Synthesis and Characterization of Magnetic-Graphene Molecularly Imprinted Polymers for Selective Recognition of L-tryptophan

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Abstract. In this paper, a novel molecular imprinted polymer using L-tryptophan(L-Trp) as the template, dopamine(DA) as both functional monomer and cross linking agent, magnetic graphene as the supporting matrix was synthesized. The prepared magnetic-graphene molecularly imprinted polymers($\text{Fe}_3\text{O}_4@\text{GO-MIPs}$) were characterized by Fourier transform infrared spectrometer(FT-IR), transmission electron microscopy (TEM), vibrating sample magnetometer (VSM), respectively. The results showed that when the molar ratio of L- tryptophan and dopamine was 1:4 and the reaction temperature was 60 °C, $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ had the best adsorption quantity of 31.9 mg/g. The rebinding experiments indicated that $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ not only have outstanding affinity and selectivity towards L-Trp over structurally related compounds but also easily reach the magnetic separation under an external magnetic field.

1 Introduction

Molecular imprinting has been widely recognized as a potential technique for the synthesis of tailor-made recognition materials by the formation of a polymer network around a template molecule. Compared with natural receptors, the molecularly imprinted polymers have advantages including good physical and chemical stability, high selectivity of recognition and its reusability. Owing to these fantastic properties, the molecularly imprinted polymers have been widely used in the fields of biosensors^[1], solid-phase extraction^[2], LC^[3], biosensors^[4], chiral recognition and separation^[5], type enzyme catalyst^[6], pharmacy^[7] and other fields.

Graphene has attracted considerable attention in recent years because of its large specific surface area and excellent mechanical, electrical, thermal, optical properties. This unique nanostructure with extremely (theoretical value $2630 \text{ m}^2 \text{ g}^{-1}$) and small dimension makes graphene an excellent candidate as supported material for preparing molecularly imprinted materials^[8]. The magnetic molecularly imprinted polymer can be influenced under an external magnetic field and it enables separation of analyte easier and faster and also provides large surface to volume ratio. $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ have both specific recognition and rapid separation characteristics for the template molecule based on integration of the virtues of graphene, magnetic separation technology and imprinted polymer. L-Trp is known to be metabolized to several neuroactive substances such as serotonin, kynurenic acid, and quinolinic

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acid^[9-11]. Analyses of L-Trp and its metabolites in biological fluids, such as serum or plasma and urine, are of clinical importance for diagnosis of several diseases in humans as several reports have indicated that concentrations of L-Trp and its metabolites are altered in various disease states^[12]. Furthermore, L-Trp is used for insomnia, sleep apnea, depression, anxiety, facial pain, a severe form of premenstrual syndrome called premenstrual dysphoric disorder (PMDD), smoking cessation, grinding teeth during sleep (bruxism), attention deficit-hyperactivity disorder (ADHD), Tourette's syndrome and to improve athletic performance^[13-15]. On the other hand, no medicinal properties for D-tryptophan has been reported^[16]. The aim of the paper is to provide a novel synthesis method of $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ that may be used for determination of L-Trp in presence of D-tryptophan.

2 Experimental

2.1 Reagents

DA and L-Trp purchased from Shanghai Macklin Biochemical Technology; Graphite powder, potassium permanganate purchased from Tianjin Hongda Chemical Reagent Factory; All other reagents and solvents were of analytical grade and used without further purification unless otherwise noted. All aqueous solutions were prepared using ultrapure water from US Millipore company.

2.2 Apparatus

The FT-IR spectra of the prepared nanospheres in the region $4000\text{-}400\text{cm}^{-1}$ were recorded using a BRUKER OPTICS FT-IR spectrometer (KBr pellets). Magnetic properties were measured on vibrating sample magnetometer (VSM, Quantum Design, Beijing). The size, morphology, and structure of the nanospheres were characterized by transmission electron microscopy (TEM, JEOL, Japan). The ground samples were dissolved in anhydrous ethanol, washed with copper mesh, dried, and then observed by transmission electron microscopy.

