A Facile Approach for Fabrication of Core-Shell Magnetic Molecularly Imprinted Nanospheres towards Hypericin

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Academic Editor: Po-Chih Yang
Received: 2 March 2017; Accepted: 3 April 2017; Published: 7 April 2017

Abstract: By taking advantage of the self-polymerization of dopamine on the surface of magnetic nanospheres in weak alkaline Tris-HCl buffer solution, a facile approach was established to fabricate core-shell magnetic molecularly imprinted nanospheres towards hypericin (Fe₃O₄@PDA/Hyp NSs), via a surface molecular imprinting technique. The Fe₃O₄@PDA/Hyp NSs were characterized by FTIR, TEM, DLS, and BET methods, respectively. The reaction conditions for adsorption capacity and selectivity towards hypericin were optimized, and the Fe₃O₄@PDA/Hyp NSs synthesized under the optimized conditions showed a high adsorption capacity (Q = 18.28 mg/g) towards hypericin. The selectivity factors of Fe₃O₄@PDA/Hyp NSs were about 1.92 and 3.55 towards protohypericin and emodin, respectively. In addition, the approach established in this work showed good reproducibility for fabrication of Fe₃O₄@PDA/Hyp.

Keywords: surface molecular imprinting; hypericin; magnetic nanospheres; dopamine; core-shell

1. Introduction

Hypericin (Hyp) is one of the principal bioactive components of the St. John’s Wort plant (Hypericum perforatum). Traditionally used as anti-bacterial, anti-inflammatory, anti-depressive, and anti-virus agents, hypericin has gained increasing interest recently due to its potential as a highly effective anti-tumor photosensitizer [1]. Though hypericin can be obtained via chemical synthesis [2], extraction from the plant itself is still an indispensable supply pathway. However, hypericin exists in a very low concentration with structural similar analogs (such as pseudohypericin) in the plant [3]. This, together with its poor solubility and degradation upon exposure to heat and light, leaves its enrichment and purification from the plant a great challenge. This greatly inhibits the wide applications of hypericin in pharmaceutical fields. Establishing a facile method for producing efficient separation sorbents with high specificity and adsorption capacity towards hypericin is of significance and much desired [4].

Molecular imprinting [5,6] is a well-known modern technology for the production of nanostructured materials capable of molecular recognition, with high selectivity and good chemical stability at a low cost. Surface molecularly imprinted polymers (SMIPs) [7,8] are synthesized by allowing polymerization to take place on substrate surface, in order to create recognition sites on/near the material surface. These recognition sites, alongside the MIPs advantages mentioned above, provide faster rebinding kinetics for the target molecules, and are effective for the separation and enrichment of natural bio-compounds, making this an attractive solution to the challenge [9]. A variety of
Traditionally, the MIP layer (shell) around MNS (core) is formed by self-assembly of functional monomers and templates, followed by copolymerization with cross-linkers. The copolymerization is normally initiated by heat or UV light, and performed in organic solvents, with the process extending over a prolonged period of time (overnight or longer) [19]. Considering the increasing likelihood of hypericin to degrade during exposure to heat/light, it is obvious that the traditional procedure is not suitable for fabricating SMIPs for hypericin. Contrarily, dopamine can be oxidized and spontaneously self-polymerize, with oxygen as the oxidant under weak alkaline solution to yield polydopamine (PDA) [20]. Though the molecular mechanism behind the formation of PDA is complicated and not well-understood, the self-polymerization of dopamine is very mild and the cross-linked PDA network can adhere to virtually any type of material surfaces. These unique features make dopamine a promising functional monomer for fabrication of core-shell structured SMIP nanoparticles without a cross-linker (also called bi- or multi-functional comonomer), which is generally required. In fact, a few SMIP nanoparticles that were successfully prepared using dopamine as a monomer have been reported [14,21], including Fe₃O₄@PDA NPs as a stationary phase for recognition of OT-CEC [15] and SiO₂@PDA nanoparticles for protein recognition and separation [13].

**Scheme 1.** (A) Illustration of noncovalent bonding of template, hypericin with the functional monomer, dopamine: hydrogen bonding and π−π interaction; (B) Schematic illustration of fabricating Fe₃O₄@PDA/Hyp.
Inspired by the above-mentioned works, we envisioned that self-polymerization of dopamine on the surface of MNSs would provide a facile approach for fabrication of core-shell structured magnetic molecularly imprinted nanospheres for selective recognition of hypericin (Fe₃O₄@PDA/Hyp NSs). Herein, we investigated the feasibility for fabrication of Fe₃O₄@PDA/Hyp NSs by using dopamine as the only functional monomer. As depicted in Scheme 1, MNSs suspended in weak alkaline Tris-HCl buffer solution was first mixed with a solution of hypericin in acetone, followed by the addition of dopamine. Dopamine self-polymerized on the surface of MNSs in the air to form a thin layer of PDA within which hypericin molecules were embedded via non-covalent hydrogen bonding and π–π interactions. Removal of hypericin from the PDA layer leaves the recognition sites behind, and Fe₃O₄@PDA/Hyp NSs were thus obtained. The recognition properties of so-prepared Fe₃O₄@PDA/Hyp NSs toward hypericin were then evaluated and screened by varying reaction conditions.

