ($0 < P/P_0 < 0.1$), the curves are biased towards the Y-axis and the adsorption rises slowly, indicating that the polymer has a strong interaction with N₂. In the medium to high pressure range, the adsorption and desorption curves of MIP and NIP do not coincide and an H₃-type hysteresis loop appears, indicating the presence of mesoporous structures in both polymers. The specific surface area of the polymers was calculated by fitting the adsorption branch data by the BET equation and the pore size of the

polymer mediated pores by fitting the desorption branch data by the BJH equation, which results are shown in Table 1. The pore volumes, mean pore sizes and specific surface areas of the MIP and NIP were differences, indicating that imprinted and non-imprinted polymers have different pore structures and thus exhibit different adsorption result. Similarly, the results (Table 2) indicated that SMIP and SNIP also exhibited different adsorption result.

Table 1. Structural parameters of MIPs (NIPs)

	surface areas ($\text{m}^2 \cdot \text{g}^{-1}$)	total pore volume ($\text{cm}^3 \cdot \text{g}^{-1}$)	pore size (nm)
MIPs	8.5554	0.015104	7.0619
NIPs	9.1901	0.013660	5.9453

Table 2. Structural parameters of SMIPs (SNIPs)

	surface areas ($\text{m}^2 \cdot \text{g}^{-1}$)	total pore volume ($\text{cm}^3 \cdot \text{g}^{-1}$)	pore size (nm)
SMIPs	9.1833	0.011890	6.0022
SNIPs	6.5881	0.008951	5.6545

2.4. Evaluation of adsorption performance

2.4.1. Dynamic adsorption

The kinetic curves of adsorption of GAS by MIPs and NIPs were researched. **Figure 17** showed that the rebound of GAS in MIPs or NIPs was increased with the growth of adsorption time. The adsorption equilibrium of MIPs and NIPs is reached at about 120 min, and the equilibrium adsorption of MIPs is much higher than that of NIPs. The imprinting

factor (IF) was calculated to be 1.26 on the basis of formula (3). The binding properties of MIPs were further evaluated, and the data obtained were coincided with the kinetics analysis. The Table S1 can be seen that the pseudo second order model ($R_{2MIP_2}=0.5894$, $R_{2NIP_2}=0.1468$) is consistent with the experiment data less than the pseudo first order model ($R_{1MIP_1}=0.835$, $R_{1NIP_1}=0.9065$). Therefore, the pseudo first order kinetic model was evaluated to be more suitable for the MIPs dynamic adsorption. The pseudo first-order dynamic model curve of MIPs was shown in the **Fig. S4**.

2.4.2. Static adsorption

The rebinding isotherm was indicated in **Fig.18(a)**. The content of GAS raised with its initial concentration until it reached the saturation state. In addition, the equilibrium adsorption capacity of GAS-NIPs ($Q=220.4719 \text{ mg}\cdot\text{g}^{-1}$) was much lower than GAS-MIPs ($Q=308.5447 \text{ mg}\cdot\text{g}^{-1}$). It indicated that the MIPs had a strong memory effect and outstanding imprinting effect for GAS. In order to further understand the binding affinity of adsorptive material, the adsorption capacity was estimated by Scatchard and Langmuir. The **Fig.18 (b)** manifested the Scatchard curve possessed two binding sites in MIPs, which was specific and non-specific binding sites. Two linear regression equations were represented as $y_1=3.2018x+168.87(R_2=0.98077)$ and $y_2=10.221x+3856.9(R_2=0.83782)$, respectively. These results demonstrated that MIPs showed high affinity for GAS.

2.5. Reusability

Reusability is one of the most significant characteristics of MIPs. On this basis, a series of MIPs were assessed through five adsorption-desorption experiments. The **Fig.19** showed that

the adsorption capacity decreases slightly, but doesn't decline obviously with the increase of recycle time, declaring MIPs possessed stability and reusability.

2.6. Application of molecularly imprinted polymers to extract of GAS

In the crude extract of *Gastrodia elata*, the separation and enrichment of GAS was restricted due to the diversity of ingredients in natural products. In this experiment, we are mainly developing an adsorbent which can effectively extract GAS from the crude extract of *Gastrodia elata*. **Fig.20** exhibited the chromatograms including the *gastrodia elata* extract and the eluent solution from MIPs. GAS standard was shown in **Fig. 20a**, and the peak of GAS is detected in 3.97 min. The sample of *gastrodia elata* extract was shown in **Fig.20b**. MIPs adsorbed with GAS were eluted with methanol acetic acid(v/v,9:1), methanol, 70% methanol, ethanol, 50% ethanol and 70% ethanol respectively. The results were shown in **Fig.20c, 20d, 20e, 20f, 20g and 20h**. It was finally watched that 70% ethanol had the best elution effectivity. **Fig.20h** observed that the content of impurities decreased significantly and the peak area of GAS was the maximal. The relative content of GAS increased obviously. The linear regression equation was expressed as $y=19803x+576.63$ and the correlation coefficient (R^2) was 0.9991. 70 % ethanol effluent was spiked at three levels of 0.1, 0.2 and 0.3 mg·ml⁻¹. Table S2 expressed that the recoveries of samples were 97.75-103.43% with RSD not higher than 2.88%. The results declared that the MIPs could be served as an effective adsorbing material to separating the GAS from *Gastrodia elata*. It could also be utilized to rapidly enrich and separate GAS from other natural medical plants.

3. Experimental

3.1. Chemical and reagents

Gastrodin(GAS) was purchased from Yuanye Biotechnology Co., Ltd (Shanghai, China). 1-Bromotetradecane & 1- N-Vinyl imidazole were purchased from Pure Reagent Co., Ltd. (Shanghai, China). Ethylene glycol dimethacrylate & N, N-azodiisobutyronitrile were bought from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Methacrylic acid & acrylic acid were bought from Saen Chemical Technology Co., Ltd (Shanghai, China). Methanol and ethanol was purchased from the Chengdu KeLong chemical reagent factory (Chengdu, China). Dehydroabietic acid [2-(acryloyloxy) ethyl] ester (DAAEE), [COOHvimBr] and [C₁₄VIM⁺] [Br⁻] were synthesized.

3.2. Instrumentation

FT-IR (Zeiss, Germany) spectra were recorded on a TENSOR 27 instrument to analyze the structure of synthetic substances. The UV Vis absorption spectrum was recorded with a UV-2550 spectrophotometer (Shimadzu, Japan) to assist in the selection of functional monomers and solvents. The structure of the functional monomer was identified by ¹H NMR spectroscopy (Bruker, Germany). Scanning electron microscope (SEM) imaging was performed on the Sigma 300 (Zeiss, Germany) to observe the shape and structure of the prepared MIP and NIP. The chemical elements of the GAS-MIP were analyzed by EDS. TG/DTA7300 (Hitachi, Japan) was used for thermo-gravimetric analysis (TGA) at a heating rate of 10°C min⁻¹. The concentrations of GAS were determined by HPLC (Agilent 1260, USA).

3.3. Computer simulation

In recent years, computer simulation method has been continuously introduced into the research of synthesis of molecularly imprinted polymers. Through computer simulation method, it can well replace conventional experiments, which greatly reduce unnecessary solvent and artificial consumption. It also gives some guidance in improving the R & D efficiency of MIPs, as well as the affinity and selectivity of MIPs.

In this paper, the computer simulation was researched by Materials Studio(MS) 2019. Firstly, the structures of GAS (template molecule) and functional monomer were respectively optimized by DMOL3 module with the force field of Compass II. Secondly, the Initial models which contained template molecules and different functional monomers (1:1) were established through Amorphous Cell module. The number of solvent molecules was set 50 in each models. The structure of the initial models was optimized by using the Forcite module. The dynamics of the model was simulated at 500ps by using the canonical ensemble (NVT) and isothermal-isobaric ensemble (NPT). After the models reached equilibrium, the binding energy between monomer & GAS (ΔE) was calculated. The lower ΔE between GAS & monomer, the better binding performance of two molecules. These also means that the formed composite becomes more stable. The calculation formula of binding energy(ΔE) is as follows (1):

$$\Delta E = E(\text{Total}) - (E(\text{template}) + E(\text{monomer}) + E(\text{solvent})) \quad (1)$$

3.4. Ultraviolet and visible spectrophotometry analysis of prepolymerization system

3.4.1. Selection of functional monomer

The same volume and concentration of functional monomer ($[C_{14}VIM^+][Br^-]$, imidazole propionate, DAAEE, MAA and AA) ethanol solutions were added into the GAS ethanol solution in turn. Then the solutions were vibrated by ultrasound for 4 h and scanned at 200-350 nm with the ultraviolet absorption spectrum. It is generally believed that the more the characteristic absorption peak moved, the stronger the binding between the template molecule & monomer.

