Chiral Recognition of Tryptophan Enantiomers Based on a Polypyrrole-Flake Graphite Composite Electrode Column

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ABSTRACT: A polypyrrole (PPy)-flake graphite composite was synthesized by *in situ* polymerization of pyrrole on flake graphite surface, and the resulting organic–inorganic composite material was developed as the conductive stationary phase in an electrode column for enantioselective recognition of tryptophan (Trp) enantiomers. Compared with the stationary phase made up with conducting PPy, the conductivity of the composite stationary phase was enhanced significantly. As a result, satisfactory enantioselectivity of Trp enantiomers can be achieved easily by modulating the potential applied on the electrode column.

Various important parameters influencing the performance of the composite electrode column were investigated to obtain the optimum recognition efficiency. A recognition efficiency of 3.7 could be achieved under the optimum conditions. Enantioselectivity of the composite material for Trp enantiomers is attributed to the reversible doping/de-doping properties of PPy. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: polypyrrole; flake graphite; electrode column; chiral recognition; tryptophan enantiomers

INTRODUCTION

Amino acids and their derivatives are chiral, and with few exceptions only one enantiomer is of interest.[1] The chiral separation of amino acids has been achieved most frequently by HPLC, since special chiral stationary phases and chiral derivatizing agents have been used in the past decade.[2–4] However, it is usually expensive and troublesome for the chiral stationary phases or derivatizing agents to be widely used in separation field.

Conventional conducting polymers such as polyaniline (PAn) and polypyrrole (PPy) have good electrochemical reversibility, and they have been used as the molecularly imprinted polymers in separation field.[5–8] More recently, PAn and PPy were applied as the conductive stationary phases in electrode columns, and amino acids enantiomers have been recognized successfully at these novel electrode columns.[9,10] The recognition procedures are as follows: first, a certain potential is applied on the column for de-doping the L-amino acid template molecules and thus molecularly imprinted conducting polymers are obtained; then another certain potential is applied when the mobile phases containing L or D-amino acids are pumped through the column. The amount of L-amino acid enriched in the imprinted column is larger than that of D-amino acid, since the shape of L-amino acid is complementary to the cavity left in the electrode column during the de-doping process. However, the low conductivity of PAn and PPy limits their practical applications as conductive stationary phases in electrode columns.

Recently, composite materials based on polymers such as PPy and poly(vinyl alcohol) and various inorganic materials such as TiO_2,[11,12] SiO_2,[13–15] Fe_3O_4,[16] carbon nanotube,[17] and graphene oxide[18] have been reported since these organic–inorganic materials have some special physical properties. Flake graphite is a kind of carbon material, and it has excellent conductivity and chemical stability. In the present work, we take advantage of the fact that PPy has reversible doping/de-doping properties and flake graphite possesses excellent conductivity creates a possibility for using this PPy-flake graphite composite as a conductive stationary phase, and thus fabricate an electrode column for the chiral recognition of tryptophan (Trp) enantiomers. Compared

with our previous work in which only PPy was used as the stationary phase,[10] an enhanced recognition efficiency can be obtained at the novel composite electrode column under the optimum conditions.

EXPERIMENTAL

Reagents

Pyrrole (Aldrich, Shanghai, China, 98%) was distilled under reduced pressure before use. L and D-Trp were purchased from Fluka (Steinheim, Germany). Natural flake graphite (99%, grain size 50 mesh) was obtained from Haida Graphite Company (Qingdao, China). All other chemicals were of analytical grade and used without further purification, and the solutions were prepared by doubly distilled water.

Instruments

The cyclic voltammetry of PPy-flake graphite composite, denoted as PPy-C, was carried out on a CHI 660D electrochemical analyzer (CH Instruments, Chenhua Co., Shanghai, China), which was also used as a potentiostat to control the potential applied on the electrode column. The fluorescence from L or D-Trp was detected with a model F-280 fluorescence spectrometer (Tianjin Gangdong Sci&-Tech. Development Co., China). The FTIR spectra of PPy-C and PPy samples were measured by using a FTIR-8400S spectrometer (Shimadzu, Japan). Morphologies of both PPy-C and PPy were investigated by using a JSM-6360LA scanning electron microscopy (Japan). Mobile phases containing L or D-Trp were pumped through the electrode column by using a model BT00-100M peristaltic pump (Changzhou Chenghe Sanitary Equipment Factory, China). The conductivity of PPy-C or PPy was measured on a pressed pellet using a four-probe technique.