2. Experimental Section

2.1. Chemicals and Instrumentation

Dopamine hydrochloride (DA, 98%), polyethylene glycol 1000 (PEG 1000), and ethanolamine were purchased from Aladdin, Shanghai, China. FeCl₃·6H₂O, ammonium hydroxide (28%), and ethylene glycol were purchased from Xilong Chemical Industry, Sichuan, China. Emodin was purchased from Xi’an Tianfeng Biological Technology Co., Ltd. (Xi’an, China) Acetone was purchased from Rionlon Bohua Pharmaceutical & Chemical Co., Tianjin, China. Anhydrous sodium acetate was purchased from Tianjin Bodi Chemical Industry Co., Ltd., Tianjin, China. Protohypericin (Protohyp) and hypericin (Hyp) were synthesized according to the procedures developed in our lab [22], and characterized by "H-NMR (See Figures S1–S3).

NMR spectra were recorded on a Bruker 500 MHz Spectrometer (Bruker, Fällanden, Switzerland) with working frequencies of 500 MHz for "H in DMSO-d₆ or MeOD-d₄. The residual signals from DMSO-d₆ ("H: δ 2.50 ppm), or MeOD-d₄ ("H: δ 3.31 ppm) were used as internal standards. Transmission Electron Microscope (TEM) images were taken by an H-600 instrument (Hitachi Ltd., Tokyo, Japan, 80 kV). The samples were prepared by dropping a droplet of the sample solution onto a TEM grid (copper grid, 300 meshes, coated with carbon film). Dynamic light scattering (DLS) measurements were performed on a DelsaTM Nano system (Beckman Coulter, Brea, CA, USA). UV-Vis spectra were recorded with Shimadzu 1750 UV-Visible spectrophotometer (Shimadzu, Tokyo, Japan) at 298 K. The surface area and the porosity of the prepared NSs were measured by nitrogen physisorption (Autosorb-iQ, Quantachrome, Boynton Beach, FL, USA), based on the Brunauer–Emmet–Teller ( BET) method (ASAP 2020, Micromeritics Inc., Norcross, GA, USA). Samples were vacuum-degassed at 50 °C for 9 h before the adsorption experiments.

2.2. Preparation of Fe₃O₄ Magnetic Nanospheres (MNSs)

The MNs were synthesized through the solvothermal approach according to the literature [23]. Briefly, 1.68 g FeCl₃·6H₂O was dissolved in 50 mL ethylene glycol with vigorous stirring until the solid was dissolved, then 4.5 g anhydrous sodium acetate and 1.25 g PEG were added to the solution, with continuous stirring for one hour. The resultant mixture was transferred into a Teflon-lined stainless steel autoclave (with a volume of 100 mL) and placed in an oven at 200 °C for 10 h, then cooled to room temperature. The obtained precipitate was washed with ethanol and deionized water several times and collected by magnet. The final product was dispersed in ethanol for further use.

2.3. Preparation of Hypericin-Imprinted Nanospheres (Fe₃O₄@PDA/Hyp) and Non-Imprinted Nanospheres (Fe₃O₄@PDA)

A typical procedure for preparation of Fe₃O₄@PDA/Hyp NSs was as following: 25 mg MNs were dispersed in 10 mM Tris-HCl solution (pH = 8.0 unless specified) by ultrasonication for 10 min.
Then hypericin dissolved in acetone was added to the suspension by mechanically stirring for 10 min, followed by the addition of dopamine. The mixture was stirred under air with a mechanical stirrer for 4 h. The solid was collected by magnetic separation and washed first with ultrapure water several times, then alternately with an acetone solution containing acetic acid (3% in volume), and ammonium hydroxide (3% in volume), to remove the embedded template, until no hypericin in the supernatant was detected using UV-vis spectrophotometer (Shimadzu, Tokyo, Japan) at 597 nm. Then the solid was treated with 2 µM ethanolamine to give Fe₃O₄@PDA/Hyp NSs. The final product was dispersed in ethanol for further use.

Fe₃O₄@PDA NSs were prepared and used as a control by following the same procedure as described for Fe₃O₄@PDA/Hyp NSs without the template.

2.4. Determination of Static Adsorption Capacity of Fe₃O₄@PDA/Hyp for Hypericin (Q)

To a centrifuge tube of 10 mL, 4 mg of Fe₃O₄@PDA/Hyp or Fe₃O₄@PDA NSs were added into a 4 mL known concentration of hypericin (59.4 µM unless specified) acetone solution. The tube was shaken at room temperature for 24 h (unless specified) in the dark. Then a magnet was used for separation, and the concentration of hypericin in the supernatant was measured with a UV-Vis spectrophotometer at 597 nm (Figures S6 and S7).

The adsorption capacity (Q, µg/g) of the NSs (Fe₃O₄@PDA/Hyp or Fe₃O₄@PDA) towards the test molecule was calculated by the following equation:

\[ Q = \frac{M(C_0 - C_e)V}{W} \]  \hspace{1cm} (1)

where \( C_0 \) and \( C_e \) represent the initial and equilibrium concentrations of the test molecule in acetone (µM), respectively; \( M \) is the molecular weight of the test molecule; \( V \) (L) is the volume of the solution, and \( W \) is the dry weight of the NSs (g).

