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A Molecularly Imprinted Copolymer Designed for Enantioselective Recognition of Glutamic Acid**

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A newly designed molecularly imprinted polymer (MIP) material was developed and successfully used as recognition element to fabricate a capacitive sensor for enantioselective recognition of glutamic acid (Glu). The MIP with a well-defined structure was synthesized on a gold electrode in one step by electrochemical copolymerization of *o*-phenylenediamine (*o*-PD) and dopamine (DA) in the presence of template molecule Glu. The resulting MIP material was characterized with a potentiostatic frequency scan method, cyclic voltammetry, capacitance measurements, atomic force microscopy, and X-ray photoelectron spectroscopy. The structure and recognition behaviour of the copolymer film to template molecule depended on its composition. The optimal composition was at the *o*-PD to DA molar ratio of 3:2. With a potentiostatic time scan method the copolymer displayed high enantioselectivity and sensitivity to the stereoselective rebinding of L- or D-Glu to their corresponding artificial receptor due to the exact definition of the imprint cavity. The capacitance response of the sensor for L-Glu or D-Glu was proportional to their concentration in the range of 16.7 to 250 μM. The enantiometric selectivity coefficients for L-Glu and D-Glu imprinted films against their respective enantiomers are 24 and 15, respectively. The resulting MIP capacitive sensors showed good reproducibility, stability and repeatability. This strategy opened a convenient way for preparation of enantioselective MIPs and recognition of enantiotropic molecules.

1. Introduction

Receptors existing in all living organisms show high selectivity towards their respective target molecules and have attracted considerable interest for analytical purposes.[1] However, the utilization of a natural product such as antibody, enzyme or microbe as recognition element often suffers from high cost and poor stability. Furthermore, some target molecules lack specific enzymes or receptors for detection need. Molecularly imprinted polymers (MIPs, also called artificial receptors or antibodies) that mimic the action of antibodies and enzymes overcome these problems and provide the desirable properties for highly selective molecular recognition, such as durability, specificity, stability at extreme conditions, ease of mass production and low cost.[2–5] Therefore, MIPs have been applied in separation,[6] artificial antibody mimics,[7,8] catalysis,[9–11] sensing devices,[12–15] and drug delivery.[16]

MIPs are frequently prepared with acrylate and vinyl derivatives polymeric materials.[1,7,10–12,14] Recently, the electrosynthesized polymers such as poly(dopamine) (PDA) and poly(*o*-phenylenediamine) (P*o*-PD) have been prepared for obtaining biomimetic recognition matrices.[17–19] These polymers are insulating and ultrathin, which are two important desirable features for the design of a MIP capacitive sensor.[20] Although the electrosynthesis leads to a zigzag chain structure of PDA and a wide variety of functional groups such as carbonylic and hydroxy groups can be introduced to the cavity of this polymer as the binding sites,[21] the polymer as MIP suffered from poor enantioselective recognition. On the other hand, the electrochemical copolymerization of *o*-PD with aniline can produce the branch chains, which make the formed film more rigid and compact.[22] This work designed a copolymer film using DA and *o*-PD as electrosynthesis elements to form a novel well-defined structure at an electrode. The designed copolymer film could be used for recognition of corresponding template analyte, leading to the capacitive sensing of target enantiomers, L- and D-glutamic acid (Glu).

Glu is a significant signal molecule in many biological activities. For example, Glu is a main excitatory neurotransmitter in the brain,[23] and increase of extracellular Glu is believed to be partially responsible for brain damage resulting from the central nervous and mental disorders.[24,25] So, the determination of Glu is of great significance in life and medical sciences. Several amperometric biosensors using an enzyme as activator have been prepared for the determination of L-Glu.[26,27] By using molecular imprinting technique and electrochemical quartz crystal microbalance (EQCM) the monitoring of enantioselective binding of L- and D-Glu has been achieved at an overoxidized polypyrrole film.[28] A molecularly imprinted TiO_2 thin film for the determination of L-Glu has also been developed as a QCM sensor.[29] However, the preparation of

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these MIPs is complicated and expensive, and they show poor specific selectivity, which limit their analytical application. Herein, the electrosynthesized copolymer of *o*-PD and DA showed excellent selectivity for recognition of template analyte. Our strategy for creating artificial Glu receptors combined electrochemical impedance spectroscopic measurements with the goal of simplifying the preparation of MIPs and lowering the cost, as well as improving selectivity and sensitivity. Thus the proposed capacitive sensors for both L- and D-Glu possessed low cost, good reproducibility, acceptable enantiometric selectivity, stability and repeatability.

