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ORIGINAL PAPER

Characterization of kinetic, thermodynamic, and binding properties of L-phenylalanine molecularly imprinted polymer

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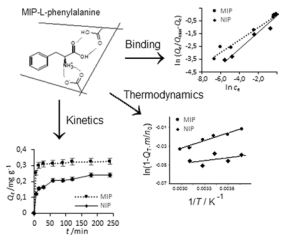
Abstract

L-Phenylalanine imprinted polymeric adsorbent was fabricated by a non-covalent approach utilizing methacrylic acid as functional monomer, ethylene glycol dimethacrylate as cross-linker, acetonitrile as porogen, and acetic acid and trifluoroacetic acid as additives (template:monomer:cross-linker ratio was 1:5:24). Adsorption equilibrium, kinetic, thermodynamic, and enantioselective studies were carried out characterization of adsorbent. Parallel studies were performed for the binding of enantiomers on non-imprinted polymer. The experimental L- and D-phenylalanine adsorption-binding isotherms were fitted with hybrid Langmuir–Freundlich model. Studies have demonstrated a heterogeneous distribution of binding sites, site with high and low binding affinity, in polymer. The thermodynamic test revealed the entropy controlled adsorption process on L-phenylalanine imprinted adsorbent. Kinetic studies indicated second-order rate adsorption process. The adsorbent is characterized by enantioselectivity for recognition of phenylalanine enantiomers.

Graphical abstract



Keywords Molecularly imprinted polymer · Adsorption isotherms · Kinetics · Thermodynamics · Phenylalanine enantiomers

Introduction

There is a growing interest in developing high selective adsorbents because of their wide applications in analytical methods, e.g., adsorbents for extraction techniques (e.g., SPE, SPME), HPLC chiral stationary phases, and sensors. Molecular imprinting is a technique for preparing synthetic polymeric materials, molecularly imprinted polymers (MIPs). Polymerization mixture consists of functional monomer, cross-linker, initiator, all dissolved in a suitable porogen solvent, and template that will be "imprinted". The prepolymerization complex of template and functional monomer is formed with the aid of covalent bonds in covalent fabrication approach. Non-covalent weak interactions, such as hydrogen bonds, van der Waals forces, π–π interactions, and electrostatic interactions, are the most widely used interactions for manufacturing MIP by non-covalent fabrication approach. The many results confirm the applicability of MIPs as selective or enantioselective valuable recognition element for separation target analyte (template) or structurally related compounds in complex mixtures. MIPs are also

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characterized high stability (chemical, thermal) and the ease of fabrication [1, 2].

The properties and diverse functions of amino acids enantiomers require development of analytical methods for their separation and determination applicable in many fields of science and industry. HPLC separations of amino acid enantiomers include indirect approach based on separation diastereoisomers of amino acid formed by derivatization with chiral reagent, and direct approach utilizing chiral stationary phases with different functionalities [3, 4] including MIP-based chiral stationary phases [5, 6]. In this study, the imprinted polymeric adsorbent, applicable in recognition of phenylalanine enantiomers, was prepared by non-covalent approach [7, 8], while L-phenylalanine was used as the template (Fig. 1). The sorption and recognition properties, and stability of MIP are affected by polymerization conditions, composition of polymerization mixture, morphology, and whole design of fabricated MIPs. The newly developed MIP are mostly characterized from the aspect their selectivity, chemical, and morphological properties. Many evaluation approaches are used for MIP characterization, e.g., expression of adsorption isotherms parameters for MIP adsorption equilibrium, description of rate of adsorbate uptake on MIP by kinetic models, and determination of thermodynamic parameters for the adsorption process [9, 10].

In the present study, we prepared and characterized MIP-L-phenylalanine, as potential enantioselective adsorbent based on its binding, kinetic, and thermodynamic properties. To better understand the mechanism of the phenylalanine imprinting process, the adsorption equilibrium data were fitted to several theoretical, homogenous (Langmuir) and heterogeneous (bi-Langmuir, Freundlich, and Langmuir–Freundlich) models. These mathematical models are based on assumption of different types of binding affinities, binding sites, and their relative populations [11]. Characterization of the binding properties of MIP, their dependence on rebinding solvent type and temperature were also documented as useful approach. The thermodynamic indicators (enthalpy and entropy parameters) associated with specific adsorption processes were evaluated. Adsorption kinetic describes the rate of phenylalanine adsorption by MIP that controls the equilibrium time and allows investigate the dynamics of adsorption. In this study, the pseudo-first-order and the pseudo-second-order kinetic models were applied to analyze the equilibrium data.

Results and discussion

MIP was fabricated based on non-covalent bulk polymerization by imprinting of template, L-phenylalanine. Based on previous study [7], methacrylic acid (MAA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as cross-linker, 2,2´-azobisisobutyronitrile (AIBN as initiator, acetonitrile as porogen, and acetic acid and trifluoroacetic acid as additives were selected as suitable constituents of polymerization mixture. Non-imprinted polymer (NIP), blank adsorbent, was synthesized to evaluate the non-specific sorption processes. FT-IR spectra, fluorescence spectra, and the swelling investigations of the MIPs and NIPs were done for characterization of adsorbents. In addition, adsorption equilibrium, and kinetic and thermodynamic studies were carried out.