The specific adsorption capacity of Fe₃O₄@PDA/Hyp NSs (Qₛ) towards hypericin is defined as the neat adsorption capacity of Fe₃O₄@PDA/Hyp over that of Fe₃O₄@PDA, and is calculated according to Equation (2):

\[ Q_s = Q_1 - Q_2 \]  \hspace{1cm} (2)

where \( Q_1 \) and \( Q_2 \) are the static adsorption capacity of Fe₃O₄@PDA/Hyp and Fe₃O₄@PDA (µg/g) towards hypericin, respectively.

2.5. Dynamic Adsorption Test

To investigate the adsorption kinetics of Fe₃O₄@PDA/Hyp (or Fe₃O₄@PDA) NSs, 4 mg of Fe₃O₄@PDA/Hyp (or Fe₃O₄@PDA) NSs were weighed into hypericin solution (59.4 µM, 4 mL) in a 10 mL centrifuge tube. The tubes were shaken at room temperature for the different time intervals (0.25, 1, 2, 4, 6, 8, and 12 h, respectively) in the dark. Then a magnet was used for the separation, and the concentration of hypericin in the supernatant was measured with a UV-Vis spectrophotometer at 597 nm.

2.6. Selectivity of Fe₃O₄@PDA/Hyp and Fe₃O₄@PDA for Hypericin

The binding selectivity of Fe₃O₄@PDA/Hyp and Fe₃O₄@PDA NSs was evaluated by measuring their binding capacities towards hypericin and two other molecules of protohypericin and emodin. 4 mg of the Fe₃O₄@PDA/Hyp or Fe₃O₄@PDA NSs were incubated respectively with 4 mL of hypericin, protohypericin, and emodin solution (59.4 µM in acetone) at 25 °C. After being incubated under continuously shaking for 24 h, the amounts of hypericin, protohypericin, and emodin bound to the Fe₃O₄@PDA/Hyp or Fe₃O₄@PDA NSs were measured, respectively. The binding selectivity of the
NSs towards different molecules was compared using the “selectivity factor” \((SF)\) and “imprinting factor” \((IF)\) \([24]\) that can be defined by the following equations:

\[
SF = \frac{Q_1}{Q_1'},
\]

where \(Q_1'\) is the adsorption capacity of the \(\text{Fe}_3\text{O}_4@\text{PDA/Hyp NSs (µg/g)}\) towards a non-template molecule.

\[
IF = \frac{Q_{\text{MIP}}}{Q_{\text{NIP}}},
\]

where \(Q_{\text{MIP}}\) and \(Q_{\text{NIP}}\) is the adsorption capacity of the \(\text{Fe}_3\text{O}_4@\text{PDA/Hyp}\) and \(\text{Fe}_3\text{O}_4@\text{PDA NSs (µg/g)}\) towards a test molecule, respectively.

2.7. Adsorption–Extraction Cycles

One adsorption–extraction cycle consisted of loading the template, reaching equilibrium adsorption, followed by the extraction of the template. For the adsorption, 4 mg of \(\text{Fe}_3\text{O}_4@\text{PDA/Hyp}\) was added to 4 mL 59.4 µM template in acetone. The suspension was incubated with a shaker at 25 °C for 8 h. Then the NSs were collected with a magnet and washed following the extraction procedure.

2.8. Preparation of the Herb Extract Solution

To prepare the herb extract, fresh flowers were picked up from \textit{Hypericum perforatum} plant just before the extraction process. The extraction was performed in the dark. The procedure was as following: 10 g fresh flowers was charged to a 2-L beaker and immersed with distilled water at 50 °C for 2 h. Then the flowers were transferred to a 1000-mL flask and refluxed with 500 mL methanol-water mixture (80:20, \(v/v\)) for 6 h. The contents in the flask were cooled to room temperature and filtered. The filtrate was dried under reduced pressure. The residue was dissolved with acetone and filtered. The filtrate was combined and the volume was adjusted with a 25 mL volumetric flask.

2.9. HPLC Analysis

A total of 8 mL of the herb extract solution was mixed with 1 mL of hypericin and 1 mL of protohypericin solution (each with a concentration of 600 µM in acetone), to obtain an original solution for adsorption. To 4 mL of this final solution, 4 mg of the NSs (\(\text{Fe}_3\text{O}_4@\text{PDA/Hyp}\) or \(\text{Fe}_3\text{O}_4@\text{PDA}\)) was added. The mixture was shaken for 8 h. The supernatant was analyzed by HPLC (Shimadzu, Tokyo, Japan), with C18 reversed-phase column (5 µm, 4.6 mm × 150 mm) at 25 °C. The mobile phase consisted of 50% acetonitrile, 50% of the mixture of ammonium acetate-acetic acid buffer (0.3 M, pH = 6.96) and methanol (1:4, \(v/v\)); detection wavelength: 590 nm; flow rate: 0.4 mL/min; injection volume: 10 µL.

