Binding and Release of Glutamate from Overoxidized Polypyrrole via an Applied Potential for Application as a Molecular Switch

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The controlled binding and release of glutamate from overoxidized polypyrrole (PPy) films via a variable potential was investigated. Glutamate-doped PPy films were electrochemically deposited from aqueous sodium glutamate electrolytes containing the pyrrole monomer. The resulting polymer films were found to have a high degree of roughness, which increased with increasing film thickness. This was also found to correspond to an increase in the glutamate content on the PPy film surface. The glutamate content on the film was in the order of 10^{-8} m/cm^2 depending on the film deposition time. Glutamate was then released from the film into the electrolyte through overoxidization of the PPy layer and an applied potential. The amount of glutamate released from the film was greater than that on the surface of the film indicating that glutamate can be released from the PPy film via an applied potential. The switching behaviour of the polymer electrode, i. e. the repeated binding and release of glutamate to/from the polymer film via a variable potential, was investigated. The glutamate content in the samples was detected via liquid scintillation counting techniques performed on samples prepared with tritium (^{3}H)-marked glutamate.

Key words: Polypyrrole; Ion Selective Electrode; Molecular Switch.

1. Introduction

Conjugated polymers are interesting candidate materials for a range of applications as polymer layers can be processed at low temperatures and easily applied to nearly any surface area. These applications include semiconducting devices [1 – 3], molecular imprinting techniques for molecular recognition [4 – 7], drug release [8, 9], and microactuators [10]. For some valuable overviews on developments in these areas, please see [11 – 13].

Polypyrrole (PPy) is a conjugated polymer that has received much interest due to its stability, high conductivity, and redox properties. PPy can be easily synthesized by means of chemical or electrochemical oxidation of the pyrrole monomer. Electrochemical synthesis of PPy results in an electrically conductive polymer film adhering to an electrode surface. The polymer film is doped during deposition as anions from the electrolyte solution are incorporated into the polymer film to preserve charge neutrality during polymer film growth. The mass of the polymer film deposited is directly proportional to the flow of charge during film growth [14]. The polymer can be converted from its conducting state to its neutral state by releasing the anion through reduction of the polymer film (Fig. 1).

The characteristics of electrochemically deposited polypyrrole films depend strongly on the deposition parameters, including the electrolyte solution (electrolyte composition, pH, temperature) used and the voltage applied during polymer film growth [11, 14, 15]. Depositing the PPy film at lower voltage results in more homogeneous and conductive films, and at higher voltage, the PPy film becomes overoxidized, resulting in a loss of conductivity [11].

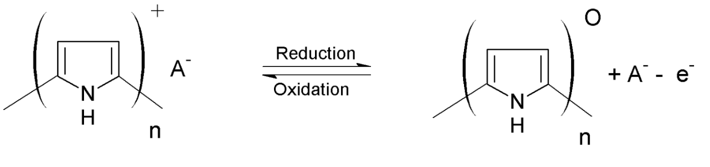

Fig. 1. Doped (conductive) polypyrrole on the left. A^- is the anion incorporated into the polymer chain. Reducing the polymer converts the polymer to its neutral state.

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Electrochemically deposited PPy is an interesting material for applications involving molecular recognition. PPy films have been observed to demonstrate a selectivity for the anion incorporated into the polymer film during deposition [16, 17], and it has been shown that overoxidation of PPy films results in the release of the anion, leaving behind selective pockets in the porous polymer film. Overoxidized PPy films electropolymerized in aqueous electrolyte solutions containing L-glutamate have been demonstrated to show chiral selectivity for L-glutamate over D-glutamate, where the potential inducing enantioselective uptake and release of glutamate by the polymer film was controlled by varying the pH value of the electrolyte [6, 7].

The selective molecular properties and stability of PPy layers are potentially interesting for the development and realization of selective molecular switches which can efficiently and reproducibly bind and release target molecules in solutions containing other species, e. g. physiological solutions. In the case of a target molecule containing a total net charge, such as glutamate which is negatively charged at neutral pH, varying the electrical potential applied to the polymer-coated electrode may be a simple method of controlling the switching behaviour of the device, i. e. the binding/release of glutamate to/from the PPy film. Such a selective molecular switch could be operated under the desired conditions simply by varying the electrical potential on the polymer electrode, without changes to the target molecule or to the solution.

