Characterization of Overoxidized Polypyrrole Colloids Imprinted with L-Lactate and Their Application to Enantioseparation of Amino Acids

Hiroyuki Okuno, Tomomi Kitano, Hidetaka Yakabe, Masayoshi Kishimoto, Bhavana A. Deore, Hiroshi Siigi, and Tsutomu Nagaoka*

Department of Applied Chemistry, Faculty of Engineering, Yamaguchi University, 2-16-1 Tokiwadai, Ube 755-8611, Japan

Overoxidized polypyrrole colloids imprinted with L-lactate were prepared to evaluate the performance of the overoxidation pseudo-template technique developed by the authors. A polypyrrole colloid that had been prepared from a mixture of monomer (pyrrole), dopant (L-lactate), steric stabilizer (poly(vinylpyrrolidone)) and oxidizing agent (peroxodisulfate) was electrochemically overoxidized at +1.5 V vs Ag/AgCl to create a complementary cavity for recognition of the molecules, which were structurally similar to dopant, through dedoping. As a target molecule for enantioselective uptake into the overoxidized colloid, we selected alanine, which is structurally different from the template (lactate) only in one side chain (alanine, −NH₂; lactic acid, −OH). The overoxidized polypyrrole colloid showed higher affinity for L-alanine than for D-alanine, and an uptake ratio (L/D) of as high as 11 ± 4 was observed under optimum conditions. Uptake reached equilibrium in 10 min, thanks to the high surface area and short diffusion length in the colloidal particle. Further, to confirm the complementarity of the cavity, the effect of side chain size on uptake of several α-amino acids was examined to indicate that the uptake amount decreased with increasing molecular volume of the L-amino acids.

Molecular imprinting techniques are becoming a powerful tool for preparing polymeric materials, which can recognize target molecules through their templated cavities. Typical procedures for preparation of such molecularly imprinted polymers (MIPs) are the formation of a complex of a template molecule with one or more functional monomers, which is then polymerized with a cross-linker to obtain a resin. Upon removal of the template, the cavities that can recognize the template molecule are produced. At present, acrylate-based, styrene-based, and silane-based polymeric materials are most frequently used as the recognition matrix.

To seek a new class of polymeric materials that possesses high target molecule selectivity, we have been studying conducting polymers as candidates for such a recognition matrix. It is rather surprising that although conducting polymers have been extensively used for a number of analytical applications, such as entrapment of biological entities and permselective coatings of a sensor surface, they have been little studied for use in molecular imprinting.

It has been known that a polypyrrole film undergoes irreversible dedoping, called overoxidation, to form a degraded polymer at potentials more positive than those for reversible doping/ dedoping, allowing cationic molecules to permeate through the film as a result. In this connection, the film has become a

* Corresponding author. E-mail: nagaoka@yamaguchi-u.ac.jp.
EXPERIMENTAL SECTION

Chemicals. Poly(vinylpyrrolidone) (PVP) K90 Mw \approx 1.2 \times 10^6 was purchased from Wako Chemicals (Wako, Japan). L-Lactic acid (95% TCI, Tokyo) and lithium \( \alpha \)-lactate (>95% Sigma) were used without further purification. An HPLC grade of o-phthalaldehyde (OPA) was purchased from Wako, and 3-mercaptopropionic acid (>97%) was supplied by Dojindo (Kumamoto, Japan). All the other chemicals used were of reagent grade, and Milli-Q water was used throughout (>12 M \( \Omega \) cm).

Preparation of Colloidal Particles. In this paper, we refer to the composite particle, polypyrrole-PVP, doped with anion \( X^- \) as PPYPVP/\( X^- \). To prepare PPYPVP/\( \alpha \)-lactate, 1.0 mL of pyrrole was added to a 100-mL aqueous solution containing 1.8 g of \( \alpha \)-lactic acid, 0.085 g of PVP, and 0.70 g of ammonium peroxodisulfate. Before adding pyrrole, the pH of the aqueous solution was adjusted by aqueous NaOH, and polymerization was carried out at 30 °C for 15 h unless otherwise stated. The details of the other preparation procedures were reported elsewhere.\(^{12}\) As a reference, a PPYPVP/\( \alpha \)-lactate colloid was prepared in the same manner as above but by using an equimolar amount of lithium \( \beta \)-lactate. Since the pH was not adjusted in this case, polymerization occurred at pH 6.9.