Figure S1a (Supplemental material) presents the infrared spectra of the MIP, NIP, and L-phenylalanine adsorbed onto MIP. As can be seen, the absence of characteristic L-phenylalanine bands in MIP spectra suggests the successful removal of template by Soxhlet extraction. The spectra of

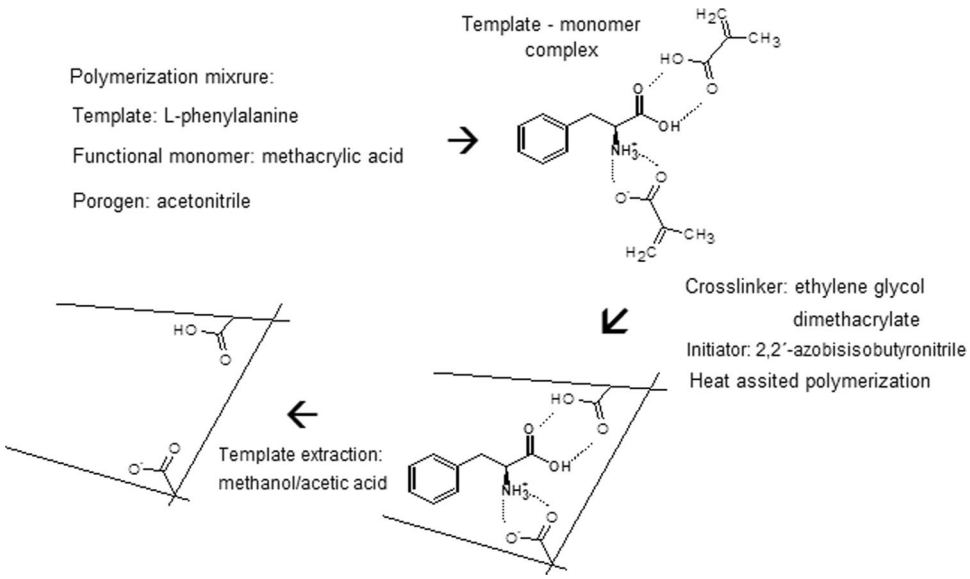

Fig. 1 The scheme of MIP-L-phenylalanine synthesis and analyte rebinding

MIP (after template removal) and NIP are similar. The bands at around 3555, 2956, 1723, 1249, and 1146 cm^{-1} represent the functional groups of –OH, –CH–, –CO–, and –COC–, respectively, attributed to EDGMA and MAA.

In addition, the fluorescence spectrometry was used for evaluation of template removal. Figure S1b (in Supplemental material) shows fluorescence spectra of MIP, NIP, and template. No band in the spectra of MIP at emission wavelength 300 nm corresponding to L-phenylalanine indicates the absence of template. The advantage of this method is higher sensitivity.

Contact of the MIP with solvent leads to the changes in sizes and shapes of the imprinted three-dimensional cavities, and therefore affects the recognition ability and selectivity of the polymer [12]. The swelling behavior of MIP was tested in two solvents, water, and methanol/water (3/7, v/v) (Table S1, Supplemental material). Both imprinted and blank polymers exhibit a degree of swelling that is in the same order of magnitude, although NIP presents a slightly higher values of swelling ratio (SR). In water, the values of SR are higher, because the ionization of the carboxylic group of MAA in the polymer occurred which led to ionic repulsion. This resulted in a more hydrophilic polymer network and contributed to higher water absorption [13].

Chiral separation of DL-phenylalanine

The separation of enantiomers by batch experiments was investigated for DL-phenylalanine solutions in water or in methanol/water (3/7, v/v). The extent of the chiral resolution was quantified based on separation and determination of L- and D-phenylalanine by chiral HPLC with spectrophotometric detection. It is evident from the collected data in Table 1 that efficiency factor (EF) values of enantiomers obtained for NIP were similar (statistically not significant differences, *t* test, 95%). Thus, NIP exhibited no enantiorecognition properties because of the absence of enantiospecific cavities. On the other hand, higher EF values were observed for MIP-L-phenylalanine batches experiments, preferring the L-enantiomer. The highest difference EF values of L- and D-form were observed for methanol/water as rebinding solvent, indicating that performed L-phenylalanine imprinting was effective in creating enantioselective cavities towards the targeted L-enantiomer.

Table 1 Efficiency of enantiomeric resolution of DL-phenylalanine using MIP and NIP

Solvent	Enantiomeric form	EF	
		MIP	NIP
Water	L	1.11	1.03
	D	1.08	1.02
Methanol/water (3/7, v/v)	L	1.36	0.97
	D	1.10	1.01

EF efficiency factor

Adsorption isotherms

Adsorption isotherm [dependence of the amount of adsorbed analyte (Q_e) vs. concentration of free analyte in solution (c_e)] is a useful tool for predicting adsorption on MIP and NIP. Generally, the MIP isotherm plot is non-linear with plateau at higher concentrations of analyte indicating saturation of active sites in the adsorbent. In the NIP the active sites interacting with analyte are randomly arranged in adsorbent that cause to some extend bindings which are non-specific and weaker than those of the MIP. On the surface of NIP, monomer self-association can occur and reduces the number of free functional groups where the analyte can be bound. Therefore, NIP is typically characterized by lower adsorption capacity and the adsorption isotherm should be near linear [11, 14, 15]. Experimental studies of L- and D-phenylalanine adsorption on MIP and NIP were obtained for two rebinding solvents, water and mixture methanol/water (3/7, v/v) [6], by batch experiments. Figure 2 shows that adsorbed amount of L- and D-phenylalanine on MIP and NIP was similar for lower concentrations of enantiomers (results for water as rebinding solvent). However, when concentration of enantiomer increased over 0.1 mg cm^{-3}, the non-specific interaction between NIP and enantiomer has greater impact on adsorption process.

Mathematical models of adsorption isotherms, including Langmuir, bi-Langmuir, Freundlich, and Langmuir–Freundlich isotherm models, were used to fit the equilibrium adsorption data for MIP and NIP.