In this study, glutamate-doped polypyrrole films were electrochemically synthesized. The amount of glutamate on the surface and within the PPy films was investigated to determine the content of glutamate that the PPy layer can potentially traffic as a molecular switch. Glutamate was released from the overoxidized PPy film via an applied potential, and repeated binding and release of glutamate from the polymer film was investigated. The content of glutamate in the samples was determined by liquid scintillation counting on known concentrations of ^{3}H-marked glutamate within the samples.

2. Experimental

2.1. Materials

Pyrrole (99% extra pure) was purchased from Acros Organics. L-Glutamic acid monosodium salt monohydrate (98% purum) was commercially obtained from Fluka. Tritium (^{3}H)-marked L-Glutamatic acid ($\geq 97\%$ pure, 1.48 – 2.96 TBq/mmol) was aquired from Hartmann Analytic. Indium tin oxide (ITO) and Pt foil (99.9% pure) were used as substrates for the working electrode. ITO was cleaned with acetone, isoproponal and water in a ultrasonic bath prior to use, and Pt with water in a ultrasonic bath. All water used in this study was purified by a Milli-Q system (Millipore).

2.2. Instrumentation

A PS5 from Sensortechnik Meinsberg instrument was used to potentiostatically deposit the PPy films and measure the current during film deposition. For liquid scintillation counting, a Quicksafe A scintillation cocktail from Zinsser Analytic was combined with samples in a scintillation vial; then the samples were placed in a Wallac 1415 liquid scintillation counter to measure the activity. The efficiency of the scintillation counter was known and corrected for in the data. Activities were measured as disintegrations per minute (DPM). Background counts were taken and subtracted from the measured sample activity.

2.3. Deposition of PPy Films

The electrolyte used for depositing polypyrrole films was aqueous 0.1 M L-glutamic acid monosodium salt monohydrate solution containing 50 mM of the pyrrole monomer. Polymer films were deposited under ambient conditions in an electrochemical cell with a fixed electrode geometry resulting in a defined area of 0.2 cm^2 for the polymer films. For investigations of the film structure, PPy films were deposited on ITO substrates. For investigations involving radioactively marked glutamate, PPy films were deposited on Pt substrates with 2 μL ^{3}H-marked glutamic acid per mL of electrolyte. All films were deposited at an anodic potential of 800 mV against an Ag/AgCl reference electrode. The PPy films were well rinsed after deposition to remove loose glutamate from the sample surface.

3. Results and Discussion

3.1. PPy Film Structure

Electropolymerization of polypyrrole occurs above an anodic potential that depends on the electrolyte. It has, however, been reported that the current-voltage characteristics of PPy films deposited under the same conditions demonstrate poor reproducibility [18],

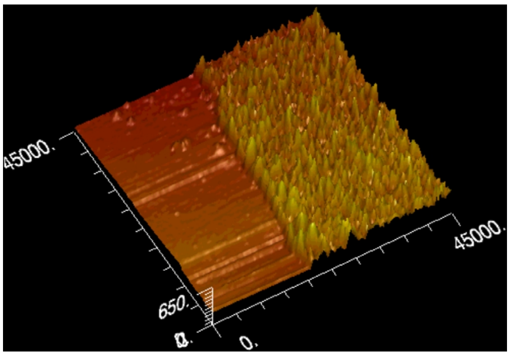

Fig. 2. AFM image of the PPy film on ITO. The ITO substrate is visible on the left, and the PPy film on the right.

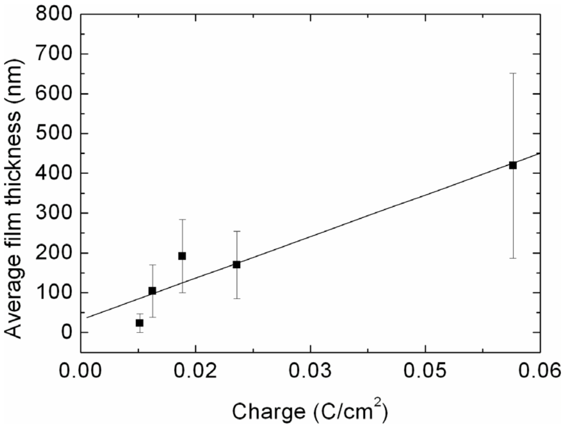

Fig. 3. Film thickness versus total charge flow during film deposition. The error bars show the RMS roughness of the films.