The resulting colloids were dispersed in aqueous 0.1 M NaOH and then overoxidized at +1.5 V vs Ag/AgCl (saturated KCl) with a flow-through electrolysis cell at a flow rate of 0.25 mL min\(^{-1}\). We obtained higher enantioselectivity by applying a more positive potential and a smaller flow rate than those used in the previous work.\(^{12}\) Figure 1 illustrates a schematic cross section of the electrolysis cell. A Vycor glass tube (Corning; i.d., 0.5 cm, length, 5.8 cm; nominal pore diameter, 10 nm), packed with a working electrode material made up of short strands of an unraveled carbon cloth (GF-8P-21E, Nippon Carbon, Tokyo), was inserted into a 5-mL disposable plastic syringe (Terumo) filled with aqueous 0.1 M NaOH. A Pt or Ta wire was used as the working electrode and counter electrode leads. The current efficiency of this cell was found to be quantitative (>98%) for reduction of 2.0 mM [Fe(CN)\(_6\)]\(^3^-\) in aqueous 0.1 M KCl at 15 mL min\(^{-1}\).

The overoxidized colloids were purified by repeated centrifugation and washing and then stored in a refrigerator after redispersion in 30 mL of water. Concentration of the colloidal dispersion was evaluated by measuring particle weight after the dispersion had been dried for 24 h at 70 °C.

Apparatus and Procedures. The uptake procedures of amino acids into colloidal particles were reported elsewhere in detail.\(^{12}\) A mixture of colloidal dispersion and an amino acid solution was shaken with a microsample-tube mixer (Iwaki Glass, Twin 3-28) for 10 min. Amino acids left in the solution phases were then determined by OPA fluorometry or high performance capillary electrophoresis (HPCE). The fluorescence from the amino acid–OPA adduct was recorded with a Hitachi model F-2500 fluorescence spectrometer; detailed procedures for the fluorometry were reported elsewhere.\(^{11,12}\) HPCE experiments were carried out using an Agilent Technologies G1602A 3D system equipped with a 50 \( \mu \)m × 64.5 cm fused-silica bubble-cell capillary at an operating voltage of 20 kV. 3D pherograms were recorded in a range 190–600 nm, and absorbance at 334 nm was used for determination of amino acids. A migration buffer used was an aqueous solution consisting of 10 mM OPA, 10 mM 3-mercaptopropionic acid, and

2 mM ethylenediaminetetraacetic acid dissolved in a pH 10.5 buffer, which involved 50 mM boric acid and 12.5 mM citric acid dissolved in a NaPO₄ solution to adjust the pH to 10.5. Phero
grams of colloidal particles were observed at 30 kV in a range 190–600 nm by using a 50 µm × 112.5 cm bubble cell capillary. As a migration buffer, an aqueous solution consisting of 50 mM boric acid and 12.5 mM citric acid was dissolved in a NaPO₄ solution to adjust the pH to 7.0. Lactate was determined with an ion chromatographic system consisting of a Shimadzu LC-6A pump, a Tosoh CM-8010 conductivity detector, a Rhodyne 3125 injector with a 5-mL sample loop, and a Tosoh IC-Anion PW column operated at 40 °C. FT-IR spectra were recorded using a Horiba FT-710 spectrometer. Colloidal particles were dried and then diluted with KBr powder to prepare a tablet for IR measurements. Thermogravimetric analysis coupled with mass spectroscopy was performed using a JEOI 220 TG/DTA-MS system under helium atmosphere. UV–vis spectra were recorded with a Shimadzu UV-2400PC. X-ray photoelectron spectra (XPS) were observed with an ESCALAB 210 spectrometer (FISIONS Instruments) with Al Kα X-radiation. MO calculation was carried out with the PM 3 approximation by using Fujitsu WinMOPAC software (v. 2.0). The molecular volume was evaluated from the calculated data by using Free Wheel (v. 0.57T, Butch Software).

