Basic Electrochemistry Meets Nanotechnology: Electrochemical Preparation of Artificial Receptors Based on a Nanostructured Conducting Polymer, Polypyrrole

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Electrochemistry and bioanalytical chemistry are fundamental topics in the majority of undergraduate courses in instrumental analysis. University students perform laboratory exercises that integrate concepts in these areas. Usually, they apply simple electrochemical detection methods or simple enzymatic-electrochemical biosensors for the detection of glucose, alcohols, and so forth. However, current progress in sensor technology requires wider knowledge in preparation of nanostructured polymers, since sensors are suitable as biological recognition systems.

Laboratory experiments utilizing electrochemical detection are widely used in the chemical education process. The application of some electrochemical techniques for laboratory experiments have been described in this *Journal*: (i) the backgrounds and laboratory equipment essential for pulsed amperometric detection (PAD) *(1)*; (ii) the fundamentals of chronoamperometry, which are closely related to PAD *(2)*; (iii) electrochemical constant-potential detection of *p*-aminophenol at carbon electrode *(3)*; and (iv) detection of sugars at copper electrode *(4)*. Some detection methods were used following liquid chromatographic *(1, 3, 4)* separation of organic compounds. Here PAD was recognized as the more useful electrochemical detection technique when compared with constant potential-based detection methods *(1)*.

In addition, a few simple techniques were also published in this *Journal*: (i) electrochemical preparation of conducting polymers, polyaniline *(5)* and polypyrrole *(6)*, (ii) bioanalytical application of electrochemically synthesized polypyrrole (PPy) in a simple chemical sensor based on PPy doped by nitrate ions *(7)*, biosensor based on glucoseoxidase entrapped within PPy *(8)*, and (iii) fundamentals related to discovery, properties, and application of conducting polymers *(9)*. The state of the art in the science and chemical education clearly shows that nanotechnologies are becoming one of the current topics. The potential of nanotechnologies *(10)* and some methods of nanostructured polymer preparation *(11)* were reviewed in this *Journal*. Caffeine is a simple organic molecule that exhibits a unique biological activity *(12, 13)*. It has attracted considerable attention in educational projects related to the extraction *(14)* and detection of this unique compound *(15, 16)*. Attempts to obtain proper caffeine-selective systems *(17)* and caffeine-molecularly imprinted polymers *(18)* have been reported.

A combination of all topics mentioned: electrochemistry, polymer chemistry, and nanotechnology can be applied to the preparation of molecularly imprinted conducting polymers (MIPs). We believe that MIPs are very promising in analytical systems, and it is reasonable to introduce the preparation of such polymeric structures into the process of chemical education.

Fundamentals of Polypyrrole Application

Polypyrrole is conductive and biocompatible. It causes minimal and reversible disturbance to the working environment and protects electrodes from fouling *(19)*. PPy is an effective immobilizing material *(20)* and is easily synthesized by chemical and electrochemical polymerization *(21)*. Electropolymerization is an elegant method of polymeric film deposition *(7, 22)* and has found an increasing use in the development of bio- and immuno-sensors *(23, 24)*. Useful copolymeric structures based on PPy have also been developed *(25)*. Artificial receptors have been gaining importance as a possible alternative to immobilized biomolecules in analytical systems. Molecular imprinting is recognized as a versatile technique for the preparation of artificial receptors based on molecularly imprinted conducting polymers (MIPs) containing tailor-made recognition sites. These highly-stable synthetic polymers possess molecular recognition properties owing to cavities in the polymer matrix that are complementary to the analyte (ligand) both in the shape and in the positioning of the functional groups (Figure 1) *(26, 27)*. Among other electrochemical binding detection techniques *(7)* pulsed amperometric detection technique is suitable for direct analyte detection *(28)*. In this method the changes in charge densities or conductivities are used for transduction *(29)* and do not need any auxiliary reaction *(30, 31)*.

Overview of the Experiment

The aim of this experiment is to apply PAD for the detection of analyte during operation of a molecularly imprinted polypyrrole (mPPy) based affinity sensor.

Chemicals, Equipment, and Electrochemical Setup

Pyrrole is purified by passing through 5-cm length column filled with 99.8% alumina (3-mm). All electrochemi-

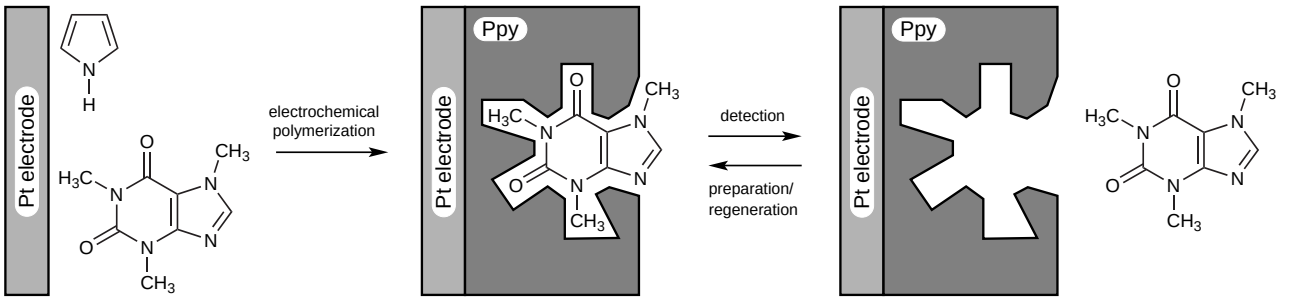

Figure 1. Formation of molecularly imprinted polypyrrole by caffeine and its application in affinity sensor design.