2. Results and Discussion

2.1. Copolymerization of *o*-PD and DA

Figure 1 shows the cyclic voltammograms of DA (A) and *o*-PD (B) at the first, second and tenth cycles obtained at pH 7.4, respectively. DA shows a pair of redox peaks at –0.225 and –0.294 V and an irreversible oxidation peak at +0.320 V. Upon the continuous sweep these peaks decrease and disappear at the tenth cycle, producing a polymer film with a polyindole-like structure (zigzag chain) via an electrochemical-chemical-electrochemical reactions mechanism.[21] Similarly, *o*-PD shows one anodic peak at +0.375 V with a shoulder peak at +0.615 V, which correspond to the oxidation of *o*-PD, as the previous report.[30] In the solution containing both DA and *o*-PD at the same concentration the cyclic voltammogram shows all these electrochemical responses (Fig. 1C), but the peak currents are slightly lower than the sum of their corresponding peak currents observed in Figure 1A and B and their peak potentials shift greatly, indicating that the copolymerization of the two monomers occurs to form a copolymer of *o*-PD and DA due to the competitive coupling of radical cations resulting from the oxidation of each monomer, which makes the peaks decrease dramatically.

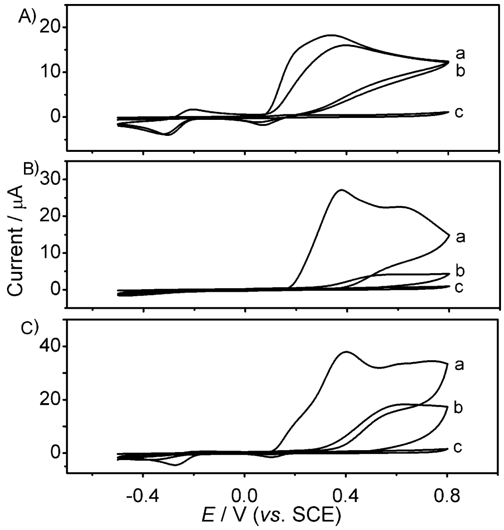


Figure 1. Cyclic voltammograms of 20 mM PBS (pH 7.4) containing A) 5.0 mM DA, B) 5.0 mM *o*-PD, and C) 5.0 mM DA and *o*-PD at a) 1st, b) 2nd, and c) 10th cycle. Scan rate: 20 mV s^{-1}.

The electrochemical copolymerization of *o*-PD and DA in the presence of template L-Glu in 20 mM phosphate buffer solution (PBS, pH 7.4) is shown in Figure 2. In comparison with Figure 1 no new peak is observed, thus Glu is electroinactive in the potential window. Dramatic decrease of these peaks cycle-by-cycle indicates that the formed film is insulating. After 10 scans an ultrathin artificial receptor layer is formed at the gold electrode, which blocks the further redox reactions of *o*-PD and DA, thus the peak currents decrease to 0. During this process the negatively charged Glu with an isoelectric point (p*I*) of 3.2[28] can be incorporated into the positively charged film[31] at pH 7.4 via both electrostatic affinity and hydrogen bonding interaction between the template molecules and the formed copolymer.

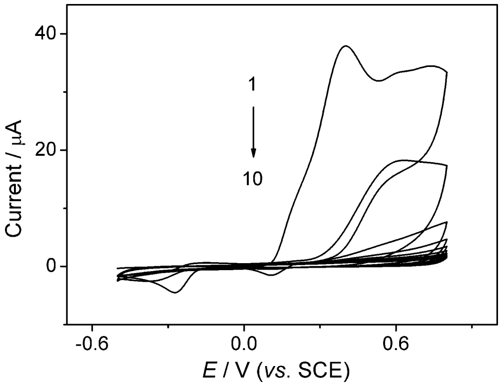


Figure 2. Cyclic voltammetric preparation of MIP on a gold electrode for 10 cycles in 20 mM PBS (pH 7.4) containing 5.0 mM *o*-PD, 5.0 mM DA and 10 mM L-Glu at 20 mV s^{-1}.

2.2. Characterization of the Copolymer

2.2.1. Surface Morphology of MIP

For sensitive capacitive transduction, the leakage and thickness of the formed film should be adequately low.[19] Thus the surface morphology of the film is very important. The root-mean-square roughnesses (RMS) of PDA, P*o*-PD, copolymer and L-Glu template incorporated copolymer are obtained from the atomic force microscopic images (Fig. 3a–d) to be 3.12, 0.78, 0.80 and 0.72 nm, indicating that the copolymer has more compact surface morphology than PDA film. The RMS value of 0.80 nm for the copolymer film is smaller than that of 1.57 nm for a MIP film believed to have a smooth and uniform surface.[32] The entrapment of the template molecules in the copolymer film does not influence its surface morphology (Fig. 3d), similar to the previous report.[32] Thus the change in capacitor behaviour upon addition of the target molecules to