Preparation of the PPy-C composite electrode column

The chemical polymerization of pyrrole was carried out in this work because a great amount of PPy was needed as the stationary phase in an electrode column, and the synthesized PPy acted as the coating polymer of flake graphite to form PPy-C. The procedures were as follows: 20-mL $(NH_4)_2S_2O_8$ solution of $1.14M$ was slowly added in 35-mL aqueous solution consisting of $0.82M$ pyrrole, 1.16-g flake graphite and 0.78 mM L-Trp, and the pH of the mixture was adjusted to 7.0 with NaOH. The mixture was continuously stirred at 0°C for 10 h. The resulting PPy-C was washed with ethanol until the filtrate was colorless, and then dried under dynamic vacuum at 60°C

for 24 h. For comparison, PPy was synthesized according to the same procedures without adding flake graphite. A porous ceramic tube was used as the electrode column, and it was packed with 0.37 g PPy-C. The procedures for packing the electrode column with PPy-C were very similar to those of PAn and PPy electrode columns.[9,10]

Potential-induced de-doping and re-binding of L-Trp

The packed column was connected to the electrochemical workstation as the working electrode of a three-electrode cell; a platinum foil and a saturated calomel electrode were served as the auxiliary and the reference electrode, respectively.

A positive potential of 0.9 V was applied on the electrode column for 2000 s to eject the L-Trp templates in the PPy-C stationary phase, and the electrolyte was $0.05M$ PBS (pH 6.95). For re-binding L or D-Trp in the mobile phase to the imprinted PPy-C, a negative potential of −0.4 V was applied on the electrode column with $0.1M$ KCl (pH 4.0) as the electrolyte and the mobile phase. To calculate the amount of L or D-Trp rebound, a potential of 0.9 V was applied on the electrode column again to eject the Trp enantiomers. The amount of ejected L or D-Trp was determined by fluorescence spectrum.

RESULTS AND DISCUSSION

Conductivity of PPy-C composite

Because of the excellent conductivity of flake graphite ($>10^4$ S/cm), the conductivity of PPy-C composite is assumed to be higher than that of PPy synthesized under the same conditions except the adding of flake graphite. Figure 1 shows the conductivity of PPy-C composite with different amount of flake graphite. It is found that the conductivity of PPy is only 0.11 S/cm, and it increases with increasing content of flake graphite. When the mass ratio of pyrrole monomer/flake graphite reaches 1 : 0.6, a maximum conductivity of 4.88 S/cm can be obtained. However, PPy cannot cover the surface of flake graphite completely if the content of flake graphite increases further, which will result in a nonuniform stationary phase. So, a mass ratio of pyrrole to flake graphite is controlled at 1 : 0.6 for preparing PPy-C with high conductivity and homogeneous nature.

Characterization of PPy-C composite

Surface morphologies of PPy-C composite and PPy are shown in Figure 2. It is observed that PPy particles, synthesized without adding flake graphite, are aggregated to form a loose, porous, and coral-like microstructure [Fig. 2(a)]. However, it is quite
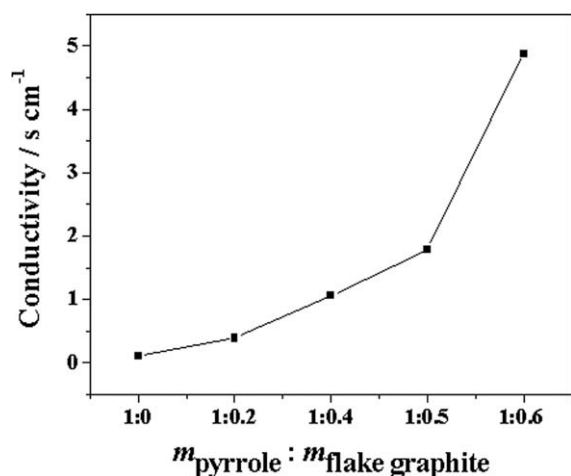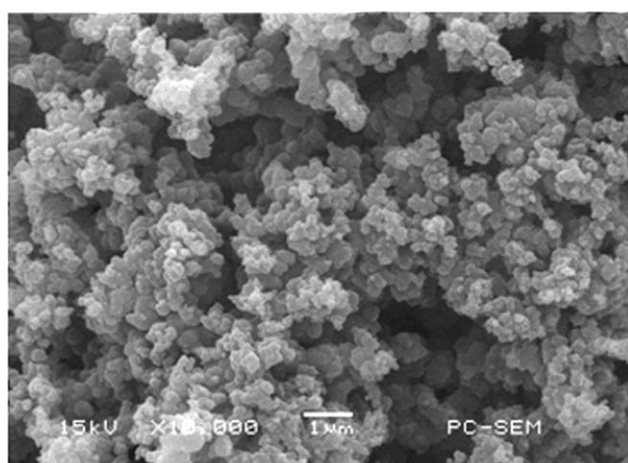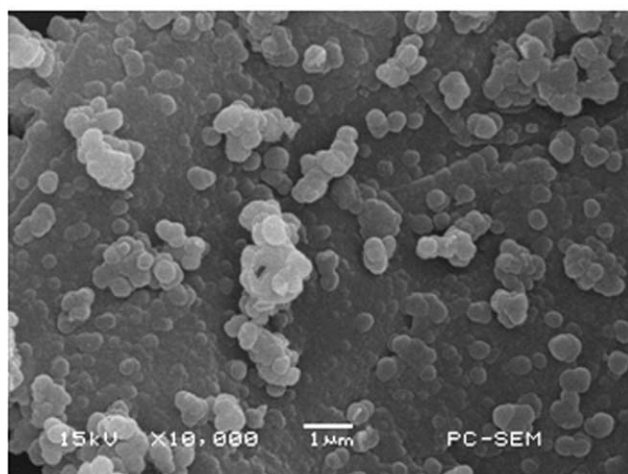
Figure 1 Effect of flake graphite content on the conductivity of PPy-C composite.