3. Results and Discussion

3.1. Synthesis of \(\text{Fe}_3\text{O}_4@\text{PDA/Hyp}\)

The synthesis of \(\text{Fe}_3\text{O}_4@\text{PDA/Hyp}\) is described in Scheme 1. Dopamine is a small molecule containing catechol and amino groups. As illustrated in Scheme 1a, when mixed with the template, dopamine formed assembly with hypericin via hydrogen bonding and \(\pi-\pi\) interaction. The stability of the assembly and hypericin under the reaction conditions was investigated by UV-Vis spectroscopy (see Figure S4). Comparing to the spectrum of hypericin in the mixture of Tris-HCl buffer/acetone without dopamine, only a blue shift was observed in the spectrum of hypericin with dopamine, even after the prolongation of time. The result indicated that both the assembly and hypericin were stable under the reaction conditions. The oxidization of dopamine under the reaction condition, alongside with its noncovalent self-assembly and covalent polymerization, led to a cross-linked PDA layer deposited on the surface of MNSs \([20]\). Removal of hypericin from the PDA layer gave \(\text{Fe}_3\text{O}_4@\text{PDA/Hyp NSs.}

3.2. Characterization of Fe₃O₄@PDA/Hyp

3.2.1. FTIR Analysis

To verify the self-polymerization of dopamine on the surface of MNSs in the absence or presence of hypericin, Fe₃O₄@PDA and Fe₃O₄@PDA/Hyp were analyzed by FTIR spectroscopy. As shown in Figure 1, two characteristic bands of 1510 and 1333 cm⁻¹ from the spectrum of PDA, ascribed to C=N and C–N–C stretching vibration [25], respectively were found on both spectra of Fe₃O₄@PDA and Fe₃O₄@PDA/Hyp; This was also the case for the characteristic band of 1261 cm⁻¹ (C–O stretching vibration) from the spectrum of hypericin on the spectrum of Fe₃O₄@PDA/Hyp. The results imply that dopamine successfully polymerized on the surface of MNSs with or without the presence of hypericin under the reaction conditions used in this work.

![FTIR spectra of Fe₃O₄@PDA/Hyp, Fe₃O₄@PDA, MNSs, Hypericin and polydopamine (PDA).](image)

**Figure 1.** FTIR spectra of Fe₃O₄@PDA/Hyp, Fe₃O₄@PDA, MNSs, Hypericin and polydopamine (PDA).

3.2.2. TEM and DLS Analysis

The morphology and the structure of Fe₃O₄@PDA/Hyp NSs were further studied by TEM and DLS. According to the TEM images (Figure 2), a thin layer around MNSs with a thickness of about 29 nm for Fe₃O₄@PDA NSs, and 23 nm for Fe₃O₄@PDA/Hyp NSs, was clearly seen. The thickness obtained in this work is similar to that reported in the literature [26]. The spherical shape of MNSs was preserved after polymerization. DLS analysis (Table 1 and Figure S5) showed the average diameter of Fe₃O₄@PDA and Fe₃O₄@PDA/Hyp NSs increased by 60 and 46 nm, to 573 and 559 nm, respectively, compared with that of MNSs (513 nm). The increased thickness in both cases coincided with the thickness of the PDA layer measured by TEM.

In addition, when Fe₃O₄@PDA and Fe₃O₄@PDA/Hyp NSs were subjected to the extraction process, the ζ potential of Fe₃O₄@PDA/Hyp NSs became more negative (from −0.86 to −9.96), while that of Fe₃O₄@PDA NSs was less affected (see Table 1). The difference of the ζ potentials of Fe₃O₄@PDA/Hyp NSs before and after extracting process may be ascribed to the removal of the templates from Fe₃O₄@PDA/Hyp NSs. All these results indicate that the desired core-shell structure of Fe₃O₄@PDA/Hyp NSs can be prepared conveniently via dopamine, self-polymerized on the surface of MNSs in the presence of hypericin.
3.3. Optimization of Preparation Conditions for Specific Adsorption Capacity of Fe₃O₄@PDA/Hyp NSs

Considering that adsorption capacity of MIPs for targeting molecules can be affected by a number of parameters, the preparation conditions of Fe₃O₄@PDA/Hyp were screened and optimized, including monomer concentration, molar ratio of template to monomer, solvent, pH value, post-treatment, etc.

3.3.1. Effect of Monomer Concentration on Specific Adsorption Capacity

For SMIP, the concentration of monomer is a key factor that affects the thickness of the polymeric layer and the number of the recognition sites formed. It has been suggested the concentration of dopamine should be at least 2 mg/mL to form a PDA film on the surface of substrate [20]. To evaluate

Table 1. The DLS data of the NSs prepared.

<table>
<thead>
<tr>
<th>NSs</th>
<th>Particle size (nm)</th>
<th>Polydispersity index</th>
<th>ζ Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNSs</td>
<td>513 ± 98</td>
<td>0.444</td>
<td>1.35 ± 0.98</td>
</tr>
<tr>
<td>Fe₃O₄@PDA</td>
<td>572 ± 85</td>
<td>0.761</td>
<td>−3.96 ± 0.87</td>
</tr>
<tr>
<td>Fe₃O₄@PDA/Hyp</td>
<td>559 ± 40</td>
<td>0.385</td>
<td>−0.86 ± 0.08</td>
</tr>
<tr>
<td>Fe₃O₄@PDA after extracting process</td>
<td>574 ± 40</td>
<td>0.374</td>
<td>−1.38 ± 0.42</td>
</tr>
<tr>
<td>Fe₃O₄@PDA/Hyp after extracting process</td>
<td>561 ± 98</td>
<td>0.338</td>
<td>−9.96 ± 0.66</td>
</tr>
</tbody>
</table>

Table 2. The pore size, surface area, and the pore volume of the NSs.

