Selective separation and enrichment of glibenclamide in health foods using surface molecularly imprinted polymers prepared via dendritic grafting of magnetic nanoparticles

In this paper, the novel surface molecularly imprinted polymers based on dendritic-grafting magnetic nanoparticles were developed to enrich and separate glibenclamide in health foods. The density functional theory method was used to give theoretical directions to the synthesis of molecularly imprinted polymers. The polymers were prepared by using magnetic nanoparticles as supporting materials, methacrylic acid as the functional monomer, and ethylene glycol dimethacrylate as the cross-linker. The characteristics of magnetic nanoparticles and polymers were measured by transmission electron microscope and SEM, respectively. The enriching ability of molecularly imprinted polymers was measured by Freundlich Isotherm. The molecularly imprinted polymers were used as dispersive SPE materials to enrich, separate, and detect glibenclamide in health foods by HPLC. The average recoveries of glibenclamide in spiked health foods were 81.46–93.53% with the RSD < 4.07%.

Keywords: Dispersive SPE / Glibenclamide / HPLC / Magnetic nanoparticles / Molecularly imprinted polymers

1 Introduction

Nowadays, over 150 million people are suffering from diabetes mellitus and the number of which is growing rapidly all over the world [1]. As an oral hypoglycemic agent of the second generation of sulphonylurea, glibenclamide (Gb) has been widely used in the treatment of type II diabetes. However, the usage of Gb is controlled because of the harmful side effects such as liver damage and thrombocytopenia. Today, the health foods (Hfs) have been successful in capturing the market under the impression that they are safe and free from any side effects. Whereas some of them have been found containing Gb as adulterants, which makes it a great challenge to detect and separate from the complex matrix.

Recently, the study of surface molecularly imprinted polymers has got great attentions due to their excellent selectivity and high affinity like antibodies [2–7]. With the characteristics of low leakage and good kinetic, the surface molecularly imprinted polymers are used in the SPE [8–11], solid-phase microextraction [12], stir bar sorptive extraction [13], and dispersive SPE (DSPE) [14, 15]. However, the imprinting efficiency of surface molecularly imprinted polymers is not satisfactory and still need to be improved [9, 16]. Currently, magnetic separation technology has showed great advantages in drug delivery [17, 18], cell separation [19], and enzyme immobilization [20, 21]. With the introduction of magnetic materials, the preparation and application of surface molecularly imprinted polymers will become time saving and convenient [22, 23].

Dendrimers, a new kind of highly branched polymers, were firstly synthesized by Tamalia in late 70s [24]. As one of the significant dendrimers, PAMAM has been used in many areas such as drug delivery application [25], electrochemistry [26–29] and so on. Due to its well controlled, highly branched structure, and enriched functional groups characteristics, the research strategy on combination of PAMAM and molecularly imprinted polymers (MIPs) will be expected to improve the imprinting efficiency.

The choice of functional monomers and proper porogenic solvents is vital to the molecular imprinting technology.
Traditional selection of functional monomers and solvents is the extensive and time-consuming experimental trials. Nowadays, the computational simulation and quantum chemical have been further studied and already applied for the rational design of MIPs. Compared with other methods, the density functional theory (DFT) method has higher accuracy and reliability [30–35].

In this paper, the high performance glibenclamide surface molecularly imprinted polymers (Gb-SMIPs) based on the PAMAM-G3-grafting magnetic nanoparticles were synthesized. In the imprinting process, Gb, MAA, and EGDMA were chosen as template molecule, functional monomer, and cross-linker, respectively. The resulting Gb-SMIPs have high adsorption capacity, quick-binding kinetics, and good selectivity for templates. They were successfully used as DSPE materials coupled with HPLC for the detection of trace Gb in Hfs, and encouraging results were obtained.