different for PPy-C composite, since PPy particles are deposited on a flat support instead of aggregation [Fig. 2(b)]. The appearance of the flat support is

(a)

(b)

Figure 2 SEM images of PPy (a) and PPy-C composite (b).

attributed to the existence of flake graphite in the organic–inorganic composite.

The FTIR spectra of PPy-C composite and PPy obtained by the KBr method are shown in Figure 3. As can be seen, all the PPy bands such as conjugated double bond at 1560 cm^{-1} and amines at 1200 cm^{-1} are presented in the spectrum for PPy-C composite.

Overoxidation of the PPy-C stationary phase

PPy-C prepared by chemical polymerization is in its oxidation state with positive charge on the backbone of PPy. The isoelectric point of Trp and the pH of the solution for synthesis of PPy-C are 5.9 and 7.0, respectively, so the dominant species of Trp in the solution is negatively charged. As a result, L-Trp anions are expected to compensate the positive charge on PPy backbone, and therefore PPy-C doped with L-Trp anions (PPy-C/L-Trp$^-$) are obtained. PPy-C/L-Trp$^-$ are packed into the ceramic tube as the conductive stationary phase, and the whole electrode column is used as the working electrode in a three-electrode system. High positive potentials usually cause the overoxidation of conducting polymers. Overoxidation process has often been regarded as an undesirable degradation of PPy, which leads to the loss of conductivity and de-doping of dopant.[7,10,19–22] Therefore, cavities complementary to L-Trp templates are formed as the result of overoxidation of PPy, and thus molecularly imprinted PPy-C are obtained.

Figure 4 shows the cyclic voltammograms of the electrode column packed with PPy-C in 0.05 H$_2$SO$_4$ solution before and after overoxidation of PPy. As can be seen, an oxidation peak at 0.62 V and a reduction peak at 0.29 V appear on the cyclic voltammogram of PPy-C (curve a), which is attributed to the redox of PPy. However, the redox peaks almost disappear after overoxidation of PPy (curve b). The disappearance of such redox peaks is caused
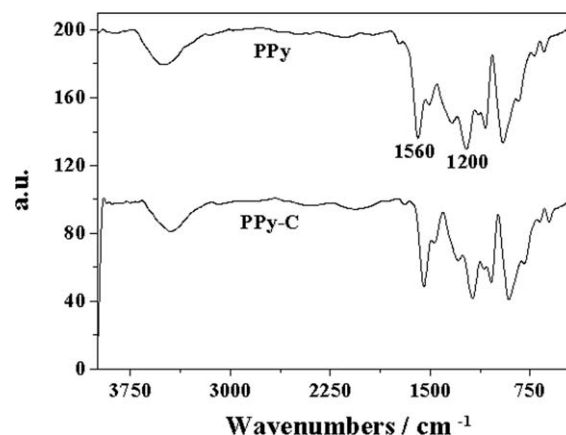