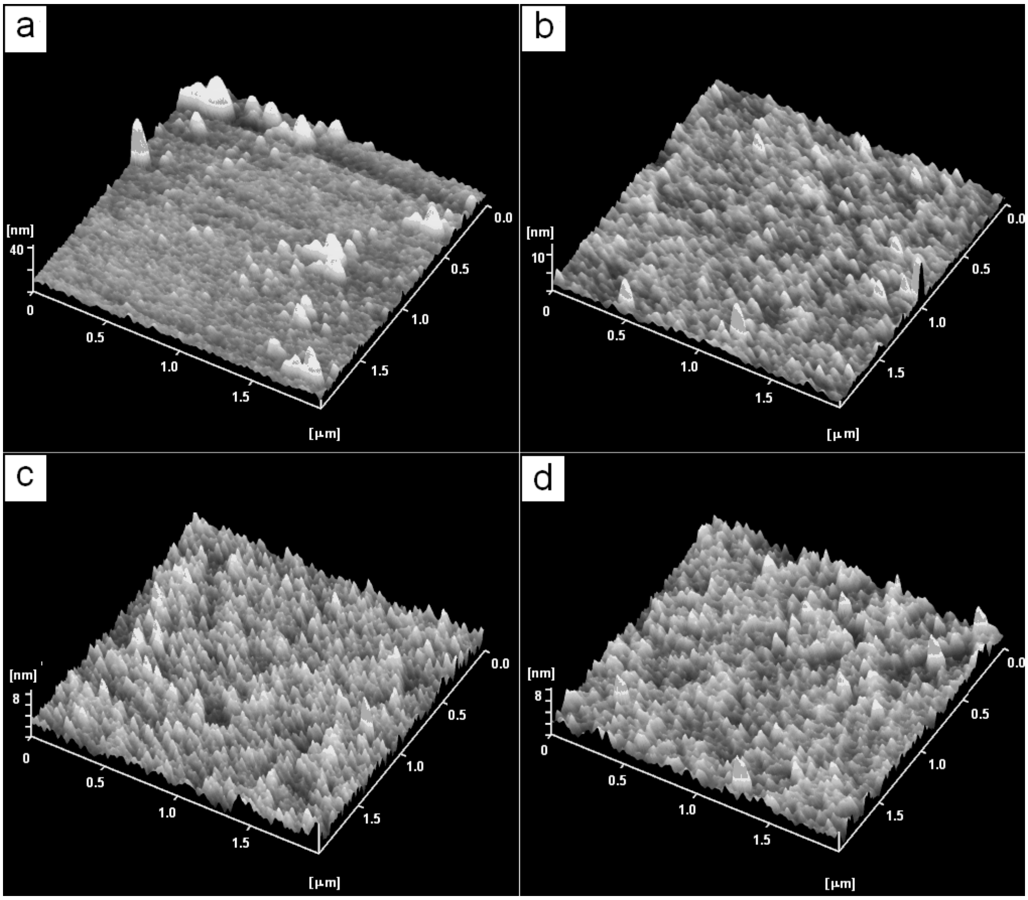


Figure 3. Tapping mode AFM images of a) PDA, b) Po-PD, c) copolymer, and d) L-Glu template incorporated copolymer.

the solution results from their entrapment into the copolymer without the change of surface morphology.

An ultrathin film has often many pinholes or defects, because film growth is favored on the top of the polymer instead of on the bare substrate.[19] These pinholes and defects also occurred in the copolymer film, which was demonstrated from the cyclic voltammetric responses and impedance measurements. At a bare gold electrode $[Fe(CN)_6]^{3-/4-}$ showed a couple of reversible redox peaks (Fig. 4a). Although the imprinted copolymer resulted in a dramatic decrease of the redox peaks (Fig. 4b), the response of $[Fe(CN)_6]^{3-/4-}$ was observable. In order to lower the leakage of the copolymer this work further used 1-dodecanethiol to fill the pinholes or defects of the copolymer film. After this procedure the cyclic voltammogram showed very low background and no response of the redox probe was observed (Fig. 4c), implying that these pinholes had been successfully filled. Thus the resulting MIP could be used for effective capacitive sensing.

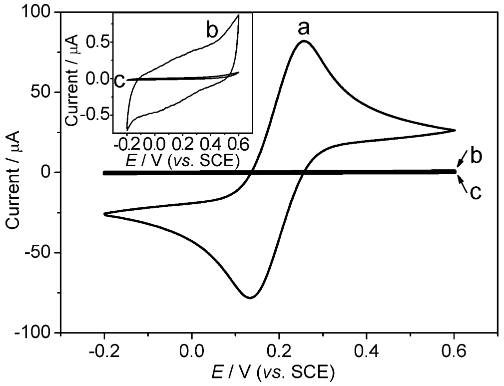


Figure 4. Cyclic voltammograms of a) bare, b) L-Glu template-copolymer modified gold electrode and c) the modified electrode treated with 1-dodecanethiol in 0.1 M KCl containing 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Scan rate: 50 mV s^{-1}. Inset: the magnification of curves b) and c).

2.2.2. Capacitive Transduction of MIP

The Bode plots of impedance magnitude and phase angle on a log scale as a function of frequency upon the treatment of 1-dodecanethiol were plotted in Figure 5. After treatment the slope of the impedance was approximately –1, and the corresponding phase angle became greater, indicating that the treatment improved the capacitive properties of the imprinted copolymer (Fig. 5, curve a versus curve b).