2.3 Preparation of $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$

Oxidized graphene was synthesized from nature graphite via an improved Hummers' method[17]. In a typical $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ Synthesis, GO(50 mg) was dispersed in 100 mL of ultrapure water by ultrasonication. Under the condition of stirring, 4 mmol of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 2 mmol of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were added into 100 mL of oxidized graphene dispersion in sequence. After the two ferric salts were dissolved respectively, the rotation speed was increased. Then, 25 mL of ammonia was added, the pH was adjusted to 9.0. After reacting for 30 min at room temperature, 50 mg of dopamine hydrochloride was added and stirring was continued for 1 h. The cooled solution was separated with a magnet, and washed with ultrapure water until the pH was stabilized to 8.0. The black synthetic products were washed several times with ethanol and ultrapure water and dried at 70°C for 12 h. The $\text{Fe}_3\text{O}_4@\text{GO}$ (400 mg) were dispersed in 80 mL of Tris buffer (10 mM, pH 8.0) by ultrasonication. L-Trp (30 mg) was then added and stirred for 0.5 h in room temperature followed by 120 mg of DA the reaction was continued at 60°C for 6 h. The obtained product was washed with water to remove the unreacted monomer, then was washed with a mixture of hydrochloric acid solution (1 mol/L) and methanol (1:4, v/v) to extract the template L-Trp, and was rewashed thoroughly with ultrapure water. For comparison, $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$ prepared for the same procedures without the addition of the template L-Trp.

Figure 1. Preparation of $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$

2.4 Binding Experiments

For the kinetics experiments, 10.0 mg of $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ or $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$ was ultrasonically dispersed in 20 mL of L-Trp solution for 1 min the mixture was shaken for 2 h, respectively. $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ or $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$ were withdrawn at appropriate time at intervals. For the adsorption isotherm experiments, an amount of 10 mg of $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ or $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$ was added to 20 mL of L-Trp solution of different concentrations. After shaking at room temperature for 1 h. The concentration of L-Trp were measured by UV-vis spectrophotometer at 278 nm. Adsorption quantity called Q was calculated from the equation: $Q = (C_0 - C)V/W$, where C_0 and C were the template concentrations ($\text{mg} \cdot \text{mL}^{-1}$) in the solutions which were measured initially and after sorption, respectively, V (mL) was the volume of the bulk solution, and W (g) was the weight of the $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ or $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$. The selectivity of the $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ was investigated using 0.04 mmol/L of D-tryptophan, L-phenylalanine, D-phenylalanine, L-tyrosine, and D-tyrosine as comparatives.

3 Results and discussion

3.1 Preparation of $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$

In order to obtain efficient L-Trp recognition, the preparation conditions of $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$, including the molar ratio of the template L-Trp to the monomer DA, reaction temperature, were optimized as discussed below in detail. The best conditions were investigated in a set of batch experiments according to the determination of adsorption quantity (Q) of L-Trp onto $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$. It can be seen from Fig.2 that when the mole ratio of L-Trp template molecule to dopamine was 1:4, the adsorption quantity of L-Trp reached the highest (about 15 mg/g). We think that the increasing DA amount could increase the thickness of PDA (polydopamine) film on the surface of RGO which can accommodate more L-Trp molecule and thus lead to the increase the recognition cavities. On the other hand, when the DA amount increases to a certain value, the over thickness of the PDA film could block the adsorption sites accessibility and result in the decrease of the binding amount. Thus, 1:4 was selected to be the optimum mole ratio in this work.

Figure 2. Effect of molar ratio on adsorption capacity

The effect of the reaction temperature on adsorption capacity was investigated from 25 °C to 80 °C . As shown in Fig.3 , with increasing temperature values between 25 °C to 60 °C, the adsorption capacity of Fe₃O₄@GO-MIPs increased gradually and then decreases slightly from 60 °C to 80 °C. It can be inferred that the synthesis temperatures have an important effect on the synthesis of Fe₃O₄@GO-MIPs from the point of increasing adsorption capacity and saving energy. Therefore, it is appropriate to use 60 °C as the optimum reaction temperature.

Figure 3. Effect of reaction temperature on adsorption capacity

3.2 Characterization of Fe₃O₄@GO-MIPs

The FT-IR spectra of GO(a), Fe₃O₄@GO(b), Fe₃O₄@GO-MIPs(c), Fe₃O₄@GO-NIPs(d) are shown in Fig.4. As shown in the spectrum of GO(a), the characteristic peak at 1740 cm⁻¹ is attributed to Cdouble bond. The feature peaks at 588 cm⁻¹ observed from spectra b-d belong to Fe₃O₄. For the FTIR spectrum of Fe₃O₄@GO-MIP(c), the appearance of the stretching vibration of -NH₂ at 3399 cm⁻¹ provides evidence of the formation of PDA layer. In addition, compared to GO(a), the disappearance of the C=O peak at 1740 cm⁻¹ provides a solid indication of GO reduction. All these bands demonstrate that Fe₃O₄@GO-MIPs were synthesized successfully.

Figure 4. FT-IR spectra of GO(a), $\text{Fe}_3\text{O}_4@\text{GO}$ (b), $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ (c), $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$ (d)

It is vitally important for magnetic separation that the sorbent is sufficiently magnetic. VSM was employed to characterize the magnetic properties of the obtained magnetic materials, and their VSM magnetization curves are shown in Fig.5. Based on the VSM data, the $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ exhibited a saturation magnetization value of 36.7 emu g^{-1} . In the absence of an external magnetic field, a black homogeneous dispersion exists. When an external magnetic field was applied, the black particles were attracted to the wall of vial in a short time.

