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Short communication

Dynamic modulation of detection window in conducting polymer based biosensors

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ABSTRACT

Here we demonstrate a novel application that employs the ion exchange properties of conducting polymers (CP) to modulate the detection window of a CP based biosensor under electrical stimuli. The detection window can be modulated by electrochemically controlling the degree of swelling of the CP associated with ion transport in and out of the polymer. We show that the modulation in the detection window of a caffeine imprinted polypyrrole biosensor, and by extension other CP based biosensors, can be achieved with this mechanism. Such dynamic modulation in the detection window has great potential for the development of smart biosensors, where the sensitivity of the sensor can be dynamically optimized for a specific test solution.

1. Introduction

Conducting polymers undergo reversible swelling and shrinking when controlled electrochemically in an electrolyte system. When a voltage is applied, the electronic charge in the conducting polymer is altered; this is compensated by the ions transport between the polymer and electrolyte. When ions enter a polymer, it swells and when they exit it shrinks. The degree of swelling or shrinkage depends on the number and the size of ion exchange (Pei and Inganas, 1993; Michalska et al., 2003). This electrically stimulated actuation has been widely used in artificial muscles (Smela, 2003; Otero, 2008) and controlled drug-delivery systems (Pyo and Reynolds, 1996; Abidian et al., 2006). In this work, we explore this electrical responsive feature of conducting polymers in biosensing, which is one of the promising applications of conducting polymers. In practice, a biosensor which is designed to facilitate detection of a specific molecule at high concentrations is insensitive when sensing at lower concentrations, or vice versa. The detection window is constrained by the physical parameters of the sensor (i.e. reactive surface area and bio-reagent loading) which are controlled during the device fabrication (Malhotra et al., 2006). However, by integrating the reversible swelling and the biosensing capabilities of conducting polymers, we demonstrate a simple technique to dynamically control the detection window of a biosensor through electrical stimuli. In other words, it becomes possible to combine both low and high detection windows in one biosensor without compromising the sensitivity of the sensor.

We first investigate this concept using a molecularly imprinted polypyrrole (MIPPy) based biosensor for caffeine detection. Polypyrrole is a biocompatible conducting polymer, where its ion exchange properties are widely used in both electrochemical actuation (Pei and Inganas, 1993; Michalska et al., 2003) and biosensing applications (Palmisano et al., 2000; Yuan et al., 2000). For a molecular (caffeine) imprinting sensor, the sensor response is based on the specific rebinding of the caffeine to its imprint sites in the polymer matrix, which have the size and shape of the caffeine molecules. The detection window of the sensor is well defined by the number of caffeine imprints created in the polymer during polymer synthesis (Ebarvia et al., 2005; MeCluskey et al., 2007), as mathematically modeled by the binding isotherm of the system (see Supplemental information). For instance, a thick MIPPy film with a large volume can accommodate a large number of caffeine imprints, which enables detection at high concentrations, whereas the detection range can be brought down when a thin polymer film with limited imprint sites is used. Therefore, adjusting the film thickness is a direct way to fine-tune the detection window of the sensor.

Here, we present a simple strategy to alter the number of imprints available for caffeine rebinding by modulating the effective thickness of the polymer film under electrical stimuli as illustrated in Fig. 1. In equilibrium (Fig. 1a), the MIPPy is essentially a closed structure, where the caffeine imprints embedded in the polymer matrix are unrecognised and inaccessible by the caffeine molecules in the solution. Hence, the MIPPy sensor is not

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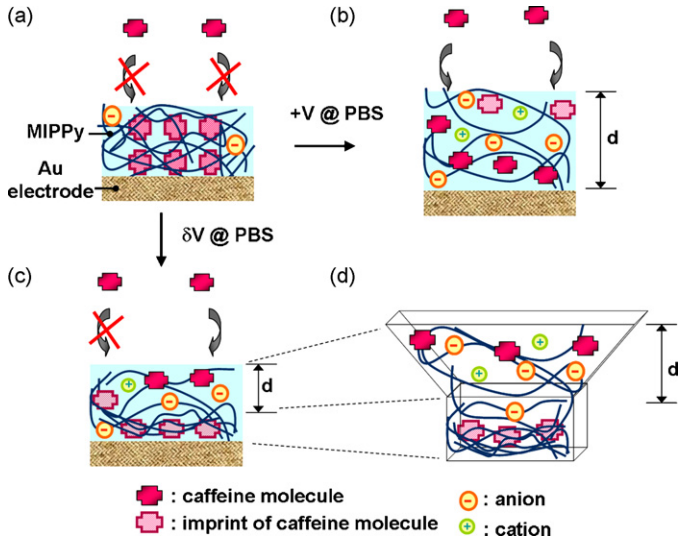