RESULTS
Structure of the Colloidal Particle. The average diameter of the particle observed by scanning electron microscope was 50–100 nm. It has been reported that the first layer of polypyrrole particles consists of surfactant stabilizer and that the thickness of the stabilizer layer in its solvated form is <25 nm.33 Elsewhere, we have pointed out from the results of FT-IR experiments that the PVP layer on the polypyrrole core is very sparse because of no distinctive sign of PVP presence in the spectra.12 Thermogravimetric analysis detected ~6 and ~2% mass decreases assigned to PVP decomposition at ~400 °C for the untreated and electrochemically treated colloidal particles, respectively. The thermogravimetric and FT-IR analyses indicate that PVP molecules are very sparsely implanted on the surface, and the smaller PVP content for the treated particle suggests that weak absorption of PVP on the polypyrrole core removed it during electrolysis and following washing procedures. Luk et al. also reported that a polypyrrole–dodecylbenzenesulfonate composite particle had a patchy or thin surfactant layer.34

The thin surfactant layer indicates that the enantioselectivity of the colloidal particles discussed later arose from the molecular recognition ability of the polypyrrole core. It has been reported that long alkyl molecules, which chemically attach onto an ITO glass or Au substrate, can create complementary cavities for hydrophobic molecules.35,36 However, the surface PVP layer in our system does not seem to be dense enough to activate this feature. Further, the sparse structure would not interrupt the diffusion of target molecules into the polypyrrole core.

Molecular Imprinting Protocols with Overoxidized Polypyrrole. Scheme 1 shows the overoxidization mechanism of polypyrrole presented by Beck et al.19 There are many lines of evidence that carbonyl groups are introduced in the polymer backbone after overoxidation.20–23 Further, the presence of carbonyl has also been suggested by the XPS analyses of deconvoluted signals and by surface derivatization with trifluoroethanol.20 Indeed, the FT-IR spectra of the colloidal particles prepared at different pH levels demonstrated increasing intensity of a carbonyl stretching vibration at 1670 cm⁻¹ with an increase in the pH on polymerization. According to Beck et al., polypyrrole salt, 2, is oxidized to undergo carboxylation, and A⁻ is dedoped as a result.19 We have utilized this feature to obtain a precisely controlled complementary cavity for A⁻.

Figure 2 illustrates a model for the molecular recognition developed here. A polypyrrole colloid doped with l-lactate is overoxidized to obtain a cavity (A), which is then used as the recognition site for structurally similar molecules (B). On the basis of this pseudocomplementary model, recognition of amino acids has been studied. We adopted this approach because of the difficulty in the preparation of polypyrrole doped with a target amino acid. Out of 21 protein amino acids, only glutamic and aspartic acids can be used as dopant, because the other amino acids do not carry a negative charge in neutral and acidic pH levels, in which pyrrole can be polymerized. Unfortunately, the formation of PPypVP/glutamate or aspartate did not occur with

Figure 2. A model of pseudomodel imprinting: (A) L-lactate is dedoped upon overoxidation of polypyrrole colloid; (B) L-alanine is taken up into the templated cavity.

Scheme 1

![Scheme 1](image-url)
appreciable rates, indicating that there is no appropriate protein amino acid as the dopant.

To confirm the feasibility of the pseudotemplate technique as an alternative approach, we have confirmed doping of l-lactate as a template for amino acid recognition. Although FT-IR spectra of PPyPVP/l-lactate did not show any distinctive vibrational features that can be assigned to lactate, probably because of overlapping with other bands from polypyrrole, chemical analysis allowed us to confirm incorporation of lactate. PPyPVP/lactate (polymerized at pH 4; 0.11 g after drying at 70 °C) dispersed in water and in 0.1 M NaOH ejected 0 and 48 μmol of lactate to the solution phases, respectively. Further, experimental evidence that neither an S(2s) nor S(2p) peak was detected in the XPS spectra of both the untreated and electrochemically treated colloids indicates that the polypyrrole cores were not doped with SO₄²⁻, which is generated as the reduction product of peroxodisulfate.

**Direct Overoxidation with Peroxodisulfate.** The inset in Figure 3 shows UV–vis absorption spectra of PPyPVP/l-lactate colloids dispersed in water. Two bands at 450 and >850 nm diminished with increased polymerization pH, the latter being assigned to electronic transition for bipolarons.37,38 Disappearance of the bipolaron band at the high pH region,39 which is caused by destruction of the conjugated structure of pyrrole as a result of the carbonyl group introduction (see Scheme 1), indicates that peroxodisulfate leads to overoxidation after polymerization (path 1 in Scheme 2).