3.4.2. Selection of the optimal molar ratio

The interaction of $[C_{14}VIM^+][Br^-]$ and GAS in different molar ratios was studied. Ultraviolet absorption spectrum was measured at 200-350 nm with an ultraviolet spectrophotometer.

3.5. Synthesis of dehydroabiatic acid [2-(acryloyloxy)ethyl] ester (DAAEE)

Dehydroabiatic acid (5 g, 0.016 mol) and sulfoxide chloride (4.76 g, 0.04 mol) were slowly dripped to toluene (50 ml) in a three-necked flask. The solution was refluxed at 110°C for 42 h and then cooled to room temperature. Finally, the mixtures were evaporated to toluene at low temperature (60°C) and the red brown liquid is acyl chloride dehydroabiatic acid.

Acyl chloride dehydroabiatic acid (6.4 g, 0.02 mol), hydroxyethyl acrylate (6.97 g, 0.06 mol), trimethylamine (2.05 g, 0.02 mol) were added to toluene (50 ml) in the 250 ml three-neck flask. The mixture was refluxed at 60°C for 12 h. The product is purified by silica gel

column chromatography (the eluent is ethyl acetate-petroleum ether=1:8). The white solid obtained is dehydroabiatic acid [2-(acryloyloxy)ethyl] ester (DAAEE). ¹H NMR were used to characterized the product structure. The synthesis route is shown in the **Fig.3**.

3.6. Synthesis of [COOHpvimBr]

Refer to the previous experiment of the research group ^[38]: Under anhydrous and oxygen free conditions, 3-bromopropionic acid (3.83 g, 0.025 mol), N-vinyl imidazole (2.35 g, 0.025 mol) were added to acetone (40 ml) in a flask. The solution was refluxed for 20 h. Subsequently, the solvent is removed to obtain a yellow solid, which was recrystallized in ethanol to obtain [COOHvimBr]. Finally, ¹H NMR were used to characterized the product. The synthesis route is shown in the **Fig.4**.

3.7. Synthesis of [C₁₄VIM⁺] [Br⁻] ionic liquid

In a three-necked flask, 1-Bromotetradecane (7.20 g, 0.026 mol) and 1-Vinylimidazole (2.45 g, 0.026 mol) were added into acetone (80 ml). The solution was refluxed for 24 h under nitrogen atmosphere at 50°C. After 24 h, the solvents were eliminated to obtain white solid, which was recrystallized in ethyl acetate and petroleum ether (volume ratio 1:1) to get [C₁₄VIM⁺] [Br⁻] ionic liquid. FT-IR and ¹H NMR were used to characterized the product. The synthesis route is shown in the **Fig.5**.

3.8. Synthesis of molecularly imprinted polymers

At room temperature, GAS (0.0867 g, 0.3 mmol), [C₁₄VIM⁺] [Br⁻] (0.48 g, 1.26 mmol) & ethanol (80 ml) were successively added into a flask under nitrogen atmosphere. The solution

was stirred for 12 h. Subsequently, the cross-linker (EGDMA, 1.2 g) and the initiator (AIBN, 0.06 g) were added into the solution. The polymerization solution is stirred in an oil bath at 70°C for 12 h. The unreacted template was repeatedly eluted with methanol-acetic acid solution (9:1, v/v) until there was no template. The preparation process of MIPs was shown in **Fig.6**. GAS-NIP was also prepared when polymerization was carried out in the absence of GAS.

3.9. Synthesis of SMIPs (SNIPs)

Synthesis of silica gel: ethanol (100 mL), H₂O (20 mL), NH₃·H₂O (10 mL) and tetraethyl silicate (10 mL) were successively added into a Round-bottom flask. The solution was stirred at room temperature for 5 h. The white solid was washed with water and ethanol respectively.

Activation of silica gel: silica gel (1.98 g), H₂O (140 mL) and 10% diluted hydrochloric acid (8 mL) were dispersed into a three necked bottle, under constant stirring for 8h at 75°C. Finally, the products were washed with water & ethanol respectively and dried with a rotary evaporator at 40°C.

Synthesis of modified silica gel: The activated silica gels (1.51 g), water (50 ml), KH570 (4 ml) and alcohol (50 ml) were added to a 250 mL three-necked flask. The reaction was stirred at 70°C for 3 h. Finally, the solid was washed with water & ethanol respectively. The white solid was obtained after dried with a rotary evaporator at 40°C.

Synthesis of SMIPs (SNIPs): At room temperature, GAS (0.0867 g, 0.3 mmol), [C₁₄VIM⁺] [Br⁻] (0.48 g, 1.26 mmol), modified silica gel (750 mg) & ethanol (100 ml) were

successively added into a three necked bottle with condensing tubes and nitrogen protection devices. The solution was stirred for 12 h after dissolving. Subsequently, the cross-linker (EGDMA, 1.2 g) and the initiator (AIBN, 0.06 g) were added. The solution was bubbled with nitrogen for 10 min to get rid of oxygen. The polymerization solution is stirred in an oil bath at 70°C for 12 hours. The template was repeatedly eluted with methanol-acetic acid solution (9:1, v/v) until there was no template. SNIPs were also prepared when polymerization was carried out in the absence of GAS.

3.10. Characterization of functional monomers

3.10.1. FT-IR studies

The template molecule(GAS), MIP, functional monomers($[C_{14}VIM^+][Br^-]$), silica gel, modified silica gel, SNIPs and MIP adsorbed with GAS were analyzed by FT-IR (4000-400 cm^{-1}) by potassium bromide tablet pressing method.

3.10.2. ^1H NMR studies

In the ^1H NMR studies, $[C_{14}VIM^+][Br^-]$ was dissolved in the D_2O , DAAEE was dissolved in the $CDCl_3$, $[COOHpvimBr]$ was dissolved in the D_2O-d_6 .

3.11. Characterization of MIPs and SMIPs

3.11.1. SEM studies

The surface morphology images of MIPs and SMIPs were recorded on SEM. In addition, the elemental composition of MIPs and SMIPs was studied through EDS.

3.11.2. Nitrogen adsorption/desorption isotherm

Through the nitrogen gas adsorption experiment, the surface areas, pore volumes & pore sizes of MIPs and SMIPs were measured. The samples of 83.6 mg were degassed for 12 h at 120°C prior to the final N₂ adsorption/desorption analysis, which was conducted at a temperature of 77.30 K.

3.11.3. Thermo-gravimetric analysis

20 mg of MIPs or SMIPs were weighed for thermo-gravimetric analysis to evaluate the thermal stability of the polymer. The test temperature range is 30-800°C and the heating rate is 10°C min⁻¹.

3.12. HPLC analysis

Agilent 1260 Series HPLC was used to detect the contents of the samples. Detection conditions of GAS: The chromatographic column was HYPURITY C18 (5μm, 4.6 × 250 mm), mobile phase contained acetonitrile and 0.2% phosphoric -aqueous solution (15:85, v/v), flow rate was 0.8 mL/min, injection volume was 10 μ, UV detector wavelength was set at 220 nm, and the GAS retention time was 3.83 min.

3.13. Adsorption experiments

3.13.1. Dynamic adsorption

The 20 mg MIPs or NIPs were accurately weighed into a 10 mL centrifuge tube. 10 mL of 0.20 mg·mL⁻¹ GAS-ethanol solution was added into centrifuge tube. After 10, 20, 30, 60, 90, 120, 180 min of vibrating at room temperature, the mixture was centrifuged for 10 min (4000

r/min), the concentrations of GAS were measured by HPLC after being filtered through microporous membrane. Finally, the adsorption capacity (Q_e) of the polymer to GAS was calculated at different times according to formula (2). The adsorption equilibrium capacity (Q_e , $\text{mg}\cdot\text{g}^{-1}$) was calculated through the follows:

$$Q_e = (C_0 - C_e)V/M \quad (2)$$

In the equation (2), The meanings and units of each symbol were shown in Table 3.

Table 3. Meanings and units of symbols in the equation (2)

Symbols	Meaning of symbols	Units
Q_e	the equilibrium adsorption capacity	$\text{mg}\cdot\text{g}^{-1}$
C_0	the initial concentration of GAS	$\text{mg}\cdot\text{mL}^{-1}$
C_e	the equilibrium adsorption concentration of GAS	$\text{mg}\cdot\text{mL}^{-1}$
V	the volume of solution	mL
M	the mass of polymers	mg

When the Imprinting Factor (IF) >1 , it indicates that MIP can recognize the template molecule. The larger the IF , the better the impression effect ^[39]. IF is calculated as follows:

$$IF = Q_{MIPS}/Q_{NIPS} \quad (3)$$

Q_{MIPS} ($\text{mg}\cdot\text{g}^{-1}$) is the adsorption quantity of MIPs, similarly Q_{NIPS} ($\text{mg}\cdot\text{g}^{-1}$) is expressed as the adsorption quantity of NIPs.