The slope of –1 and the phase angle close to –90° demonstrated the treated copolymer possessed good capacitive behavior over the frequency range from 10 to 1000 Hz, and an equivalent circuit of the MIP film modified electrode could be

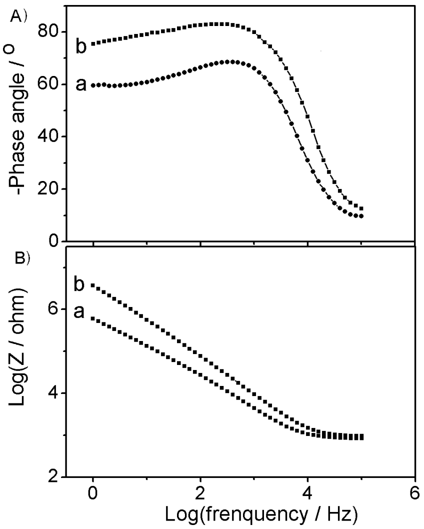


Figure 5. Bode plots of A) phase angle and B) logarithm of impedance versus logarithm of frequency in 20 mM PBS (pH 7.4) for a) L-Glu template-copolymer modified gold electrode and b) the modified electrode treated with 1-dodecanethiol.

analogous to the ideal Randles cell.[33] Thus the capacitance of this system could be measured with the potentiostatic time scan method and the following equation:[34]

$$C = -\frac{1}{2\pi f Z_{\mathrm{im}}} \tag{1}$$

Here, C is capacitance, f is AC frequency (Hz), and Z_{im} is the imaginary part of the impedance. The preparation of the electrosynthesized copolymer imprinted with template molecules and the enantioselective capacitive sensing process were outlined in Scheme 1.

It can be seen from Figure 5A that the L-Glu template-copolymer film shows the maximum phase angle at the frequency of 398 Hz, thus this frequency was chosen for capacitance measurements, at which the proposed template-copolymer film showed the best response. After the template molecules were removed from the imprint cavities, these cavities were occupied by the water and buffer species. Upon addition of L-Glu into the buffer solution the L-Glu molecules rebound to the imprinted film, which was driven by the electrostatic interaction between the copolymer and negatively charged L-Glu, the hydrogen bonding formed between the amino and oxygen-containing groups of L-Glu and nitrogen- and oxygen-containing groups in the copolymer and the structural complementarity of the cavity and L-Glu molecule. The rebinding of the L-Glu molecules decreased the dielectric constant ε of the MIP film due to the exclusion of water and buffer species, thus decreased the capacitance following the equation:[35]

$$C = \frac{\varepsilon \varepsilon_0 A}{d} \tag{2}$$

where C and ε is the capacitance and dielectric constant of the imprinted film, respectively, ε_0 is the permittivity of free space (8.85×10^{-14} F cm^{-1}), A is the electrode area (cm^2) and d is the imprinted film thickness (cm).

P*o*-PD and PDA have been used as artificial receptor layers for recognizing different molecules.[17–19] Here, the capacitance decrease $-\Delta C$ relative to the initial value C_0, $-\Delta C/C_0$, is used to evaluate the recognition behaviors of these polymer films. As seen from Figure 6, at all L-Glu concentrations, both the imprinted P*o*-PD and the imprinted PDA give relatively low capacitance changes upon the rebinding of L-Glu, which is disadvantageous to capacitive sensing. The reason may be assigned to the structures and functional groups of these polymers, which affect greatly the formation of imprint cavities. The electropolymerization of DA has the most likelihood to form a zigzag chain structure polymer,[21] which leads to a poor definition of imprint cavities. Moreover, a coarse-grain surface morphology of PDA observed in Figure 3a also has a negative influence on the film behavior.[36,37] The relatively poor recognition of P*o*-PD to L-Glu results from the lack of oxygen-containing group in the polymer and weaker interaction of the imprinted P*o*-PD than the imprinted copolymer with the template molecules. The exact definition of imprint cavity in the imprinted copolymer and its strong electrostatic and hydrogen bonding interactions lead to excellent recognition of the copolymer to L-Glu. Therefore, the prepared imprinted copolymer possesses high selectivity and significant affinity.