Fig. 1. Schematic illustration of the mechanism in modulating the effective thickness of the polymer film, *d*, under electrical stimuli. (a) In equilibrium, there is no swollen volume in the MIPPy to allow the access of caffeine molecules into the polymer. (b) Upon application of a positive potential, the polymer swells and opens up all the imprints for caffeine rebinding. The effective thickness *d* is smaller in (c) a partially swollen MIPPy compared to (b) a MIPPy which swells over the entire volume. (d) A three-dimensional enlargement of the partially swollen MIPPy under a small, positive potential pulse. The caffeine imprints in the swollen outer zone (marked by *d*) are opened up for caffeine rebinding but those embedded inside the non-swollen part near to the Au electrode are not.

able to detect any caffeine in this state. When a positive potential is applied, ions in the electrolyte penetrate the polymer and the polymer swells (Fig. 1b); this porous structure allows the caffeine molecules in the solution to enter the MIPPy and rebind to its imprint sites. In this phase, all imprints created during polymer synthesis are practically available for caffeine rebinding/detection, therefore the MIPPy sensor offers a detection window at high concentration range. However, by applying a series of well-defined potential pulses, the MIPPy partially swells (Fig. 1c and d); ions in the electrolyte possess only sufficient energy to enter the polymer volume adjacent to the electrolyte. Therefore, the part of the polymer at the electrolyte interface swells more than the inner one (Fig. 1d). The imprints in the swollen layer are freely accessible by the caffeine in the electrolyte, which defines the effective thickness of the MIPPy, *d*. In contrast, the polymer near to the Au electrode remains in a closed form which impedes the penetration of caffeine in the solution. In this phase, caffeine rebinding is limited to those imprints at the swollen outer zone of the polymer resulting in a smaller effective thickness *d*; the number of accessible imprints is much smaller than the created imprints, thus virtually turning the polymer into an ultrathin MIPPy film which enables sensing with a low detection limit.

2. Materials and methods

2.1. Synthesis of caffeine imprinted polypyrrole sensor

A solution containing pyrrole (100 mM), KCl (50 mM) and caffeine (2 mM) was used for electrochemical formation of caffeine doped polypyrrole films. Caffeine (98.5–101.5%, Acro), pyrrole monomer (99%, Acros Organics) and potassium chloride (>99%, Sigma) were all used as purchased. Distilled water was used for preparation of electrolyte solutions. Prior to electrodeposition, the electrochemical solution was degassed with nitrogen. Electrochemical experiments were performed in a single compartment cell with a three-electrode setup using an Autolab PGSTAT12 system. The working electrode (WE) consisted of an Au electrode (0.1 cm × 0.3 cm), whereas a graphite electrode and a saturated calomel electrode (SCE) served as the counter (CE) and the reference electrode, respectively. The electrochemical potential pulse technique has been applied for fabrication of caffeine imprinted polypyrrole on an Au substrate as described in Ramanaviciene and Ramanavicius (2004) and Ramanaviciene et al. (2004). The electrochemical formation of the caffeine doped polypyrrole film was carried out by applying 5 potential pulses between 0.3 V (1 s) and 0.7 V (10 s) vs. SCE, to allow the caffeine and the pyrrole monomer to equilibrate in the neighborhood of the Au electrode while depositing a conformal film of non-overoxidised PPy on the Au electrode. Non-imprinted PPy thin film electrodes (without caffeine imprints) were prepared using the same deposition protocol in a solution of pyrrole and KCl, but without caffeine molecules. During the final preparation step, all the samples were rinsed with DI water, and then soaked in ethanol for 30 s followed by a thorough wash with DI water in order to remove the caffeine templates and pyrrole residues. These MIPPy sensors with caffeine imprints were then ready for caffeine rebinding/detection.