## 2 Materials and methods

### 2.1 Materials

Glibenclamide was purchased from Sigma-Aldrich, 2,2’-azobisobutyronitrile (AIBN) was purchased from Shanghai No.4 Reagent & H.V Chemical Co., γ-methacryloxypropyl trimethoxysilane (KH570) and 3-triethoxysilylpropylamine (KH550) were obtained from Diamond Advanced Material of Chemical, ethylenediamine (EDA), methyl acrylate (MA), and methacrylic acid (MAA) were purchased from Shanghai Longfeng, acrylic acid (AA), acrylamide (AM) and 4-vinylpyridine (4VP) were purchased from TCI (Shanghai) Development Co., ethylene glycol dimethacrylate (EGDMA) was purchased from Sigma-Aldrich, triethoxysilane (KH570) and 3-triethoxysilylpropylamine (KH550) were obtained from Diamond Advanced Material of Chemical, ethylenediamine (EDA), methyl acrylate (MA), and methacrylic acid (MAA) were purchased from Shanghai Longfeng, acrylic acid (AA), acrylamide (AM) and 4-vinylpyridine (4VP) were purchased from TCI (Shanghai) Development Co., ethylene glycol dimethacrylate (EGDMA) was purchased from Sigma-Aldrich, tetraethyl orthosilicate (TEOS) was from Sinopharm, all other chemicals used were of analytical grade and obtained commercially. Hfs (S.1 and S.2) were purchased from market.

### 2.2 Equipment

HPLC was performed with Shimadzu (Japan) system comprising LC-10ATVP pump, SPD-10AVP UV-detector, and HW-2000 chromatographic work station. The identification of target compounds was performed using and Agilent 1200 liquid chromatograph that was coupled to an Agilent 6410B Triple Quad mass spectrometer. Other instruments included HZ-9211KB rocking bed (Hualida Laboratory Equipment Co.), Milli-Q® (Millipore, Milford, MA, USA) water purification system, DZG-6020 vacuum driving oven (Shanghai Huaqi Laboratory Equipment Co., China), C-MAG HS 7 Temperature magnetic mixer (IKA® Processing Equipment, Germany), and KQ5200 Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co., China). The characteristics of Gb-SMIPs were measured by transmission electron microscope (TEM) (JEM1010, JEOL Ltd., Japan). X-ray diffraction (Dmax22500, Rigaku, Japan), vibrating sample magnetometry (Lake Shore), and SEM (JSM-5900, JEOL, Japan).

### 2.3 Preparation of magnetic nanoparticles

The Fe₃O₄ nanoparticles were synthesized by coprecipitation method, and magnetic nanoparticles (MNs) were synthesized by hydrolysis of TEOS with aqueous ammonia as follows: 1.72 g FeCl₃ · 6 H₂O and 4.72 g FeCl₃ · 6 H₂O were dissolved in 80 mL of water. The mixture was magnetic stirred and purged with nitrogen gas, and then 10 mL aqueous ammonia were added. The reaction was stirred for ½ h at 80°C. After finished, the Fe₃O₄ nanoparticles were washed by deionized water until neutral. Then 0.3 g neutral Fe₃O₄ nanoparticles were dispersed in 50 mL of 2-propyl alcohol, 2 mL aqueous ammonia, and 2 mL deionized water were added, subsequently, 5 mL TEOS were added. The mixture was magnetic stirred for 12 h at room temperature. The obtained MNs were collected by an external magnetic field, and dried in vacuum.

### 2.4 Preparation of MNs@PAMAM

1. 1.0 g MNs were dispersed in 100 mL of toluene, each of 10 mL KH570 and KH550 were added in the solution under the nitrogen gas. The mixture was stirred for 24 h at 120°C. After finished, MNs@PAMAM(G0) were collected by an external magnetic field and dried in vacuum.

2. 0.5 g MNs@PAMAM(G0) were dispersed in 100 mL methanol and 3.75 mL MA were added. The solution was stirred for 24 h at 50°C with the nitrogen gas. The MNs@PAMAM(G0.5) were obtained and dried. Then the products were dispersed in 100 mL methanol with 3.00 mL EDA. The reaction was stirred for 24 h at 50°C under nitrogen gas, and MNs@PAMAM(G1) were obtained. The amounts of MA and EDA were doubled once when the above reaction recycled once to get MNs@PAMAM(G2). Then, MNs@PAMAM(G3) were obtained by repeating above reaction. And amounts of MA and EDA were doubled once more.

