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Molecularly imprinted electrochemical sensor, formed on Ag screen-printed electrodes, for the enantioselective recognition of D and L phenylalanine

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ABSTRACT

In this study, electrochemical sensors for the enantioselective recognition of D and L phenylalanine were prepared using a molecular imprinting technique in which the electro-polymerization of pyrrole was carried out by Chronopotentiometry(CP) with the target molecules being present on a Ag screen printed electrode's (SPE) surface. The sensing performance was evaluated by multi-potential steps at 0 and 2 V(vs. Ag/AgCl) held for 1 s and 2 s, respectively, for 20 cycles (with the two enantiomers being present at the same concentration). The individual selectivity's for L and D- phenylalanine on their respective imprinted films were estimated to be L/D = 23.480 ± 2.844/1 and D/L = 19.134 ± 1.870/1 respectively, based on the current change between 0 and 2 V (vs. Ag/AgCl) with the two enantiomers being present at the same concentration (10 mM). Several parameters affecting recognition ability were investigated including: cross-selectivity of D and L- phenylalanine imprinted film, phenylalanine concentration effects, interfering species, deactivation and the storage life of electrode. The phenylalanine imprinted films were also characterized by AC impedance, chronoamperometry, Fourier-transform infrared spectroscopy (FTIR), Scanning Electron Microscope(SEM), and Energy Dispersive X-Ray Spectroscopy (EDS). Finally, a recognition mechanism for the interaction of the polypyrrole film with its template under the influence of applied negative and positive potentials is proposed.

1. Introduction

Screen-printed electrodes (SPEs) are widely used as sensing element components in analyte-specific biosensors, in which the advantages of miniaturization, design flexibility, process automation, good reproducibility, and a wide choice of available materials are all factors able to be exploited. Enzymes, microorganisms, antibodies, nucleic acids and receptors can be employed in the construction of screen-printed biosensors (Alonso-Lomillo et al., 2010; Moreira et al., 2016; Tsai et al., 2016). Such species can be immobilized onto the surface of the working electrode, which can be formed from materials such as carbon, silver or gold; through adsorption, entrapment, microencapsulation, cross-linking or covalent attachment. Disposable SPEs biosensors are commonly used as practical devices for the rapid, easy-to-use and low cost determination of many substances of analytical interest (Ma et al., 2016; Hayat and Marty, 2014).

Molecular imprinting is a popular method of creating molecular recognition sites that has attracted growing interest for the preparation of 'complementary impressions' of target molecules since the early development of the technique in ~1972 (Wulff and Sarhan, 1972). The method relies on the molecular 'recognition' shown by the imprint for the imprint template or target molecule. A pre-complex is first formed by the template, functional monomers and cross-linkers; which after polymerization is completed, results in interactions that maintain the position of the functional groups relative to the functional sites on the template, resulting in (after template extraction) selective molecular recognition sites. At the end of the polymerization stage, the polymeric matrix has specific recognition sites; which after template removal, with suitable desorption agents, results in molecularly imprinted polymers (MIPs) able to bind the template with higher selectivity than competing species (Algieri et al., 2014). MIPs can be produced using various polymerization methods with differing combinations of cross-linkers, functional monomers and solvents (Mosbach, 2006; Saylan et al., 2017; Vasapollo et al., 2011). The quality of the resulting MIPs, and their binding features, results from the combination of specific reagents, and also the experimental conditions employed, e.g. the initiator used and its quantity, the polymerization temperature etc, (Sharma et al., 2012; Zaidi and Shin, 2014; Turco et al., 2015). The preparation of polymeric materials with cavities that have the ability to selectively distinguish target molecules, by size, shape and functional

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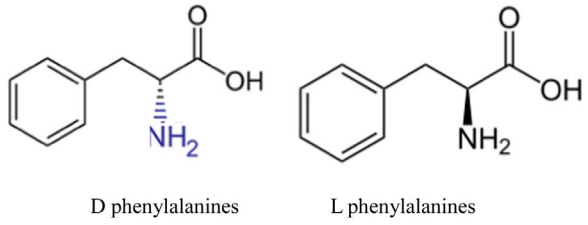

Fig. 1. The structures of D and L phenylalanine.

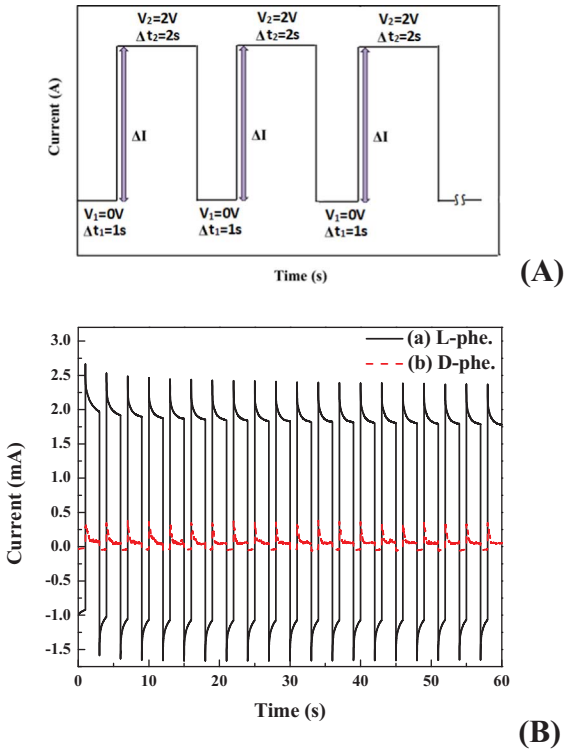

Fig. 2. (A) Multi-potential steps method by applying potentials V_1 and V_2 respectively kept for the time t_1 and t_2 for obtaining the current change ΔI between them periodically. (B) Typical rebinding test of L-phenylalanines imprinted/SPE for 10 mM (a) L- phenylalanines (b) D- phenylalanines solution.