2.2. Application of pulsed potential for MIPPy actuation and caffeine detection

The pulsed potentials were performed for 10 min in phosphate buffer solution (0.1 M, pH 7.0) with caffeine. A sequence of 5 potential pulses with a two-step potential waveform was applied: 1 s at 0 V and 1 s at 0.6 V (or 0.5 V or 0.4 V) vs. SCE. The sum of both anodic and cathodic peak currents (amplitude of peak-to-peak current) at the 5th pulse was then recorded as ΔI_0. After that, the same protocol was repeated every minute: 5 potential pulses were applied and the peak-to-peak current was recorded (ΔI_t). The sensor response was then obtained from the current change ($\Delta I_0 - \Delta I_t$) as shown in Fig. 2b.

2.3. Regeneration of actuated MIPPy sensor

After the caffeine detection, the sensor was soaked in phosphate buffer solution (0.1 M, pH 7.0) for 3 min to remove the caffeine molecules from their bound imprint sites. This was then followed by soaking the MIPPy in DI water for 5 min, allowing the ions incorporated during actuation to diffuse out from the MIPPy, and allow it to resume its equilibrium condition. Note that it is important to perform the caffeine extraction before the ion removal so that the MIPPy is still in a swollen state after the actuation and the porous structure of the MIPPy allows the bound caffeine to penetrate out from the polymer matrix.

3. Results and discussion

3.1. Response of MIPPy sensor under electrochemical actuation

A thin film of about 20 nm thick caffeine imprinted, chloride anion (Cl^-) doped polypyrrole was deposited on an Au electrode using an electrochemical potential pulse technique. The caffeine molecules served as the template for creating the imprint sites whereas the chloride ions were incorporated into the polypyrrole film to balance the positive charge on the polymer chain. During the electropolymerization, the potential pulses applied were controlled well below the overoxidation range of polypyrrole, to prevent polypyrrole from losing its actuation properties due to the broken polymer chains which hinder the swelling behavior of polypyrrole.

The electrochemical actuation of the MIPPy was performed by applying a sequence of 5 potential pulses at 0 V (1 s) and 0.6 V (1 s) vs. SCE periodically. The pulsed potential method requires

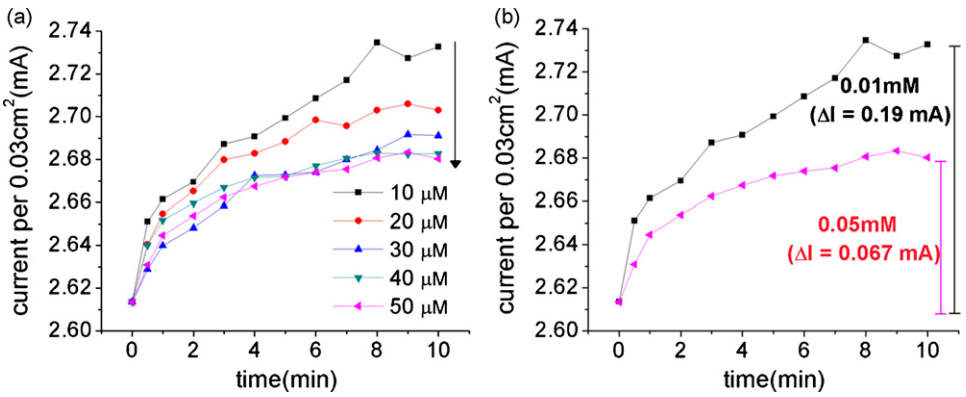

Fig. 2. (a) Sensor response monitored for 10 min in 0.1 M, pH 7.0 phosphate buffer solution with caffeine at different concentrations. (b) Data in 10 and 50 μM caffeine solutions (extracted from (a)) to show the current change in 10 min due to rebinding of caffeine molecules to MIPPy.