The increasing overoxidation degree with an increase in the polymerization pH (curve A in Figure 3) shows that an OH-associated intermediate, such as 3 (Scheme 1), is involved in the overall mechanism. Cyclic voltammetric investigation of a polypyrrole/lactate film deposited on a glassy carbon electrode in order to obtain information about the oxidation mechanism was not successful because of the absence of well-defined peaks assigned to overoxidation. However, Beck et al. reported that a peak for overoxidation of a polypyrrole/BF₄ film shifted negatively with increasing pH (ca. −20 mV/pH in a pH region <10).38 The shift agrees with the participation of an OH intermediate, suggested by curve A in Figure 3, during polymerization.

**Overoxidation with Electrochemical Treatment.** More effective overoxidation of the colloids, which had already been overoxidized in some degree during polymerization, as stated in Section iii, was electrochemically carried out (curve B in Figure 3). The standard redox potential of peroxodisulfate (+1.81 V vs Ag/AgCl) is much higher than the potential for electrochemical treatment (+1.50 V), and therefore, it is not apparent why the electrochemical treatment was more effective than oxidation with peroxodisulfate (cf. curves A and B in Figure 3). This contradiction may be explained in terms of the difference in the pH, where two reactions took place (electrochemical treatment in 0.1 M NaOH; peroxodisulfate overoxidation at pH 2–6). The evidence that the voltammetric peak for overoxidation of a polypyrrole/BF₄ film shifts about −0.5 V as the pH changes from 4 to 13 indicates that overpotential for electrochemical overoxidation in 0.1 M NaOH is −0.2 V as high as that for peroxodisulfate overoxidation at pH 4.40 Thus, higher overoxidation efficiency for the electrochemical treatment can be concluded if the same overoxidation potential vs pH relationship applies to our colloid system. The identical vibrational frequencies observed in the FT-IR spectra of the untreated and treated colloids suggest that both the reaction paths, 1 and 2, lead to the same product, such as 8 (Scheme 2).

**Charge Accumulation on the Colloid.** The electropherogram of an untreated colloid polymerized at pH 4 consisted of many sharp peaks reflecting various sizes and charged states of the colloidal particle (Figure 4 A). The sharp dip (N) in the middle of the peaks, which is due to neutral species, such as water, in the injected sample, divided the charged states of the colloidal particle. The peaks appearing at migration times shorter than the dip can be assigned to positively charged colloids, and the peaks after the dip can be attributed to negatively charged colloids. In the inset, the ratio of the areas for these divided sections is plotted as a function of the pH on polymerization, where the areas corresponding to the positively and negatively charged species are defined as areas 1 and 2, respectively. These results demonstrate that as the polymerization pH increases, colloids are more extensively overoxidized (Scheme 2, path 1) to accumulate the negative charge. However, with the direct overoxidation, a positive charge of 20% was still present, even at pH 6.

The electrochemically treated colloids gave pherograms consisting of very broad peaks (Figure 4 B), and a peak instead of the dip was observed as the neutral species, probably due to electrolysis of impurities. However, the pherogram, solely composed of the post-dip peaks, shows that the electrochemical

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**Scheme 2**

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\[
\begin{align*}
\text{S}_2\text{O}_8^{2-} + \text{A} & \rightarrow 2\text{SO}_4^{2-} + \text{A}^+ \\
\text{SO}_4^{2-} + \text{OH}^- & \rightarrow \text{SO}_3^{2-} + \text{H}_2\text{O}
\end{align*}
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**Figure 3.** Effect of pH on absorbance of 0.14 g L⁻¹ colloids (A) untreated and (B) treated electrochemically. Inset: UV–vis spectra of aqueous dispersions of untreated polypyrrole colloids (0.14 g L⁻¹) prepared at different polymerization pHs. The reference was a filtrate of the colloidal dispersion.

