Molecularly Imprinted Polypyrrole Prepared by Electrodeposition for the Selective Recognition of Tryptophan Enantiomers

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ABSTRACT: Herein we report the electrosynthesis of polypyrrole with L-tryptophan (L-Trp) as a template to prepare molecularly imprinted polymers (MIPs). Overoxidized polypyrrole films with cavities complementary to the template were used for the enantioselective detection of L-Trp and D-tryptophan (D-Trp). Important parameters, such as the electropolymerization potential, overoxidization potential and time, thickness of the polypyrrole films, and scanning rate of the stripping voltammetric experiments, were varied to achieve an optimum sensor response. We found that L-Trp was inserted about 2 times higher into the imprinted polymer film than D-Trp. Also, in this study, an electrochemical quartz crystal microbalance technique was used to investigate the performance of overoxidized polypyrrole films. The enantioselectivity of the MIPs was attributed to the cavities in the imprinted films, which were complementary to the target molecules, both in shape and in positioning of the functional groups. The results also suggest the feasibility of preparing MIPs by electropolymerization for the enantioselective recognition of other amino acid enantiomers. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 1952–1957, 2010

Key words: biological applications of polymers; chiral; conducting polymers; electrochemistry; polypyrroles

INTRODUCTION

Molecularly imprinted polymers (MIPs) have been studied extensively in recent years.[1,2] The preparation of MIPs is a popular technique for enantiomer separation,[3] artificial antibodies,[4] sensors and assaysm,[5,6] and solid phase extraction.[7,8] Recently, MIPs have also been used for the recognition of proteins.[9,10] The general procedure of molecular imprinting is accomplished by either reversible covalent bonding or noncovalent interactions between monomers and imprint molecules. Preparation methods of MIPs have been reported and have included electropolymerization,[11] soft lithography,[12] molecular self-assembly,[13] and chemical grafting.[14] Not much work has been carried out to study the formation of MIPs by the electrochemical synthesis of conducting polymers; the electrochemistry technique has many advantages, including easy adherence of the polymer films to the surface of metal electrodes of any shape and size and the ability to control the thickness of the films under different electrochemical conditions.[15]

Among various types of conducting polymers, polypyrrole (PPy) is one of the best candidates for the preparation of molecular imprinting systems because its excellent biocompatibility and easy pathways for the immobilization of various biologically active compounds.[3] On the other hand, overoxidized polypyrrole (oPPy) exhibited improved selectivity, which was attributed to the removal of positive charges from the PPy backbone because of the introduction of oxygen functionality, such as with carbonyl groups.[16] Ozcan et al.[10] reported the determination of paracetamol based on molecularly imprinted PPy-modified electrodes. Chen et al.[17] used oPPy colloids to detect alanine, cysteine, and phenylalanine enantiomers; they also reported the enantioselective detection of L-glutamic acid and D-glutamic acid with oPPy.[18] More recently, Syritski et al.[19] synthesized molecularly imprinted PPy films for the enantioselective recognition of L-aspartic acid through a constant-current technique. However, research on the selective recognition of Trp enantiomers has not been reported until now.

In this article, we first report the feasibility of preparing highly enantioselective L-tryptophan (L-Trp) imprinting PPy films with electrodeposition. Thin films of PPy, electropolymerized in the presence of L-Trp as a template molecule with subsequent overoxidation to create shape-complementary cavities, were evaluated as MIPs. For optimum selectivity, the electropolymerization potential, overoxidation potential and time, conditions of enrichment, scanning rate of stripping voltammetry, and thickness of the imprinted oPPy films were considered at film synthesis and detection.

EXPERIMENTAL

Materials and reagents

Pyrrole (Py; Aldrich, St. Louis, MO; 98%) was purified by vacuum distillation before use. L-Trp and D-tryptophan (D-Trp; ≥99%) were purchased from Fluka (Steinheim, Germany). Sodium chloride, sodium hydroxide, and hydrochloric acid were from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China). All solutions were prepared with deionized water from a Millipore system.