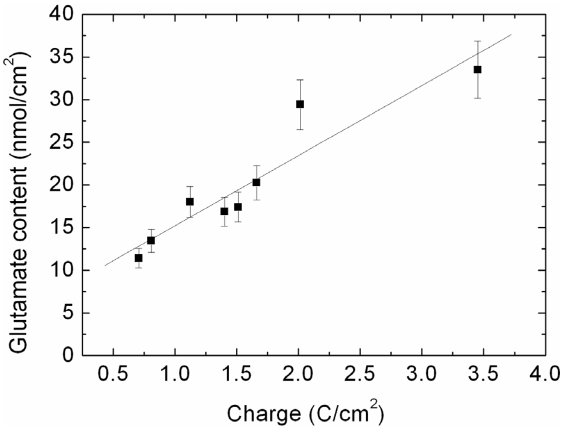

Fig. 4. Glutamate content on the PPy film versus total charge flow during film deposition.

something that we also observed for films deposited in sodium glutamate electrolytes. For this reason, the polymer films in this study were deposited potentiostatically (constant potential) rather than galvanostatically (constant current) as the conductivity and structure of the resulting PPy film is known to depend on the potential used for film deposition [11], while the magnitude of the current during growth influences only the thickness of the deposited film. We found that films could be deposited at potentials $\geq$ 750 mV, although below 800 mV sometimes no film was observed to grow on the ITO substrate.

Atomic force microscopy (AFM) images were taken of the PPy films to investigate the polymer film structure. The PPy films were observed to be relatively rough. The roughness increased with increasing film thickness, corresponding to an increase in the surface area of the film, which may be a useful property for increasing the amount of glutamate which the film can hold or traffic.

Figure 2 shows the surface of a PPy film on an ITO substrate. On the left, the bare ITO substrate is visible, and on the right the PPy film. Film thicknesses were in the order of a few hundred nm, depending on the deposition time, with patches of substrate visible between film peaks for thinner films. Figure 3 shows the average film thickness versus total charge flow during film deposition for a series of films. The error bars give the root mean square (rms) roughness of the film surfaces. Film thickness and roughness increased with total charge flow during deposition.

3.2. Glutamate Content on the PPy Film Surface

Liquid scintillation counting was performed on PPy films grown in electrolytes containing ^{3}H-marked glutamate for longer times, resulting in thicker films (in the order of μm) to investigate the glutamate content and controlled release from the PPy films. It was observed that the activity on the PPy films also increases proportional to the total charge flow during film deposition, again indicating an increase in the surface area with charge flow for thicker films. From the known activity of the ^{3}H-marked glutamate and the concentration of the electrolyte, the amount of glutamate on the film surface could be calculated. It was assumed that the activity measured from the PPy substrates represents half of the actual activity on the film surface as the substrate absorbs all radiation released in its direction. Figure 4 shows the glutamate content on the PPy films versus total charge flow during film deposition.

This corresponds to a glutamate content in the order of 10^{-8} mol/cm^2 on the film surface.

It was found that scratching the films (not shown here) leads to even higher activity measurements indicating that more glutamate is contained within the film than just on the surface. The thick PPy films absorb the activity resulting in activity readings only representing glutamate contained on or near the surface of the polymer film. In the next section, the release of glutamate from overoxidized PPy films is investigated.

4. Binding and Release of Glutamate from the PPy Electrode via an Applied Potential

4.1. *Controlled Release of Glutamate from the Overoxidized PPy Film*

The investigations of the potential-induced release of glutamate using liquid scintillation techniques are not trivial as glutamate contained on and within the overoxidized PPy films is loosely bound and rincing techniques can result in lower glutamate contents in the samples. For this reason, the effect of rincing as well as of glutamate diffusion from the PPy film into the electrolyte and vice versa in the absence of an applied voltage was first investigated.