Langmuir isotherm

Langmuir, the simplest, adsorption isotherm assumes that adsorbent contains one type of binding sites. The adsorption process is homogeneous with monolayer coverage due to the equal binding affinity of all adsorption sites to adsorbate. This reduces the adsorption processes after saturation of adsorbent surface. For this reason, Langmuir model is more suitable to describe of adsorption on MIP at higher concentration levels, where saturation is reached [16, 17]. Linearized form of isotherm model is expressed by Eq. (1)

$$c_e/Q_e = 1/\left(Q_{max} \times K_L\right) + c_e/\left(Q_{max}\right), \quad (1)$$

where Q_e (mg g^{-1}) is amount of analyte adsorbed on 1 g of MIP/NIP at equilibrium concentration of analyte, c_e (mg cm^{-3}) is equilibrium concentration of free analyte in solution, K_L (cm^3 mg^{-1}) is the Langmuir constant, and Q_{max} is the theoretical maximum adsorption capacity (mg g^{-1}). The

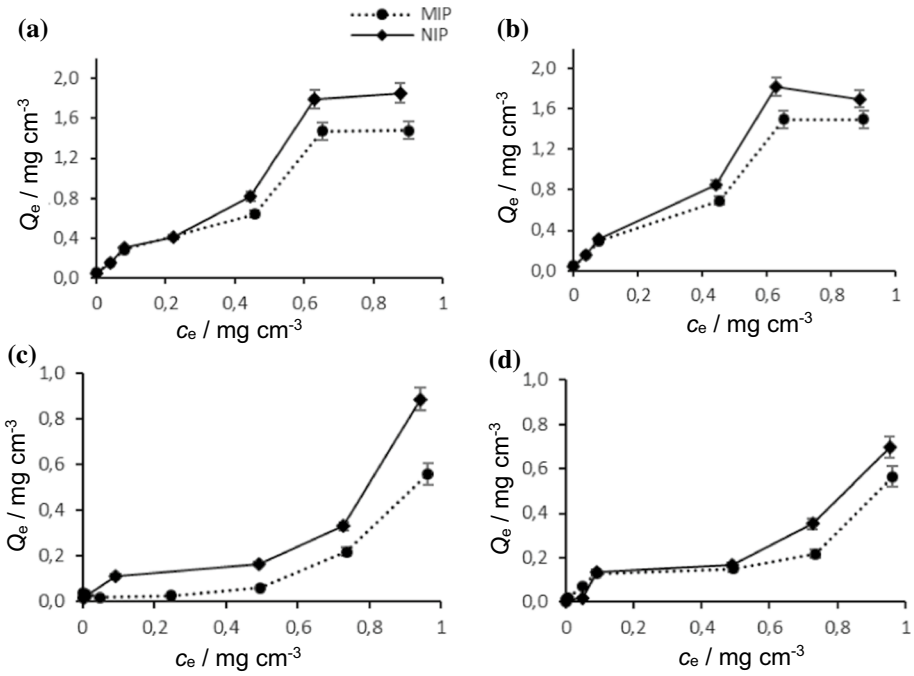

Fig. 2 Experimental adsorption isotherms of MIP and NIP for L-phenylalanine (**a**), D-phenylalanine (**b**), adsorption from water and L-phenylalanine (**c**), and D-phenylalanine (**d**) from methanol/water (3/7, v/v)

dependences of $c_e/Q_e = f(c_e)$ for adsorption of L- and D-phenylalanine and both rebinding solvents are shown in Fig. S2 (Supplemental material). The Langmuir model parameters, binding constant and theoretical maximum adsorption capacity values, for both MIP and NIP adsorbents are tabulated in Table 2. Higher values of binding constant were observed for MIP indicating formation of specific binding sites during imprinting. Moreover, higher values were obtained for imprinting template, L-phenylalanine. For MIP and NIP, the coefficients of determination were lower (0.42–0.85), indicating that the homogenous Langmuir isotherm model was less suitable for characterization of fabricated polymers, because of heterogeneous surface and different types of binding sites occur in polymers synthetized by non-covalent approach. In next step, heterogeneous isotherm models were tested.

Bi-Langmuir isotherm

Bi-Langmuir isotherm model is suitable for characterization of binding sites distribution and their binding affinity. Model assumed two types of binding sites in adsorbent, the high-affinity binding sites with Q_{max1} and K_{L1} and low-affinity binding sites with Q_{max2} and K_{L2}. The isotherm parameters for the adsorption of L- and D-phenylalanine on MIP and NIP were calculated by Eq. (2) [11, 18]

$$c_e/Q_e = \left(1/\left(Q_{max1} \times K_{L1}\right) + c_e/\left(Q_{max2}\right)\right) + \left(1/\left(Q_{max2} \times K_{L2}\right) + c_e/\left(Q_{max2}\right)\right). \quad (2)$$

Figure S3 in Supplemental material shows that the MIP and NIP contain two type of binding sites. The MIP Langmuir constant K_{L1} (Table 2) is higher than thus of NIP obtained for both rebinding solvents, indicating presence of high-affinity specific binding sites on MIP. Type of rebinding solvent affects specific interactions between enantiomers and MIP, which results in different values of affinity constant for both enantiomeric forms. For low-affinity binding sites, the results are not unambiguous, as reflected by the low value of R^2. Although bi-Langmuir isotherm is applicable for interpretation of different types of binding sites in adsorbent, by utilizing separate binding isotherm parameters, it is not always an unambiguous determination which polymer or experimental conditions (e.g., rebinding solvent) are better, because it is unclear which binding parameter is more important, affinity constant or number of binding sites. Another drawback of this model is poor characterization of heterogeneous structure of MIP [19]. Based on lower values of coefficient of determination (0.293–0.566 for high-affinity binding sites, 0.009–0.866 for low-affinity binding sites), it was determined that bi-Langmuir isotherm model was not suitable for evaluation of MIP-L-phenylalanine adsorption properties.