### 2.5 Molecular modeling studies

Computational simulation was employed to optimize structure of the complex and to calculate the binding energy (ΔE) between templates and monomers. The full geometry optimization of template, monomer, and template-monomer complex was carried with DFT method at B3LYP/6–31 + G (d, p) level by Gaussian 09 program. The ΔE was calculated by the following generic formula:

\[
\Delta E = |E_{\text{template--monomer complex}} - E_{\text{template}} - E_{\text{monomer}}|
\]
2.6 Preparation of Gb-SMIPs and Gb-SNIPs

Fifty microgram MNs@PAMAM(G3), 128.44 mg Gb, 0.78 mmol MAA were dispersed into 50 mL toluene solution, the mixture was magnetic stirred at room temperature. Then 3.12 mmol EGDMA and 30 mg AIBN were added as cross-linker and initiator, respectively. The mixture was magnetic stirred for 6 h at 50°C, 24 h at 60°C, 6 h at 85°C under the protection of nitrogen. After the reaction completed, polymers were separated by an external magnetic field and eluted by a mixture of 2.0 mol L\(^{-1}\) hydrochloric acid–methanol (1:5, v/v) to remove templates. The polymers were finally rinsed with methanol to remove the remaining acid and then dried in vacuum. The Gb-SMIPs were obtained. The glibenclamide surface none molecularly imprinted polymers (Gb-SNIPs) were prepared by the same manner in the absence of templates. The Gb-SMIPs and Gb-SNIPs based on MNs@PAMAM(G0, G1, G2) nanoparticles were also prepared by the same manner above. The probable preparation process is presented in Supporting Information Fig. S1.

2.7 Characterization of Gb-SMIPs

The size of Gb-SMIPs was measured by SEM and TEM. The identification of crystalline phase of Gb-SMIPs was performed by an X-ray diffraction over the 20 range of 10–80°. The magnetic properties of Gb-SMIPs were measured by vibrating sample magnetometry. Also, FT-IR spectra were recorded in the range of 4000–400 cm\(^{-1}\).

2.8 Adsorption experiment

The adsorption experiments were carried out as follows: 20 mg Gb-SMIPs/Gb-SNIPs were added into 5.0 mL Gb standard solutions (0.05–10 mM) that were prepared in toluene–methanol (9:1, v/v) and pH was 3.5 (adjusted by acetic acid). And the flow rate was 1.0 mL min\(^{-1}\). The injection volume was 20 μL, and detection wavelength was at 300 nm.

2.9 Chromatographic condition

A diamosil® C18 column (5 μm, 150 × 4.6 mm) was used for chromatographic experiments. The mobile phase consisted of methanol–water (80:20, v/v) and pH was 3.5 (adjusted by acetic acid). And the flow rate was 1.0 mL min\(^{-1}\). The injection volume was 20 μL, and detection wavelength was at 300 nm.

2.10 Extraction procedure and calibration curve

Hundred milligrams of S.1 (or S.2) were dispersed in 10 mL methanol and ultrasound for 25 min. Then, the mixture was separated by filtrating. Two milliliters of filtrate was evaporated to dryness by nitrogen gas at room temperature, and then 20 mg Gb-SMIPs and 1 mL of toluene–methanol (9:1, v/v) were added to the residues shaken at room temperature for 25 min. The Gb-SMIPs were separated by magnetic and then added 1 mL of eluent solution by sonication for 15 min. Five hundred microliters supernatants were obtained and evaporated to dryness under a stream of nitrogen at 25°C. Finally, the residues were redissolved in 500 μL mobile phase. For recovery studies, real samples spiked with Gb at five different levels were tested by the method built above (n = 5).

The calibration curve in the range of 5–2000 ng mL\(^{-1}\) for Gb was obtained. The LOD and LOQ were defined as three and ten times ratio of signal to noise (S/N = 3, S/N = 10), respectively.

3 Results and discussion

3.1 The choice of MNs@PAMAM generation

The generations of MNs@PAMAM had been investigated by imprinting effect of Gb-SMIPs and Gb-SNIPs prepared with different generations of MNs@PAMAM(G0, G1, G2, G3), respectively. The results demonstrated that MNs@PAMAM(G3) were expected to be the ideal supporting materials (Supporting Information Table S1).