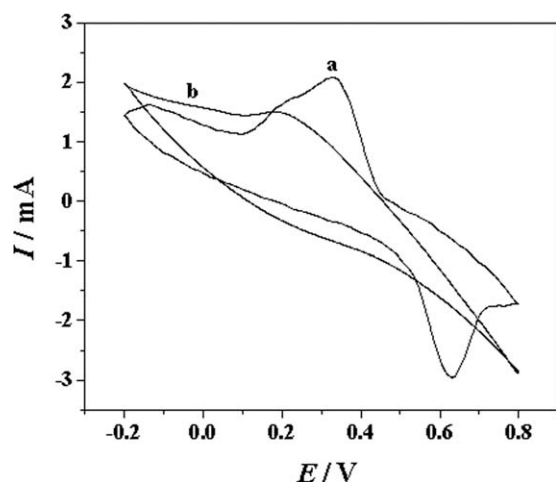**Figure 3** FTIR of PPy and PPy-C composite obtained by the KBr method.

Figure 4 Cyclic voltammograms of the electrode column packed with PPy-C in 0.05 H_2SO_4 solution before (a) and after overoxidation of PPy (b). Scan rate: 10 mV/s.

by the decrease of the electrochemical activity of PPy after overoxidation.

The overoxidation of PPy-C stationary phase is also monitored with FTIR (figure omitted), and the results are quite similar to our previous report.[10] Compared to the FTIR spectrum of PPy, a sharp peak at 1728 cm^{-1} is observed in the FTIR spectrum of overoxidized PPy. The peak at 1728 cm^{-1} is attributed to the carbonyl groups, which is introduced to PPy backbone during the overoxidation. The mechanism for PPy overoxidation is shown in Scheme 1, where X^- represents L-Trp anions.

Effects of potential and time on the de-doping of L-Trp

Different positive potentials are applied on the electrode column for the overoxidation of PPy-C, and the amount of L-Trp ejected from the PPy-C stationary phase is calculated from the fluorescence spectrum. Figure 5 shows the relationship between the overoxidation potential and the amount of L-Trp de-doped. As can be seen, a maximum amount of L-Trp de-doped from the PPy-C stationary phase is obtained when the potential for PPy overoxidation is controlled at 0.9 V. If the potential increases further, a decrease in the amount of L-Trp de-doped is observed, which is attributed to the oxidation of L-Trp templates. Our previous work has pointed out that a potential of 1.0 V is needed for the overoxidation of PPy stationary phase.[10] Compared with PPy
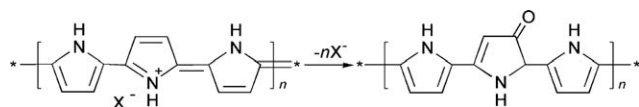
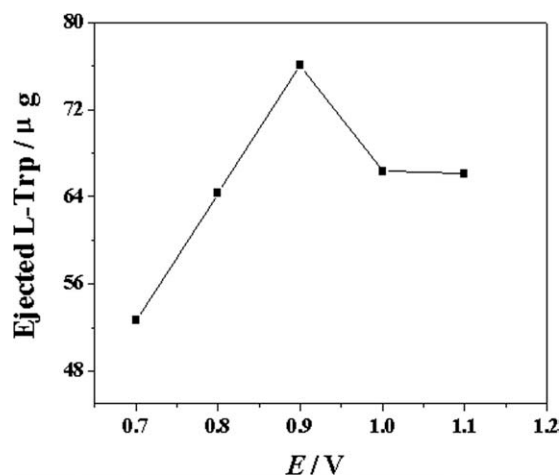**Scheme 1** The mechanism of PPy overoxidation.

Figure 5 The relationship between the overoxidation potential and the amount of L-Trp de-doped from the PPy-C stationary phase.

stationary phase, the potential for the overoxidation of PPy in the PPy-C composite is lowered by 0.1 V. The decrease in the overoxidation potential is attributable to the improved conductivity of PPy-C composite.