Freundlich isotherm

The Freundlich isotherm describes the adsorption processes that occur on heterogonous polymeric surfaces and is more accurate in the lower concentrations of analyte. In

Table 2 Parameters for L- and D-phenylalanine adsorption on MIP and NIP according to different isotherm models

	MIP				NIP			
	Rebinding solvent							
	Water		Methanol/water[a]		Water		Methanol/water[a]	
	L-Phe	D-Phe	L-Phe	D-Phe	L-Phe	D-Phe	L-Phe	D-Phe
Langmuir isotherm								
K_a/cm^3 mg^{-1}	3.05	2.78	32.46	24.394	2.12	2.42	7.46	2.45
Q_{max}/mg g^{-1}	2.06	2.15	0.05	0.20	2.90	2.63	0.31	0.43
R^2	0.814	0.832	0.767	0.848	0.713	0.785	0.783	0.423
Bi-Langmuir isotherm	High-affinity binding sites							
K_{L1}/cm^3 mg^{-1}	14.04	12.51	11.97	9.28	13.13	12.13	7.66	6.39
Q_{max1}/mg g^{-1}	0.001	0.003	0.008	0.002	0.002	0.004	0.03	0.07
R^2	0.739	0.739	0.585	0.566	0.740	0.739	0.734	0.293
	Low-affinity binding sites							
K_{L2}/cm^3 mg^{-1}	0.26	0.10	5.20	5.73	0.45	3.86	3.57	9.81
Q_{max1}/mg g^{-1}	2.60	5.77	1.51	1.69	1.29	0.44	0.76	1.20
R^2	0.039	0.009	0.858	0.710	0.207	0.416	0.866	0.776
Freundlich isotherm								
K_F/cm^3 mg^{-1}	1.16	1.37	0.37	0.35	1.45	1.70	0.47	0.44
m	0.45	0.57	0.45	0.60	0.56	0.62	0.60	0.71
R^2	0.897	0.958	0.939	0.920	0.918	0.966	0.923	0.894
Langmuir–Freundlich isotherm								
K/cm^3 mg^{-1}	0.98	1.37	0.88	0.84	1.13	1.58	0.76	0.82
m	0.46	0.60	0.49	0.58	0.59	0.63	0.64	0.77
Q_{max}/mg g^{-1}	1.41	1.53	0.76	0.67	1.21	1.35	0.60	0.52
R^2	0.931	0.976	0.973	0.959	0.947	0.968	0.965	0.897

K_L, K_F, K_{LF} Langmuir, Freundlich, and Langmuir–Freundlich constants, m heterogeneity factor, Q_{max} theoretical maximum adsorption capacity

[a]Methanol/water (3/7, v/v)

addition, the advantage in comparison with other binding models results from its unsuitability for binding saturation (i.e., it predicts indefinitely increasing of the amount of analyte bounded to the polymer when analyte concentration increases) [11, 17, 20, 21].

The constant representing adsorption capacity (K_F, Freundlich constant) and heterogeneity factor (m, $m \sim 1$ corresponds to homogeneous sites) for the adsorption on MIP and NIP were calculated from linear equation of Freundlich isotherm model

$$\log Q_e = \log K_F + m \times \log c_e. \quad (3)$$

The plots of log Q_e vs. log c_e are shown in Fig. S4 (Supplemental material) and corresponding isotherm parameters are listed in Table 2. Freundlich constants, referring the intensity of interaction between enantiomer and polymer, were higher for NIP. The NIP possess more homogenous surface (values of $m \sim 0.6$–0.7) than MIP (higher values of $m \sim 0.4$–0.6, indicating more type of binding sites). According to higher values of coefficient of determination ($R^2 > 0.894$), Freundlich model better fits experimental data in comparison with Langmuir and bi-Langmuir models; however, obtained results could be skewed, since this model is more accurate for low concentration range.

Langmuir–Freundlich isotherm

The Langmuir–Freundlich isotherm (Sips isotherm) is hybrid model that combines Langmuir and Freundlich isotherms. Usually, the MIP prepared by non-covalent approach cannot be properly described with Langmuir or Freundlich isotherms, due to the limited concentration ranges, as was mentioned in the previous parts. The Langmuir–Freundlich model describes both the saturation and sub-saturation concentration ranges, and can be applied for characterization of MIP with homogenous or heterogeneous distribution of binding sites. In this work, the linear form of isotherm was used [11, 17]

$$\ln Q_e/\left(Q_{max} - Q_e\right) = \ln K_{LF} + m \times \ln c_e, \quad (4)$$

where m is heterogeneity factor and Q_{max} is maximum analyte amount bounded per 1 g of polymer (theoretical maximum adsorption capacity) at very high concentration of free analyte. The Q_{max} is unknown and needs to be optimized. The optimization was done by plotting the graph ln Q_e/(Q_{max}—Q_e) = f (ln c_e) and fitting the parameter Q_{max} when the plot gives the best linearity (the highest R^2) [22]. We tested Q_{max} interval from 0.2 to 3.0 mg g^{-1}, based on results obtained for Langmuir isotherm model. The isotherm model parameters of MIP and NIP for adsorption of phenylalanine enantiomers are listed in Table 2. Higher theoretical values of Q_{max} observed for water rebinding solvent indicate that water contributes to L- and D-phenylalanine adsorption onto MIP. Both, K_{LF} and m values correspond with results obtained for Freundlich isotherm model. Higher values of m in both rebinding solvents (water ~ 0.6; methanol/water ~ 0.7) observed for NIP indicate more homogenous surface in comparison with MIP (m ~ 0.5) (the MIP structure contains non-specific binding sites and specific imprinting cavities). Based the highest values of coefficient of determination (R^2 0.897–0.976), the Langmuir–Freundlich isotherm was selected as the best model fitting the experimental results of L- and D-phenylalanine adsorption on MIP. The linear Langmuir–Freundlich isotherms are shown in Fig. 3.