The MNs were modified by KH570 and KH550 at the ratio of 1:1. Then, a Michael addition reaction took place between the preexisting amino groups (from KH550) and MA with the ratio of two propionate ester groups to one amino group. Subsequently, ester groups reacted with EDA to obtained terminal amino groups. The desired MNs@PAMAM(G3) were produced by repeating above two steps twice.

3.2 Molecular modeling of template monomer

In our work, some widely used functional monomers (AA, MAA, AM, 4VP) were elected as candidates. In order to confirm the molar ratio of the interaction between template and monomers, the obtained ∆E was illustrated in Table 1. This showed that the ∆E of Gb-MAA complex was higher than Gb-AA, Gb-AM, and Gb-4VP. Meantime, the adsorption capacities of Gb-SMIPs synthesized using AA, AM, MAA, 4VP were 130.56, 100.11, 235.12, 80.27 μmol g\(^{-1}\), respectively. So the MAA was chosen as the optimal functional monomer.

Moreover, the molar ratio of interaction between Gb and MAA can be chosen according to binding energies and adsorption capacities. The detailed geometric parameters and adsorption capacities were given in Supporting Information Table S2. The average hydrogen bond length (angle) was 1.67 Å (156.1°), 1.64 Å (153.2°), 1.63 Å (150.3°), 1.63 Å (151.2°), and
The binding energies of Gb with different monomers and adsorption capacities of Gb-SMIPs prepared using various monomers

<table>
<thead>
<tr>
<th>Molecules</th>
<th>$\Delta E$ (a.u.)</th>
<th>$\Delta E$ (kJ mol$^{-1}$)</th>
<th>Adsorption capacity$^a$ (µmol g$^{-1}$)</th>
</tr>
</thead>
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<tr>
<td>Gb-MAA</td>
<td>0.03857</td>
<td>101.2445</td>
<td>235.12</td>
</tr>
<tr>
<td>Gb-AA</td>
<td>0.02955</td>
<td>77.5674</td>
<td>130.56</td>
</tr>
<tr>
<td>Gb-AM</td>
<td>0.02319</td>
<td>60.8727</td>
<td>100.11</td>
</tr>
<tr>
<td>Gb-4VP</td>
<td>0.01903</td>
<td>49.9529</td>
<td>80.27</td>
</tr>
</tbody>
</table>

$^a$The adsorption capacities of Gb-SMIPs to Gb were measured in 5.0 mL of Gb standard solution (6.0 mM).

Figure 1. (A) Vibrating sample magnetometry analysis of Gb-SMIPs and (B) X-ray diffraction patterns of Gb-SMIPs (a), MNs@PAMAM(G3) (b), and Fe$_3$O$_4$ (c).

1.64 Å (150.4°) with molar ratio of 1:1, 1:2, 1:3, 1:4, 1:5, respectively. The results illustrated that strong hydrogen bonds were existed between Gb and MAA. The $\Delta E$ of Gb and MAA were also presented as follows: $\Delta E$ (1:1) $< \Delta E$ (1:2) $< \Delta E$ (1:3), and the molar ratio of 1:4 and 1:5 did not lead to a greater level of $\Delta E$. At the same time, the adsorption capacities of Gb-SMIPs using Gb-MAA complex (1:3) was the highest. Herein, a complex of Gb-MAA (1:3) was chosen in the following experiments. We suggested that Gb-SMIPs in the molar ratio of 1:3 contained the most effective sites, which led the templates in and out easily.

3.3 Characterization

Vibrating sample magnetometry was employed to measure the magnetic properties of Gb-SMIPs. Figure 1A shows the magnetic hysteresis loops of Gb-SMIPs. And the saturation magnetization of Fe$_3$O$_4$, MNs@PAMAM(G3), Gb-SMIPs were 80.2, 30.6, 13.4 emu g$^{-1}$, respectively. Fast separation of dispersed Gb-SMIPs from the solution in the presence of an external magnetic field was also easily visible (in Fig. 1A).

The X-ray diffraction patterns of Gb-SMIPs (curve a), MNs@PAMAM(G3) (curve b), and Fe$_3$O$_4$ (curve c) are displayed in Fig. 1B. The results showed that the main crystalline structure of the Fe$_3$O$_4$ was retained, and the amorphous diffraction of SiO$_2$ was in the range of 10–30°.