<table>
<thead>
<tr>
<th>NSs</th>
<th>Average pore diameter (nm)</th>
<th>Surface area (m²/g)</th>
<th>Pore volume (cm³/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe₃O₄@PDA/Hyp</td>
<td>8.095 ± 0.409</td>
<td>54.764 ± 4.189</td>
<td>0.111 ± 0.003</td>
</tr>
<tr>
<td>Fe₃O₄@PDA</td>
<td>9.185 ± 1.086</td>
<td>12.176 ± 2.166</td>
<td>0.028 ± 0.001</td>
</tr>
</tbody>
</table>

Figure 2. TEM images of the nanospheres (NSs) prepared: (a) Fe₃O₄@PDA NSs; (b) Fe₃O₄@PDA/Hyp NSs; (c,d) are the enlarged images corresponding to (a,b), respectively. Scale bar: 200 nm.
the effect of dopamine concentration on specific adsorption capacity, a series of Fe₃O₄@PDA/Hyp NSs were prepared by varying the concentration of dopamine in a range of 0.5–5 mg/mL. As shown in Figure 3, \( Q_s \) increased gradually when the concentration of dopamine increased from 0.5 to 2 mg/mL, and reached a plateau with further increase of dopamine concentration. Interestingly, self-polymerization of dopamine in the system could take place either on the surface of the MNSs or in the solution. When the concentration of dopamine was lower than 2 mg/mL, self-polymerization of dopamine mainly took place on the surface of the MNSs, which led to the formation of a PDA layer around the MNSs. The higher the concentration, the thicker the layer, and therefore the more recognition sites led to a higher \( Q_s \) value. However, a concentration of dopamine higher than 2 mg/mL increased the opportunity of its self-polymerization in solution and led to the formation of pure PDA nanoparticles. Hence, we chose 2 mg/mL as the optimal concentration of dopamine for further study.

![Figure 3. Effect of concentration of dopamine on specific absorption capacity (\( Q_s \)).](image)

### 3.3.2. Effect of the Ratio of Hypericin to Dopamine on Specific Adsorption Capacity

The ratio of template to monomers (including comonomer) has long been recognized as one of the crucial parameters for selective adsorption capacity of MIPs. The optimal ratio can vary in a wide range. For example, a value of 1/155 was reported for a MIP towards EA9A [27], while in another system, 1/41 was used to prepare a MIP for protonated primary alkylamine [24]. The effect of the ratio of hypericin to dopamine (in mole percentage) on specific adsorption capacity was studied by varying the ratio between 0.16% and 3.2%. The results were summarized in Figure 4. It can be seen that the \( Q_s \) value increased with the ratio of hypericin to dopamine, and reached to a vertex at a ratio of 0.82% (equals to 1/122). Further increase of the ratio led to a slight decrease of the \( Q_s \). Therefore, the best ratio to acquire an optimal \( Q_s \) was determined to be 0.82%.

![Figure 4. Effect of amount of hypericin (% of dopamine in mole) on specific absorption capacity (\( Q_s \)).](image)
3.3.3. Effect of the Amount of Acetone on Specific Adsorption Capacity

Considering hypericin is insoluble in water, it is necessary to dissolve it in a suitable organic solvent before the imprinting process. Acetone, as one of the few organic solvents which can dissolve hypericin and is miscible with water, was chosen and its influence on $Q_s$ was investigated accordingly. As shown in Figure 5, the amount of acetone used during imprinting affected the polymerization rate of dopamine and nanostructure of the polymer network.

As shown in Table 3, as mentioned before, self-polymerization of dopamine is induced by oxygen in a weak alkaline solution; therefore, the effect of pH of Tris-HCl on $Q_s$ at 7.5, 8.0 and 8.5 was explored. It was found that $Q_s$ obtained at pH 8.0 is the highest within the pH range studied. Imprinting at a lower temperature resulted in the decrease of $Q_s$, for instance from 16.40 mg/g at 25 °C to 10.12 mg/g at 0 °C. The change in the order of addition of hypericin and dopamine (i.e., mixing dopamine with MNSs before the addition of hypericin) led to a significant decrease of $Q_s$ from 16.40 to 6.44 mg/g. Moreover, the post-treatment of Fe$_3$O$_4$@PDA/Hyp with ethanolamine nearly doubled the $Q_s$ from 8.02 to 16.40 mg/g. The increase of $Q_s$ might be ascribed to the deactivation of the surface of PDA by ethanolamine via Schiff base reaction and/or Michael addition [28].