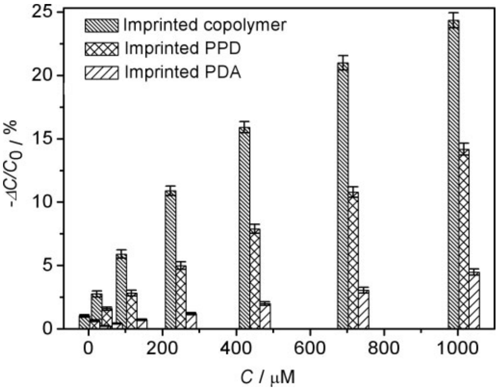
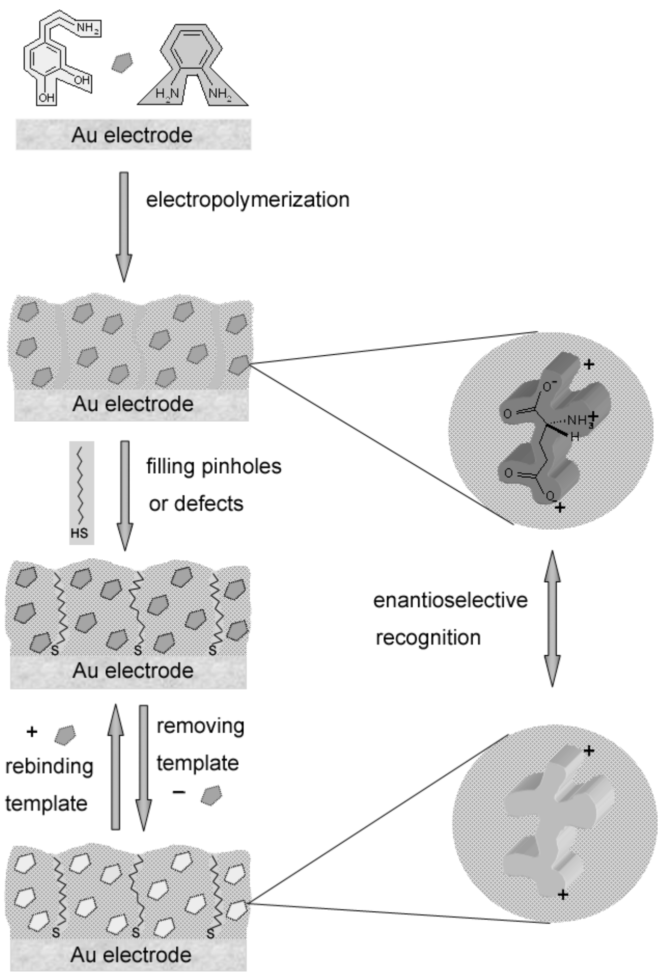


Figure 6. Graphical comparison among L-Glu-imprinted copolymer, P*o*-PD or PDA towards L-Glu. Error bars represent RSD, $n = 4$, measurements are carried out at the same films.

2.2.3. XPS Characterization of MIP

X-ray photoelectron spectroscopy (XPS)[32] was used to investigate the surface composition of the copolymer film prepared at the optimal experimental conditions in the absence of the template without treatment with thiol. As shown in Figure 7A, the XPS survey shows intense signals of C1s at 285 eV, N1s at 398 eV and O1s at 531 eV. The atomic ratio of carbon to nitrogen can be used to estimate the surface composition of the copolymer film. Providing the polymer surface contains X moles of *o*-PD ($C_6H_8N_2$) and Y moles of DA ($C_8H_{11}NO_2$).



Scheme 1. Fabrication process of MIP film.

Then, Equation 3 can be deduced to estimate the ratio of *o*-PD to DA:

$$X/Y = (8 - C/N)/(2C/N - 6) \quad (3)$$

Where C and N represent the XPS corrected peak areas of carbon and nitrogen. The ratio of peak areas is 4.25:1, thus an actual *o*-PD to DA molar ratio of 3:2 is obtained. The fitted C1s XPS spectrum (Fig. 7B) displays three peaks at 284.6, 285.6 and 287.9 eV attributed to C–C, C–N (C=N, C–N$^+$) and C=O groups, respectively, which are in good accordance with the assumed chemical composition of the copolymer film. While the XPS spectrum of P*o*-PD film shows the ratio of 3.0:1 of C1s to N1s peak areas, suggesting the nonexistence of monomer DA, which reveals a significant difference between P*o*-PD and the copolymer film. These results indicate that both P*o*-PD and PDA are actually incorporated into the copolymer film.

2.2.4. Effect of Chemical Composition on Recognition Behavior of MIP

The recognition behavior of the copolymer films towards the template (L-Glu) depends on the initial ratios of *o*-PD to DA

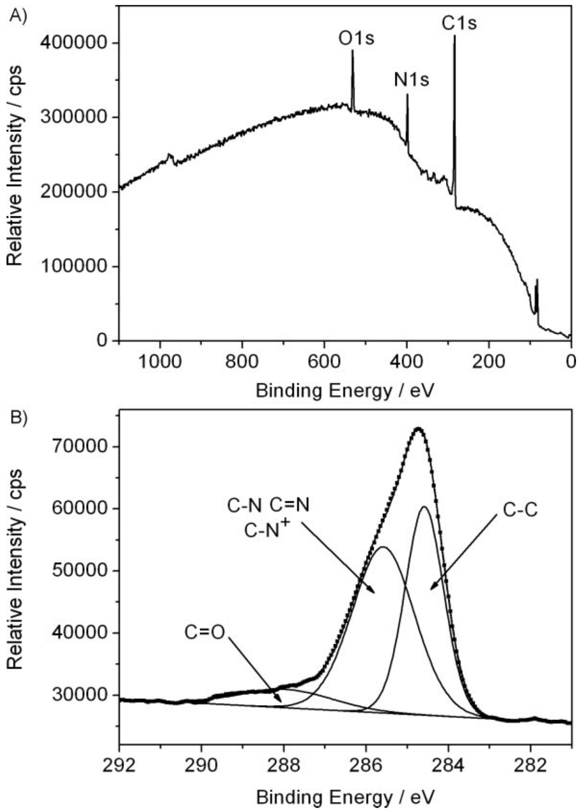


Figure 7. A) XPS survey and B) fitted C1s XPS spectrum of the copolymer film without the template.