The pseudo-first-order kinetic equation (formula (4)) and pseudo-second-order kinetic equation (formula (5)) were used to evaluated the adsorption characteristics of polymers ^[40].

The adsorption kinetics was estimated as follows:

$$\ln(Q_e - Q_t) = \ln Q_e - k_1 t \quad (4)$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + t/Q_e \quad (5)$$

The meanings and units of each symbol in formulas (4) and (5) were shown in Table 4.

Table 4. Meanings and units of symbols in formulas (4) and (5)

Symbols	Meaning of symbols	Units
Q_e	the equilibrium adsorption capacity	$\text{mg} \cdot \text{g}^{-1}$
Q_t	the adsorption capacity of the adsorbent at time t	min
K_1	the pseudo-first-order rate constant	min^{-1}
K_2	the pseudo-second-order rate constant	$\text{g} \cdot \text{mg}^{-1} \text{ min}^{-1}$

3.13.2. Static adsorption

In the equilibrium adsorption, 20 mg of MIPs or NIPs was weighed in a centrifuge tube. 10 mL GAS ethanol solution of various concentrations ($0.05\text{-}0.5 \text{ mg} \cdot \text{mL}^{-1}$) was added in the centrifuge tube. After shaking for 3 h, the supernatant was obtained by high-speed centrifugation for 15 min. The content of GAS was measured by HPLC. Meanwhile, NIPs were used in parallel experiments. Through the Scatchard equation (formulas (6)), we evaluated the adsorption characteristics of MIPs.

$$\frac{Q_e}{C_e} = \frac{Q_m}{K_d} - Q_e/K_d \quad (6)$$

The meanings and units of each symbol in formulas (6) were shown in Table 5.

Table 5. Meanings and units of symbols in formulas (6)

Symbols	Meaning of symbols	Units
Q_e	the equilibrium adsorption capacity	$\text{mg} \cdot \text{g}^{-1}$

Q_m	the maximum adsorption capacity	$\text{mg}\cdot\text{g}^{-1}$
C_e	the equilibrium adsorption concentration of GAS	$\text{mg}\cdot\text{mL}^{-1}$
K_d	the dissociation constant of the equilibrium state	$\text{mg}\cdot\text{mL}^{-1}$

3.14. Solid phase extraction on molecularly imprinted polymers(MISPE)

2.0 g of dried Gastrodia elata root powders were extracted in 40 mL of ethanol (50%) for 30 min. The extract solution was filtered and concentrated to 10 ml as loading solution. 500 mg of MIPs were packed into a SPE column (6.0 mL). 5 ml of the loading solution was added to the MIPs-SPE column. MIPs-SPE was eluted with 5 mL of ethanol, 50% ethanol, 70% ethanol, methanol and 70% methanol respectively. Finally, the eluents were analyzed by HPLC.

4. Conclusions

On the basis of the results of computer molecular simulation and UV vis spectrum, $[\text{C}_{14}\text{VIM}^+][\text{Br}^-]$ was finally selected as the functional monomer compared with AA, MAA, Imidazole propionate and DAAEE. According to computer molecular simulation, $[\text{C}_{14}\text{VIM}^+][\text{Br}^-]$ may interact with template molecules through van der waals force interaction. The adsorption performance showed that the MIP($[\text{C}_{14}\text{VIM}^+][\text{Br}^-]$ as monomer) had a large adsorption capacity for GAS ($Q_{\text{max}}=151.84 \text{ mg}\cdot\text{g}^{-1}$, $\text{IF}=1.26$). After repeated use for 5 times, no obvious difference in maximum adsorption capacity was detected. By Molecular Imprinting-SPE extraction(HPLC), the content of gastrodin in eluent (70% ethanol) was significantly increased compared with it in plant extract, which is also better

than that of SMIPs-SPE. The recoveries of GAS spiked samples were 97.75-103.43% with RSD (the relative standard deviations) lower than 2.88%. The MIPs using 1-vinyl-3-tetradecyl imidazolium bromide ionic liquid as monomer was a potential separating materials for gastrodin.

Table

Table 1. Structural parameters of MIPs (NIPs)

	surface areas ($\text{m}^2\cdot\text{g}^{-1}$)	total pore volume ($\text{cm}^3\cdot\text{g}^{-1}$)	pore size (nm)
MIPs	8.5554	0.015104	7.0619
NIPs	9.1901	0.013660	5.9453

Table 2. Structural parameters of SMIPs (SNIPs)

	surface areas ($\text{m}^2\cdot\text{g}^{-1}$)	total pore volume ($\text{cm}^3\cdot\text{g}^{-1}$)	pore size (nm)
SMIPs	9.1833	0.011890	6.0022
SNIPs	6.5881	0.008951	5.6545

Table 3. Meanings and units of symbols in the equation (2)

Symbols	Meaning of symbols	Units
Q_e	the equilibrium adsorption capacity	$\text{mg}\cdot\text{g}^{-1}$
C_0	the initial concentration of GAS	$\text{mg}\cdot\text{mL}^{-1}$
C_e	the equilibrium adsorption concentration of GAS	$\text{mg}\cdot\text{mL}^{-1}$
V	the volume of solution	mL
M	the mass of polymers	mg

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Table 4. Meanings and units of symbols in formulas (4) and (5)

Symbols	Meaning of symbols	Units
Q_e	the equilibrium adsorption capacity	$\text{mg}\cdot\text{g}^{-1}$
	the adsorption capacity of the adsorbent at	
Q_t	time t	min
K_1	the pseudo-first-order rate constant	min^{-1}
K_2	the pseudo-second-order rate constant	$\text{g}\cdot\text{mg}^{-1}\text{ min}^{-1}$

469

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Table 5. Meanings and units of symbols in formulas (6)

Symbols	Meaning of symbols	Units
Q_e	the equilibrium adsorption capacity	$\text{mg}\cdot\text{g}^{-1}$
Q_m	the maximum adsorption capacity	$\text{mg}\cdot\text{g}^{-1}$
C_e	the equilibrium adsorption concentration of GAS	$\text{mg}\cdot\text{mL}^{-1}$
K_d	the dissociation constant of the equilibrium state	$\text{mg}\cdot\text{mL}^{-1}$

471

Tab. S1 simulation parameters of pseudo-first-order dynamics and
pseudo-second- order dynamics

	$Q_{e(\text{exp})}$ (mg/g)	the pseudo-first-order model			the pseudo- second -order model		
		R_1^2	K_1 (min^{-1})	$\ln Q_e$ (mg/g)	R_2^2	$K_2(\text{g} \cdot \text{mg}^{-1} \cdot \text{min}^{-1})$	$\ln Q_e$ (mg/g)
MIP	151.8424	0.835	0.0222	5.6032	0.5894	-0.0042	0.3204
NIP	120.3517	0.9065	0.0239	5.0973	0.1468	-0.0016	0.9631

Table S2 Recoveries of GAS at different spiked levels in samples(n=3)

Spiked levels(%)	Recovery (%)	Mean recovery (%)	RSD (%)
80%	97.75	0.99092	2.23
	101.64		
	97.89		
100%	102.15	1.004249333	1.53
	99.88		
	99.24		
120%	103.43	1.001487667	2.88
	99.03		
	97.98		

Figure

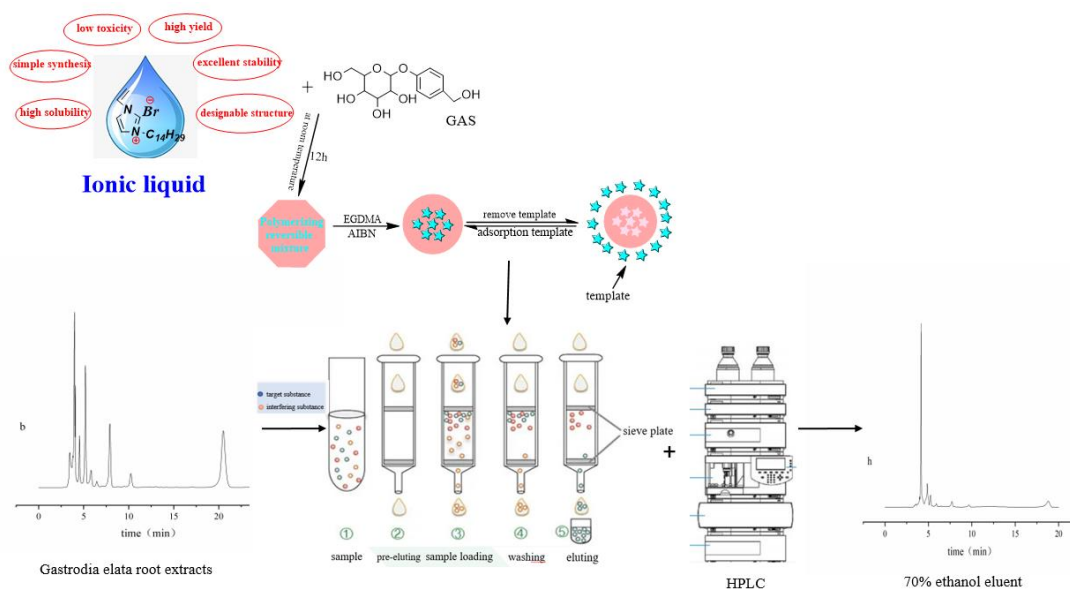


Fig.1 graphical abstract.