3.3.4. Effect of Other Parameters on Specific Adsorption Capacity

Besides the parameters discussed above, some other parameters (such as pH, temperature, mixing order, post-treatment, etc.) greatly influence $Q_s$, as shown in Table 3. As mentioned before, the ratio of acetone to Tris-HCl buffer ($v/v$) varied from 1/24 to 1/2. $Q_s$ was found to reach a maximum at the ratio of acetone to Tris-HCl around 1/6, and drop dramatically afterwards. It was deduced that acetone affected $Q_s$ by altering the aggregation status of hypericin in the system and affecting the polymerization rate of dopamine and nanostructure of the polymer network.

Table 3. $Q_s$ of Fe$_3$O$_4$@PDA/Hyp NSs synthesized with different conditions $^a$.

<table>
<thead>
<tr>
<th>Entry</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>$Q_s$ (mg/g)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.5</td>
<td>25</td>
<td>15.23 ± 0.08</td>
<td>0.005</td>
</tr>
<tr>
<td>2</td>
<td>8.0</td>
<td>25</td>
<td>16.40 ± 0.23</td>
<td>0.014</td>
</tr>
<tr>
<td>3</td>
<td>8.5</td>
<td>25</td>
<td>15.41 ± 0.52</td>
<td>0.034</td>
</tr>
<tr>
<td>4</td>
<td>8.0</td>
<td>0</td>
<td>10.12 ± 0.89</td>
<td>0.088</td>
</tr>
<tr>
<td>5 b</td>
<td>8.0</td>
<td>25</td>
<td>6.44 ± 0.19</td>
<td>0.029</td>
</tr>
<tr>
<td>6 c</td>
<td>8.0</td>
<td>25</td>
<td>8.02 ± 0.51</td>
<td>0.005</td>
</tr>
</tbody>
</table>

$^a$ A total of 25 mg MNSs suspension in Tris-HCl were mixed with 2.7 mg hypericin in acetone under mechanical stirring in 50 mL solvent (acetone/Tris-HCl = 1/6 in volume) for 10 min, followed by the addition of 100 mg dopamine; $^b$ 25 mg MNSs suspension in Tris-HCl were mixed with 100 mg dopamine under mechanical stirring for 10 min, followed by the addition of 2.7 mg hypericin dissolved in acetone (total volume is 50 mL, acetone/Tris-HCl = 1/6 in volume); $^c$ Fe$_3$O$_4$@PDA/Hyp prepared according to Entry 2, but without post-treatment with ethanolamine.
Due to the before-mentioned results, we concluded that Fe₃O₄@PDA/Hyp NSs with an optimal \( Q_s \) value were prepared under the conditions used in Entry 2—i.e., 25 mg MNSs suspended in 41.67 mL Tris-HCl buffer solution was mixed with 2.7 mg hypericin dissolved in 8.33 mL acetone under mechanical stirring (acetone/Tris-HCl = 1/6 in volume, pH = 8.0) for 10 min, followed by the addition of 100 mg dopamine. The polymerization was run at room temperature in the atmosphere for 4 h. The nanospheres were subjected to the extracting process for removal of the template, followed by post-treatment with ethanolamine to obtain the best Fe₃O₄@PDA/Hyp NSs.

3.4. Dynamic Adsorption Study

The equilibrium adsorption isotherms of Fe₃O₄@PDA/Hyp and Fe₃O₄@PDA NSs for the binding of hypericin were studied, and the results are shown in Figure 6. As observed in Figure 6, the adsorption process of the Fe₃O₄@PDA/Hyp NSs displayed two periods: the adsorption amount increased quickly and reached to about one third of the total adsorption capacity during the first hour. After this period, adsorption rate slowed down, and the equilibrium was achieved at 8 h. The explanation for this phenomenon is that the recognition sites located on the surface of PDA layer was ascribed to the binding of hypericin molecules in the first period, and then the diffusion of hypericin into the internal binding sites resulted in the slow adsorption rate, when the surface recognition sites became saturated. While the binding of Fe₃O₄@PDA towards hypericin saturated quickly after 0.25 h, the prolongation of contact time did not have a positive effect on the absorption. This may be explained by the much smaller pore volume and surface area of Fe₃O₄@PDA (proved by BET), where the non-specific adsorption of hypericin mainly occurred on the surface of Fe₃O₄@PDA, and the mass transfer for hypericin into the interior of the PDA was inhibited. Compared to Fe₃O₄@PDA, a much stronger adsorption was observed on Fe₃O₄@PDA/Hyp NSs, which demonstrated the specific recognition sites were well-formed on the surface of Fe₃O₄@PDA/Hyp NSs.

![Figure 6. Dynamic adsorption of hypericin on Fe₃O₄@PDA/Hyp and Fe₃O₄@PDA NSs.](image)

3.5. Maximum of Specific Adsorption Capacity

To investigate the binding performance of Fe₃O₄@PDA/Hyp NSs prepared under the optimized conditions, binding experiments were conducted with different concentrations of hypericin, varying in a range of 0 to 100 \( \mu \)M.