Figure 5. The hysteresis loop of $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ beads. The insert shows the separation and redispersion process of a solution of $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ in the absence (left) and presence (right) of an external magnetic field.

The morphological structures of $\text{Fe}_3\text{O}_4@\text{GO}$ (a), $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ (b), $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$ (c) were examined by TEM. It can be seen from Fig.6 that $\text{Fe}_3\text{O}_4@\text{GO}$ (a) has a spherical structure with a diameter of about 10 nm, which is evenly distributed on the graphene oxide, with some slight agglomeration and overlap. $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ has some larger pores on the basis of the original $\text{Fe}_3\text{O}_4@\text{GO}$. The pore size is about 20 nm, which is larger than that of $\text{Fe}_3\text{O}_4@\text{GO}$. It could be concluded that L-Trp left imprinted sites in the polymer, indicating that the imprinted polymer was modified on $\text{Fe}_3\text{O}_4@\text{GO}$ surface. $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$ is similar to $\text{Fe}_3\text{O}_4@\text{GO}$ in the size of about 10 nm magnetic microspheres, while it is quite different from that of $\text{Fe}_3\text{O}_4@\text{GO-MIPs}$ due to the absence of the template in $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$. Owing to that no L-Trp template molecules were added in the synthesis of $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$, the agglomeration of $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$ was greater than that of $\text{Fe}_3\text{O}_4@\text{GO}$. Furthermore, $\text{Fe}_3\text{O}_4@\text{GO-NIPs}$ (Figure (c)) were in a similar "tribal" form.

Figure 6. TEM images of Fe₃O₄@GO(a), Fe₃O₄@GO-MIPs(b), Fe₃O₄@GO-NIPs(c)

3.3 Absorption isotherms and Adsorption Kinetics

The L-Trp recognition ability of the Fe₃O₄@GO-MIPs was investigated by the static absorption experiments. The binding isotherms of L-Trp onto Fe₃O₄@GO-MIPs and Fe₃O₄@GO-NIPs are shown in Fig.7. It can be seen that the Fe₃O₄@GO-MIPs superior to those of most imprinted polymers using L-Trp as the template as reported in the literature. This remarkably high binding capacity may lie in a combination of the thin PDA layers on the surface of graphene and the large surface area of graphene. Due to the presence of Graphene, more recognition sites could be created at its surface, whereas the thin PDA layer ensures the complete removal of the template molecules. The adsorption kinetics process of L-Trp was investigated by varying the adsorption time from 1 to 700 min, and the initial concentration of L-Trp was kept at 1.0 mmol/L. As shown in Fig.8 , the Fe₃O₄@GO-MIPs took up 88% of the equilibrium amount of the template molecules from solution within a period of 40 min, and the adsorption equilibrium was reached within 100 min. The results revealed a remarkable rapid adsorption dynamics of L-Trp molecules. This fast dynamics adsorption might originate from the high ratio of surface-imprinted sites, large surface-to-volume ratio, and complete removal of the L-Trp templates.

Figure 7. Absorption isotherms of L-Trp on Fe₃O₄@GO-MIPs and Fe₃O₄@GO-NIPs

Figure 8. Adsorption kinetics of L-Trp on Fe₃O₄@GO-MIPs

3.4 Selectivity of Fe₃O₄@GO-MIP

The experiments were carried out using D-tryptophan(D-Trp), L-phenylalanine(L-Phe), D-phenylalanine(D-Phe), L-tyrosine(L-Tyr), and D-tyrosine(D-Tyr) as control molecules. Fig.9. shows the rebinding capacities of the Fe₃O₄@GO@MIPs and Fe₃O₄@GO-NIPs for these amino acid with a feed concentration of 0.4 mg/mL of. It is obvious that Fe₃O₄@GO@MIPs exhibits a much higher adsorption amount of L-Trp than other analogues suggesting a better adsorption and binding capacity toward the template molecules. In addition, the imprinting factor α , taken as the binding ratio of Fe₃O₄@GO@MIPs/ Fe₃O₄@GO@NIPs, was determined as 2.3 for binding the template L-Trp, whereas the values of α for binding D-Trp, L-Phe, D-Phe and L-Tyr, D-Tyr were 1.2, 1.1, 1.0, and 1.0, respectively, showing that the adsorbed amounts of the non-template amino acid did not have great differences between the imprinted Fe₃O₄@GO-MIPs and Fe₃O₄@GO-NIPs.