The relationship between the overoxidation time and the amount of L-Trp ejected is shown in Figure 6. When the potential for PPy-C overoxidation is set as 0.9 V, the amount of L-Trp de-doped increases with increasing time until 2000 s. When the overoxidation time exceeds 2000 s, the de-doped amount of L-Trp remains almost constant. It indicates that an overoxidation time of 2000 s can make a complete de-doping of L-Trp templates. It is interesting to notice that for PPy stationary phase without the adding of flake graphite, an overoxidation time as long as 5000 s is needed for the de-doping of templates,[10] which is much longer than that for the PPy-C stationary phase. The significant decrease in the
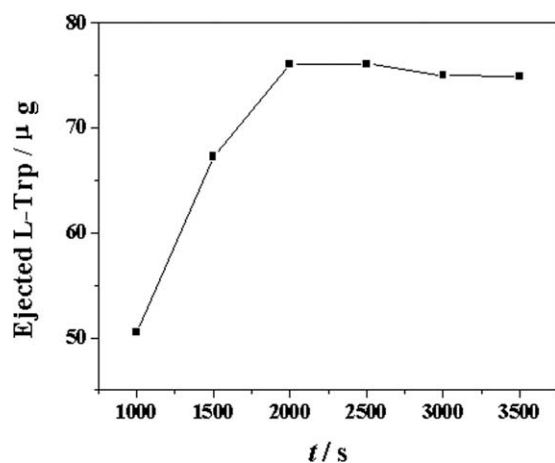

Figure 6 The relationship between the overoxidation time and the amount of L-Trp de-doped from the PPy-C stationary phase at a potential of 0.9 V.
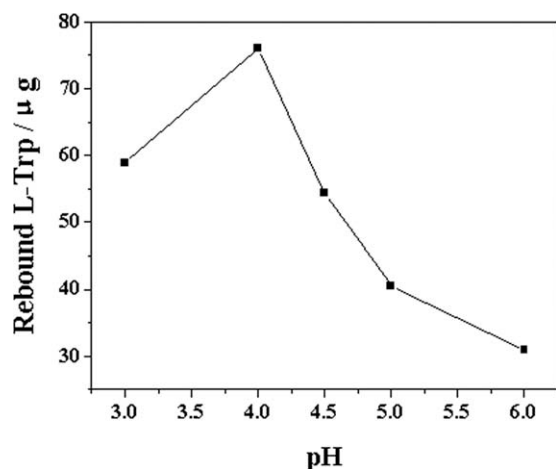
Figure 7 The relationship between the pH value for enrichment and the enrichment amount of L-Trp in the mobile phase at a potential of −0.4 V.

de-doping time is also assigned to the improved conductivity of PPy-C composite, and it is easier for L-Trp template molecules to be ejected from PPy-C stationary phase than from PPy stationary phase.

So the potential and the time for the overoxidation of PPy-C are controlled at 0.9 V and 2000 s in the following experiments.

Effect of pH value on the enrichment of L-Trp

Overoxidized PPy has cavities complementary to L-Trp molecules both in shape and in positioning of functional groups, which is beneficial to the enrichment of L-Trp in the mobile phase to diffuse into the inner cavities of the polymer.[7,10] A negative potential is applied on the electrode column packed with the imprinted PPy-C when a mobile phase containing L-Trp is pumped through the column. L-Trp in the mobile phase can be rebound to the imprinted composite since chirality matched up with the cavities in the composite. The pH value of the mobile phase plays an important role in the enrichment of L-Trp, which is presented in Figure 7. It deserves noting that for estimating the amount of L-Trp rebound, a potential of 0.9 V is applied on the electrode column again to eject L-Trp and the eluted L-Trp is determined by fluorescence spectrum. When the pH of the solution is above 5.9 (isoelectric point of Trp), Trp exists as anions and they can hardly be rebound to the imprinted PPy-C stationary phase with a negative potential applied. As a result, the pH for L-Trp enrichment must be below 5.9. It can be seen from Figure 7 that the enrichment amount of L-Trp increases with decreasing pH values and it reaches the maximum when the pH is 4.0. However, the amount of L-Trp rebound to the imprinted PPy-C decreases if the pH value decreases further, which may be caused by the partial damage of the imprint-

ing cavities at strong acidic solution. As a result, the pH value of the mobile phase is controlled at 4.0 in the following experiments.

Effect of potential on the enrichment of L-Trp

Figure 8 shows the effect of potential on the enrichment of L-Trp cations in the mobile phase (pH 4.0). It is observed that the enrichment amount of L-Trp increases with increasing negative potentials. This is caused by the electrostatic interaction between the positively charged Trp and the PPy-C stationary phase with negative potential applied, though molecularly imprinting plays a dominant role in the enrichment of L-Trp. The more negative potential, the stronger the electrostatic interaction occurs. When the negative potential applied on the electrode column exceeds −0.4 V, the enrichment amount of L-Trp changes little. So, the potential applied on the electrode column is set at −0.4 V for the enrichment of L-Trp.