Adsorption kinetics of polymers

The adsorption kinetics of MIP and NIP were performed using DL-phenylalanine solution in water or methanol/water (3/7, v/v) at time interval from 5 to 240 min. The result for water as rebinding solvent (Fig. 4a, b) showed a faster adsorption of both enantiomers in 5 min and then reached equilibrium after 30 min. Although fast absorption and high adsorption capacity were obtained for MIP, the non-specific interactions were dominant, related from similar profile of NIP kinetic curve. In the case of methanol/water rebinding solvent (Fig. 4c, d), the equilibrium adsorption capacity increased rapidly in the first 15 min, and reached equilibrium in 60 min. It is evident, that in the early stage, the template molecules were captured in the cavities on the surface of the MIP (MIP possessed a larger amount of empty binding sites, which enabled enantiomers to be easily adsorbed). Over time, the adsorption rate slowed down, because the binding sites on the surface are mostly occupied and the target molecules had to be penetrated into the deeper part of the polymer, which would be more time-consuming. The experimental equilibrium time, adsorption capacities, and imprinting factors (IF) of MIP for tested rebinding solvents are summarized in Table 3. The values of IF for methanol/water (more than 1.5) were higher than for water (IF = 1.0 and 1.06) indicating a better imprinting effect due to the suppression of interactions water-analyte by methanol and thus allows transfer of analyte to the binding sites. Moreover, the

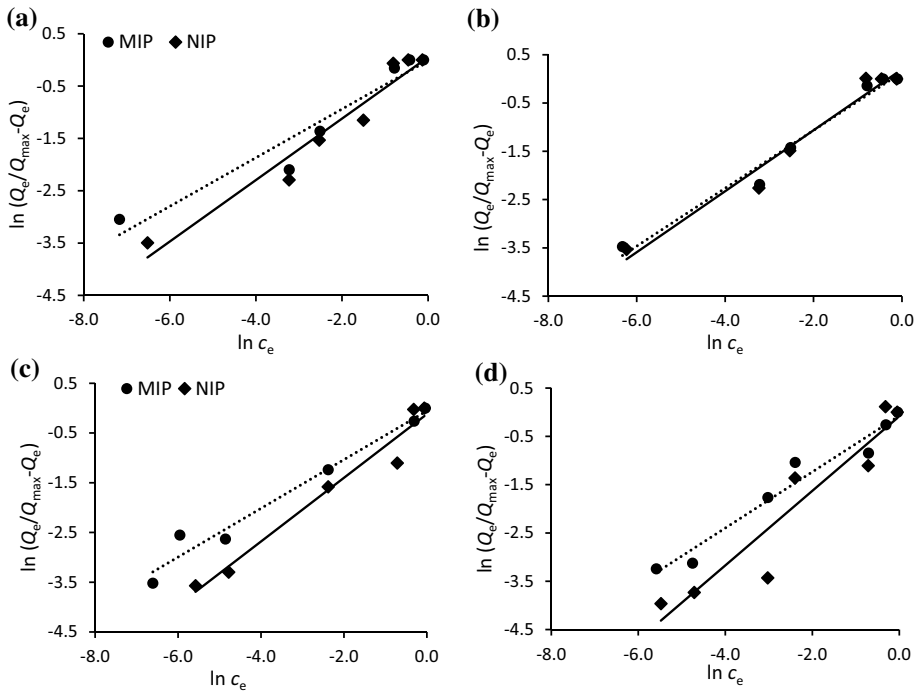

Fig. 3 Langmuir–Freundlich isotherms of MIP and NIP for L-phenylalanine (**a**), D-phenylalanine (**b**), adsorption from water and L-phenylalanine (**c**), and D-phenylalanine (**d**) adsorption from methanol/water (3/7, v/v)

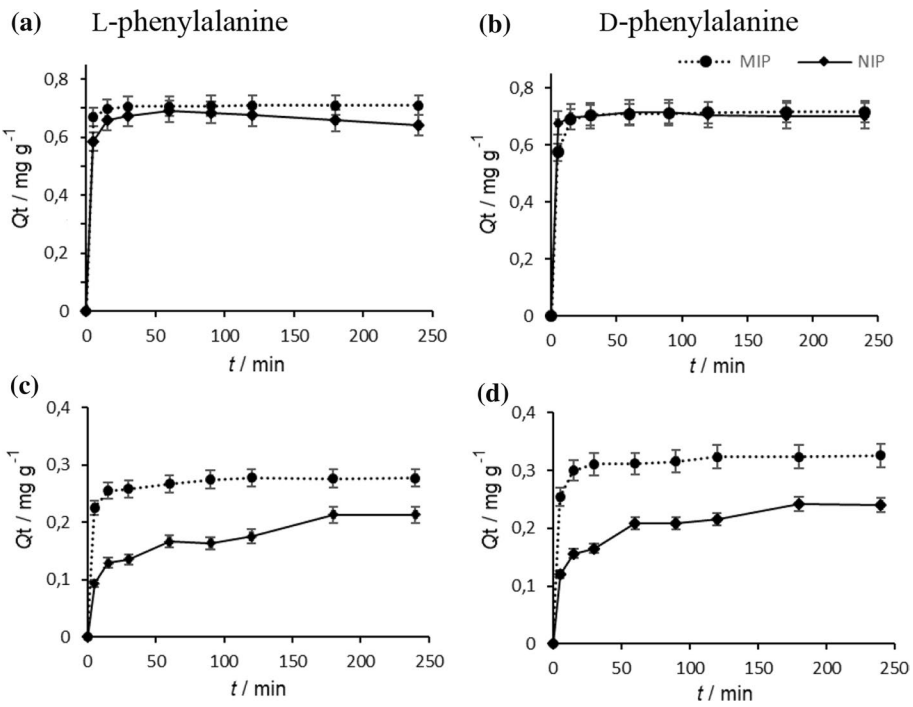

Fig. 4 Adsorption dynamic curves of MIP and NIP in rebinding solvent water (**a**, **b**) and methanol/water (3/7, v/v) (**c**, **d**)

Table 3 The values of experimental adsorption equilibrium times, adsorption capacities, and imprinting factors of MIP and NIP for phenylalanine (Phe) enantiomers and different rebinding solvents

	Rebinding solvent	Phe form	Experimental values		
			t_e/min	Q_e(exp)/mg g^{-1}	IF[a]
MIP	Water	L	30	0.70	1.06
		D	30	0.70	1.00
	Methanol/water (3/7, v/v)	L	60	0.29	1.50
		D	60	0.33	1.61
NIP	Water	L	30	0.66	
		D	30	0.70	
	Methanol/water (3/7, v/v)	L	60	0.18	
		D	60	0.22	

[a]Imprinting factor: IF = Q_e(MIP)/Q_e(NIP)

specific adsorption capacity ($Q_{e(MIP)} - Q_{e(NIP)}$) was found to be higher for methanol/water solutions (0.11 mg g^{-1}).