Synthesis of the molecularly imprinted PPy

A single-compartment, three-electrode cell was used to perform the electrochemical experiments. It consisted of two platinum foils and one saturated calomel electrode (SCE). The apparatus was an electrochemistry analyzing system (model CHI 410A) (Chenhua, Instrument Co., Ltd., China) equipped with a quartz crystal microbalance. The preparation of molecularly imprinted PPy was as follows. First, the electrodeposition of Py on a platinum working electrode was performed at a constant potential of 0.8 V in a solution containing 0.1M Py, 0.1M NaCl, and 4 mM L-Trp, and the deposition of the films was carried out for 150 s. After that, for the formation of complementary cavities, the prepared PPy/L-Trp films were overoxidized in a 0.1M NaOH solution by the application of a constant potential of 1.0 V for 10 min. The PPy films before overoxidation were investigated by scanning electron microscopy (model JSM-6360LA) (JEOL Ltd., Tokyo, Japan). Nonimprinted PPy was prepared according the same protocol; only, the presence of L-Trp was excluded during the electrochemically induced polymerization procedure.

Enantioselectivity of the oPPy films

The enantioselectivity of the oPPy films was determined as follows. First, the imprinted oPPy-modified electrode was immersed in a 0.1M NaCl aqueous solution (pH 5.0) containing 2 mM L-Trp (or D-Trp) under open-circuit conditions for 25 min to make L-Trp (or D-Trp) enriched. Second, stripping voltammetry experiments were used to detect Trp enriched in the imprinted oPPy films.

Also, in this study, electrochemical quartz crystal microbalance (EQCM) experiments were performed to characterize the enrichment of Trp in the oPPy films. A Teflon electrochemical cell was used for the synthesis and electrochemical measurements. A PPy film consisting of L-Trp templates was formed on an 8-MHz, gold-coated crystal (mirror finished; exposed area = 0.196 cm^2), whereas a platinum foil was used as the counter electrode, and SCE was used as the reference electrode. Before electrodeposition of the PPy films, activation of the Au surface was carried out by cyclic voltammetry in 1M H$_2$SO$_4$ in the potential between −0.2 and 1.5 V versus SCE until a reproducible voltammogram was obtained.[20] The crystal was then rinsed several times with deionized water. After electrosynthesis of PPy, overoxidation was carried out to obtain the imprinted oPPy films. The time dependence of the frequency for the crystal in 2 mM L-Trp and D-Trp was monitored at pH 5.0.

RESULTS AND DISCUSSION

Electrodeposition of the PPy films with the L-Trp template

The synthesis of the PPy/L-Trp films was carried out in an aqueous solution containing 0.1M Py, 0.1M NaCl, and 4 mM L-Trp. The isoelectric point of Trp was 5.8, which was lower than the pH value of the synthesis solution (pH 6.5). Therefore, the dominant L-Trp species in the solution was negatively charged Trp$^−$. During the electrochemical polymerization of Py, L-Trp$^−$ anions were expected to compensate the
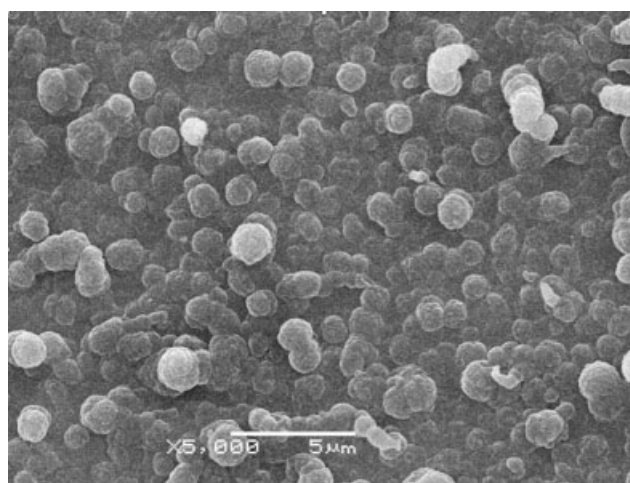

Figure 1 Scanning electron microscopy image of the PPy films electrosynthesized on a platinum electrode.
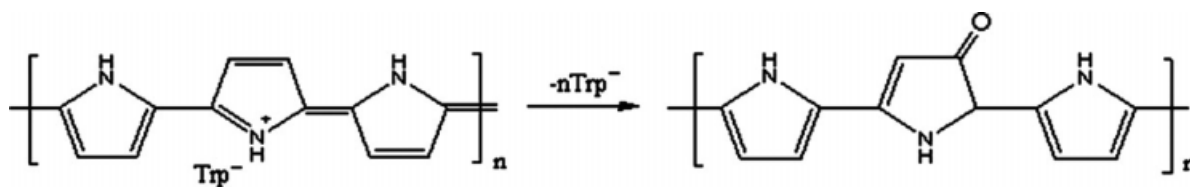
Figure 2 Chemical or electrochemical overoxidization of PPy to cause dedoping.