(Fig. 8), indicating the microstructures of the copolymer films obtained at different initial molar ratios of *o*-PD to DA are different. Optimal recognition behavior is achieved at the initial *o*-PD and DA concentrations of 5.0 mM, corresponding to a microstructure of the copolymer film with an actual *o*-PD to DA ratio of 3:2. The results also support the conclusion that both *o*-PD and DA are actually incorporated into the copolymer film.

2.3. Selective Response of L-Glu and D-Glu Imprinted Electrodes

Considering the good capacitance performance of the copolymer film, a capacitive sensor for L-Glu based on the L-Glu

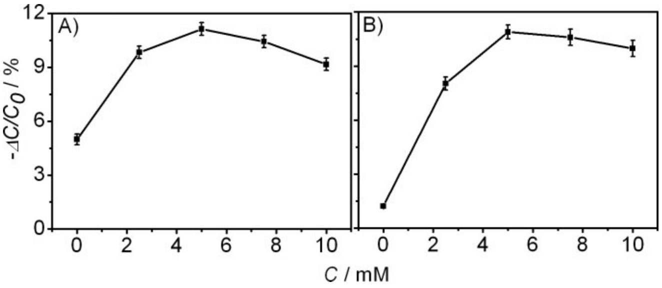


Figure 8. Effects of A) DA concentration in the presence of 5.0 mM *o*-PD and B) *o*-PD concentration in the presence of 5.0 mM DA on the selective recognition behavior of the copolymer films towards 250 µM L-Glu.

imprinted copolymer film was fabricated. Figure 9A shows the capacitance response of the sensor upon addition of L-Glu. With the increasing concentration of L-Glu the capacitance decreases, which can be ascribed to the occupation of imprint sites in the imprinted film by L-Glu molecules. At each addition the capacitance trends to a stable value in about 10 min. When the rebinding trends to saturation of imprint sites the capacitance trends to a constant value. The plot of the relative capacitance decrease ($-\Delta C/C_0$) versus L-Glu concentration is shown in Figure 9B. The plot shows a linear response in the concentration range from 16.7 to 250 μM with the regression equation of $-\Delta C/C_0 = 0.042c$ (μM) + 0.613 and a correlation coefficient of 0.9976. The detection limit was 4.7 μM ($S/N = 3$).

At the non-imprinted copolymer modified electrode prepared by electrosynthesis in the same PBS containing DA and *o*-PD without the presence of template molecule, no significant capacitance change was observed upon injection of L-Glu (Fig. 9b). The small capacitance change produced at high Glu concentration was due to the weak interaction of L-Glu with the functional groups that distributed randomly through the film, which led to nonspecific binding of some L-Glu molecules. These results demonstrated that the capacitance change at the imprinted copolymer modified electrode resulted from the entrapment of the template L-Glu molecules into the cavities in the imprinted copolymer.

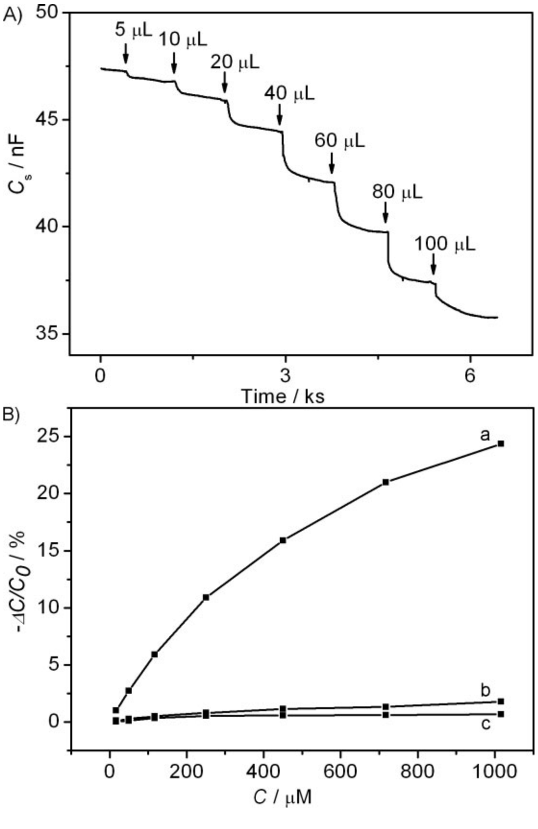


Figure 9. A) Capacitance change of L-Glu-imprinted copolymer film upon successive injection of 10 mM L-Glu with marked volumes into 3.0 mL deaerated 20 mM PBS containing 0.1 M KCl (pH 7.4) and B) dependences of relative capacitance change on the concentrations of L-Glu at a) L-Glu-imprinted and b) non-imprinted copolymer films, and c) D-Glu at the same L-Glu-imprinted copolymer film.

Similarly, the electrode modified with the D-Glu imprinted copolymer also presents good selectivity towards D-Glu recognition. The real-time response of the proposed MIP capacitive sensor to the introduction of D-Glu is shown in Figure 10A. A notable decrease in the capacitance is observed after injecting D-Glu into the detection solution, revealing the insertion of D-Glu into the imprinted copolymer film. In the concentration range of 16.7 to 250 μM the relative capacitance decrease is proportional to the D-Glu concentration with a linear regression equation of $-\Delta C/C_0 = 0.045c$ (μM) + 1.16 and a correlation coefficient of 0.9924 (Fig. 10B). The detection limit was 5.9 μM ($S/N = 3$).

