

The Synthesis of L-Phenylalanine-Imprinted Polymers and Studies on Its Performance

1st Bo Gao
School of Sciences
Xijing University
Xi'an, China
gaoboo_1989@126.com

2nd Yuzhen Zhao
School of Sciences
Xijing University
Xi'an, China
zhaoyuzhen@xijing.edu.cn

3rd Jiao Liu
School of Sciences
Xijing University
Xi'an, China
jingjingliuajiao@163.com

Abstract—L-Phenylalanine (L-Phe) imprinted polymers (L-MIPs) were prepared via precipitation polymerization using a novel biocompatible and polymerizable ionic liquid (3-aminopropyl-1-vinylimidazole chloride, [AVIM]Cl) as functional monomer, which could interact with L-Phe through hydrogen-bonding, π - π and electrostatic interactions. The [AVIM]Cl designed had an outstanding contribution to polymerize of the L-MIPs. The L-MIPs prepared under optimized conditions were subsequently used in the adsorption isotherm, adsorption dynamics and the reusable test. The adsorption isotherm revealed that the L-MIPs showed the best imprinted effect at L-Phe concentration of 0.1 mg·mL⁻¹ and their imprinting factor at 3.03. The adsorption dynamic studies revealed that the adsorptive rate of the L-MIPs was much faster than the L-NIPs, and both of them could be equilibrated in 8h. The cyclic regeneration capability of L-MIPs was relatively slightly, after five cycles and the adsorption rate lost only 22%.

Keywords—L-Phenylalanine, Molecularly imprinting, Selectivity, Ionic liquids

I. INTRODUCTION

Molecularly imprinted polymer (MIPs) are biomimetic functional polymers possessing specific cavities and binding sites complement with the template molecule, that capable to optionally recognize and bind the target molecular with affinities comparable to those of natural receptors[1-3]. Synthesized by copolymerizing functional monomers and crosslinkers in the presence of a template molecule, then the template was removed by efficient eluent, there are specific recognition elements leaved on the MIPs complementary binding the target molecule. These tailor-made synthetic receptors have a promising development prospect for their considerable advantages, such as their greater chemical and physical stability, ease for mass preparation, excellent heat resistance and organic solvent resistance.

L-Phenylalanine (L-Phe) is a kind of essential amino acids since the body cannot synthesize itself. It is often applied in the fields of food additives and pharmaceuticals as nutritional supplement or precursor. For instance, L-Phe is an important precursor to synthesize aspartame (L-aspartyl-L-Phenylalanine methyl ester). At present the methods to obtain L-Phe are mainly chemical synthesis and biosynthesis. Although biosynthesis could obtain pure L-Phe by using recombinant microorganisms, the yield is low and the period is long. Compared with biosynthesis, chemical synthesis of L-Phe usually employed organic raw material, there will be harmful residues in the process of preparation and the products were mixtures of D-Phe and L-Phe isomers mostly, which make the purification processes complex and the cost was very high.

Ionic liquids (ILs) are also known as room temperature molten salts, which consist of nitrogen-containing organic cations and inorganic anions. ILs have many favorable properties including negligible vapor pressure, good thermal stability, a wide liquid range, low flammability, powerful dissolution ability, high ionic conductivity and design ability[4-6]. Ionic liquids are considered to be one kind of new environment-friendly solvent which can substitute the traditional organic solvent[7], now have become a research hotspot in catalyst, reactive monomer, adsorbing material, fluorescence modified material, polymerization and biomaterial separation. In addition, ionic liquids have also been used as solvent or porogen to prepare MIPs. Furthermore, it should be noted that more researchers synthesize new kinds of imidazole ILs, which could be used as the functional monomer for preparation of MIPs in aqueous media through hydrogen-bonding, π - π and electrostatic interactions with template [8-9]. Thus, this paper explores the advantages of ionic liquids as functional monomers.

II. EXPERIMENTAL

A. Experimental Material

L-Phenylalanine (L-Phe, $\geq 99.5\%$) was bought from Xian Wolsen Biological Co., Ltd. 3-aminopropyl-1-vinylimidazole chloride, ([AVIM]Cl) was synthesized according our group work. Ethylene glycol dimethacrylate (EGDMA) was bought from Shanghai Aladdin Reagent Company. Azobisisobutyronitrile (AIBN, 98%) used for polymer synthesis was purchased from Merck (Darmstadt, Germany). AIBN was recrystallized from methanol before used. All the other chemicals were used as received unless otherwise stated. Other Chemical reagents, including ethyl acetate, ethanol and sodium chloride were analytical grade and were purchased from Tianjin Tianli Chemical Reagent Co., Ltd.

