Creating magnetic ionic liquid molecularly imprinted polymers for selective extraction of lysozyme

Wei Xu\textsuperscript{a}, Qingzhou Dai\textsuperscript{a}, Yuzhi Wang\textsuperscript{a*}, Xiaojian Hu\textsuperscript{b*}, Panli Xu\textsuperscript{a}, Rui Ni\textsuperscript{a}, Jiaojiao Meng\textsuperscript{a}

\textsuperscript{a} State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P.R. China

\textsuperscript{b} Department of Chemistry, School of Basic Medicine, Changsha Medical University, Changsha, 410219, P.R. China

Corresponding author: Professor Yuzhi Wang; Associate professor Xiaojian Hu

State Key Laboratory of Chemo/Biosensing and Chemometrics
College of Chemistry and Chemical Engineering
Hunan University
Changsha 410082

P. R. China

Phone: +86-731-88821903

Fax: +86-731-88821848

E-mail: wyzss@hnu.edu.cn
Table S1 Preparative composition of different MIPs.

<table>
<thead>
<tr>
<th>MIPs (mg)</th>
<th>Fe₃O₄@VTEO (mg)</th>
<th>IL (mg)</th>
<th>Lys (mg)</th>
<th>MBAA (mg)</th>
<th>20%APS (μL)</th>
<th>20%TEMED (μL)</th>
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<tbody>
<tr>
<td>MIP1</td>
<td>100</td>
<td>100</td>
<td>25</td>
<td>20</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>MIP2</td>
<td>100</td>
<td>150</td>
<td>25</td>
<td>20</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>MIP3</td>
<td>100</td>
<td>200</td>
<td>25</td>
<td>20</td>
<td>75</td>
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</tr>
<tr>
<td>MIP4</td>
<td>100</td>
<td>250</td>
<td>25</td>
<td>20</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>MIP5</td>
<td>100</td>
<td>300</td>
<td>25</td>
<td>20</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>NIP1</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>20</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>NIP2</td>
<td>100</td>
<td>150</td>
<td>-</td>
<td>20</td>
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<tr>
<td>NIP3</td>
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<tr>
<td>NIP5</td>
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<td>300</td>
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<td>20</td>
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Table S2 Langmuir isotherm constants of the magnetic materials.

<table>
<thead>
<tr>
<th>Magnetic materials</th>
<th>$^aK_L$ (mL·mg$^{-1}$)</th>
<th>$^bQ_{\text{max}}$ (mg·g$^{-1}$)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$_3$O$_4$@VTEO@IL-MIPs</td>
<td>116</td>
<td>213</td>
<td>0.9985</td>
</tr>
<tr>
<td>Fe$_3$O$_4$@VTEO@IL-NIPs</td>
<td>299</td>
<td>121</td>
<td>0.9993</td>
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</table>

Where $^aK_L$ is the equilibrium constant of Langmuir adsorption, $^bQ_{\text{max}}$ is the theoretical maximum adsorption capacity.
Characterization of Fe₃O₄@VTEO@IL-MIPs

The FT-IR spectra of [AAPIM]Cl, Fe₃O₄, Fe₃O₄@VTEO, Fe₃O₄@VTEO@IL-NIPs and Fe₃O₄@VTEO@IL-MIPs were illustrated in Fig. S1. The characteristic peak at 573 cm⁻¹ (Fig. S1) is assigned to the stretching vibration of Fe-O.¹ The spectrum of Fe₃O₄@VTEO in Fig. S1b contains peaks at 1653 and 1130 cm⁻¹ corresponding to C=C stretching vibration and Si-O-Si asymmetric stretching vibration, respectively. The phenomenon indicates that the successful modification of VTEO.² In the spectrum of Fe₃O₄@VTEO@IL-NIPs (Fig. S1d), the characteristic peaks of polymer layer are not clearly observed. However, double peaks of amino group at 3400-3100 cm⁻¹ are observed in Fe₃O₄@VTEO@IL-MIPs (stretching vibrations of N-H) (Fig. S1c), which confirms the presence of the NH-IL monomer and the successful modification of polymer layer. In the spectrum of [AAPIM]Cl (Fig. S1e), the stretching vibrations of N-H at 3430 and 3365 cm⁻¹ are presented. Moreover, the characteristic bands of C=C are observed at 1680 and 1630 cm⁻¹, and the characteristic signals of imidazolium C=N stretching vibration are observed at 1570 and 1454 cm⁻¹, which indicates the successful synthesis of IL.
The FT-IR spectra of Fe$_3$O$_4$ (a), Fe$_3$O$_4$@VTEO (b), Fe$_3$O$_4$@VTEO@IL-MIPs (c), Fe$_3$O$_4$@VTEO@IL-NIPs (d) and [AAPIM]Cl (e).