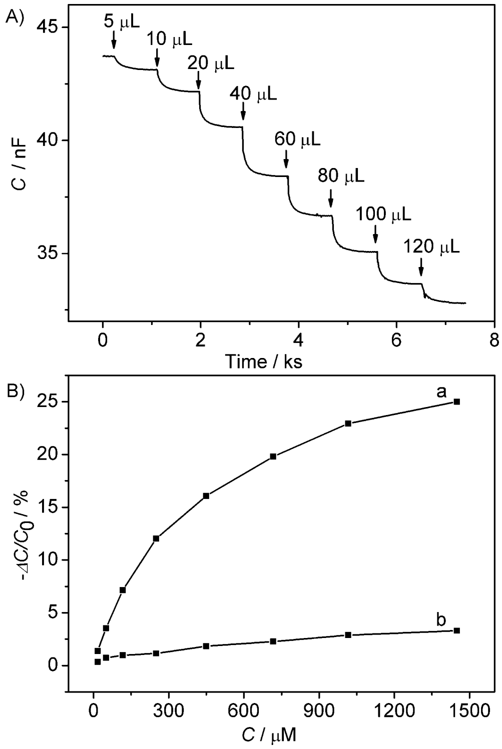


Figure 10. A) Capacitance change of D-Glu-imprinted copolymer film upon successive injection of 10 mM D-Glu with marked volumes into 3.0 mL deaerated 20 mM PBS containing 0.1 M KCl (pH 7.4) and B) dependences of relative capacitance change on the concentrations of a) D- and b) L-Glu at the same D-Glu-imprinted copolymer film.

2.4. Selectivity, Reproducibility, and Repeatability of L-Glu MIP Capacitive Sensor

To evaluate the selectivity of the MIP capacitive sensor for L-Glu, the interference of amino acids with different structures, such as D-Glu, L-glutamine, L-aspartic acid, L-serine, L-cysteine, L-glycine, L-leucine, L-tyrosine, L-phenylalanine and L-lysine, were examined. Figure 11 indicates the selectivity of MIP capacitive sensor is closely associated with molecular structure (size and shape), functional group and charge of the interfering species. At L-Glu imprinted copolymer L-glutamine shows

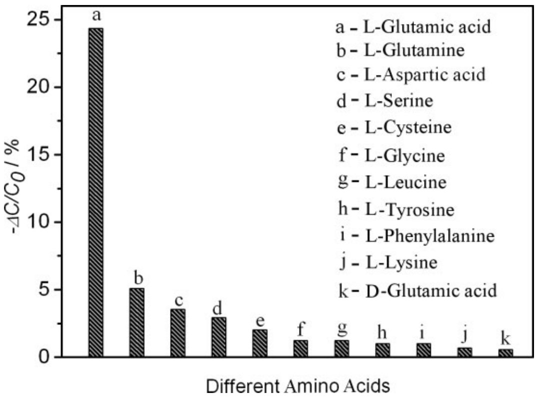


Figure 11. Relative capacitance change of the MIP capacitive sensor for 1.0 mM L-Glu and other amino acids at the same L-Glu-imprinted copolymer film.

some apparent capacitance change due to its analogous structure and negative charge, which makes it easily enter the recognition sites applicable for L-Glu. Only small quantities of capacitance changes are observed for other substrates at a high concentration of 1.0 mM. This confirms that the sensor based on the artificial L-Glu receptor has a good selectivity for its recognition.

When the size of interfering species was smaller than the cavity, the capacitance change decreased with the decreasing molecular size, while larger molecule produced smaller capacitance change when the size of interfering species was larger than the cavity. This phenomenon could be visually explained as a sieve-like effect in view of steric limitations for large molecules. Cationic species such as L-lysine hardly diffused into the imprint cavities due to an electrostatic repulsion effect between the positively charged polymer chain and the positively charged molecules.

The enantioselectivity of the artificial receptor towards the template molecules was also very excellent. As seen from Figure 9B the L-Glu imprinted copolymer showed very weak capacitance response to D-Glu. The linear slope for L-Glu response was 24 times larger than that for D-Glu response. Thus the enantiometric selectivity coefficient for L-Glu imprinted sensor was 24, which was 5 times higher than that previously reported.[29] Similarly, the enantiometric selectivity coefficient for D-Glu imprinted sensor was 15 (Fig. 10B). The higher enantiometric selectivity coefficient of L-Glu imprinted sensor may be assigned to the difference in film thickness. As previously reported,[28] the film thickness has great influence on the enantiomorphic property of the template molecule, and D-Glu imprinted film is prone to be more thicker than L-Glu imprinted film. The increase of film thickness has a negative effect on the recognition behavior of MIP capacitive sensor for template molecule due to the increasing nonspecific adsorption, leading to lower enantioselectivity of the D-Glu imprinted sensor than the L-Glu imprinted sensor.