B. Experimental Facilities

The concentration of L-Phe was determined by Ultraviolet spectrophotometer (Shimadzu UV-2550, Japan), SHA-CA Cyclotron Oscillator (Jintan Fuhua Instrument Co., Ltd, China) was applied for the adsorption of prepared polymers.

C. Molecular Synthesis

The solution containing L-Phe (66 mg) and [AVIM]Cl (300 mg) which was dissolved in mixed solvent (60 mL) of water-ethanol (1:3, V/V) was displaced in a 100 mL three-necked flask equipped with a reflux condenser and a nitrogen inlet. After pre-polymerization at the temperature of 4°C for 12h, initiator (AIBN, 45.0 mg) and crosslinker (EGDMA, 1.2 mL) were added to the above mixture. After deoxygenated with nitrogen for 30 min at 25°C, the three-necked flask was sealed and put in a thermostat-controlled water bath with magnetic stirring (350 r·min⁻¹) at 70°C for 24h until the polymerization was completed. The obtained L-Phenylalanine imprinted polymer particles were washed by 0.6 mol·L⁻¹ sodium chloride until no L-Phe could be detected by UV-visible spectrophotometer. Subsequently, the products were washed by 300 mL water three times and then were dried in a vacuum at 50°C for 24h.

The corresponding non-imprinted polymer (L-NIPs) was generated in the same way, but in the absence of L-Phe.

III. ADSORPTION EXPERIMENTS

The binding experiments involve the adsorption isotherm and adsorption dynamic. 0.1g L-MIPs and L-NIPs were dispersed in 10 mL L-Phe solution within a series of different concentrations from 0.1 g·L⁻¹ to 1.0 g·L⁻¹. After the incubation, the supernatant solutions were collected by centrifugation, determined using a UV spectrophotometer. The amount of the L-Phe adsorbed on L-MIPs and L-NIPs was calculated based on the following Eqn[10] (1).

$$Q = \frac{(C_0 - C_e)V}{m} \quad (1)$$

Where Q (mg·g⁻¹) is the amount of the L-Phe adsorbed, C₀ (mg·mL⁻¹) and C_e (mg·mL⁻¹) are the initial and final L-Phe concentrations respectively, V (mL) is volume of L-Phe solution, and m (g) is the weight of the polymeric microspheres.

To estimate the specific recognition property of L- MIPs, imprinting factor (IF) was evaluated according to the Eqn [11] (2):

$$IF = \frac{Q_{MIPs}}{Q_{NIPs}} \quad (2)$$

A. Adsorption kinetics of MIPs and NIPs

The adsorption dynamic curves of L--MIPs and L-NIPs were investigated. The profile of adsorption dynamics was shown in Figure 1. As Figure 1 illustrates, the equilibrium adsorption of L-Phe on L-MIPs was more than that of L-NIPs, which was about three times as much as that of L-NIPs. It could be seen that an initially increase of adsorption capacity occurred in the first 6 h, and the adsorption capacity slowly increased as time went on. And adsorption process reached equilibrium at 8 h, since L-MIPs possessed a large amount of specific recognize binding sites and matching cavity. In the adsorption process, this rapid adsorption behavior could be explained by multiple interactions (hydrogen bonds interactions, π - π interactions and electrostatic interactions) between [AVIM]Cl and L-Phe, because multiple interactions were more efficient. For the absence of template L-Phe, there is no imprinting cavity on the L-NIPs that the imidazolium and amino groups were embedded in the polymer, even if the cavities were created, the functional groups would distribute randomly. This result leads to the much higher adsorption capacity of L-MIPs than that of L-NIPs.