The adsorption kinetics of D- and L-phenylalanine onto MIP was fitted by two kinetic models [23, 24]. The pseudo-first-order model Eq. (5) assumes the monomolecular adsorption of analyte on adsorption sites, unlike the pseudo-second-order model Eq. (6). The linear forms of equations are as follows:

$$\ln\left(Q_e - Q_t\right) = \ln Q_e - k_1 t \tag{5}$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{1}{Q_e} t, \tag{6}$$

where Q_e and Q_t (mg g^{-1}) are the amounts of phenylalanine enantiomer adsorbed on 1 g of MIP or NIP at equilibrium concentration of analyte and at specific time t (min), k_1 (min^{-1}), and k_2 (g mg^{-1} min^{-1}) are the pseudo-first-order and pseudo-second-order kinetic constants, respectively. Kinetic parameters and coefficients of determination of both kinetic models for L- and D-phenylalanine adsorption on MIP are summarized in Table 4. The pseudo-second-order kinetic model better fits the experimental data (higher values of R^2).

Thermodynamic study of adsorption

The thermodynamic parameters, Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS) are the thermodynamic indicators of adsorption. Since the adsorption process of target analyte on MIP is the result of the induced molecular memory, therefore the change of ΔH is related to the MIP–analyte interaction. Higher values of ΔH exhibit a stronger MIP–analyte interaction (the mechanism of adsorption process is based on physical adsorption if the ΔH value is less than 80 kJ mol^{-1} or is based on chemisorption if the ΔH value ranged from 80 to 400 kJ mol^{-1}) [25]. The ΔS relates with state disorders before and after adsorption and reflects the change of analyte state from solution (free state) to the MIP surface and cavity. The thermodynamic parameters (Table 5) of L- and D-phenylalanine adsorption on MIP were obtained from intercept and slope of the dependence

Table 4 Kinetic parameters of L- and D-phenylalanine adsorption on MIP according to pseudo-first-order and pseudo-second-order kinetic model

Rebinding solvent	Phe form	Pseudo-first-order			Pseudo-second-order		
		k_1/min^{-1}	Q_e/mg g^{-1}	R^2	k_2/g mg^{-1} min^{-1}	Q_e/mg g^{-1}	R^2
Water	L	0.03	0.02	0.941	0.64	0.71	0.997
	D	0.01	0.03	0.847	1.70	0.72	0.999
Methanol/water (3/7, v/v)	L	0.02	0.03	0.873	73.38	0.28	0.998
	D	0.01	0.03	0.985	56.19	0.33	0.999

k_1 and k_2 pseudo-first-order and pseudo-second-order kinetic constants, Q_e amount of phenylalanine enantiomer adsorbed on 1 g of MIP at equilibrium concentration of analyte, *Phe* phenylalanine

Table 5 Thermodynamic parameters for adsorption of phenylalanine enantiomers on MIP

Rebinding solvent	Phe form	R^2	ΔH/J mol^{-1}	ΔS/J mol^{-1} K^{-1}	ΔG/J mol^{-1}
Water	L	0.919	894.86	3.54	− 148.78
	D	0.926	634.17	2.72	− 166.93
Methanol/water (3/7, v/v)	L	0.959	523.91	2.09	− 93.45
	D	0.965	335.88	1.28	− 41.40

ΔH adsorption enthalpy, ΔS adsorption entropy (calculated for $T = 294.65$ K), R^2 correlation coefficient, *Phe* phenylalanine

ln(1-Q_T.m/n_0) versus 1/T (Fig. S5, Supplemental material). The ΔH values indicate that the adsorption process occurs by the physical adsorption for both rebinding solvents. This phenomenon assumes electrostatic and hydrogen interactions for template bonding (Fig. 1). Positive values of ΔH confirm endothermic nature of the process of enantiomers adsorption on MIP. Positive values of ΔS indicate an increasing in the number of degrees of freedom of the system. In this study, the higher values $T \times \Delta S$ were obtained compared to ΔH values, which indicate entropy-driven adsorption on MIP. The values of ΔG suggest a physical adsorption process (values of ΔG ranged from − 20 to 0 kJ mol^{-1}), and also, negative values of ΔG indicate spontaneous adsorption process. For studied systems MIP-rebinding solvent (MIP-water or MIP-methanol/water), it is possible to observe an increase of spontaneity with increasing temperature (results not shown; calculated for temperature interval 10–50 °C).

Comparison with some related imprinted polymers

The main objective of the work was to examine the properties of L-phenylalanine imprinted adsorbent. MIP was prepared by block polymerization method. Although this method has several disadvantages (irregular particles obtained after block crushing, deformation of cavities during crushing), it is a simplest experimental approach useful for adsorbent application in extraction techniques [5]. Polymerization methods, such as suspension [26], emulsion [27], precipitation [28] polymerization, or surface molecularly imprinting [29, 30], were introduced for adsorbents fabrication applicable as HPLC chiral stationary phases to increase efficiency of column and enantioseparation. Imprinting the corresponding template derivative was effective to increase imprinting effect of functionally simple and small templates, e.g., dansyl L-phenylalanine [28], *S*-mandelic acid-*p*-aminophenol amide [31]. The results showed that our MIP-L-phenylalanine exhibits comparable HPLC enatioresolution characteristic (chromatographic resolution of enantiomers: 1.49) [7] to MIP-L-phenylalanine prepared by suspension polymerization (chromatographic resolution of enantiomers: 1.46) [26]. These results are slightly higher in comparison with enantioresolution characteristic obtained for MIP-L-phenylalanine fabricated by block polymerization with toluene porogen (chromatographic resolution of enantiomers: 1.38) [9].