a longer time to actuate a thick MIPPy compared to a constant potential method. However, in this case where nano-actuations (actuate a few nanometer thickness of a MIPPy film) are required, pulse potential becomes a better choice. Further, the recorded currents from the potential pulses provide a direct means to detect the amount of caffeine rebinded to the polymer (Ramanaviciene and Ramanavicius, 2004; Ramanaviciene et al., 2004). The sensor response was monitored during 10 min of incubation in 0.1 M, pH 7.0 phosphate buffer solution with caffeine at different concentrations (Fig. 2a). As explained in Fig. 2b, when the sensor was placed in a 50 μM caffeine solution, the recorded currents of the sensor were substantially lower than in a 10 μM caffeine solution. This is attributed to the higher impedance of electron flow when larger amounts of neutral caffeine molecules were rebinded into the MIPPy.

3.2. Dynamic modulation of detection window

In our case where a thin MIPPy film was used, periodical potential pulses at 0.6 V vs. SCE for 10 min was sufficient to induce a complete swelling over the entire volume of the polymer. This opened up the passage for caffeine to penetrate into the polymer matrix and rebind to its imprint sites. Under this condition, every caffeine imprint in the polymer matrix was ready for caffeine rebinding. As shown in the calibration curve at 0.6 V in Fig. 3a, the current of the sensor, ΔI, with a maximum value at 10 μM of caffeine, decreased exponentially, reaching saturation after 40 μM. Therefore, the detection limit of this sensor is 10 μM, whereas the concentration range of 10–40 μM is defined as the detection window. Conversely, the sensor was insensitive to caffeine rebinding at concentrations lower than 10 μM; the sensor response recorded showed no particular trend. The exponential decay in current over the detection window indicates an increase of caffeine molecules into the polymer matrix when sensing at higher caffeine concentrations, and can be attributed to the recognition and rebinding of caffeine to the imprinted polypyrrole. The current saturated when all the caffeine imprinted sites on the MIPPy film were re-occupied by the caffeine in the solution. This exponential decay to minimum is recognised as the sensor response of specific binding of caffeine to its imprinted sites. As opposed to the specific rebinding of MIPPy, the non-imprinted PPy (a PPy film with no caffeine imprints) shows insensitivity to caffeine molecules over the detection range used here (Fig. 3b).

In order to reach a lower detection limit (<10 μM), according to our proposed mechanism, a lower positive potential (<0.6 V) has to be used. Note that the step-down pulse potential was fixed at 0 V in all cases. A similar exponential decay response was observed when applying potential pulses at 0.5 V (Fig. 3c). However, the entire calibration curve was shifted to the left, bringing the detection limit down to 5 μM. In addition, the sensor response saturated after 30 μM, as compared to 40 μM recorded at 0.6 V. The improvement in detection limit indicates that the sensor operated at this phase has a smaller capacity of caffeine binding sites, which is due to the reduction in the effective thickness d of the MIPPy. When the potential pulses were reduced to 0.4 V (Fig. 3d), the entire calibration curve was further shifted to the left, enabling detection down to 3 μM. A linear sensor response was obtained over the range of 3–10 μM. This narrow detection window verifies our hypothesis that the caffeine rebinding was limited to the shallow, swollen outer zone of the polymer, and the polymer was virtually an ultrathin MIPPy film at this point. Furthermore, the noise in the saturation range (10–40 μM) can be explained as an unstable rebinding of caffeine molecules to their imprint sites at the surface of the polymer, where the caffeine molecules have a tendency to un-bind from the imprint sites and return to the caffeine solution. Therefore, we can conclude that the applied potential pulses have effectively actuated and modulated the effective thickness of the MIPPy, thereby the detection level of the sensor. In contrast, the non-electrical actuated sensor would yield calibration curves which exhibit the same detection level regardless of the applied potential; the applied potential does not actuate the polymer but solely enhances or reduces the current flow within the conducting polymer.