The adsorption isotherm of Fe₃O₄@PDA/Hyp NSs towards hypericin is shown in Figure 7a. It can be seen that \( Q_s \) increased with the concentration of hypericin before the saturation concentration \( C_S \) (59.4 \( \mu \)M), and reached a maximum adsorption at \( C_S \). Further increase in concentration did not cause...
any difference in adsorption process. Obviously, it fits Langmuir adsorption isotherm. The Langmuir equation is expressed as following:

$$\theta = \frac{Q}{Q_{\text{max}}} = K C^m$$  \hspace{1cm} (5)

where $\theta$ equals to $Q/Q_{\text{max}}$. The fitting plot is shown in Figure 7b. The fact that $m$ value was close to 1 implied that the binding sites of Fe₃O₄@PDA/Hyp were homogeneous. The associated constant obtained was $1.367 \times 10^{-2} \text{ M}^{-1}$. The $R^2$ value of 0.9915 showed a good consistency of the Langmuir equation with the measured data.

Figure 7. (a) The adsorption isotherm of Fe₃O₄@PDA/Hyp NSs towards hypericin; (b) The fitting plot of the adsorption isotherm of Fe₃O₄@PDA/Hyp NSs towards hypericin by Langmuir isotherm.

The specific adsorption of Fe₃O₄@PDA/Hyp NSs towards hypericin ($Q_s$), as shown in Table 4, also increased with the concentration of hypericin, and achieved a maximum value of 16.30 mg/g at $C_5$. It is worth mentioning that Fe₃O₄@PDA/Hyp NSs prepared under the optimized conditions in this work has a much higher specific absorption capacity for hypericin than that reported MIP via bulk polymerization (0.646 mg/g) [29].

Table 4. The effect of the concentration of hypericin on $Q_s$.

<table>
<thead>
<tr>
<th>Concentration of hypericin (µM)</th>
<th>$Q_1$ (mg/g)</th>
<th>RSD</th>
<th>$Q_s$ (mg/g)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.62 ± 0.03</td>
<td>0.006</td>
<td>0.92 ± 0.80</td>
<td>0.814</td>
</tr>
<tr>
<td>25</td>
<td>7.21 ± 0.71</td>
<td>0.110</td>
<td>3.72 ± 0.68</td>
<td>0.067</td>
</tr>
<tr>
<td>40</td>
<td>10.89 ± 0.71</td>
<td>0.095</td>
<td>8.10 ± 0.27</td>
<td>0.031</td>
</tr>
<tr>
<td>59.4</td>
<td>18.28 ± 0.73</td>
<td>0.047</td>
<td>16.30 ± 0.12</td>
<td>0.007</td>
</tr>
<tr>
<td>75</td>
<td>19.06 ± 0.02</td>
<td>0.015</td>
<td>10.58 ± 1.11</td>
<td>0.105</td>
</tr>
<tr>
<td>100</td>
<td>19.00 ± 0.04</td>
<td>0.096</td>
<td>14.96 ± 1.32</td>
<td>0.077</td>
</tr>
</tbody>
</table>

3.6. Binding Selectivity

In order to examine the selectivity of Fe₃O₄@PDA/Hyp NSs toward template hypericin, protohypericin and emodin were chosen as competitors of hypericin they are all quinone-type structures containing common hydroxyl groups, similar to hypericin. Fe₃O₄@PDA/Hyp or Fe₃O₄@PDA NSs were incubated respectively with the same amount of hypericin, protohypericin, and emodin under the same conditions. The adsorption capacity of Fe₃O₄@PDA/Hyp and Fe₃O₄@PDA NSs towards the three quinone compounds was summarized in Figure 8b. Fe₃O₄@PDA/Hyp NSs showed a higher adsorption for hypericin (18.2 mg/g) than that for protohypericin and emodin (9.4 and 5.1 mg/g, respectively). The binding selectivity of the polymer particles was evaluated with $SF$ and $IF$, respectively. The results are listed in Table 5. $SF$ of Fe₃O₄@PDA/Hyp NSs towards protohypericin (a structurally similar molecule) and emodin (a structurally dissimilar molecule), was 1.92 and 3.55,
compounds. In addition, the IF value of Fe$_3$O$_4$@PDA/Hyp NSs towards hypericin in this work was much higher than that reported [29,30].

The reproducibility of the approach for fabrication of Fe$_3$O$_4$@PDA/Hyp NSs under the optimized conditions was evaluated by three parallel experiments. As is shown in Figure 9, similar specific adsorption capacities of Fe$_3$O$_4$@PDA/Hyp NSs prepared individually at the optimal conditions were measured with 16.68, 16.41 and 16.22 mg/g, respectively. The results indicate that the approach established in this work for fabrication of core-shell Fe$_3$O$_4$@PDA/Hyp NSs for selective recognition of hypericin is reproducible.

3.7. Reproducibility and Reusability Evaluation

The reproducibility study of the approach for fabrication of Fe$_3$O$_4$@PDA/Hyp NSs prepared in this work showed that their reusability is not optimal. As shown in Figure S8, the absorption capacity decreased by 35% at the second cycle, and ~50% at the fourth cycle.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hypericin</th>
<th>Protohypericin</th>
<th>Emodin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF</td>
<td>/</td>
<td>1.92</td>
<td>3.07</td>
</tr>
<tr>
<td>IF</td>
<td>8.01 (3.56 $^a$, 4.5 $^b$)</td>
<td>3.07</td>
<td>1.36</td>
</tr>
</tbody>
</table>

$^a$ The value was from Ref. [29]; $^b$ The value was from Ref. [30].