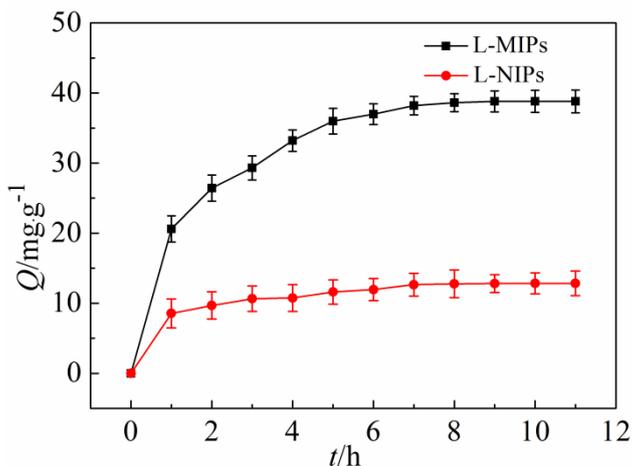


Figure 1. Adsorption dynamic curves of L-MIPs for L-Phe

B. Adsorption Isotherm of the L-MIPs and L-NIPs

In order to characterize the adsorption behaviors of L-MIPs, adsorption isotherm experiments were performed at a series of L-Phe solutions with different initial concentrations from 0.1 to 1.2 mg·mL⁻¹, the result was showed in figure 2. The molecularly imprinted polymers were first immersed and shaken gently in L-Phe solutions for 8h to reach the adsorption equilibrium. The equilibrium concentration of L-Phe in solution was measured by UV-vis spectrophotometer at 254 nm. The adsorption amount was calculated in accordance with Eqn (1).

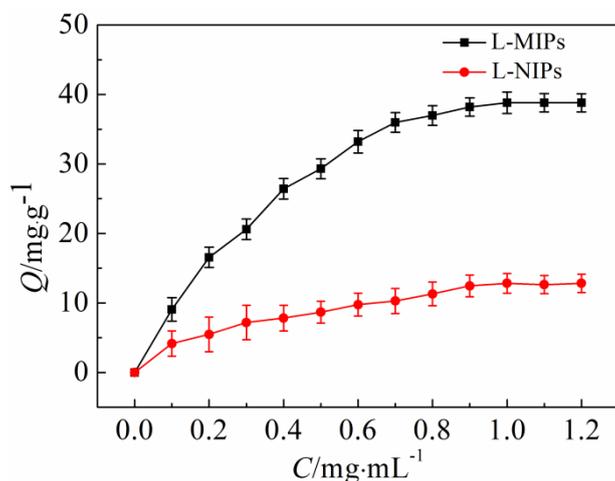


Figure 2. Adsorption isotherms of L-MIPs for L-Phe

From Figure 2, we could see the adsorption isotherms of L-MIPs and L-NIPs in different L-Phe solutions. The adsorption capacity of L-MIPs exhibited higher than that of L-NIPs. As it demonstrated, the amount of L-Phe bound to L-MIPs and L-NIPs increased with the initial concentrations of L-Phe at low concentrations, mean that the amount was not enough to bind the specific sites and occupied the cavities. And finally the saturation value was achieved at 1.0 mg·mL⁻¹. The maximum adsorption capacities of L-MIPs and L-NIPs were 38.8mg·g⁻¹ and 12.8 mg·g⁻¹ respectively, and the imprinting factor was calculated to be 3.03, which implied that the binding capacity of L-MIPs was much higher than that of L-NIPs. The above indicated L-Phe was more accessible to the surface of polymers using [AVIM]Cl as functional monomer through multiple interactions.

C. The Reusability of L- MIPs and L- NIPs

With the increase of times of desorption, the change of the adsorption rate and desorption rate of L-Phe on the L-MIPs were shown in Figure 3. It exhibited that the adsorption capacity of L-MIPs have a slight decrease from 38.8 mg·g⁻¹ to 30 mg·g⁻¹ after five cycles and the adsorption rate lost only 22%, which indicated that the adsorption capacity of L-MIPs was at an almost constant value. This reusability study certified that the L-MIPs have a good performance in the regeneration study, which revealed that the L-MIPs prepared by this way have a potential application prospect in practice.

