

Introduction

Patulin is a polyketide lactone mycotoxin that is produced by *Aspergillus*, amongst other fungi. The toxin is commonly associated with apple juice prepared from contaminated fruit although is considered a bioterrorism threat due to the ease with which it can be mass produced. Patulin is highly stable and can accumulate within the body causing acute or chronic illness when ingested. If present, patulin cannot be readily des

stroyed and hence there is a strong reliance on screening products to detect contaminated batches. The regulatory limit for patulin is 50 parts per billion (ppb) although given that apple juice is consumed by the young there is consideration for reducing this level to 25 ppb. Quantification of ppb levels of patulin can be routinely performed using laboratory based techniques, such as High Pressure Liquid Chromatography. However, a major limitation of laboratory based techniques is the time delay incurred in obtaining the results. In contrast, on-site testing is rapid thereby enabling immediate corrective action to be implemented should contaminated batches be detected. The ideal on-site sensor requires to be sensitive (detect ppb levels) and require minimal user input by the operator. Enzyme Linked Immuno Sorbant Assay (ELISA) are commercially available for patulin screening although are costly and require multiple step protocols. The following reports on the fabrication of a patulin sensor based on molecular imprinted polypyrrole. Molecular imprinted (MI) films are artificial antibodies which are produced by forming a polymer around the analyte (or analog) template. Upon completion of the polymerization the template is removed leaving voids which exhibit high affinity towards the analyte. Unlike biological agents such as antibodies, MIs are robust, stable, cheap and conducive to mass manufacturing. Polypyrrole is a semi-conductor that can switch between conducting and insulating states under the influence of external stimuli. The main advantage of using polypyrrole for MI fabrication is that the binding of target analyte and be instantly transduced into an electrical signal thereby enabling reagentless sensing.

Methodology

Fabrication of patulin imprinted polypyrrole films

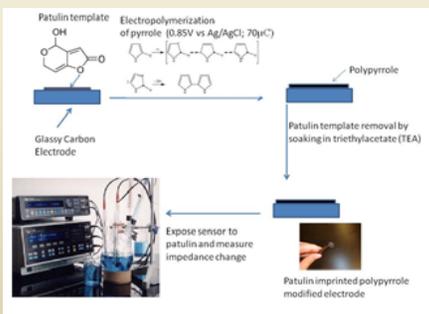


Figure 1: Fabrication of patulin imprinted polypyrrole films. Patulin (200 µg) was adsorbed onto the surface of a glassy carbon electrode for 20min. The electrode was then placed in a 0.5M pyrrole solution containing 0.1M TBAP and 0.2M EDGMA and electropolymerization performed galvanostatically at 0.85V vs Ag/AgCl until the accumulated charge reached 70µC. The electrode was soaked for 10min in a 3% v/v TEA solution to release the patulin template. The electrode was subsequently exposed to patulin (20-200 ppb) and changes in polypyrrole impedance recorded.

Results

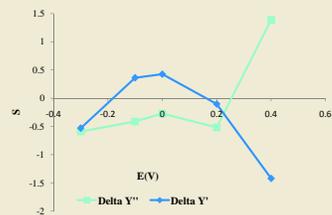


Figure 2: Change in Y' and Y'' by the addition of patulin (200ppb) to a patulin imprinted polypyrrole modified glassy carbon electrode poised at different bias potential. Background impedance spectra were obtained by placing the electrode in 0.5M NaCl along with a platinum mesh counter and Ag/AgCl reference. The impedance sweep was performed from 0.1 – 100kHz with an amplitude of 40mV and at the appropriate bias potential. The electrode was then removed and 20µl aliquots of patulin dispensed onto the polypyrrole surface. The electrode was returned to the electrochemical cell and impedance sweeps repeated. The response was represented by the change in Y' and Y'' with respect to background values.

• Exposure of the modified electrode to patulin resulted in a significant ($P < 0.05$) change in Y' (conductance) and to a lesser extent Y'' (susceptance) of the supporting polypyrrole film (Fig 2).

• The magnitude of the response was dependent on the oxidative state of the film. The change in Y' was maximal between -0.1 – 0 V vs Ag/AgCl where charge carrying polaron dominate. At 0.4V, where bipolarons predominate, the interaction resulted in over-oxidation of polypyrrole (decrease in Y' and increase in Y'' ; Fig 2).

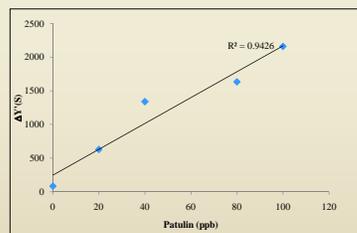


Figure 3: Response of imprinted polypyrrole modified electrodes to patulin. Impedance spectroscopy was performed on the modified electrodes over a frequency range of 0.1 – 100kHz using a bias potential of 0 V vs Ag/AgCl. The electrode was then exposed to solutions containing different patulin concentrations for 10min and changes in Y'' determined.