positive charge of the PPy backbone and result in the formation of a PPy film doped with L-Trp (PPy/L-Trp). A scanning electron microscopy image of the synthesized PPy films is shown in Figure 1; we observed that the PPy particles were uniform in size, which was about 1–2 μm. However, the electrodeposition rate of PPy/L-Trp was much smaller than that of the PPy film without the L-Trp template under identical electrodeposition conditions, and this may have been caused by the different dopants. When amino acids are doped into a PPy texture, more energy is required than for inorganic anions.[17,21]

Effects of the electropolymerization potential

The electropolymerization potential was one of the important factors that affected the synthesis of the PPy films. Py could not be electropolymerized on the platinum electrode when the potential was lower than 0.7 V; only soluble Py oligomers were formed under low-potential conditions. In this study, we chose a potential of 0.8 V to electrosynthesize the PPy films because a too-high electropolymerization potential would have caused overoxidation of the PPy films during the electrodeposition process, which would have reduced the recognition efficiency of the Trp enantiomer.

Overoxidation of the PPy films: The formation of complementary cavities

The purpose of overoxidation was to form complementary cavities for the recognition of the Trp enantiomer in the PPy films. Because oxygen-containing groups, such as carbonyl groups, were introduced during overoxidation, along with the loss of the cationic charge on the PPy backbone, L-Trp$^-$ was excluded from the oPPy films (Fig. 2). Also, during overoxidation, PPy lost its electroactivity in parallel with the ejection of the dopants. As a result, cavities complementary to the template, both in shape and in positioning of functional groups, were left in the oPPy films. This made selective recognition of the Trp enantiomer possible. The overoxidization potential and time were two important parameters in the overoxidation process. A potential that was too high or a time of oxidation that was too long would have caused structural damage to the cavities. On the other hand, an inadequate overoxidation potential

and time would have made the numbers of recognition sites not high enough for the recognition of the Trp enantiomer. The relationship between the overoxidation time and recognition effect is shown in Figure 3. Here, the peak current ratio of L-Trp to D-Trp in the stripping voltammetry was used to evaluate the recognition effect of the oPPy films. As shown, the optimum overoxidation time was 10 min. A similar experimental result indicated that 1.0 V was the most suitable overoxidation potential (figure omitted).

Enrichment of L-Trp

The oPPy films had cavities complementary to L-Trp molecules both in shape and in positioning of the functional groups, which benefited L-Trp in the enrichment solution to diffuse into the inner cavities of the polymer. However, for the nonimprinted oPPy films, nonspecific adsorption (hydrogen bonding) played a major role because of the lack of recognition sites, and there were not suitable cavities in the oPPy films. Sreenivasan[22] reported that when aniline was used as monomer to synthesize MIPs for nuclei acid bases, hydrogen bonding had a dominant effect. As shown in Figure 4, in this study,
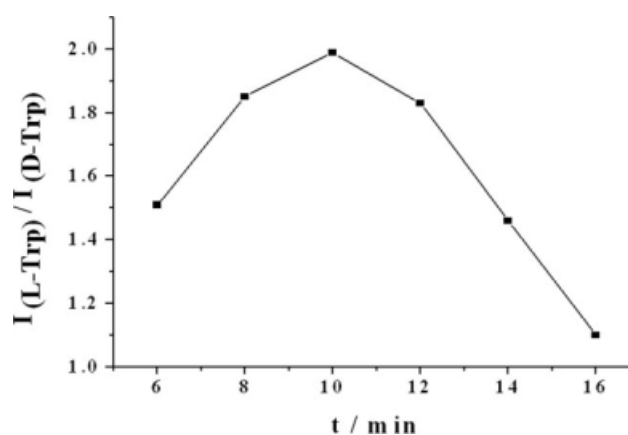