Figure 8. (a) The chemical structures of hypericin, protohypericin, and emodin; (b) Selective bindings of Fe$_3$O$_4$@PDA/Hyp (black) and Fe$_3$O$_4$@PDA (red) NSs toward to hypericin, protohypericin, and emodin, respectively.

Figure 9. Reproducibility study of the approach for fabrication of Fe$_3$O$_4$@PDA/Hyp NSs under the optimized conditions.
The reusability of MIPs is crucial in developing applications that are reliable, economic and sustainable [31]. Unfortunately, the evaluation on the Fe₃O₄@PDA/Hyp NSs prepared in this work showed that their reusability is not optimal. As shown in Figure S8, the absorption capacity decreased by 35% at the second cycle, and ~50% at the fourth cycle.

3.8. Adsorption of Fe₃O₄@PDA/Hyp NSs from the Herb Extract

To test the selective adsorption ability of Fe₃O₄@PDA/Hyp NSs towards hypericin in a real sample, a herb extract was prepared and mixed with equal amount of hypericin and protohypericin. After adsorption, the supernatant was analyzed by HPLC, and the results are shown in Figure 10 and Table 6. According to the HPLC chromatogram of the herb extract, both peaks of hypericin and protohypericin were found (Figure 10a) and were well-separated under the LC conditions used for the analysis. From Table 6, it can be seen that 47.8% of hypericin in the initial sample was absorbed by Fe₃O₄@PDA/Hyp, while only 8.3% was absorbed by Fe₃O₄@PDA. Compared to Fe₃O₄@PDA, Fe₃O₄@PDA/Hyp showed nearly 6 times higher adsorption toward hypericin. On the other hand, Fe₃O₄@PDA gave similar adsorption to both hypericin and protohypericin (8.3% and 11.5%, respectively), which implied that the adsorption was non-specific.

![HPLC chromatograms](image)

**Figure 10.** HPLC chromatograms. (a) the herb extract; (b) the mixture of the extract, hypericin and protohypericin before adsorption; (c) the supernatant after the adsorption of Fe₃O₄@PDA NSs; (d) the supernatant after the adsorption of Fe₃O₄@PDA/Hyp NSs.

**Table 6.** The adsorption capacity of hypericin for hyperforin perforatum.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak area (Hyp)</th>
<th>Peak area (Protohyp)</th>
<th>Adsorption of Hyp (%)</th>
<th>Adsorption of Protohyp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>676,506.3</td>
<td>364,871.0</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Fe₃O₄@PDA</td>
<td>620,591.5</td>
<td>322,926.9</td>
<td>8.3</td>
<td>11.5</td>
</tr>
<tr>
<td>Fe₃O₄@PDA/Hyp</td>
<td>353,391.0</td>
<td>246,750.0</td>
<td>47.8</td>
<td>32.4</td>
</tr>
</tbody>
</table>

4. Conclusions

This work has for the first time established a facile approach to fabricate core-shell magnetic molecularly imprinted nanospheres for selective recognition of hypericin (Fe₃O₄@PDA/Hyp) NSs via self-polymerization of dopamine on the surface of MNSs. The core-shell structure of the synthesized Fe₃O₄@PDA/Hyp NSs was confirmed by TEM. The reaction conditions for adsorption capacity were screened, which revealed that the ratios of acetone to Tris-HCl buffer and hypericin to dopamine, and post-treatment with ethanolamine, are crucial in maximizing the imprinting efficiency. Fe₃O₄@PDA/Hyp NSs prepared under the optimized conditions demonstrate a high adsorption
capacity ($Q = 18.28 \text{ mg/g}$), and possess good binding selectivity towards hypericin. In addition, the approach established in this work has good reproducibility for fabrication of Fe$_3$O$_4$@PDA/Hyp.

**Supplementary Materials:** The following are available online at www.mdpi.com/2073-4360/9/4/135/s1.

- Figure S1: The $^1$H-NMR spectrum of emodin anthrone;
- Figure S2: The $^1$H-NMR spectrum of protohypericin;
- Figure S3: The $^1$H-NMR spectrum of hypericin;
- Figure S4: UV-Vis spectra of hypericin in Tris-HCl and acetone ($v/v = 6:1$, pH = 8.0) with or without the presence of dopamine;
- Figure S5: DLS histograms of MNs and Fe$_3$O$_4$@PDA and magnetic response of MNs;
- Figure S6: (a) UV-Vis spectra of hypericin, protohypericin and emodin, respectively, (b–d) Standard curves of hypericin, protohypericin and emodin;
- Figure S7: TEM images of MNs (a) and their response to magnet in 10 s (b,c);
- Figure S8: Reusability study of Fe$_3$O$_4$@PDA/Hyp NSs.

**Acknowledgments:** This research work was supported by the Project of Science and Technology of Social Development in Shaanxi Province (2016SF-029) and the National Natural Science Foundation of China (21174113).

**Author Contributions:** Wenxia Cheng designed and performed the experiments, analyzed the data, and drafted the experimental part. Fengfeng Fan performed the experiments. Ying Zhang synthesized hypericin and other molecules. Zhichao Pei assisted with data analysis and the revision of the manuscript. Wenji Wang performed the BET surface area analysis. Yuxin Pei directed the entire research work and drafted the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**