The sizes and morphologies of Fe$_3$O$_4$, Fe$_3$O$_4$@VTEO and Fe$_3$O$_4$@VTEO@IL-MIPs were determined by TEM which was shown in Fig. S2. The average diameter of Fe$_3$O$_4$ is in the range of 10-20 nm (Fig. S2a). It is clear that the Fe$_3$O$_4$ nanoparticles conglomerated seriously. After coating with VTEO, the diameter of Fe$_3$O$_4$@VTEO particles is higher than Fe$_3$O$_4$ nanoparticles obviously (Fig. S2b). It can be seen from Fig. S2c that a gray shell is observed on the surface of the Fe$_3$O$_4$@VTEO particles, which offers strong evidence of the successful formation of IL imprinting layer.
The particles size were measured by dynamic light scattering (DLS) study (Fig. S3). Compared with TEM pictures, the measurements of the size obtained were bigger, which could be explicated by agglomeration of the particles in aqueous solution and the hydratization of particles. It is clear that the VTEO layer and the MIPs layer make the particle size bigger than Fe$_3$O$_4$. However, some particles agglomerated after coating with VTEO and MIPs layer also. This is because some Fe$_3$O$_4$ particles may be crosslinked by VTEO or MIPs, even Fe$_3$O$_4$@VTEO and Fe$_3$O$_4$@VTEO@IL-MIPs particles may agglomerate in aqueous.

**Fig. S2** The TEM images of Fe$_3$O$_4$ (a), Fe$_3$O$_4$@VTEO (b) and Fe$_3$O$_4$@VTEO@IL-MIPs (c).
Fig. S3 DLS analysis of Fe$_3$O$_4$(a), Fe$_3$O$_4$@VTEO(b) and Fe$_3$O$_4$@VTEO@IL-MIPs (c).
The magnetic properties of Fe₃O₄, Fe₃O₄@VTEO and Fe₃O₄@VTEO@IL-MIPs were evaluated by VSM, and the magnetic hysteresis curves were shown in Fig. S4A. The saturation magnetization of Fe₃O₄, Fe₃O₄@VTEO and Fe₃O₄@VTEO@IL-MIPs are 68.56, 42.27 and 39.09 emu/g, respectively. The reducing of saturation magnetization in Fe₃O₄@VTEO and Fe₃O₄@VTEO@IL-MIPs is caused by the successful modification of VTEO and polymer layer. This is consistent with the observation from TGA. As illustrated in Fig. S4B, the MIPs particles can be separated rapidly by a magnet about 6 s, which contributes to the separation of Lys in real sample application.

Fig. S4 The magnetic hysteresis loops of Fe₃O₄ (a), Fe₃O₄@VTEO (b) and Fe₃O₄@VTEO@IL-MIPs (c).
The relative composition of Fe$_3$O$_4$, Fe$_3$O$_4$@VTEO and Fe$_3$O$_4$@VTEO@IL-MIPs were characterized by TGA. As displayed in Fig. S5, the weight loss of pure Fe$_3$O$_4$ nanoparticles is only 4.6% when the temperature increases to 1000 °C. The Fe$_3$O$_4$@VTEO particles presented a weight loss of 0.2% in the temperature range of 0-200 °C, which is the release of water molecules. When the temperature increased to 1000 °C, the other decline in weight (about 18.2%) is resulted from the decomposition of VTEO coated on Fe$_3$O$_4$. For Fe$_3$O$_4$@VTEO@IL-MIPs, a weight loss of about 1.4% is discovered at 200 °C, which is because the existence of imprinted cavities absorb more water than Fe$_3$O$_4$@VTEO. At 200-1000 °C, Others decrease in weight is about 27.9% for the decomposition of MIPs coating, which confirms the formation of the IL polymers layer.

![Weight loss curves of Fe$_3$O$_4$ (a), Fe$_3$O$_4$@VTEO (b) and Fe$_3$O$_4$@VTEO@IL-MIPs (c).](image)
The phases and the structures of Fe$_3$O$_4$, Fe$_3$O$_4$@VTEO and Fe$_3$O$_4$@VTEO@IL-MIPs were investigated by XRD, which were illustrated in Fig. S6. Six characteristic diffraction peaks of Fe$_3$O$_4$ can be observed in Fig. S6a, which are assigned to (220), (311), (400), (422), (511) and (440) planes of Fe$_3$O$_4$ crystal. According to Fig. S6b-c, the similar diffraction peaks are detected in patterns of Fe$_3$O$_4$@VTEO and Fe$_3$O$_4$@VTEO@IL-MIPs. Compared with the Fe$_3$O$_4$ reflection, there are obvious broad peaks appear from $2\theta=20^\circ$ to $30^\circ$ (Fig. S6b-c). It is resulted from the amorphous peak of silica. Apparently, XRD patterns provide a strong evidence that microspheres contain Fe$_3$O$_4$ with cubic inverse spinel structure and the synthesis processes do not change the phase of Fe$_3$O$_4$.