Figure 3 Relationship between the overoxidation time and peak current ratio of L-Trp to D-Trp [overoxidation potential = 1.0 V versus SCE; enrichment time = 25 min; enrichment solution = 0.1M NaCl (pH 5.0) containing 2 mM L-Trp (or D-Trp); stripping solution = 0.1M NaCl (pH 6.0)]. I$_{(L-Trp)}$ and I$_{(D-Trp)}$ represent the oxidation peak current of L-Trp and D-Trp on oPPy, respectively.
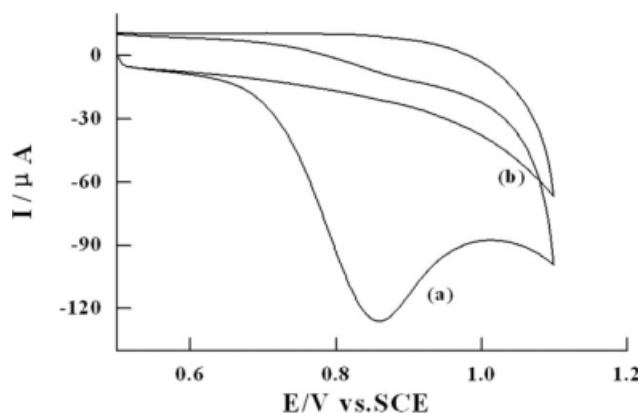
Figure 4 Cyclic voltammograms of (a) imprinted oPPy and (b) nonimprinted oPPy films in stripping solutions after the enrichment of L-Trp (for the other conditions, see Fig. 3; E = potential, I = current).

complementary cavities were more important than nonspecific adsorption for the enrichment of L-Trp molecules because the oxidation peak current of Trp at imprinted oPPy (0.85 V) was larger than that of nonimprinted oPPy in the cyclic voltammograms.

The effect of L-Trp concentration on the cyclic voltammograms was also examined in our study, and the results are shown in Table I. The peak current of L-Trp in the cyclic voltammograms increased with L-Trp concentration up to 4 mM. In this study, the concentration of L-Trp template molecules during the electrodeposition of PPy was 4 mM, so the maximum concentration of L-Trp, shown in Table I, was 4 mM. Although the peak current of D-Trp increased with D-Trp concentration, it was much smaller than that of L-Trp under the same concentration conditions. We concluded from the results that L-Trp was enriched through molecular imprinting; however, D-Trp was enriched only by nonspecific adsorption.

Both the acidity of the enrichment solution and the enrichment time had a significant impact on the recognition efficiency of the Trp enantiomer. Figure 5 indicates the relationship between the acidity of the enrichment solution and the recognition effect. As shown, the highest peak current ratio of L-Trp to D-Trp appeared when the pH value was 5.0. This was caused by the zwitterionic Trp and the

TABLE I
Effect of the L-Trp Concentration on the Cyclic Voltammograms

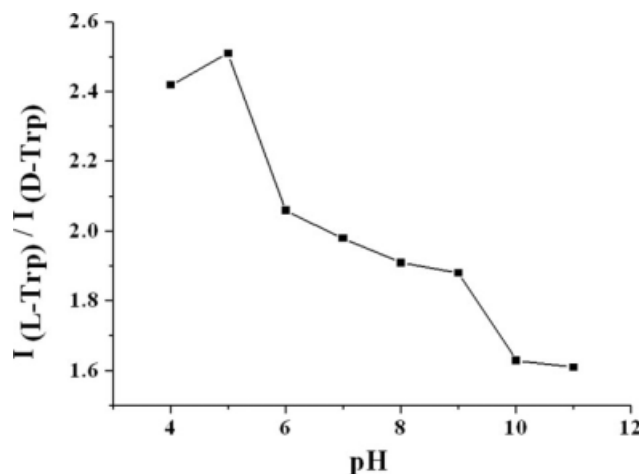| L-Trp concentration (mM)	Peak current (μA)	D-Trp concentration (mM)	Peak current (μA)
1	33.8	1	19.5
2	125.0	2	14.2
3	377.0	3	35.8
4	380.0	4	67.6

Figure 5 Relationship between the acidity of the enrichment solution and peak current ratio of L-Trp to D-Trp (for the other conditions, see Fig. 3). $I_{(L-Trp)}$ and $I_{(D-Trp)}$ represent the oxidation peak current of L-Trp and D-Trp on oPPy, respectively.