In the experimental synthesis of MIP-L-phenylalanine, methacrylic acid was used as a functional monomer to easily bound the template in prepolymerization complex through hydrogen bond. Similarly, methacrylic acid has been used in other studies [5, 9, 26], but the mixture of monomers (acrylamide and methacrylic acid) was used for MIP synthesis in a study of Qiu et al. [32]. Our previous study show that acrylamide functional monomer did not produce a block of solid polymer [7].

Conclusion

In the present work, the binding, kinetic, and thermodynamic properties of MIP designed for template L-phenylalanine were investigated. From batch rebinding studies and application of different models of adsorption isotherms to experimental data fitting, information about heterogeneity of polymeric surface was obtained. Based on the results,

Langmuir–Freundlich isotherm model well describes the binding behavior of MIP-L-phenylalanine. This can be explained as the fact that the homogenous Langmuir and heterogeneous bi-Langmuir and Freundlich isotherms are two-parameter models which are limited by concentration range, while Langmuir–Freundlich isotherm is three-parameter model and present binding behavior over the entire concentration range. Langmuir–Freundlich model provides information about theoretical maximum adsorption capacity, heterogeneity, and binding affinity. It was found that selective rebinding of enantiomers was observed when methanol/water as rebinding solvent was used. Experimental results demonstrated that MIP-L-phenylalanine exhibits specific binding sites and a heterogeneous-binding sites' distribution. The kinetic study indicated that the pseudo-second-order kinetic model represented adsorption process on MIP. Thermodynamic test showed endothermic adsorption and increased randomness of the molecules of adsorbate on the MIP surfaces than in the solution. In addition, chiral resolution experiments confirming the enantioresolution abilities of fabricated MIP were done. This enantioselective material could by useful for different applications, e.g., as adsorbent in the sample pre-treatment extraction techniques, chiral stationary phase for HPLC separation of phenylalanine enantiomers, removing the target enantiomeric compounds from food and pharmaceutical samples.

Experimental

DL-Phenylalanine (99%) and L-phenylalanine (99%) were purchased from Sigma-Aldrich (Germany). Reagents for polymer synthesis, 2,2´-azobisisobutyronitrile (AIBN), ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA), and trifluoroacetic acid were synthesis grade (all purchased from Merck, Germany), and acetic acid was analytical grade (purchased from CentralChem, Slovak Republic). Organic solvents, acetonitrile and methanol (for HPLC, gradient grade) were purchased from VWR International, Slovak Republic.

Synthesis of molecularly imprinted polymer

Molecularly imprinted polymer was prepared according the work Hroboňová and Lomenova [7]. Functional monomer MAA (0.34 cm^3, 4.54 mmol), 0.16 g template L-phenylalanine (0.97 mmol), and 3 cm^3 porogen acetonitrile were mixed in glass test tube and final mixture was stirred in ultrasonic bath for 10 min. Afterwards, 3.96 cm^3 cross-linker EGDMA (20.98 mmol), 0.19 g initiator AIBN (1.16 mmol), 0.6 cm^3 acetic acid, 0.12 cm^3 trifluoroacetic acid, and 2.0 cm^3 acetonitrile were added and the mixture was homogenized via sonication for 5 min. Polymerization was thermally initiated (60 °C, 24 h) and the resulting solid block of polymer was crushed, sieved through 44 µm sieve and washed with acetone to remove fine particles. The template was removed by Soxhlet extraction with mixture methanol/acetic acid (9/1, v/v) (200 cm^3 of extraction solvent, 24 h). A blank polymer (NIP; non-imprinted polymer) was prepared by the same procedure but without the template presence in polymerization mixture.

Characterization

FT-IR analysis was performed on Shimadzu IRSpirit spectrophotometer using the attenuated total reflectance (ATR) technique. FT-IR absorption spectra were recorded in the region from 4000 to 400 cm^{-1} using an ATR diamond crystal. Before each measurement, the crystal was washed with distilled water and acetone, respectively. Solid samples were analyzed directly by placing the sample onto the ATR crystal to provide an active sampling area by creating a uniform layer. 45 sample interferograms were acquired at a resolution of 16 cm^{-1}.

Fluorescence analysis was performed on a Perkin-Elmer LS 50 Luminescence spectrofluorometer equipped with a xenon lamp. In all cases, a fluorescence holder for solid sample was used. The excitation wavelength was 260 nm and fluorescence spectra were obtained in wavelength range of 270–400 nm. Spectra of L-phenylalanine was recorded with a 3.0 nm slit width and 2% filter. Spectra of MIP and NIP were recorded with a 5.0 nm slit width. The ambient temperature was about 25 °C during the experiment and all samples were analyzed in triplicate. The spectrometer was connected to a computer supplied with FL Data Manager Software (Perkin-Elmer) for spectra acquisition and data processing. The software Origin 8.5 was used for handling spectra.