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treatment can quantitatively convert the colloids to negatively charged particles. An electrochemically treated colloid, which had been polymerized at pH 4, showed a longer migration time than those for pHs 5 and 6; the centers of the gravity (moments) of the peaks were evaluated as 22.7, 21.2, and 19.8 min for the colloids polymerized at pH of 4, 5, and 6, respectively. However, it is not very clear yet why the greatest charge accumulation occurred at a polymerization pH of 4 in the case of electrochemical treatment. Expecting electrostatic interactions with the colloids, we have carried out all the uptake experiments at pH 1.0 to convert amino acids into cationic molecules (cf. Scheme 3 for acid/base equilibria of alanine).

**Scheme 3**

![Image](image.png)

**Figure 4.** Electropherograms of (A) an untreated colloid polymerized at pH 2.0 and (B) an electrochemically treated colloid polymerized at pH 4.0. A dispersion of 7 g L⁻¹ was injected for 60 s at 50 mbar (1 bar = 10⁵ Pa). Polarity of the electrode in the exit reservoir was negative. Inset: relative areas of pre- and postdip peaks for the untreated colloid as a function of polymerization pH.

**Enantioselectivity of the Electrochemically Treated and Untreated Colloids.** Both the untreated and electrochemically treated colloids exhibited high enantioselectivity for L-alanine (Figure 5). The untreated colloid showed enantioselectivity, which arose from the partially overoxidized structure, as discussed in Section iii. Although electrochemical treatment did not have an appreciable effect on the D-alanine uptake, L-alanine demonstrated substantially higher uptake with the treatment. As seen in Table I, polymerization temperature is an important factor for optimization of enantioselectivity: lower temperature was preferred for obtaining a precisely controlled complementary cavity, but we failed to synthesize a stable colloid at 15 °C because of macroscopic precipitation after electrochemical treatment. Thus, a temperature of 30 °C was selected here for the preparation of colloidal particles. The polymerization pH was also an important factor; pH dependence of uptake of alanine is listed in Table 2. The untreated colloid showed increasing uptake for both the enantiomers with an increase in the polymerization pH, and in contrast, the treated colloid exhibited a maximum for uptake of L-alanine at the polymerization pH of 4, probably resulting from the highest degree of overoxidation and negative charge accumulation at this pH (Figure 3). However, such a clear tendency was not found for uptake of D-alanine into the treated colloid. At pH 4, the quantitative conversion of polypyrrole (20% see inset in Figure 4) to the overoxidized form by electrochemical treatment led to the enantioselectivity increasing from 3.9 to 6.9 (Table 2).

Opposite enantiomeric uptake profiles were found for an overoxidized colloid using a D-lactate template, which had been polymerized at pH 6.9 (Table 1). The amounts of enantiomer uptake with the D-imprinted colloid were oppositely comparable to those for an L-template overoxidized colloid prepared at pH 6.0. The observed uptake symmetry with respect to the dopant chirality shows concrete evidence that this imprinting technique works as exactly as proposed in Figure 2.

**Size Effect on the Uptake Selectivity.** As is seen in Figure 6, uptake of an α-amino acid (Scheme 4) shows a clear tendency with its molecular volume. Glycine exhibited the greatest uptake amount due to the smallest side chain, whereas L-glutamic acid was scarcely taken up. Optically, inactivity of glycine, which adsorbed onto nonspecific sites on the colloid surface, might give rise to the greatest uptake.