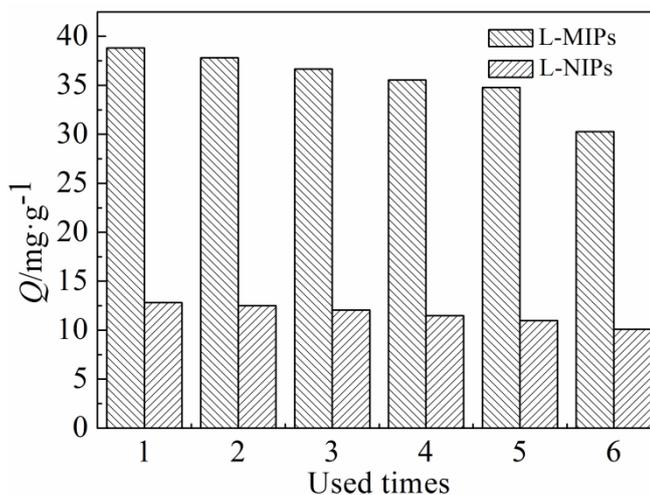


Figure 3. Curve of used times and adsorption amount of L-MIPs

IV. CONCLUSIONS

Ionic liquid has great potential for practical application due to its excellent physical and chemical properties, and alterability of the structure of cation and anion. In this study, we have successfully synthesized a novel L-MIPs using ionic liquid (3-aminopropyl-1-vinylimidazole chloride) as water-soluble functional monomer. The L-MIPs presented quite a good recognition performance to L-Phe, the experimental results of adsorption isotherms and dynamic showed that L-MIPs exhibited improvement in adsorption capacity and efficiency for L-Phe compared to that of on L-NIPs. Respectively, the adsorption capacity and imprinting factor could reach 33mg·g⁻¹ and 3.03. Besides, objective to investigate the reusability, the results indicated that L-MIPs/L-NIPs possess excellent stabilities over several adsorptions and desorption cycles and maintained 77% of the initial value. The property exhibited its potential application in L-Phe rapid separation and purification.

ACKNOWLEDGEMENTS

This work was supported by Scientific Research Program Funded by Shaanxi Provincial Education Department (18Jk1205), special fund for high-level talents of Xijing University (Grant No. XJ180101), the National Natural Science Foundation of Shaanxi (Grant No. 2017JQ2037), Scientific Research Program Funded by Shaanxi Provincial Education Department (Program No. 17JK1160).

REFERENCES

- [1] Abbate V, Bassindale AR, Brandstadt KF, Taylor PG. Biomimetic catalysis at silicon centre using molecularly imprinted polymers. *J.Catal.* 2011; 284: 68-76.
- [2] Hoshino Y, Koide H, Urakami T, Kanazawa H, Kodama T, Oku N, Shea KJ. Recognition, neutralization, and clearance of target peptides in the bloodstream of living mice by molecularly imprinted polymer nanoparticles: a plastic antibody. *J. Am. Chem. Soc.* 2010; 132: 6644-6645.
- [3] Huang B Y, Chen Y C, Wang G R, et al. Preparation and evaluation of a monolithic molecularly imprinted polymer for the chiral separation of neurotransmitters and their analogues by capillary electro-chromatography[J]. *Journal of Chromatography A*, 2011, 1218(6): 849-855.
- [4] Seddon K R. Ionic liquids for clean technology [J]. *Journal of Chemical Technology and Biotechnology*, 1997, 68(4): 351-356.
- [5] Yan H, Liu S, Gao M, et al. Ionic liquids modified dummy molecularly imprinted microspheres as solid phase extraction materials for the determination of clenbuterol and clorprenaline in urine[J]. *Journal of Chromatography A*, 2013, 1294: 10-16.
- [6] Cole AC, Jensen JL, Ntai I, Tran KLT, Weaver KJ, Forbes DC, Davis JH. Novel Brønsted acidic ionic liquids and their use as dual solvent-catalysts. *J. Am. Chem. Soc.* 2002; 124: 5962-5963.
- [7] Wang H F, Zhu Y Z, Yan X P, et al. A Room Temperature Ionic Liquid (RTIL)-Mediated, Non-Hydrolytic Sol-Gel Methodology to Prepare Molecularly Imprinted, Silica-Based Hybrid Monoliths for Chiral Separation [J]. *Advanced Materials*, 2006, 18(24): 3266-3270.
- [8] Fan J P, Tian Z Y, Tong S, et al. A novel molecularly imprinted polymer of the specific ionic liquid monomer for selective separation of synephrine from methanol-water media [J]. *Food chemistry*, 2013, 141(4): 3578-3585.
- [9] Guo L, Deng Q, Fang G, et al. Preparation and evaluation of molecularly imprinted ionic liquids polymer as sorbent for on-line solid-phase extraction of chlorsulfuron in environmental water samples [J]. *Journal of Chromatography A*, 2011, 1218(37): 6271-6277.
- [10] Wang H, Li W, He X, et al. m-Aminophenylboronic Acid as a Functional Monomer for Fabricating Molecularly Imprinted Polymer for the Recognition of Bovine Serum Albumin.[J]. *Reactive & Functional Polymers*, 2008, 68(9):1291-1296.
- [11] Qian L, Hu X, Guan P, et al. Preparation of bovine serum albumin imprinting sensitive hydrogels using ionic liquid as co-monomer and stabilizer[J]. *Talanta*, 2014, 121:56-64.