Effect of enrichment time on the re-binding of L-Trp

The relationship between the time and the enrichment amount of L-Trp in the mobile phase is shown in Figure 9. It is found that when the enrichment time is less than 3000 s, the amount of L-Trp rebound to the imprinted PPy-C stationary phase increases with time. However, the enrichment amount of L-Trp remains almost unchanged after 3000 s. It is because that the number of the cavities complementary to L-Trp in the imprinted PPy-C stationary phase is limited after the de-doping of L-Trp templates, and L-Trp rebound to the imprinted PPy-C reaches its saturation when enrichment time exceeds 3000 s. So when enrichment time is longer
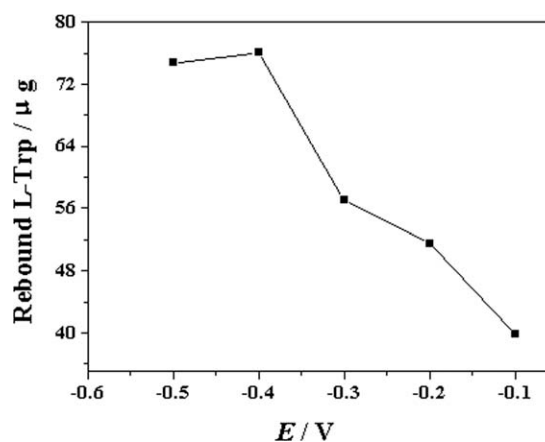

Figure 8 Effect of potential applied on the imprinted electrode column on the enrichment of L-Trp in the mobile phase.
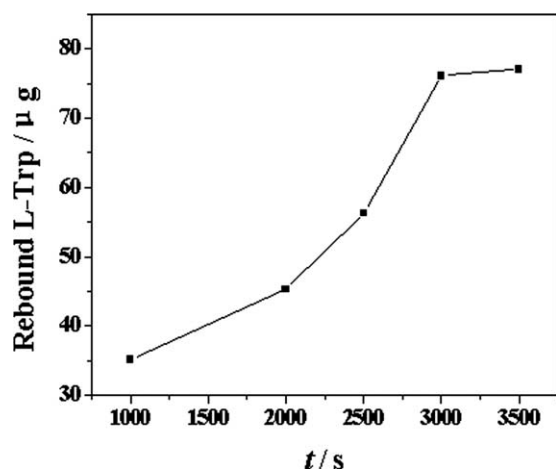
Figure 9 The relationship between enrichment time and enrichment amount of L-Trp in the mobile phase at a potential of −0.4 V.

than 3000 s, the amount of L-Trp rebound to the imprinted PPy-C varies little.

Recognition efficiency of the imprinted PPy-C for Trp enantiomers

To test the recognition efficiency of the imprinted electrode column for Trp enantiomers, the experiments for the re-binding of D-Trp to the L-Trp imprinted PPy-C stationary phase is also carried out in the same procedures as those of L-Trp. Fifty-milliliter mobile phase (pH 4.0) containing 9.8 μM L or D-Trp is pumped through the L-Trp imprinted electrode column when a potential of −0.4 V is applied on the column for the enrichment of L or D-Trp. L or D-Trp rebound to the column is de-doped again at 0.9 V and the amount of L or D-Trp is calculated by fluorescence spectrum. From the calibration plots, it is calculated that the mass of L and D-Trp rebound to the L-Trp imprinted PPy-C stationary phase are 76.09 and 20.60 μg, respectively. The corresponding recognition efficiency is 3.7. However, a recognition efficiency of 3.0 is obtained if the imprinted PPy is used as the stationary phase in the electrode column,[10] which is much inferior to that of the imprinted PPy-C composite stationary phase. The significant increase in the recognition efficiency of the imprinted PPy-C stationary phase is obviously attributed to the excellent conductivity of this composite.

CONCLUSIONS

The PPy-C composite, synthesized by *in situ* polymerization of pyrrole on the surface of flake graphite with L-Trp as templates, is packed into a ceramic tube as the conductive stationary phase to form a novel electrode column. Trp enantiomers can be successfully recognized at this electrode column by modulating the potential applied on it. The recognition of Trp enantiomers based on the electrode column is obviously superior to that on chiral stationary phases or chiral derivating agents, since only a simple potential-induced technique is needed for the recognition. Compared with our previous work in which only imprinted PPy is used as the stationary phase, the recognition efficiency is improved because of the excellent conductivity of PPy-C composite. It is clear that the novel electrode column based on the composite stationary phase is one of the most challenging applications in the enantioselective recognition of other amino acids.

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