3.3. Mechanism of electrochemically tunable detection window

In this section, we interpret the mechanism of the electrochemically tunable detection window based on the ion transport inside the MIPPy, which is accompanied by polymer swelling. Potential pulses were applied to the MIPPy in 0.1 M, pH 7.0 phosphate buffer solution without adding caffeine molecules. Caffeine exists as a neutral molecule at pH 7.0, thus its transport in both the buffer solution and inside the polymer matrix is solely by diffusion, as opposed to migration of ions induced by the applied potential.

In the current vs. time response as shown in Fig. 3e, the current recorded every minute is the sum of both anodic and cathodic peak currents (amplitude of peak-to-peak current) in the amperogram when a two-step potential waveform was applied: 1 s at 0.6 V (or 0.4 V or 0.5 V) and 1 s at 0 V vs. SCE. The positive potential was mainly used to actuate the MIPPy film by drifting the anions from the buffer solution (such as HPO_4^-, HPO_4^{2-} and Cl^- ions) into the polymer matrix, causing the polymer to swell; this resulted in anodic current (or positive current) flow. The potential was then stepped down to 0 V to reload the ions in the vicinity of the electrode surface before starting the next cycle of actuation.

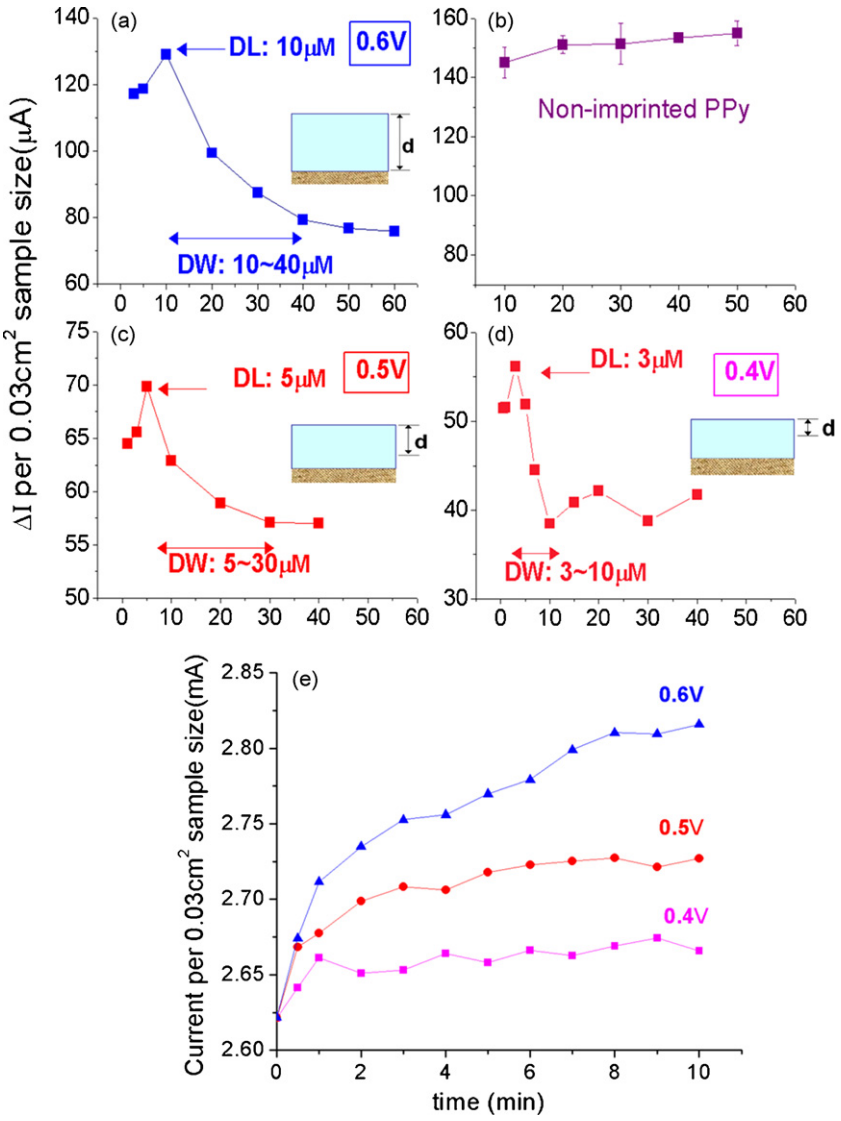

Fig. 3. (a–d) Calibration curves of the MIPPy sensor at different caffeine concentrations, showing the shift in detection window (DW) and detection limit (DL) when varying the applied potential pulses at (a) 0.6 V, (c) 0.5 V and (d) 0.4 V, respectively. The schematic illustrations show the effective thickness of the MIPPy film, *d*, when operating at that specific potential. (b) 0.6 V when using the non-imprinted PPy. (e) Current vs. time response monitored for 10 min in 0.1 M, pH 7.0 phosphate buffer solution with no caffeine, illustrating the penetration depth of ions in the MIPPy film at different applied potential.

At this stage, the current flow was reversed (cathodic or negative current), releasing some of the anions in the polymer to the buffer solution. However, the pulse at 0 V was insufficient to completely reverse the former positive pulse, therefore, a small amount of the anions was still trapped in the polymer, and the polymer swelled partially. Due to the ions insertion, the MIPPy became more conductive and generated a higher current spike in the subsequent pulse. Part of the increase in cathodic current can be associated with the insertion of a small amount of cations (e.g. K^+ ions).

As shown in Fig. 3e, overall, the sensor current increased over time for a specific potential, which implies that every cycle of the potential pulses added a net amount of ions to the MIPPy. Therefore we can imagine that these ions entered the outer zone of the polymer, the existing ions were then being pushed one step forward inside the MIPPy, thereby gradually opened up the polymer structure. The accumulative current over time provides insight into the penetration depth of ions inside the MIPPy, which is defined as the effective thickness *d* in