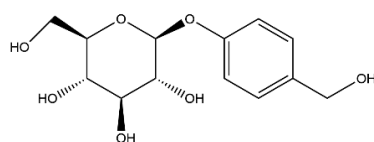


Fig.2 P-hydroxymethylbenzene-β-D-glucopyranoside structural formula.

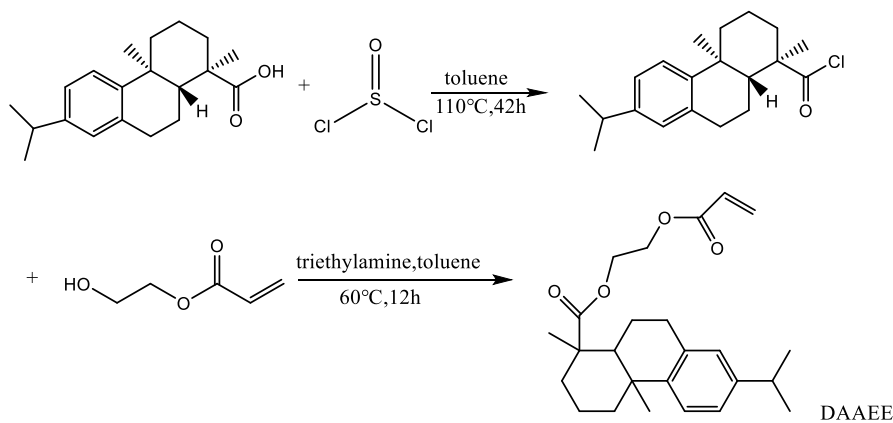


Fig.3 Synthesis of dehydroabietic acid [2-(acryloyloxy)ethyl] ester (DAAEE).

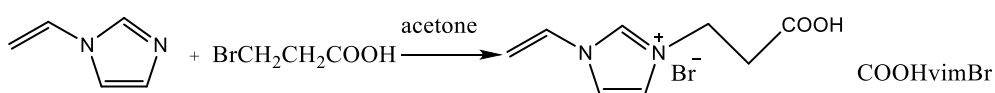


Fig.4 Synthesis of [COOHvimBr] ionic liquid.

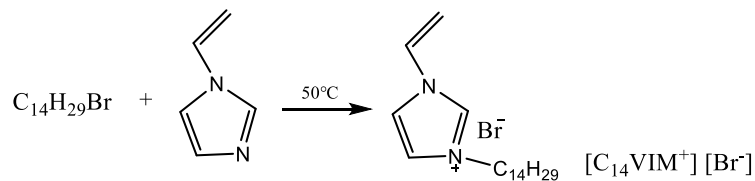


Fig.5 Synthesis of $[\text{C}_{14}\text{VIM}^+][\text{Br}^-]$ ionic liquid.

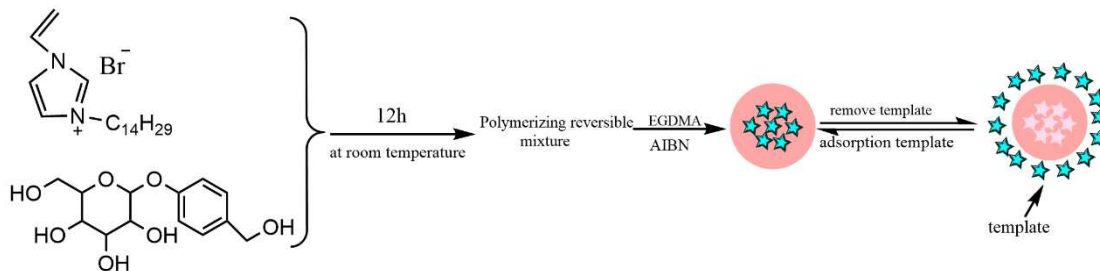


Fig.6 Synthesis of MIPs.

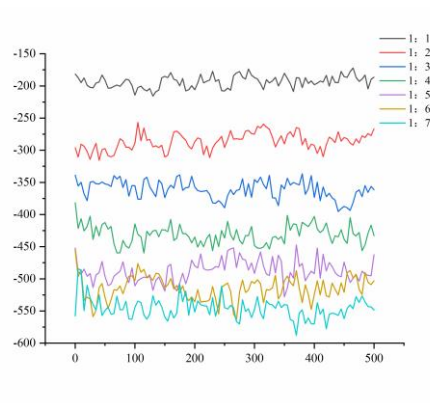
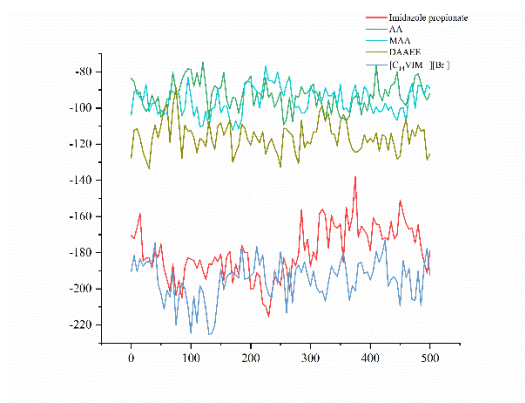


Fig.7 binding energy of functional monomer and GAS(a) and molar ratio of functional monomer to template molecule(b).

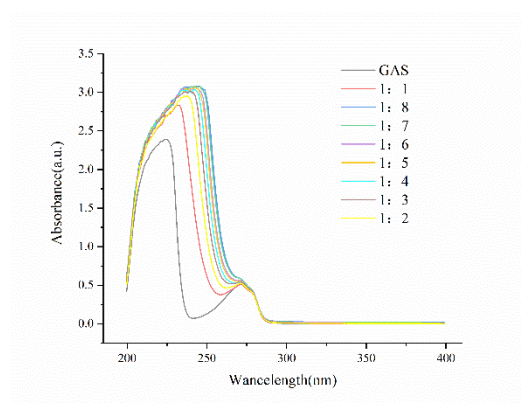
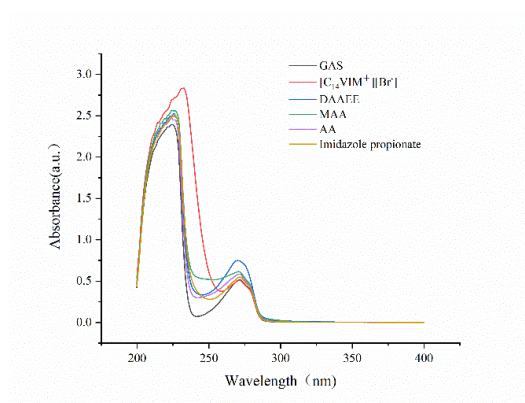


Fig.8 The Ultraviolet and visible spectrophotometry of different functional monomers (a) and GAS in ethanol with addition of different concentrations of $[C_{14}VIM^+][Br^-]$ (b).

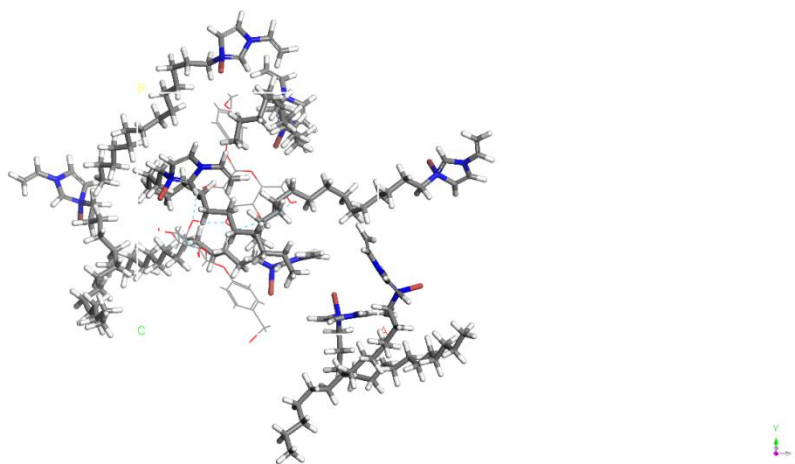


Fig.9 Interaction between $[C_{14}VIM^+][Br^-]$ and GAS.