The preparation of the imprinted copolymer film with electrosynthesis method is controllable and simple, thus it can be produced in batch. Four capacitive sensors for L-Glu prepared using L-Glu imprinted films, prepared independently, showed a relative standard deviation (RSD) of 3.8 % for measurements of relative capacitance change at 250 μM L-Glu, indicating a good fabrication reproducibility. At the same time the sensors showed good stability. After they were stored in a desiccator at room temperature for one month no obvious change in the capacitance response could be observed.

After the sensor recognized the target molecules, it could be restored by washing repeatedly with alternate 50 mM HCl and doubly distilled water. The regeneration of L-Glu MIP capacitive sensors for L-Glu showed good repeatability with a RSD of 3.5 % ($n = 4$). Moreover, the repeatability could maintain for more than 30 times regeneration at each sensor.

3. Conclusion

The designed molecularly imprinted copolymer of *o*-PD and DA has successfully been used as recognition element for chiral discrimination of Glu. The proposed electrosynthesis method for preparation of the MIP is simple, convenient and low-cost. The imprinted copolymers show excellent affinity and high enantioslectivity towards enantiomers of Glu due to the exact definition of imprint cavities and the strong interactions between the template molecules and their artificial receptors. It is the first molecularly imprinted material prepared with the copolymerization of *o*-PD and DA. The sensors with acceptable sensitivity, stability, good reproducibility and repeatability are also the first MIP capacitive sensors for both Glu enantiomers. The proposed copolymer of *o*-PD and DA could be further imprinted as artificial receptors for enantioselective recognition of other enantiotropic amino acids, drugs and proteins.

4. Experimental

Reagents: Dopamine hydrochloride and L-glutamic acid (Glu, 99.5 %) were purchased from Fluka (Buchs, Switzerland). D-Glu (99 %) and L-glutamine were purchased from Sigma (St. Louis, MO, USA). L-aspartic acid was purchased from Amresco (USA). L-glycine, L-serine, L-cysteine, L-leucine, L-lysine, L-phenylalaine, L-tyrosine and *o*-phenylenediamine were obtained from Shanghai Reagent Factory (Shanghai). *o*-PD was recrystallized twice prior to use. 1-Dodecanethiol was from Songon (Shanghai, China). Phosphate buffer solution (PBS, pH 7.4) was prepared by mixing 20 mM aqueous solution of NaH_2PO_4 and 20 mM aqueous solution of Na_2HPO_4. All other chemicals were of analytical reagent grade, and doubly distilled water was used throughout.

Apparatus: All electrochemical measurements were performed on an AutoLab electrochemical workstation (ECOCHEMIE BV Utrecht, The Netherlands) controlled by an FH560-compatible computer. A bare or film-coated gold electrode (2.0 mm in diameter, CH Instruments, Inc. USA) was used as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the counter electrode. All measurements were carried out at 25 ± 0.1 °C. Atomic force microscopic measurements were done with a Molecular Imaging Pico SPM (USA) in tapping mode with a 10 μm scanner. X-ray photoelectron spectroscopic measurements were performed with an ESCALAB 250 spectrometer (Thermo-VG Scientific) with ultra-high vacuum generators. A monochromatic Al K_a X-ray source was operated in the CAE (constant analyzer energy) mode (CAE = 70 eV for survey spectrum and CAE = 20 eV for C1s spectrum).

Fabrication of Imprinted Copolymer Film of o-PD and DA: Prior to use, the gold electrodes were polished to a mirrorlike finish with 0.3 and 0.05 µm alumina slurry followed by sequential sonication in ethanol and doubly distilled water to remove the possible contamination. The electrodes were then cyclically scanned between 0 and 1.5 V in 0.5 M H_2SO_4 until the reproducible cyclic voltammogram were obtained. Electrochemical polymerization was performed in deoxygenated PBS (20 mM, pH 7.4) containing 5.0 mM *o*-PD and 5.0 mM DA in the presence of 10 mM L- or D-Glu with cyclic voltammetry in the potential range from –0.5 to 0.8 V at 20 mV s^{-1}. The obtained copolymer modified electrode was immersed in an ethanol solution of 10 mM 1-dodecanethiol for 12 h to fill the pinholes or defects. Finally, the modified electrodes were washed with 50 mM HCl and doubly distilled water alternately to remove the template molecules and adsorbed thiol. The non-imprinted electrodes used as control were prepared under the same experimental conditions without the template.

Electrochemical Measurements: Cyclic voltammetric measurement was performed in 0.1 M KCl solution containing 10 mM $K_3Fe(CN)_6$/$K_4Fe(CN)_6$ at 50 mV s^{-1}. Bode plots for capacitance measurement were obtained in 20 mM PBS (pH 7.4) using a potentiostatic frequency scan method, and the capacitance change of the artificial receptor modified electrode was evaluated with a potentiostatic time scan method. The capacitance values were collected at a frequency of 398 Hz. The time interval for the addition of Glu was about 10 min. After each measurement, the sensor was regenerated by washing it with 50 mM HCl and doubly distilled water alternately to remove the template.

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