Figure 9. Binding amount of different amino acid on Fe₃O₄@GO-MIPs and Fe₃O₄@GO-NIPs

4 Conclusion

In summary, a novel strategy of synthesizing Fe₃O₄@GO-MIPs for selective recognition of L- tryptophan has been described. The prepared Fe₃O₄@GO-MIPs exhibited high adsorption capacity and good selectivity that showed the formation of the specific binding sites. The above-mentioned synthetic method of Fe₃O₄@GO-MIPs was rather simple and environment friendly, because of the little solvent consumption and the nontoxic stabilizers. Thus it may have a prosperous application because of its simpleness and low cost. We also visualize that the as-produced strategy could be applied in various fields such as bioseparation and food safety.

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References

1. R.J. Ansell, O. Ramström, K. Mosbach. *Towards artificial antibodies prepared by molecular imprinting*. Clin. Chem. 42(9): 1506-1512 (1996)
2. Behbahani, M., Bagheri, S., Amini, M. M., Abandansari, H. S., Moazami, H. R., Bagheri, A. Application of molecular imprinted polymer nanoparticles as a selective solid phase extraction for preconcentration and trace determination of 2,4-dichlorophenoxyacetic acid in the human urine and different water samples. Journal of Environmental Health Science and Engineering. 37: 1610-1612 (2014)
3. O. Baltrons, M. Lopez-Mesas, C. Palet, F. Le Derf, F. Portet-Kotalo. Molecularly imprinted polymer-liquid chromatography/fluorescence for the selective clean-up of hydroxylated polycyclic aromatic hydrocarbons in soils. Anal. Method, 5(22): 6297-6305 (2013)
4. A. Aghaei, M.R.M. Hosseini, M. Najafi. A novel capacitive biosensor for cholesterol assay that uses an electro polymerized molecularly imprinted polymer. Electro him. Acta, 55: 1503-1508 (2010)
5. W.J. Cheong, F. Ali, J.H. Choi, J.O. Lee, K.Y. Sung. Recent applications of molecular imprinted polymers for enantio-selective recognition. Talanta, 106: 45-59 (2013)
6. Wulff, G. Enzyme-like catalysis by molecularly imprinted polymers. Chem. Rev, 102(1):1-28 (2002)
7. P. Bures, Y. Huang, E. Oral, N.A. Peppas. Surface modifications and molecular imprinting of polymers in medical and pharmaceutical applications. J. Control. Release, 72:25-33 (2001)
8. Stoller, M. D, Park, S. J, Zhu, Y. W, An, J. H, Ruoff, R. S. Graphene-Based Ultracapacitors. Nano Lett. 8:3498–3502 (2008)
9. T. W. Stone. Endogenous neurotoxins from tryptophan. Toxicon, 39: 61-73 (2001)
10. T. W. Stone, G.M. Mackay, C.M. Forrest, J. Clark, L.G. Darlington. Tryptophan metabolites and brain disorders, Clin. Chem. Lab Med, 41: 852-859 (2003)
11. T. W. Stone, C.M. Forrest, G.M. Mackay, N. Stoy, L.G. Darlington. Tryptophan, adenosine, neurodegeneration and neuroprotection. Metab. Brain Dis, 22: 337-352 (2007)
12. C. J. McDougle, S.T. Naylor, D.J. Cohen, G.K. Aghajanian, G.R. Heninger, L.H. Price. Effects of trptophan depletion in drug-free adults with autistic disorder. Archives of General Psychiatry, 53: 993–1000 (1996)
13. N Hrboticky , LA Leiter & GH Anderson. Effects of L-tryptophan on short-term food-intake in lean men. Nutrition Research, 5: 595–607 (1985)
14. M. Garrido, A.B. Rodríguez, M.P. Terrón , Chapter 13 – tryptophan and melatonin-enriched foodstuffs to improve antioxidant status in aging. V.R. Preedy (Ed.), Aging, Academic Press, San Diego 129–136 (2014)
15. W. Kochen , H. Steinhart. L-tryptophan—Current Prospects in Medicine and Drug Safety. Walter de Gruyter, Berlin (1994)
16. H. Bagheri, M. Ahmadi, T. Madrakian, A. Afkhami. Preconcentration and spectrofluorometric determination of L-tryptophan in the presence of d-tryptophan using a chiral magnetic nanoselector. Sensors Actuators B Chem, 221: 681–687 (2015)
17. Marcano, D. C , Kosynkin, D. V , Berlin, J. M , Sinitskii, A , Sun, Z , Slesarev, A , Alemany, L. B , Lu, W , Tour, J. M , *Improved synthesis of graphene oxide*. ACS Nano 4(8): 4806– 4814 (2010)