large electronegativity of the oxygen atom of the carbonyl group in the oPPy films. When the pH value of the enrichment solution was above 5.8 (isoelectric point of Trp), Trp existed as anions, and they could hardly diffuse into the oPPy films, so the pH value must have been below 5.8. However, when the pH value was below 5.0, the peak current ratio of L-Trp to D-Trp decreased. This may have been caused by some damage of the cavities' structure at strong acidic conditions. So 5.0 was chosen as the optimum pH value. The relationship between the enrichment time and peak current ratio of L-Trp to D-Trp is shown in Figure 6. When the enrichment time was 25 min, the peak current ratio was the highest. This
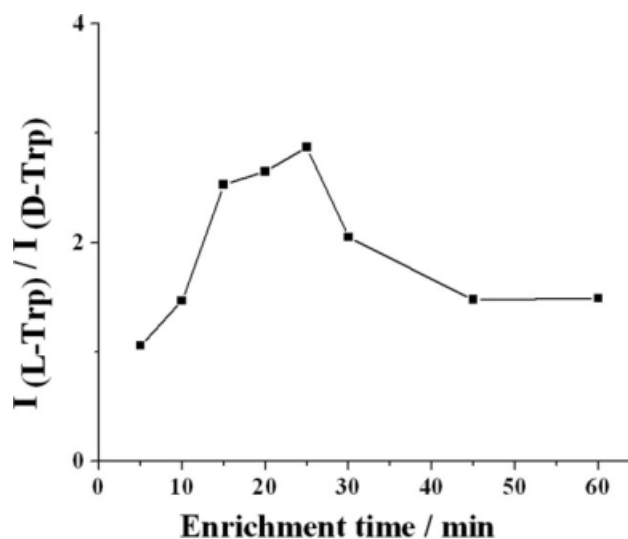

Figure 6 Relationship between the enrichment time and peak current ratio of L-Trp to D-Trp (for the other conditions, see Fig. 3). $I_{(L-Trp)}$ and $I_{(D-Trp)}$ represent the oxidation peak current of L-Trp and D-Trp on oPPy, respectively.
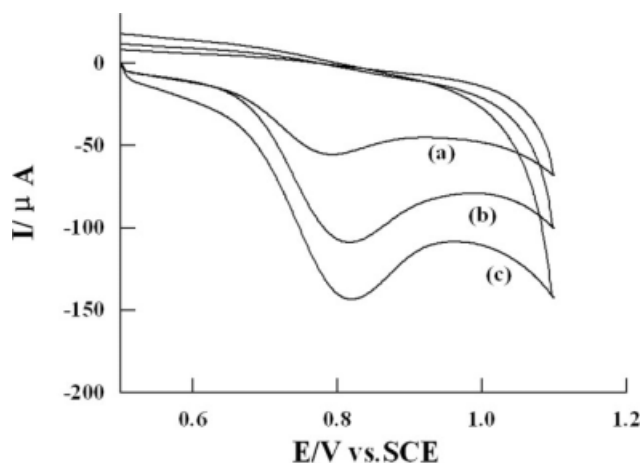
Figure 7 Cyclic voltammograms of imprinted oPPy films in stripping solutions after the enrichment of L-Trp at scan rates of (a) 50, (b) 100, and (c) 150 mV/s (for the other conditions, see Fig. 3; E = potential, I = current).

was because of the fact that when enrichment time was shorter than 25 min, the amounts of L-Trp diffused into the oPPy films increased with time. L-Trp in the oPPy films reached saturation when the enrichment time reached 25 min. Nonspecific adsorption occurred when the enrichment continued, which caused a decrease in the peak current ratio. A time of 25 min was chosen as the enrichment time throughout our experiments.

Effects of the scanning rate of stripping voltammetry

In our experiments, we observed that the scanning rate of stripping voltammetry influenced the oxidation peak current and the peak shape of L-Trp. Figure 7 shows the cyclic voltammograms of the imprinted oPPy films in the stripping solution after the enrichment of L-Trp at different scanning rates. When the scanning rate was set at 150 mV/s, the peak current of L-Trp was much larger than those at 50 and 100 mV/s. Although the peak current increased a little as the scanning rate continued to increase, a poor peak shape was obtained at a faster scanning rate. So the choice of 150 mV/s as the scan rate was very important for obtaining a large oxidation peak current and for preventing peak deterioration

Effects of the thickness of the imprinted oPPy films

The optimum thickness of the imprinted oPPy films was determined from a series of experiments in which the PPy films were electropolymerized for different times. We concluded that with too little electrodeposition time, the template would not be adequately embedded for the resulting surface, after

overoxidation, to have enough recognition sites. On the other hand, too much time would no doubt lead to L-Trp becoming permanently entrapped in the polymer. The first of these situations would lead to minimal recognition, whereas the latter would potentially increase the difficulty of the exclusion of L-Trp during the overoxidation process. Shown in Figure 8 are the peak current ratios of L-Trp to D-Trp associated to different electrodeposition times to form the PPy films. We observed that 150 s was the optimum electrodeposition time to get the highest peak current ratio.