Films deposited in solutions containing ^{3}H-glutamate were placed in non-radioactive rince solutions with the same concentration of sodium glutamate as the electrolytes used for film deposition. The rince solutions demonstrated activities in the same order as that found on the film surfaces. Similarly PPy films deposited in non-radioactive sodium glutamate electrolytes were then placed in rince solutions containing ^{3}H-marked glutamate. These films also demonstrated activity in the order of those films deposited in the electrolytes containing ^{3}H-glutamate. This is understood to be an effect of diffusion of loosely bound glutamate molecules from the film surface into the electrolyte and vice versa.

A series of PPy films were then deposited in sodium glutamate electrolytes containing ^{3}H-glutamate. The films were cut in half after deposition. The other half of the film was placed back into the electrochemical cell with a non-radioactive sodium glutamate solution. The PPy films were overoxidized by sweeping the voltage between 1200 mV and -1000 mV for 10 cycles. -1000 mV were used to electrostatically repel the negatively charged glutamate molecules from the electrode surface and overcome the diffusion processes. The activities in both film halves and in the sodium glutamate solution used for the release process were measured.

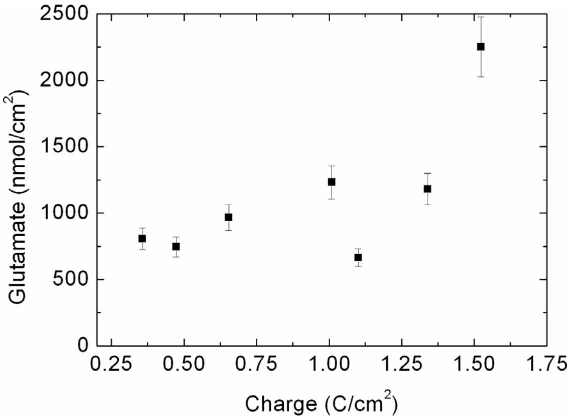

Fig. 5. Amount of glutamate released from the PPy film versus total charge flow during film deposition.

It was found that the activities in the as-grown and in the overoxidized film sections were very comparable. Some as-grown films showed slightly higher activities than the complementary overoxidized films while some of the overoxidized films showed higher activities. This is understood to be a result of incomplete dedoping of glutamate from the PPy film as the voltage dependence of the release process has yet to be investigated. The solutions used for film overoxidation and glutamate release showed the highest activities from all samples. Glutamate contents in the order of 10^{-6} mol/cm^2 of PPy film were determined from the scintillation measurements on the electrolytes used for the release process. Figure 5 shows the content of glutamate released from the film into the electrolyte against total charge flow during film deposition to illustrate the dependence of released glutamate content on film thickness. The amount of glutamate released from the PPy film increased roughly with the thickness of the PPy film investigated.

4.2. *Switching Behaviour: Repeated Binding and Release of Glutamate from the PPy Electrode*

The repeated binding and release of glutamate from the overoxidized PPy films deposited in non-radioactive sodium glutamate electrolytes was then investigated. The films were overoxidized and then placed in a sodium glutamate electrolyte containing ^{3}H-marked glutamate. Glutamate was bound to the film by applying 500 mV to the PPy-coated electrode for 5 min. The films were then transferred to a non-radioactive solution, and −500 mV were applied for 5 min to release the glutamate from the film. The electrolytes used for the release process again showed elevated activities indicating the the polymer film could bind/release glutamate due to the applied voltage, although the reproducibility of the process was poor, and some of the films no longer adhered to the substrates after several cycles.

These results indicate that the release of glutamate can be controlled from overoxidized PPy films with an applied potential. For applications of molecular trafficking, the voltage dependence and time scale of the release process as well as the effect of the film thickness on the efficiency of the switching behaviour of the device are points that will be addressed in future studies.

5. Conclusions

We investigated the controlled release of glutamate from overoxidized PPy films using an applied potential. The film surfaces were investigated and found to be rough. The roughness of the films increased with increasing film thickness, also corresponding to an increase in the amount of glutamate on the PPy film surface. The glutamate content in the samples was quantitized using liquid scintillation techniques on ^{3}H-marked glutamate. The glutamate content on the film surfaces was found to be in the order of 10^{-8} mol/cm^2 for film thicknesses in the order of μm. It was found that glutamate could be released from the overoxidized film using an applied potential, and that the glutamate content in the electrolytes used for the release process was significantly higher than that on the polymer films.

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