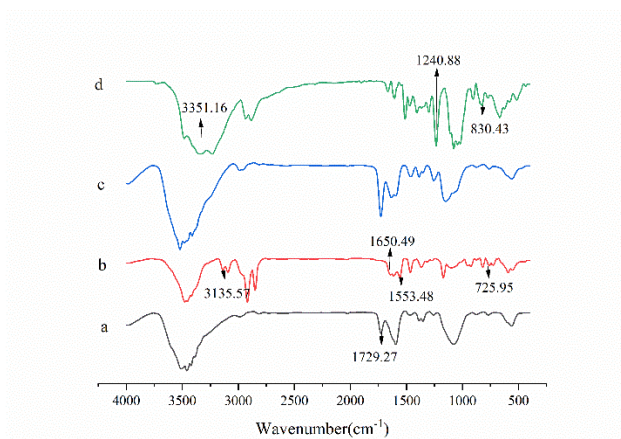


Fig.10 The FT-IR spectrum of MIP (a), $[C_{14}VIM^+][Br^-]$ (b), MIP after the adsorption of GAS(c), GAS(d).

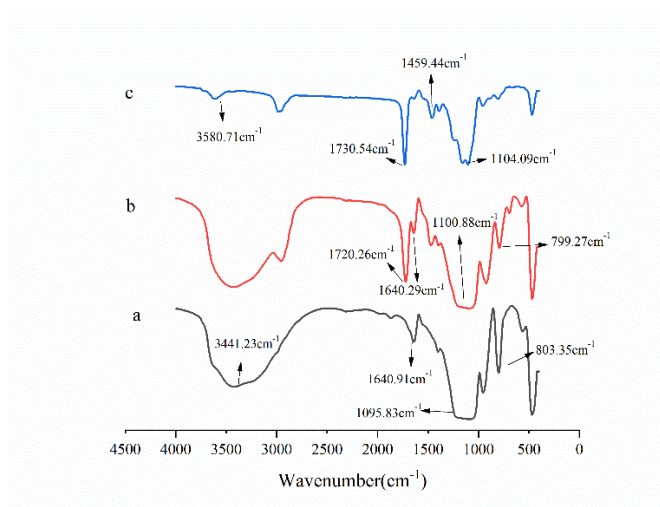
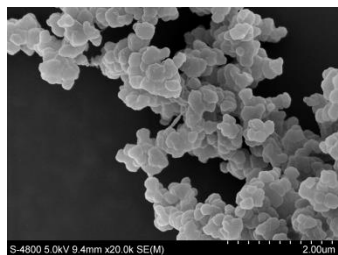


Fig.11 The FT-IR spectrum of silica gel(a), modified silica gel (b) and SNIPs(c).

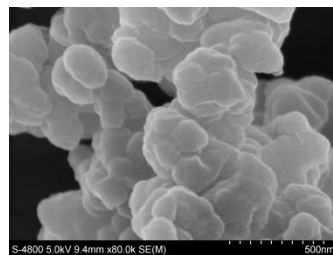
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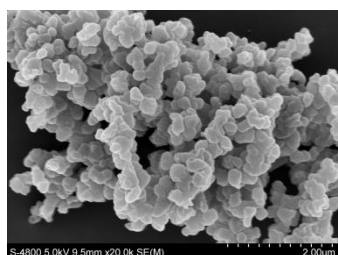
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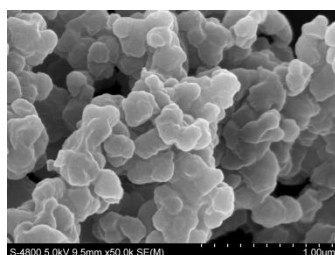
a

b

c



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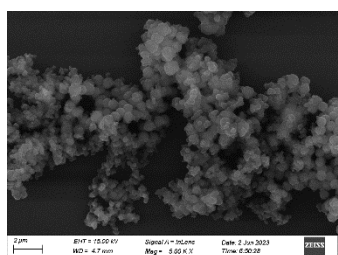
d

e

f

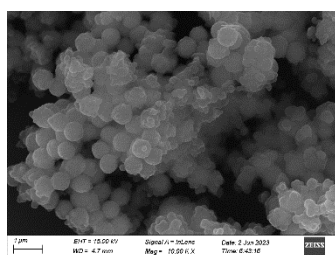
519 **Fig.12** The scanning electron microscopy of MIPs (a, b, c) and NIPs (d, e, f).

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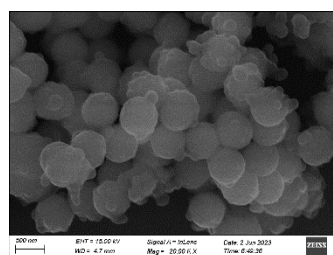
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a



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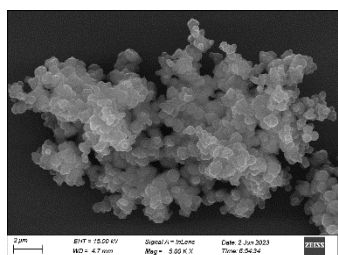
b



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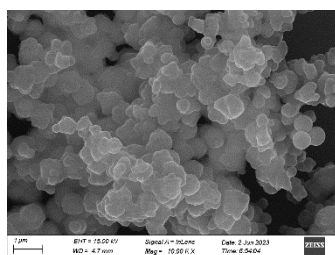
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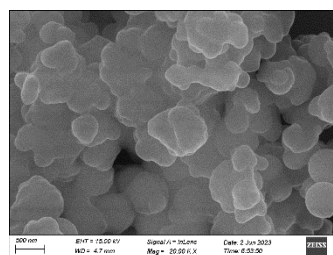
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d



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e

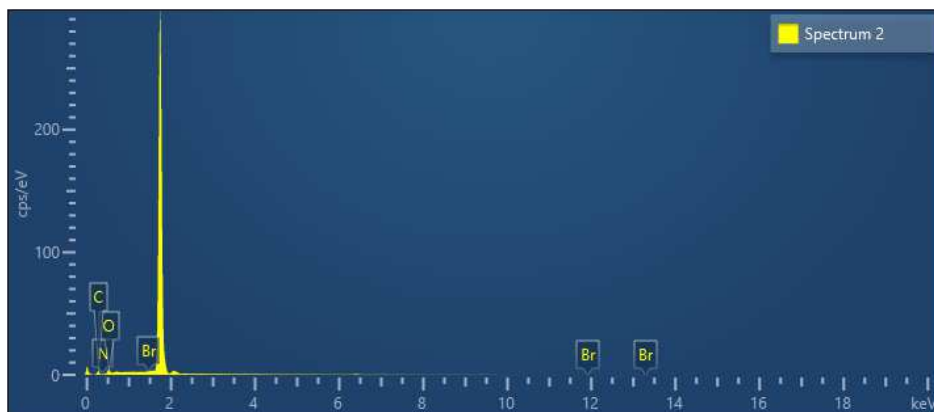


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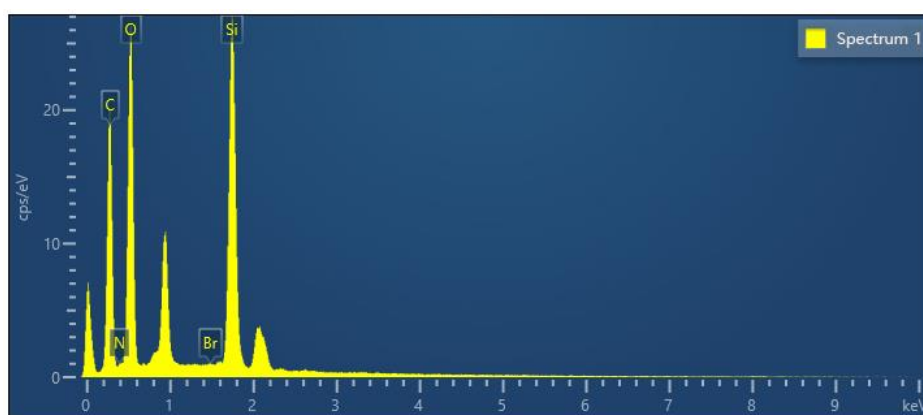
f

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529 **Fig.13** The scanning electron microscopy of SMIPs (a, b, c) and SNIPs (d, e, f).