Swelling test

Swelling studies were conducted in water or mixture methanol/water (3/7, v/v). First, amount of 0.1 g of MIP or NIP were weighed for their dry weight (w_{dry}). These adsorbents were then allowed to swell to equilibrium in 1.5 cm^3 of solvent (2 h, 23 °C, stirring at 300 rpm). The swollen particles of wet sorbent were collected and weighed (w_{wet}). The swelling ratio (SR) was calculated according to Eq. (7)

$$SR = \left[\left(w_{wet} - w_{dry}\right)/w_{dry}\right] \times 100\%. \quad (7)$$

Chiral extraction of DL-phenylalanine

Chiral separation of DL-phenylalanine was carried out utilizing batch adsorption experiment. Amount of 0.1 g of dried polymer (MIP or NIP) was suspended in 5 cm^3 of DL-phenylalanine solution (concentration 0.05 mg cm^{-3}, solvent water or methanol/water 3/7 v/v). The suspension was equilibrated on the shaker for 2 h at 300 rpm and 23 °C, and then, the particles were separated after centrifugation at 3000 rpm. The residual concentrations of D- and L-enantiomer in the loading solution were determined by chiral HPLC method. The enantiorecognition efficiency of MIPs was expressed by efficiency factor (EF). The EF values were calculated according to the Eq. (8)

$$\mathrm{EF} = c_0/c_e, \tag{8}$$

where c_0 is the initial concentration of enantiomer (mg cm^{-3}) and c_e is the residual concentration of enantiomer (mg cm^{-3}).

Adsorption, kinetic and thermodynamic binding experiments

Standard solutions of DL-phenylalanine and L-phenylalanine for adsorption, kinetic and thermodynamic binding tests were prepared in water or mixture methanol/water (3/7, v/v) (concentration 10 mg cm^{-3}). The working solutions were prepared by appropriate dilution of standard solution with water or mixture methanol/water (3/7, v/v). Solutions were found to be stable for 7 days when stored at 4 °C.

The adsorption of phenylalanine enantiomers onto MIP and NIP was evaluated by batch adsorption experiments. All experiments were realized in triplicate. Amount of 0.1 g of MIP or NIP adsorbent was added to 1.5 cm^3 of DL-phenylalanine solution (concentration of DL-phenylalanine 0.01, 0.02, 0.1, 0.2, 0.5, 1.5, and 2.0 mg cm^{-3} which correspond to a range from 0.06 to 12.11 mmol dm^{-3}; water or mixture methanol/water (3/7, v/v) were used as solvents). The mixtures were shaken on laboratory shaker for 2 h (25 °C, 300 rpm). Afterwards, mixture was centrifuged (5 min, 3000 rpm) and the supernatant was analyzed by chiral HPLC to determine D- and L- phenylalanine. The equilibrium adsorption capacity (Q_e, mg g^{-1}) was calculated according to Eq. (9)

$$Q_e = \left(c_0 - c_e\right) \times V/m, \tag{9}$$

where c_0 is the initial concentration of enantiomer (mg cm^{-3}), c_e is the residual concentration of enantiomer (mg cm^{-3}), V is the volume of DL-phenylalanine solution (cm^3), and m is the weight of adsorbent (g).

The kinetic adsorption experiments were carried out with 0.1 g of MIP or NIP. Adsorbent was mixed with 1.5 cm^3 of DL-phenylalanine solution in water or mixture methanol/water (3/7, v/v) at concentration 0.5 mg cm^{-3}. The solutions were stirred for different time intervals ranging from 5 to 240 min (25 °C, 300 rpm). Afterwards, mixture was centrifuged (5 min, 3000 rpm) and the concentrations of enantiomers in supernatant were determined by chiral HPLC. The adsorption capacity at time (Q_t, mg g^{-1}) was calculated according to Eq. (10)

$$Q_t = \left(c_0 - c_t\right) \times V/m, \tag{10}$$

where c_0 is the initial concentration of enantiomer (mg cm^{-3}), c_t is the concentration of enantiomer at given time (mg cm^{-3}), V is the volume of DL-phenylalanine solution (cm^3), and m is the weight of adsorbent (g).

For thermodynamic study, 0.1 g of MIP or NIP was placed in 1.5 cm^3 of DL-phenylalanine solution in water or mixture methanol/water (3/7, v/v) at concentration level of 0.5 mg cm^{-3} and maintained in water bath at different temperatures (10, 20, 30, 40, 50, and 60 °C). The mixture was shaken for 2 h. Afterwards, mixture was centrifuged (5 min, 3000 rpm) and concentrations of individual enantiomers in supernatant were determined by chiral HPLC. The equilibrium adsorption capacity at given temperature ($Q_{e(T)}$, mg g^{-1}) was calculated according to Eq. (9). The dependence $Q_{e(T)}$ versus thermodynamic parameters expresses Eq. (11) [33]

$$\ln\left(1 - \frac{Q_{e(T)} \times m}{n_0}\right) = \frac{\Delta H}{RT} - \frac{\Delta S}{R}, \tag{11}$$

where ΔH (J mol^{-1}) and ΔS (J mol^{-1} K^{-1}) are the adsorption enthalpy and the entropy, respectively, m is the weight of the adsorbent, n_0 is the amount of the adsorbate (mol), T (K) is the absolute temperature, and R is the Gas constant ($R = 8.3144598$ J mol^{-1} K^{-1}).

Chiral HPLC analysis

The HPLC separation was carried out using a liquid chromatograph Agilent 1100 Series consisting of binary pump (G1312A), degasser (G1379A), autosampler (G1313A), thermostat (G1364C), and DAD detector (G1365B HP). Chromatographic column Chirobiotic T (250 mm × 4 mm I. D., 5 µm, teicoplanin chiral selector) was used for separation of enantiomers. The mobile phase consisting of methanol and water (75/25, v/v) was pumped at flow rate 0.8 cm^3 min^{-1}. The column temperature was maintained at 30 °C. The spectrophotometric detector was set at 210 nm. The chromatographic characteristics, resolution value, and elution times of L- and D-phenylalanine were 1.59, 11.5 ± 0.2 min and 13.0 ± 0.2 min, respectively. The validation parameters include LOD values of 0.1 µg cm^{-3}

(0.0006 mmol dm^{-3}) for both enantiomers, linearity concentration range 0.3–20.5 μg cm^{-3} (0.0018–0.1513 mmol dm^{-3}).

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