FT-IR spectra of each generation of MNs@PAMAM are shown in Supporting Information Fig. S3. For all the generations of MNs@PAMAM, the characteristic bands at 469, 800, and 1100 cm$^{-1}$ corresponding to Si-O, Si-O-H, and Si-O-Si were observed, which indicated the existence layer of SiO$_2$. Moreover, the band at 3080 cm$^{-1}$ suggested the alkynyl groups of KH570 in all MNs@PAMAM. The C=O stretching of ester groups at 1741 cm$^{-1}$ were easily found in all half generations of MNs@PAMAM. After the half generation react with EDA to obtain the full generation, the bands at 1741 cm$^{-1}$ disappeared, demonstrating that reaction has took place. Apparently, the adsorption band at 3410 cm$^{-1}$ was attributed to the NH$_2$ stretching of the amine groups, the peak at 2926 and 2850 cm$^{-1}$ was due to the stretching C–H, the band at 1648 cm$^{-1}$ = C=O of –CONH– and the stretching of secondary amine groups was at 1539 cm$^{-1}$. All data above confirmed the successful modification of PAMAM and double bonds on the surface of MNs.

The SEM and TEM images in Fig. 2A show that MNs@PAMAM(G3) nanoparticles were uniform and about 500 nm. In comparison with Fig. 2A, the SEM in Fig. 2B shows relatively rough, which resulted from the introduction of imprinting layers. TEM image in Fig. 2B also apparently shows the core-shell structure of Gb-SMIPs.

3.4 Adsorption isotherm

The isothermal adsorption of Gb-SMIPs and Gb-SNIPs are shown in Fig. 2C. The maximum adsorption capacities of Gb-SMIPs and Gb-SNIPs were 263.26 and 66.54 µmol g$^{-1}$, respectively. The Gb-SMIPs showed much stronger memory function and higher adsorption capacities for Gb than Gb-SNIPs.

Freundlich isotherm is used for the evaluation of adsorption ability [36, 37], which was obtained from Eq. (1). Where $Q$ was the adsorbed amount and $C$ was the concentration of free analyte in solution. The $\alpha$ and $m$ were the Freundlich isotherm constants to measure the capacity and heterogeneity that could be calculated by plotting log$Q$ versus log$C$ by a linear regression (Eq. (2)). Additional, the number of binding sites ($N$) and association constant ($K$) can be estimated by Eqs. (3) and (4), where $N_{k_{\min} - k_{\max}}$ was the number of binding sites, and $K_{k_{\min} - k_{\max}}$ was the apparent average association constant.

$$Q = \alpha C^m$$  
(1)

$$\log Q = \log \alpha + m \log C$$  
(2)

$$N_{k_{\min} - k_{\max}} = \alpha(1 - m^2)(k_{\min}^{-m} - k_{\max}^{-m})$$  
(3)
Figure 2. The SEM and TEM images of MNs@PAMAM(G3) (A) and Gb-SMIPs (B); the adsorption isotherm of Gb-SMIPs and Gb-SNIPs (C), and dynamic isotherm of Gb-SMIPs in 6.0 mM Gb solution at different time (D).

All the data of the Freundlich isotherm are presented in Table 2. In the results, parameter $m$ was 0.6455 for Gb-SMIPs and 0.4746 for Gb-SNIPs, which demonstrated the existing heterogeneous-binding sites in Gb-SMIPs. Also, the numbers of binding sites measured by $N_{K_{min}-K_{max}}$ were 89.01 and 29.17 mg g$^{-1}$ for Gb-SMIPs and Gb-SNIPs, respectively.

MNs nanoparticles were first modified by KH570 and KH550. KH570 with terminal double bond could make the surface MIPs directionally attached to the surface of nanoparticles by cross-linking, and KH550 with terminal amino groups was a basement for the synthesis of PAMAM dendrimers. PAMAM were suggested to make a great contribution to producing dense, effective imprinted cavities, and binding sites, and the higher adsorption capacity of Gb-SMIPs was given. The surface MIPs’ properties were directly affected by the functional groups and structural dimensions of the dendrimers, and more in-depth studies would be expected.