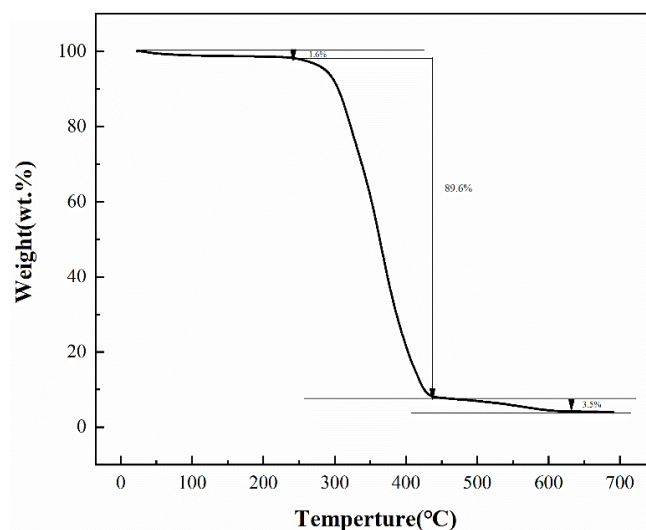


a



b

Fig.14 EDS energy spectrum of MIPs(a)and SMIPs(b).



a

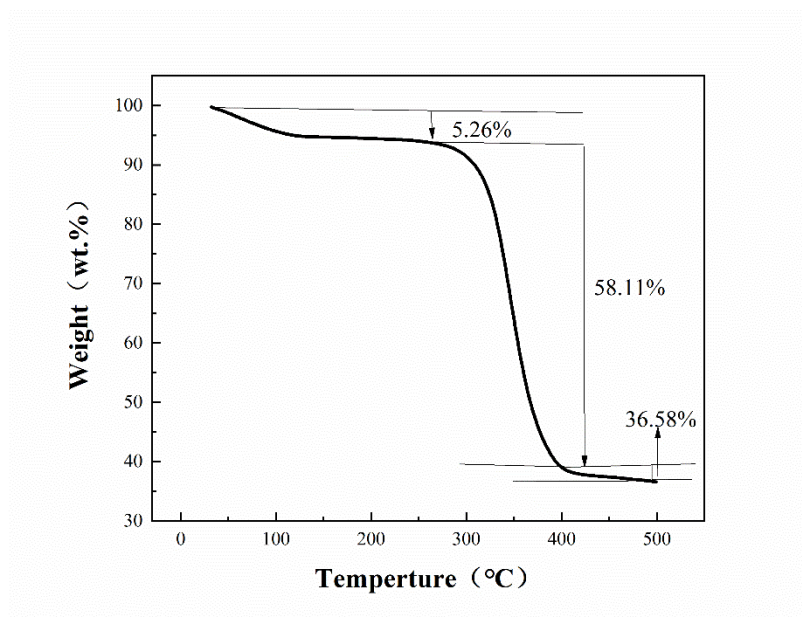


Fig.15 The TG analysis curve of MIPs(a) and SMIPs(b).

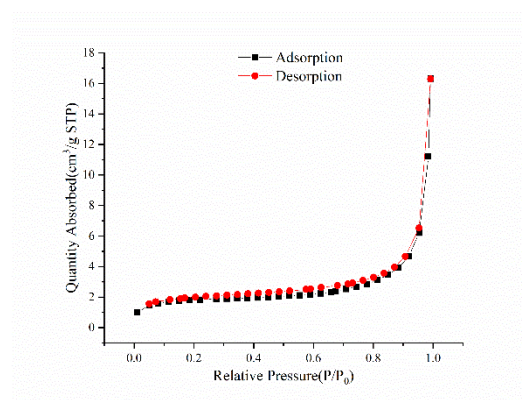
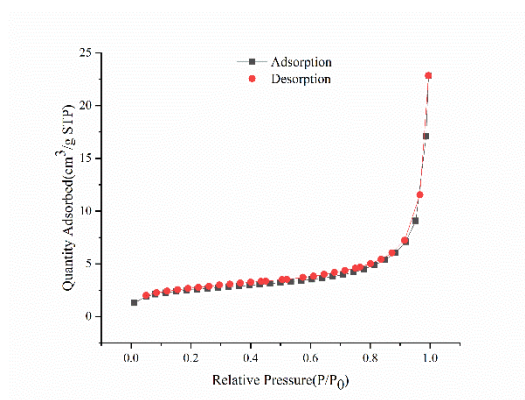
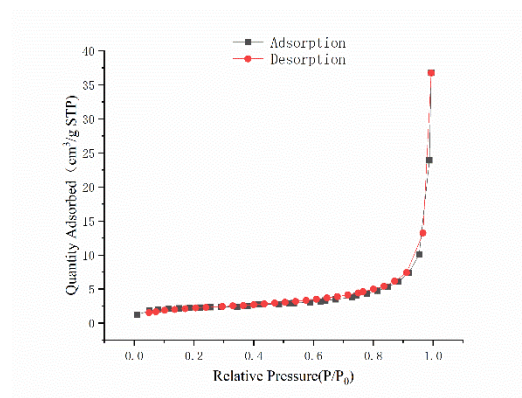
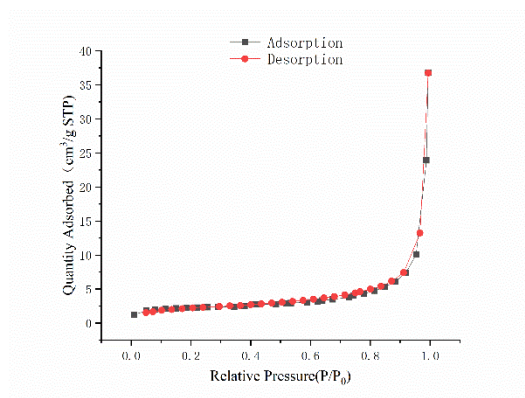
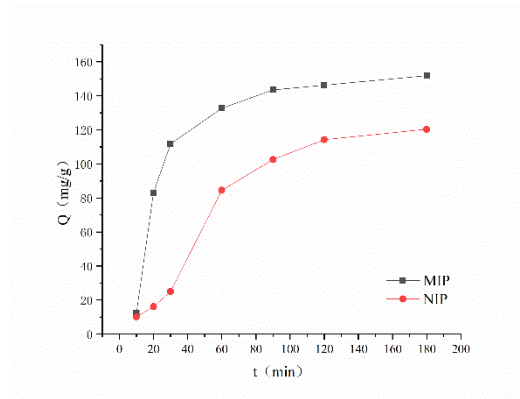
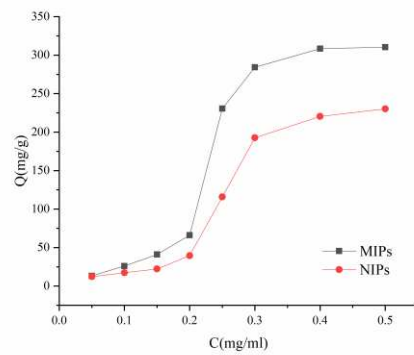


Fig.16 Nitrogen adsorption and desorption of MIP (a) & NIP (b) and SMIP (c) & SNIP (d).

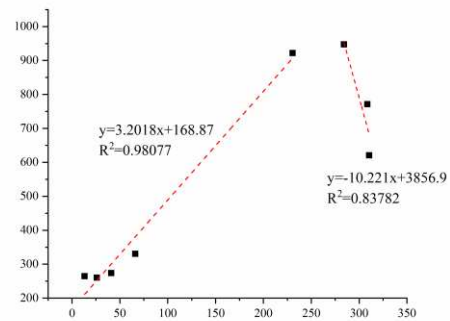


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548 **Fig.17** Adsorption kinetics curves of MIPs and NIPs.

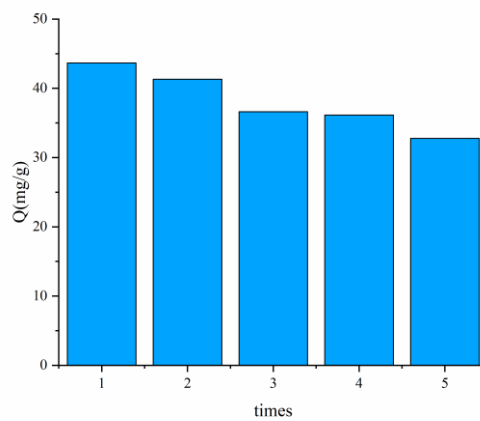


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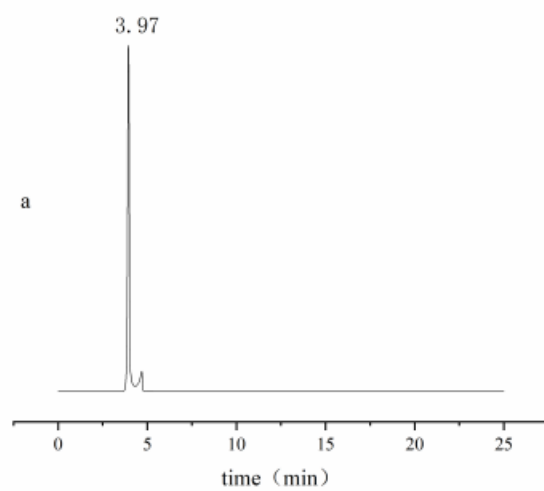
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551 **Fig.18** The Static adsorption of MIP and NIP (a), Scatchard plot for MIP (b).