**DISCUSSION**

There have been extensive efforts to develop MIP receptors that discriminate enantiomers. Amino acids frequently have been...
selected as model templates for evaluating the resolving power of such receptors against small molecules.41–46 Mosbach et al. have reported on an MIP-based sensing device for monitoring the fluorescence from dansyl-L-phenylalanine, in which the polymer can discriminate the D-enantiomers with a selectivity ratio of ~1.7.41 Liao et al. have presented that a D-tryptophan-imprinted MIP transporter, which is photopolymerized at 0 °C, preferentially transports the template molecule to the receiving solution with D/l concentration ratios ranging from 1.3 to 3.8.42 Yoshida et al. have reported a moderately high enantioselectivity, ~1.4, by using a MIP receptor prepared with the surface molecular imprinting technique for recognition of optically active tryptophan methyl ester.43 These published results certainly allow ranking overoxidized polypyrrole (the L/D concentration ratio, 11 ± 4) among the most attractive MIP materials. Further, it should be noted here that a bulky side chain of α-amino acid used in the above literature can lead to higher enantioselectivity. In our previous work, we observed an uptake ratio of ~2 toward L-alanine over D-alanine with an overoxidized L-lactate-imprinted colloid, which led to a relatively poor enantioselectivity arising from the unoptimized preparation conditions.43 However, we found much higher selectivity, even in the same preparation condition, when the template was changed to a more bulky molecule. With the colloid imprinted with L-phenyllactate prepared under the same unoptimized conditions, an observed selectivity ratio of 7 toward L-phenylalanine over the D-enantiomer suggested that an enhanced asymmetry in the molecular structure caused by a bulky side chain can lead to higher recognition performance. From these results, we believe that the overoxidized polypyrrole creates a much more precisely controlled cavity for optical resolution than previously reported MIP receptors. Another important advantage of overoxidized polypyrrole is the straightforward synthetic procedures: A tailor-made MIP receptor can be obtained with a two-step procedure (photopolymerization and electrochemical overoxidation) or even with the one-step procedure owing to a coupled polymerization and overoxidation.

Slow binding of a template to polymeric recognition sites often causes serious problems for practical applications of MIP receptors. Chromatographic applications of MIPs to stationary phases are apt to cause peak broadening and tailing, hence decreasing the separation efficiency.1,2 Enantioseparations in batchwise modes and in transport experiments often require >24 h until those systems fully develop substantial differences in enantiomer concentration.12,43 Therefore, it is worth mentioning here that the active surface area of colloidal particles, which is much greater than those for commonly used ground MIPs when compared by unit mass, allowed the system to reach absorption equilibrium in <10 min. The extremely fast uptake can be attributed not only to a large active area of the colloid surface but also to a short diffusion.

Table 2. Enantioselective Uptake of L-Alanine (ala) into Untreated and Electrochemically Treated Colloids as a Function of Colloid Polymerization pH a,b,c

<table>
<thead>
<tr>
<th>Polymerization pH</th>
<th>Untreated</th>
<th>Electrochemically Treated</th>
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<tr>
<td></td>
<td>PPyPVP/L-ala</td>
<td>OPPyPVP/L-ala</td>
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<tr>
<td>2</td>
<td>36</td>
<td>13</td>
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<tr>
<td>3</td>
<td>75</td>
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<td>4</td>
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<td>6</td>
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a Uptake was conducted by the addition of 10 mg of colloid in a 1.0 × 10⁻⁴ M alanine (5 mL) solution at pH 1.0 at 20 ± 1 °C. b n = 1. c Untreated colloid dispersed in water.

d Electrochemically overoxidized PPyPVP/L-ala.

Figure 6. Uptake of (●) L- and (○) D-amino acids into the treated colloid as a function of the molecular volume of amino acids at pH 1.0 on a colloid addition of 11 mg. Δ(Molecular volume) is the difference in the molecular volume between an amino acid and a lactate. The other experimental conditions are the same as those for Figure 5. Abbreviations: gly = glycine (optically inactive), ala = alanine, ser = serine, thr = threonine, val = valine, asn = asparagine, asp = aspartic acid, and glu = glutamic acid.
distance until a target molecule reaches a complementary cavity inside the colloid.

As a model of overoxidation, insertion of carbonyl groups frequently has been considered to be an important factor for describing the overoxidized polypyrrole property. However, the dipolar but basically electroneutral carbonyl group would not interact with cationic species strongly enough to explain the accumulative property of overoxidized polypyrrole. For example, overoxidized polypyrrole films have been used as a substitute for Nafion films; dopamine in the presence of ascorbate in biological samples has been successfully determined with this charge-selective electrode coating.47–49 If an overoxidized polypyrrole film works as a substitute for Nafion, then it would be more appropriate to consider that the film is ionized. Accordingly, we propose here from the results of the HPCE experiments that an anionic functionality such as carboxylate is created in overoxidized polypyrrole as well as nonionic dipolar groups, such as carbonyl groups (Figure 2). Zambonin et al. suggested the presence of carboxylate by the XPS analyses of deconvoluted signals and by surface derivatization with trifluoroethanol.20 Substantial uptake of a cationic amino acid at pH 1 indicates that such functionality should be highly acidic, but the absence of the sulfur signal in the XPS spectra of colloids makes insertion of sulfonate unlikely. The exact structure of the functionality is not clear yet, but –COOH as acidic as that of oxalic acid (pKₐ 1.3) might be created by overoxidation.