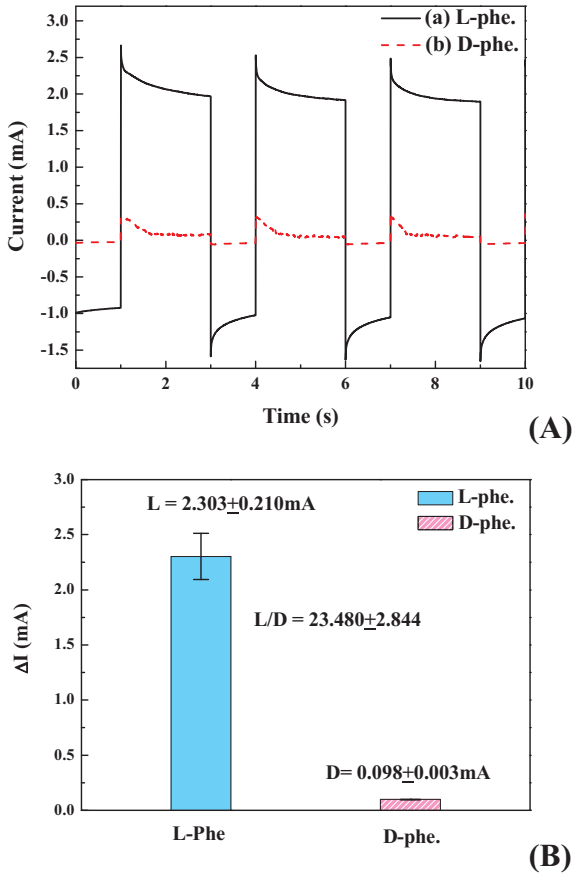

Fig. 3. Selectivity test of L- phenylalanine MIP/SPE (A) current response by multi-potential steps method applying potentials 0 and 2 V (vs. Ag/AgCl) respectively kept for 1 and 2 s for obtaining the current change ΔI between them periodically. (B) current change of rebinding test of L- phenylalanine MIP/SPE for 10 mM (a) L- phenylalanine (b) D-phenylalanine solution in phosphate buffer at pH 7.0.

group recognition, is an area of fast growing interest

Phenylalanine is an important amino acid that is a precursor to key neurotransmitters e.g. dopamine and epinephrine (Fernstrom and Fernstrom, 2007). HPLC is commonly used to analyze physiological phenylalanine concentrations, but the procedure is costly and time consuming (Silva et al., 2010). Because of the importance of these compounds, there is a growing need for innovative devices to directly monitor them in the food and pharmaceutical industries as well as for in vivo measurements in clinical settings.

Electrochemical sensors based on current measurements, derived from 'electro-generated' forces are commonly used to monitor reduction–oxidation reactions (Liang et al., 2005; J. Liu et al., 2016; B. Liu et al., 2016). Improving the sensitivity and selectivity of electrochemical biosensors is currently an important area of research.

In this study, an electrochemical phenylalanine sensor, based on molecularly imprinted polypyrrole, was fabricated and evaluated. Structurally, polypyrrole can provide a free electron for transfer in a polymer chain during an electrochemical reaction. Polypyrrole films possessing a positive charge readily allow the formation of imprints with anionic template molecules that result in good selectivities (Takeda et al., 2008; Schweiger et al., 2015; Zeng et al., 2017; Kong et al., 2010; Jara-Ulloa et al., 2013; Turco et al., 2015). It has been reported that the rebinding of amino acids on imprinted polypyrrole films can be achieved by applying a suitable potential to induce template adsorption (Liang et al., 2005). When the pH value of the rebinding solution is greater than the isoelectric point of the amino acid (A^-), the amino acid adopts an anionic form. Theoretically, the adsorption or reaction of an anion on a polypyrrole film involving the release and adoption of electrons can be expressed as in Eq. (1):

$$PPy + A^- \leftrightarrow [(PPy)^+A^-] + e^- \quad (1)$$

The adsorption and desorption reactions can be followed by monitoring current changes in an electrochemical system that incorporates the materials. We attempted to create shape complementary cavities in polypyrrole films, using electro-polymerization, for the selective recognition of the two enantiomeric amino acids D and L Phenylalanine, see Fig. 1. Recognition was quantitatively evaluated using a rapidly changing potential steps method to determine the stepped current changes.

Amino acid rebinding at the polypyrrole imprinted sites was induced by applying a positive potential; interestingly, we found that the imprinted materials were able to distinguish the D and L forms of phenylalanine. By using the same metal (Ag) as the electrode material and the same polymerization method to prepare the MIP, this study unintentionally found that the MIP coated on a SPE showed a significantly higher selectivity than a similar MIP formed on a flat plan electrode (FPE). Although polypyrrole based MIPs films have shown high selectivity in previous studies, little research has been directed towards investigating their long-term stability or their shelf life for biomarker applications (Kupai et al., 2017). In this study, conductive polypyrrole polymer MIP films, made without the use of a cross linking agent, were