this work. In this way, we can relatively compare the penetration depth of ions at different potential. At 0.4 V, the current saturated after 1 min, reflecting the shallow penetration of ions. The current saturated after 5 min at 0.5 V; the larger drifting force drove more ions towards the MIPPy and promoted ion migration deeper into the polymer matrix. The unsaturated current at 0.6 V even after 10 min indicates that the ions possess sufficient energy to penetrate through the entire thickness/volume of the polymer. This result correlates well with the change of the MIPPy effective thickness *d* in response to the applied potential as deduced in the previous section. In comparison, the current response is highest at 0.6 V, followed by 0.5 V then 0.4 V. Since the current response is the result of incorporation of ions into the MIPPy, we can deduce that the degree of polymer swelling also follows this order: MIPPy swells more at 0.6 V > 0.5 V > 0.4 V. This relationship between the actuation height thickness and the applied voltage has been visually observed by Higgins et al. (2009) using an electrochemical atomic force microscopy.

The study shows that the degree of polymer swelling can be actuated on the nanoscale by manipulating the penetration depth of the ions inside a thin MIPPy film. It should be noted that this electrical actuation is reversible for at least 20 detections; the current vs. time response at the 20th detection reproduced the sensor

response at the 1st detection (data not shown here). The sensor regeneration can be easily achieved by soaking the MIPPy in DI water for 5 min. During the soaking, the concentration difference drives the ions out from the MIPPy, the polymer shrinks and resumes its equilibrium condition, which is a closed structure. In addition, the sensors are able to differentiate caffeine from theophylline, a molecule which has a chemical structure very similar to caffeine (Fig. S1 (see Supplemental information)). We postulate that the selectivity of MIPPy is improved by swelling the polymer, leading to a porous structure which enhances the permeability of the caffeine molecules inside the polymer.

4. Conclusion

In conclusion, we demonstrated for the first time a simple mechanism to reversibly modulate the detection window of a MIPPy based biosensor under electrical stimuli. The detection window can be adjusted accordingly to optimize the sensitivity of the sensor to a specific test solution. More importantly, this approach can be explored for lowering the detection limit of a biosensor. It should be noted that this concept is not limited to molecular imprinted based biosensors, but can be extended to other biosensors which employ the finite volume of a conducting polymer as the bio-reaction center. Future experimental work will include the study of various parameters which govern the swelling behavior of conducting polymers in order to develop a quantitative model for the electrochemical tunable detection window.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.03.023.

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