As seen in Figure 6, uptake of L-enantiomers vanished at a Δ(molecular volume) of 35 × 10⁻³ nm³, which corresponds to the expansion of a hemisphere from 0.16 nm (the van der Waals radius of the methyl group of lactate, approximated as a sphere) to 0.28 nm (see the inset). With this simplified model, an increase in the side chain radius of 0.12 nm completely suppresses the amino acid uptake. From the molecular volume dependence and enantioselectivity discussed here, we can safely conclude that lactate leaves a shape-complementary cavity after overoxidation dedoping in a precisely controlled fashion.

As for the interaction model with overoxidized polypyrrole, it is almost certain that the uptake of amino acid mainly relies on the anionic charge of the overoxidized polypyrrole. In fact, substantial uptake of alanine occurs at pH values less than the pKₐ (pKₐ = –COOH) of alanine,12 and lactic acid acting as either an anion or neutral molecule (pKₐ = 3.9) was not taken up into an L-lactate-imprinted colloid as much as cationic alanine (eg. uptake of L-lactic acid at pH 1.3 and L-alanine at pH 1.0 was 39 and ~110 nmol at a colloid amount of 4 mg). The anionic site, which mainly interacts with the –NH₃⁺ group of an amino acid, might contribute to the major part of enantioselectivity; if a cationic amino acid is taken up in the L-template cavity, then one of a side chain, –H or –COOH should interact with the anionic site instead of –NH₃⁺, hence reducing the interaction energy. However, it seems difficult to explain why the anionic site is preferentially created at a position close to the OH group of dopant lactate upon overoxidation (see Figure 2). Therefore, it seems more likely that the van der Waals repulsive force the polymer wall generates merely works as the main source of the structural discrimination, whereas the anionic charge randomly distributed in the colloid acts as only a driving force for uptake.

Although conducting polymers have been used as a molecular recognition matrix through doping of anionic recognition elements, they scarcely ever have been selected as a MIP. Recently, there have appeared some attempts at using conducting polymers as MIP receptors.10–13,17,18,50 The preparation and characterization of electrosynthesized poly(phenylenediamine) entrapping a neutral template, glucose, were presented,17 but a similar approach that used the same polymer with a phenylalanine dipolar (neutral) ion template found little enantioselectivity.18 It is not very certain at this moment that a molecule entrapped by this technique leaves a highly precise complementary cavity. Therefore, the anion doping/dedoping technique presented here seems to produce MIP receptors in more effective and well-defined manners through electrochemical control. Although successful attempts with this technique had not been found in the literature until we recently discovered high enantioselectivity of a polypyrrole film doped with L-glutamate.10–13 A similar approach based on a different concept has been found. Though conducted in a qualitative manner, Kaner et al. reported that polyaniline, doped with either of (R)- or (S)-camphorsulfonic acid, exhibited enantioselectivity for uptake of amino acid enantiomers after being dedoped with NH₃. However, the formation of a chiral structure induced in the polymer backbone may be responsible for the enantioselectivity, and it is not certain at this moment that the camphorsulfonic acids left the complementary cavity in the polyaniline texture because of the lack of available sufficient experimental evidence.50

CONCLUSIONS

It has been found that the new molecular imprinting technique presented in this paper is useful and highly efficient with respect to enantioselective separations. This technique is based on a simple polymerization procedure to obtain a tailor-made soluble polymer with recognition template. The results of HPCE experiments demonstrated that the overoxidized colloid particle is negatively charged, which can be a driving force for uptake of cationic species. The overoxidized polypyrrole colloid with a L-lactate template showed higher affinity for L-alanine than for D-alanine with an uptake ratio (L/D) of as high as 11 ± 4 under optimum conditions. Further, it is worth noting here that the extremely fast enantioselective uptake was attained owing to the short diffusion length for an amino acid to reach the complementary cavity in the colloid.

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