Changes in polypyrrole conductance (Y') could be correlated to patulin concentration with a lower detection limit of 20 ppb (Fig 3).

Equivalent circuit analysis

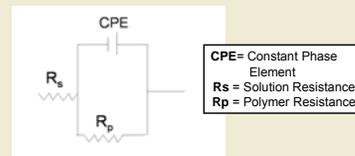


Figure 4: Equivalent circuit of patulin imprinted polypyrrole modified glassy carbon electrodes. The equivalent circuit was constructed and verified by comparing actual spectra with simulated impedance profiles. The circuit was then used to extract the resistive (R_p and R_s) and capacitive (CPE) elements from impedance spectra.

MIP: Patulin imprinted polypyrrole NIP: Non-imprinted polypyrrole

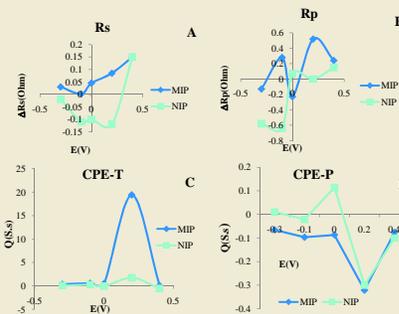


Figure 5: Changes in R_s (A), R_p (B), CPE-T (C) and CPE-P (D) upon interaction of patulin with a patulin imprinted polypyrrole modified glassy carbon electrode. Reaction conditions as described in the Legend to Figure 3. The individual circuit element values were derived from the equivalent circuit illustrated in Figure 4.

• Change in R_s : R_s represents the solution resistance between the modified electrode and counter. The observed change in R_s of MIP films with bias potential can be interpreted in terms of the outer layer of the film becoming increasingly resistive due to over-oxidation by the interaction with patulin.

• Change in R_p : R_p represents the polymer resistance which in turn is dependent on the abundance of charge carrying polarons and bipolarons. The change in polymer resistance (R_p) upon addition of patulin fluctuated depending on the applied bias potential (Fig 5). As the film became oxidized (0.2-0.4V) the presence of patulin increased the measured resistance through interacting with bipolarons. The negligible changes observed in the NIP polypyrrole at bias potentials $< 0.4V$ would indicate that the imprinted film exhibited enhanced affinity for patulin (Fig 5).

• Change in CPE-P and CPE-T: The CPE-T is an indirect measure of the film capacitance and the higher change in the MIP film compared to the non-imprinted (NIP) control would suggest that the interaction with patulin increased the capacitive (dielectric) character of the film (Fig 5). This was supported by the decrease in CPE-P at 0.2V which represents the ion diffusion within the film (Fig 5).

Possible interaction scheme for the interaction of patulin with polypyrrole

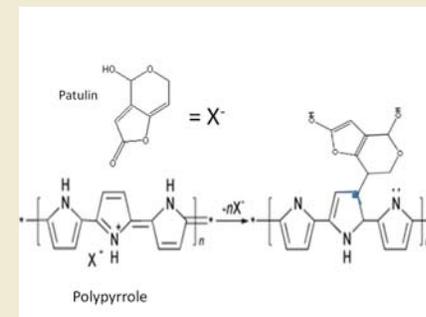


Figure 6: Schematic diagram of the interaction of patulin with charge carrying polarons/bipolarons on the polypyrrole chains. Patulin undertakes a nucleophilic attack thereby oxidizing the pyrrole units. Subsequent oxidation of the bipolarons results in covalent attachment of the patulin thereby disrupting chain conjugation resulting in an increase in film resistance.

Collectively the results from impedance analysis would suggest that patulin reacts with charge carrying polarons/bipolarons to form an adduct. Consequently polymer chain conjugation is lost resulting in an increase in film resistance.

Conclusions

• Patulin modified films can be fabricated by electropolymerization of pyrrole in the presence of the mycotoxin.

• The change in polypyrrole conductance could be correlated to patulin concentration with a lower detection limit of 20ppb.

• Imprinted films exhibited selectivity for patulin compared to non-imprinted polypyrrole at intermediate oxidation states (-0.1-0.2V) although less so at higher bias potentials (0.4V).

• It is hypothesized that the electrochemical changes induced in polypyrrole can be attributed to the formation of a covalent pyrrole-patulin adduct.

• Although imprinted films were more responsive to patulin this effect could not be attributed to a true imprinting effect (i.e. voids within the polymer having high affinity towards patulin). It is possible that the patulin adducts could form during the pyrrole electropolymerization thereby altering the polymer conformation which exhibited affinity for the mycotoxin.

Significance of the Research

The work has demonstrated proof-of-principle of fabricating reagentless sensors for detection of patulin outside the laboratory environment. The patulin sequestering property of polypyrrole could find utility in developing smart materials for detection and bioremediation applications. However, further work is required to characterize the reaction between patulin and polypyrrole, in addition to assessing the performance in food matrices.

Acknowledgments

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