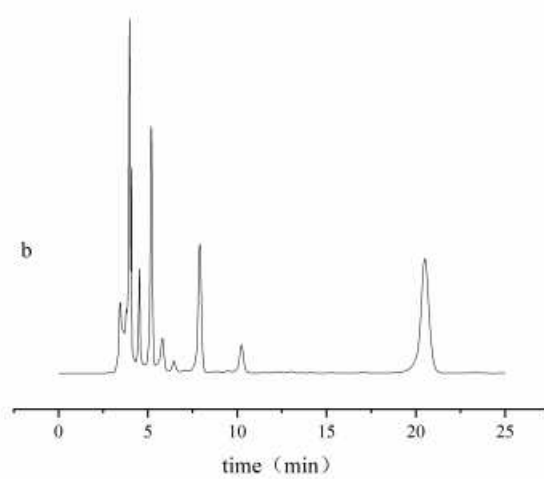


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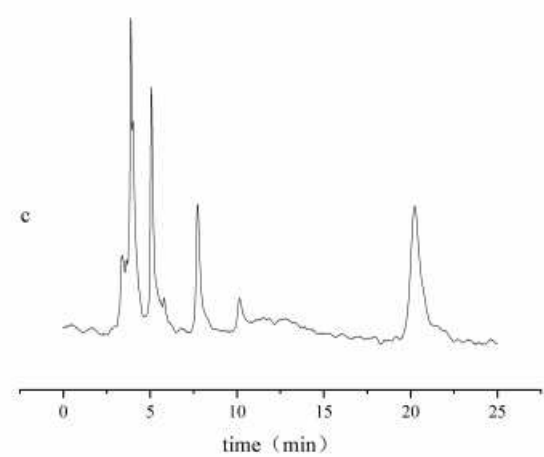
553 **Fig.19** Repeated regeneration performance of MIPs.



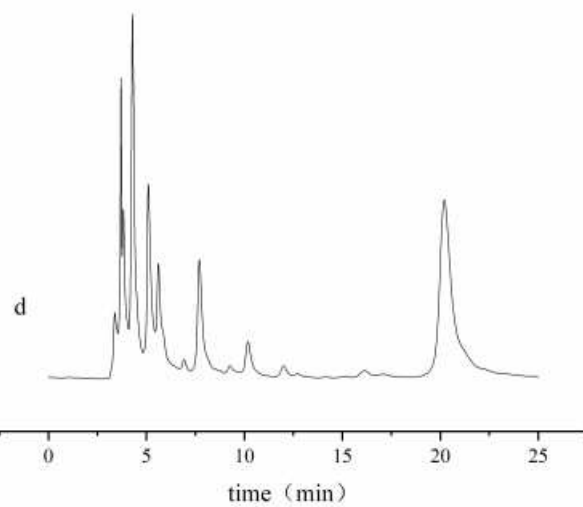
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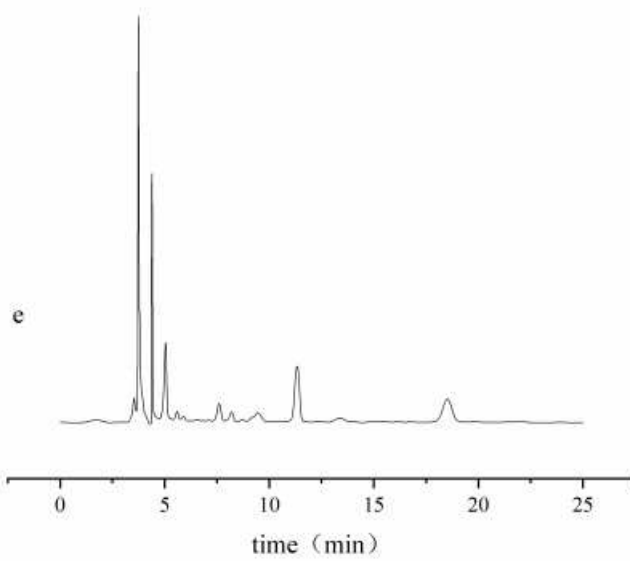
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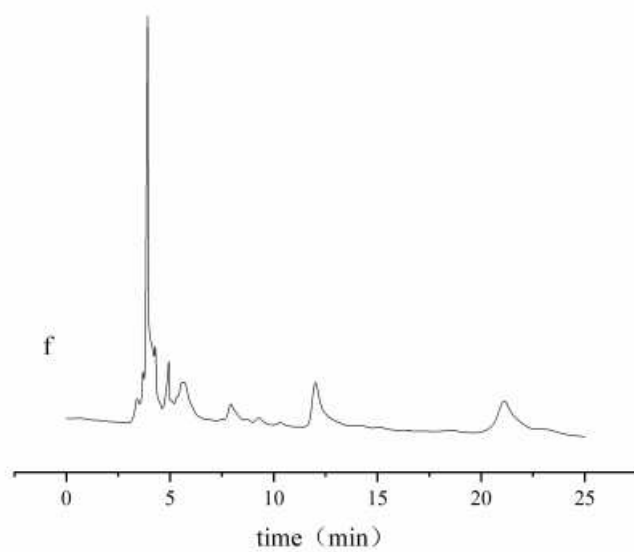
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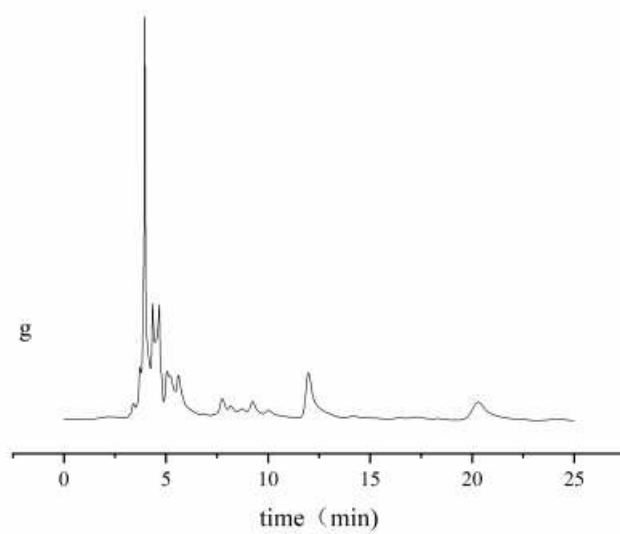
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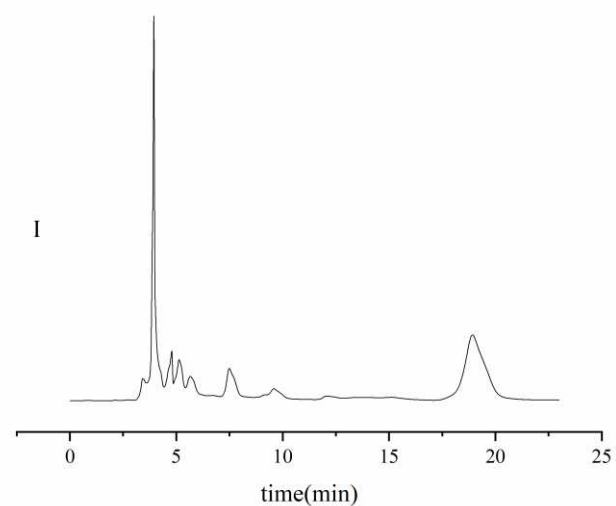
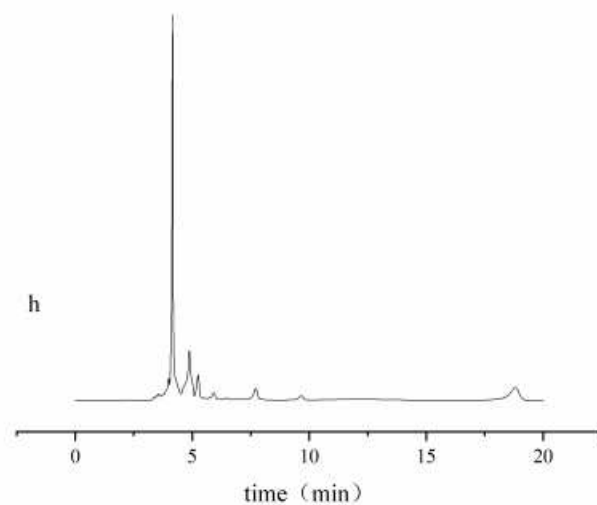


Fig.20 HPLC spectrum of GAS standard (a), *Gastrodia elata* root extracts before percolating through SPE column (b), methanol acetic acid eluent (c), methanol eluent (d), 70% methanol eluent (e), ethanol eluent (f), 50% ethanol eluent (g), 70% ethanol eluent (h), SMIPs-SPE-70% ethanol eluent (I).