### 3.5 Adsorption and desorption kinetics

The adsorption kinetics of Gb-SMIPs was investigated in the concentration of 6.0 mM Gb standard solutions at different time. In Fig. 2D, the Gb-SMIPs reached adsorption equilibrium in 25 min, which demonstrated the fast adsorption kinetics ability. The data of desorption kinetics experiments showed that desorption could reach equilibrium in 15 min (Supporting Information Fig. S2A). The short equilibrium time was mainly attributed to the densely, uniform nanostructures and effective-binding sites of MNs@PAMAM(G3). We suggested that Gb-SMIPs were orderly fixed on the surface of the carrier by self-assembly process and open cavities of MNs@PAMAM(G3) made the templates easily in and out from the uniform-binding sites, which made Gb-SMIPs get good adsorption and desorption kinetics.

### 3.6 Selectivity study

Five analogs (glimepiride, metformin, pioglitazone, glitazide, and estradiol) of Gb were investigated in selectivity study by the adsorption amount of Gb-SMIPs and Gb-SNIPs (Supporting Information Fig. S2B). The result told that the

<table>
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<tr>
<th></th>
<th>m</th>
<th>$\alpha$ (mg g$^{-1}$)</th>
<th>$R^2$</th>
<th>$N_{K_{min}-K_{max}}$ (mg g$^{-1}$)</th>
<th>$K_{min}-K_{max}$ (mg L g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gb-SMIPs</td>
<td>0.65</td>
<td>56.25</td>
<td>0.99</td>
<td>89.01</td>
<td>2.11</td>
</tr>
<tr>
<td>Gb-SNIPs</td>
<td>0.47</td>
<td>19.20</td>
<td>0.98</td>
<td>29.17</td>
<td>3.01</td>
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</table>
adsorption capacities of Gb-SMIPs for all the analogs were higher than that of Gb-SNIPs. It was interesting that although the structures of glimepiride and gliclazide were more similar to the Gb, the adsorption capacities were low. Moreover, Gb-SMIPs had even lower affinity to other three analogs. This strongly demonstrated the high selective-binding sites in Gb-SMIPs.

### 3.7 Method validation

Good linearity was achieved in range of 5–2000 ng mL⁻¹ and the regression was \( A = 907846C + 40998 \) for Gb with correlation of 0.9992, where \( A \) was the peak area, and \( C \) was the concentration of the compounds. The LOD obtained for Gb was 1.56 ng mL⁻¹, and the LOQ was 5.49 ng mL⁻¹.

The repeatability and accuracy were collected by the Hfs spiked with Gb at three different levels. The results were illustrated that average recoveries of Gb were 85.75–93.53\%, and RSD% was 2.16–4.07 in S.1, and the recoveries of S.2 were 81.46–90.87\% with the RSD% of 2.32–3.89 (Table 3). All above data presented a reliable, accurate and practical method for the determination and separation of trace level of Gb in Hfs.

### 3.8 Analysis of Gb in real samples

HPLC chromatograms of Gb in Hfs are presented in Fig. 3A. The targets with liquid–liquid extraction could not be detected by HPLC in curve a–b. Apparently, compared with Gb-SNIPs, Gb-SMIPs showed great advantages in the determination of Gb as selective sorbents in curve c–d and curve e–f. And in curve g–h, the spiked Hfs were extracted by Gb-SMIPs. In Fig. 3B, structure of Gb in Hfs had been identified by HPLC-MS/MS after extracted by Gb-SMIPs.

### 4 Concluding remarks

An efficient and novel method for enrichment and separation of Gb from Hfs by Gb-SMIPs based on MNs@PAMAM(G3) coupled with HPLC was developed. With the help of time-saving DFT method, the optimal functional monomers were selected as a function of the Mulliken charges and binding energies. Then, highly branched and well-controlled MNs@PAMAM(G3) nanoparticles were synthesized and firstly used in the preparation of Gb-SMIPs as supporting materials, which showed good adsorption, desorption, and selective abilities. The strategy was novel, inexpensive, and easy to operate, which demonstrated that Gb-SMIPs could be used as DSPE materials and be an effective tool for monitoring of trace level of adulterants in the complex matrix. This research strategy will be of great significance for study on separation science.

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5 References