Proof of the recognition characteristics of the imprinted oPPy films by EQCM

After overoxidation and enrichment, the mass change (Δm) of the imprinted oPPy films resulted in the conversion of the resonant frequency shift (Δf), which was calculated according to the Sauerbrey equation:

$$\Delta m = -\Delta f / C_f$$

where C_f is the sensitivity factor for the quartz resonators.

The EQCM frequency changed with the addition of 2 mM L-Trp and D-Trp at pH 5.0 for gold surfaces modified with imprinted oPPy; this is shown in Figure 9. As expected, L-Trp was embedded more into the gold surfaces modified with imprinted oPPy than D-Trp. When L-Trp was added, the EQCM frequencies fell sharply in the first 250 s and continued to decrease almost linearly with the time after
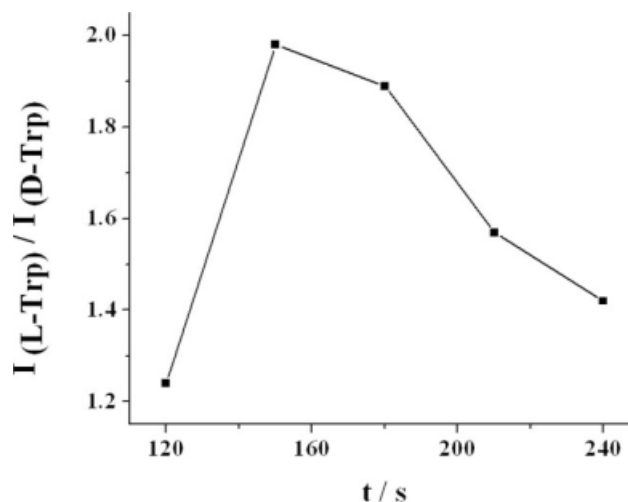

Figure 8 Relationship between the electrodeposition time and peak current ratio of L-Trp to D-Trp (for the other conditions, see Fig. 3). $I_{(L-Trp)}$ and $I_{(D-Trp)}$ represent the oxidation peak current of L-Trp and D-Trp on oPPy, respectively.
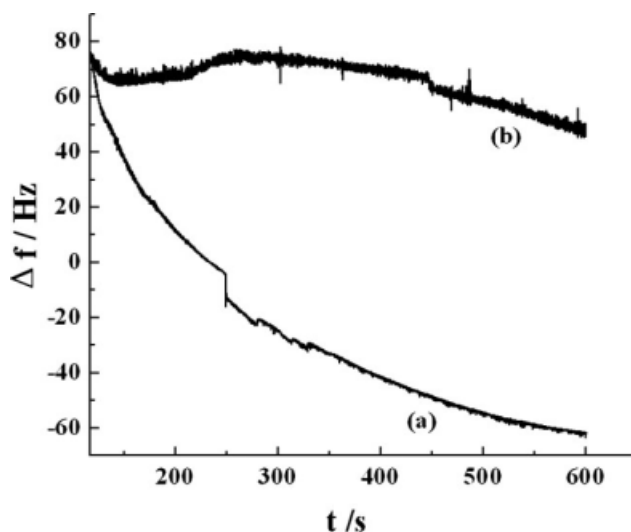
Figure 9 EQCM frequency changes with the addition of (a) 2 mM L-Trp and (b) D-Trp at pH 5.0 for gold surfaces modified with imprinted oPPy.

this period. However, when D-Trp was added, the EQCM frequencies remained almost unchanged. This was powerful evidence that the L-Trp-imprinted oPPy films had more complementary cavities for L-Trp than for D-Trp, which made the MIPs effective for the selective recognition of the Trp enantiomer.

CONCLUSIONS

In this study, thin films of PPy, electrosynthesized in the presence of L-Trp as template molecules with subsequent overoxidation to create shape complementary cavities, were evaluated as MIPs. The optimized conditions for the fabrication and discrimination properties of the oPPy films were studied systematically by EQCM and other electrochemical techniques. We found that synthesis parameters such as overoxidation potential and time and the thickness of the imprinted oPPy films strongly influ-

enced the enantioselectivity of the resulting oPPy films. The higher response and selectivity of the oPPy films for L-Trp over D-Trp were assigned to the complementary cavities generated during overoxidation. Future work should focus on improving the enantioselectivity of the imprinted oPPy films, and this method puts forward a new approach for chiral discrimination and can be generalized for other amino acid enantiomers.

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