![X-ray diffraction patterns of Fe$_3$O$_4$ (a), Fe$_3$O$_4$@VTEO (b) and Fe$_3$O$_4$@VTEO@IL-MIPs (c).](image)

**Fig. S6** X-ray diffraction patterns of Fe$_3$O$_4$ (a), Fe$_3$O$_4$@VTEO (b) and Fe$_3$O$_4$@VTEO@IL-MIPs (c).
Adsorption isotherms

\[
\frac{C_e}{Q_e} = \frac{1}{Q_{\text{max}} K_L} + \frac{C_e}{Q_{\text{max}}}
\]

Where \( C_e (\text{mg} \cdot \text{mL}^{-1}) \) is the concentration of Lys in the final equilibrium solution, \( Q_e (\text{mg} \cdot \text{g}^{-1}) \) and \( Q_{\text{max}} (\text{mg} \cdot \text{g}^{-1}) \) are the experimental adsorption capacity and the theoretical maximum adsorption capacity of \( \text{Fe}_3\text{O}_4@\text{VTEO}@\text{IL-MIPs (NIPs)} \) for the template protein, respectively. \( K_L (\text{mL} \cdot \text{mg}^{-1}) \) is the equilibrium constant of Langmuir adsorption. The linear fitting curves of the Langmuir model were plotted in \( \frac{C_e}{Q_e} \) versus \( C_e \), which were shown in Fig. S7 and Table S2. From the linear fitting curves, it can be seen that \( \frac{C_e}{Q_e} \) and \( C_e \) has a good linear relationship. The \( R^2 \) of both \( \text{Fe}_3\text{O}_4@\text{VTEO}@\text{IL-MIPs} \) and \( \text{Fe}_3\text{O}_4@\text{VTEO}@\text{IL-NIPs} \) are higher than 0.99, which proves the Langmuir equation fits well for Lys adsorption within the studied concentration range.\(^3\)
Fig. S7 Langmuir adsorption models of Lys on Fe₃O₄@VTEO@IL-MIPs and Fe₃O₄@VTEO@IL-NIPs.
Fig. S8 The results of SDS-PAGE analysis for the purification of Lys from a binary protein mixture. Lane 1, protein molecular weight marker; Lane 2, protein mixture solution after the adsorption by Fe$_3$O$_4$@VTEO@IL-NIPs; Lane 3, protein mixture solution after adsorption by Fe$_3$O$_4$@VTEO@IL-MIPs; Lane 4, protein mixture solution containing Lys and BHb.
Fig. S9 The results of SDS-PAGE analysis for the separation of Lys from practical sample. Lane 1, protein molecular weight marker; Lane 2, 10-fold diluted chicken egg white; Lane 3, 10-fold diluted chicken egg white after adsorption by \( \text{Fe}_3\text{O}_4@\text{VTEO}@\text{IL-MIPs} \); Lane 4, 10-fold diluted chicken egg white after adsorption by \( \text{Fe}_3\text{O}_4@\text{VTEO}@\text{IL-NIPs} \).
The structures of the protein could be described by the circular dichroism spectra (CD spectra), which can further evaluate the conformational changes of Lys in the adsorption process by comparing the CD spectra before and after the elution. The magnetic Fe₃O₄@VTEO@IL-MIPs were eluted by SDS-HAc (2% W/V: 2% V/V) solution. As presented in Fig. S10, all the curves show similar shape and characteristic peak at 208 nm and 222 nm, which suggests that the secondary structures of Lys had no change after adsorption and elution.

![CD spectra](image)

**Fig. S10** The CD spectra of Lys in buffer buffer solution (pH = 7.1) before adsorption (a), the supernatant after adsorption (b); in the SDS-HAc (2% W/V: 2% V/V) solution eluted from Fe₃O₄@VTEO@IL-MIPs (c).
In addition, as illustrated in Fig. S11, UV-visible spectra of Lys in buffer solution (pH = 7.1) before adsorption (a), the supernatant after adsorption by Fe₃O₄@VTEO@IL-MIPs (b), Fe₃O₄@VTEO@IL-NIPs (c) and SDS-HAc (2% W/V: 2% V/V) solution eluted from Fe₃O₄@VTEO@IL-MIPs (d) have similar shape and characteristic peak at 280 nm. The result proves the structures of Lys doesn’t be changed too much during adsorption.

**Fig. S11** The UV-visible spectra of Lys in buffer solution (pH = 7.1) before adsorption (a), the supernatant after adsorption by Fe₃O₄@VTEO@IL-MIPs (b); the supernatant after adsorption by Fe₃O₄@VTEO@IL-NIPs (c); in the SDS-HAc (2% W/V: 2% V/V) solution eluted from Fe₃O₄@VTEO@IL-MIPs (d).
References