cal experiments are performed using a conventional three-electrode system. PAD is applied to the detection of dedoping–redoping mPPy by caffeine. Caffeine can be purchased or extracted from coffee or tea *(13)*. The experiments are performed during three 2-hour laboratory periods.

Preparation of Artificial Receptors

Electrodes are pretreated following the protocol described in the Supplementary Material.[W] Once the solution of H_2SO_4 is determined to be free of oxygen, a series of voltammograms are recorded. Potential cycling should continue until no differences in the voltammograms are detected. Platinization is recommended to improve the adhesion of the conducting-polymer film and simultaneously increase the number of catalytic-active sites on the electrode. For platinization, oxygen-free H_2PtCl_6 should be used. During platinization the reduction peak, indicating the surface covered by platinum clusters, should increase slightly during each of the five cycles applied. Note, that oxygen-free solutions should be used during polymerization. If oxygen is present only short pyrrole oligomers are synthesized and they will not form a polymeric layer or the layer formed will be unstable. Depending on the success of the polymerization, a distinct, homogenous black or brown polymeric layer of overoxidized PPy will be synthesized on the surface of the electrode. The appearance of black (overoxidized) polypyrrole is more reliable and desirable at the proposed polymerization potentials. Caffeine plays a role during formation of the molecular template. After the elution of caffeine from the template, complementary binding sites are revealed allowing specific rebinding of the analyte.

Investigation of Artificial Receptors

The recognition sites obtained possess sufficient binding affinities to enable detection of caffeine. PAD is applied to investigate the elution of caffeine from the polypyrrole matrix (dedoping) and detect binding of analyte to molecularly imprinted polypyrrole (redoping). The differences in PAD signals are used to indicate redoping–dedoping of mPPy. The analyte desorption–adsorption processes can be described by:

(i) exponential rise to maximum during the elution of caffeine by blank buffer solution

$$y = c + a\left(1 - e^{-bx}\right) \quad (1)$$

where a, b, and c are constants (see the discussion in the Supplemental Material[W] for specific descriptions), y is the value of analytical signal , and x is time

(ii) exponential decay to minimum during the detection of caffeine by mPPy-based sensor in the sample because caffeine is binding to specific recognition sites of mPPy (all data presented in the Supplementary Material[W]).

$$y = c + ae^{-bx} \quad (2)$$

Application of Sensor for Determination of Caffeine Concentration in Beverages

Two methods for the estimation of analyte concentration are recommended: (i) using a simple calibration curve or (ii) the calculation of analyte concentration from the data obtained by standard addition method. The calibration of caffeine sensor is performed by measuring the analytical signal after incubation of the mPPy-modified electrode in different concentrations of caffeine-containing samples. This is approximated well by a hyperbola equation:

$$y = \frac{c + ab}{b + x} \quad (3)$$

(Data and derivation of equation are presented in the Supplementary Material.[W])

PAD measurement is performed before and after the incubation of the mPPy-modified electrode in the sample containing caffeine. The concentration of caffeine in the sample is obtained from the calibration plot. It is possible that the coffee solution contains some other compounds that can specifically interact with mPPy or directly electrochemically interfere with the analytical signal. To minimize this influence, the standard addition method is applied where known caffeine quantities are added into the same diluted coffee solution and analytical signals are measured after incubation. The points are approximated by eq 3 and constants are calculated. Then caffeine concentration in the sample is calculated by

$$|x| = \frac{-b + ab}{(y - c)} \quad (4)$$

The caffeine concentrations calculated by using the two methods slightly differ. In the case of the standard addition method, the caffeine concentration is lower by approximately 10–15%. By using the standard addition method, it is possible to estimate and eliminate the influence of other materi-

als distorting the analytical signal. The individual analytical parameters (detection range, PAD currents, signal evaluation time, etc.) of sensors can significantly differ. These parameters are dependent on mPPy layer thickness, morphology, and the surface volume concentration of individual sensors.

Hazards

Sulfuric acid and sodium hydroxide combines exothermically with water. Pyrrole monomer 98% is harmful by inhalation, ingestion, and skin absorption. Caffeine at high concentration is biologically active.

Summary

The protocol described is adopted for the preparation of mPPy during educational projects with limited resources. Other organic template molecules can be imprinted instead of caffeine. Additional analytical characteristics, such as selectivity, reusability, stability, statistical characterization of analytical method also can be measured.

Acknowledgment

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[w]Supplemental Material

Student handouts, notes for the instructor, extended laboratory protocols, and extended literature review are available in this issue of *JCE Online.*

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