Supplementary information

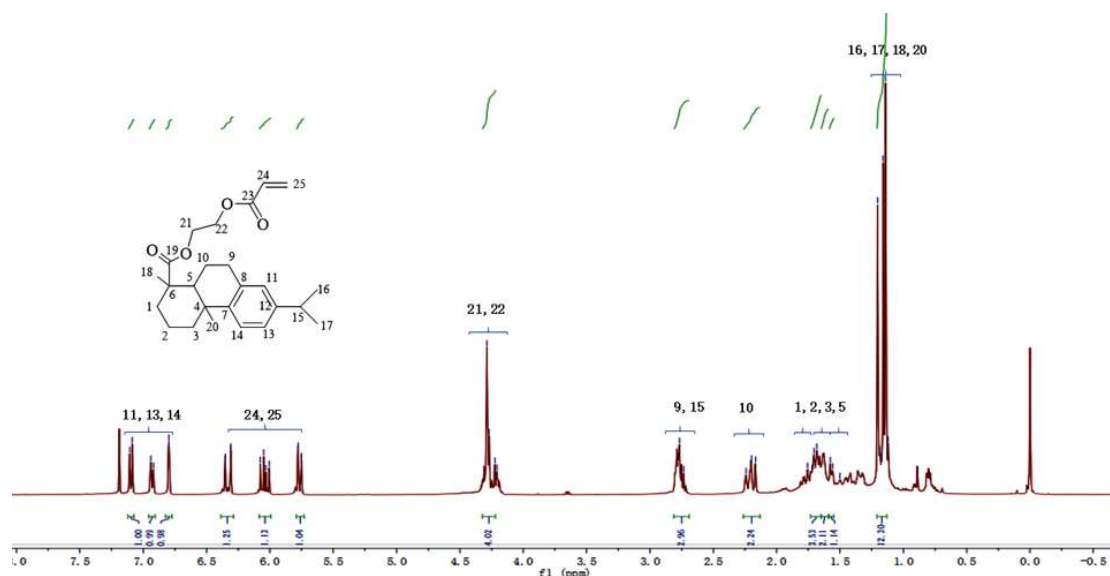


Fig. S1 ^1H -NMR of DAAEE.

^1H -NMR(400MHz; CDCl_3); δ (ppm)=7.10(d,1H),6.93(d,1H),6.80(s,1H),6.39-6.29
(m,1H),6.09-5.99(m,1H),5.77(1H,dd),4.32-4.22(m,4H),2.81-2.69(m,3H),2.26-2.13(m,2H),
1.73-1.65 (m,4H), 1.63 (s,2H), 1.57 (d,1H), 1.21-1.13 (m,12H).

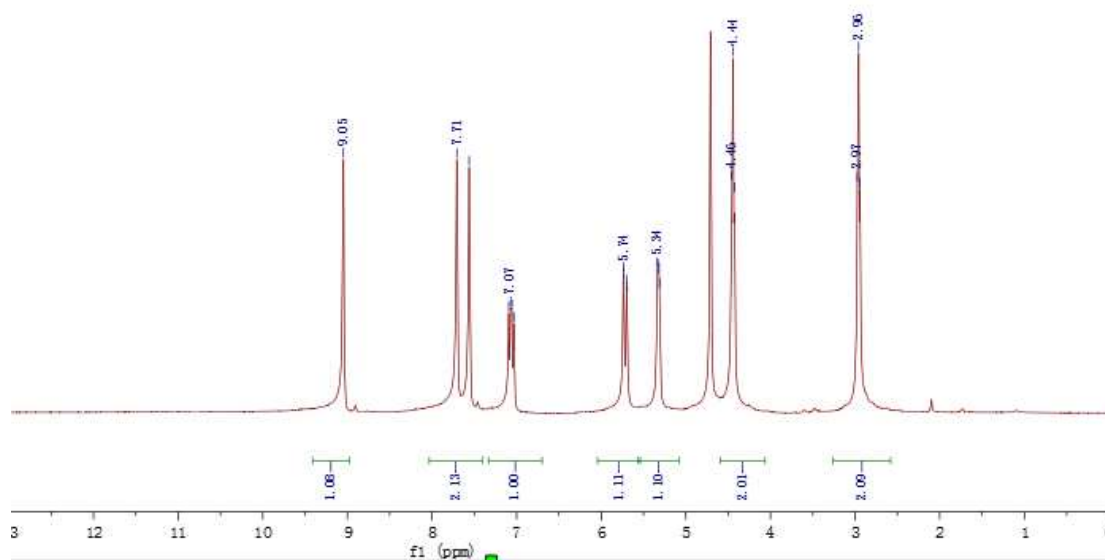


Fig. S2 ^1H -NMR of [COOHvimBr].

^1H NMR(400MHz; $\text{D}_2\text{O}-d_6$;MeSi $_4$); δ (ppm)=2.9(t,2H),4.44(t,2H),5.34(d,1H),5.74(d,
1H),7.07(m,1H),7.58(s,1H),7.71(s, 1H),9.05(s,1H).

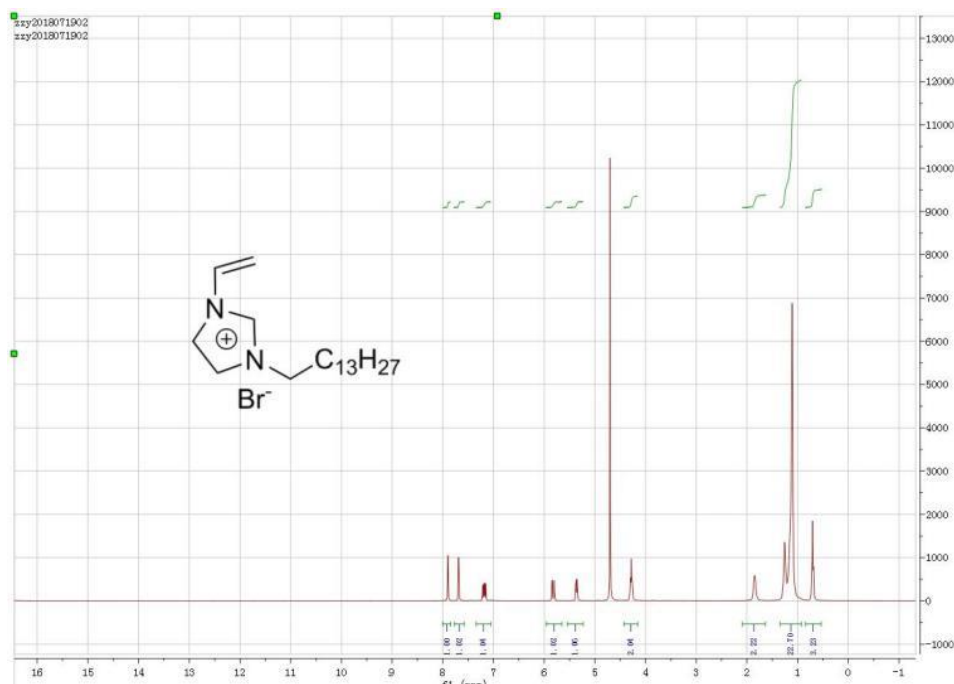


Fig. S3 ^1H -NMR of $[\text{C}_{14}\text{VIM}^+]$ $[\text{Br}^-]$.

^1H NMR(400MHz; D_2O); δ (ppm)=7.85(s,1H),7.65(s,1H),7.15(s,1H),5.85(d,1H), 5.45(s,1H), 4.25(s,2H), 1.75(s,2H), 1.15(s,23H),0.80(s,3H).

Statements & Declarations

Funding

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Competing Interests

Financial interests: Zhenyu Zuo and Jin Li declare they have no financial interests. Zhenyu Zuo has received research funding from General projects of Shaanxi provincial key R& D

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Author Contributions

Zhenyu Zuo and Jin Li contributed to the research design. Material preparation, data collection and analysis were performed by Congcong Zhao and Zhenyu Zuo. Application of Materials Studio(MS) 2019 was guided by Jin Li. The experiment and first draft of the manuscript were operated by Congcong Zhao and guided by Zhenyu Zuo. All authors read and approved the final manuscript.

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