

# Layer by Layer Fabrication of an Amperometric Nanocomposite Biosensor for Sulfite

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## ABSTRACT

A layer by layer strategy is described for the galvanostatic fabrication of a polypyrrole nanocomposite amperometric biosensor for sulfite. The strategy can be used to fabricate bilayer and trilayer sulfite biosensors, consisting of nanolayers of polypyrrole-sulfite oxidase, polypyrrole-dextran-sulfite oxidase, polypyrrole-chloride and/or polypyrrole-nitrate films. It has been demonstrated that the nature of the outer nanolayer has a significant influence on the selectivity and sensitivity of the amperometric nanocomposite biosensor. The presence of interferants, such as oxalic and tartaric acids at levels usually present in wine and beer did not interfere with the nanocomposite biosensor. The successful application of the nanolayer biosensor to sulfite determination in some wine and beer samples without sample pre-treatment is demonstrated.

**Keywords:** Nanocomposite, biosensor, polypyrrole, sulfite, layer-by-layer

## 1 INTRODUCTION

Traditionally, layer-by-layer assembly (LBL) is used for fabrication of films with molecular order and stability, based on alternating adsorption of oppositely charged macromolecules, such as biomacromolecules and polymers.<sup>1-3</sup> The resulting assembly of these ultrathin films (5-500 nm in thickness) is usually held together by electrostatic forces, covalent bonding, and, to a lesser extent, hydrogen bonding and hydrophobic interactions. This approach has gained considerable interest and has been used to prepare layered nanocomposites with high degree of organization from polymers and different nanocolloids such as nanoparticles, nanowires, nanotubes, clay platelets, and proteins. Recently, the use of conducting polymer polypyrrole (PPy) for layer-by-layer nano-assembly has been reported for the fabrication of highly sensitive and fast response humidity sensors. Also, layer-by-layer nano-assembly of polystyrenesulfonate (PSS) and PPy, as polyanion and polycation, respectively, on glass substrate has been reported. In general, most of the reported LBL methods to date rely on alternating adsorption of oppositely charged macromolecules or similar processes. However, where the use of such an adsorption process is not possible, electrochemical deposition can provide an

alternate effective strategy for achieving LBL assemblies at the nanoscale level.

This paper presents the results of a layer by layer strategy that we have developed for the fabrication of a polypyrrole nanocomposite amperometric biosensor for sulfite. The use of this strategy to fabricate bilayer and trilayer sulfite biosensors, consisting of nanolayers of polypyrrole-sulfite oxidase, polypyrrole-dextran-sulfite oxidase, polypyrrole-chloride and/or polypyrrole-nitrate films will be discussed. In particular, the influence of the outer nanolayer on the selectivity and sensitivity of the amperometric nanocomposite biosensor will be highlighted. The performance of sulfite nanocomposite biosensor in the presence and absence of interferants, such as oxalic and tartaric acids will also be discussed. Furthermore, the successful application of the nanolayer biosensor to sulfite determination in some wine and beer samples without sample pre-treatment will be demonstrated.

## 2 EXPERIMENTAL

### *Reagents*

All reagents used were AR grade unless otherwise stated. Pyrrole was obtained from Merck-Shuchardt (Sydney, Australia) and was distilled prior to use and stored covered with aluminium foil in the refrigerator to prevent UV degradation. Potassium chloride and potassium nitrate were from BDH Laboratory Supplies and sodium sulfite was obtained from Ajax Chemicals. Sulfite solution was prepared fresh prior to use. Dextran (average MW 40,000 g/mol) was obtained from ICN Biomedical and sulfite oxidase (SOx) was obtained from Sigma-Aldrich. All solutions were prepared with Milli-Q water.

### *Instrumentation*

The conducting polymer nanolayers were prepared galvanostatically with a potentiostat/galvanostat. Voltammetric and amperometric measurements were performed with a MacLab 4s (ADInstruments Pty Ltd) connected to a computer and a printer.

### *Layer-by-Layer Galvanostatic Assembly*

Nanolayers of polypyrrole-dextran-sulfite oxidase (PPy-Dex-SOx) was grown as previously described.<sup>4</sup> The desired outer nanolayers were deposited galvanostatically onto PPy-Dex-SOx inner layer, as either bilayer or trilayer. Prior

to the deposition of each layer, the monomer solutions were purged with nitrogen for 10 mins to remove oxygen. A three-electrode cell which consists of a platinum working electrode, Ag/AgCl reference electrode and a platinum auxiliary electrode was used for all electropolymerisation.

#### Characterisation of Nanolayered Films

After galvanostatic deposition of the nanolayers, the electrodes were rinsed several times with Milli-Q water to remove loosely bound molecules. Cyclic voltammetry and amperometry were then performed in phosphate buffer (pH 7.2) with a conventional three-electrode cell.

### 3 RESULTS AND DISCUSSION

The layer-by-layer galvanostatic assembly of the nanolayers, as bilayers or trilayers, is shown in Figure 1. The use of three different outer nanolayers for the fabrication of bilayer biosensors, based on galvanostatic assembly in layer-by-layer modes resulted in substantial improvement in the amperometric response for sulfite. As shown in Figure 2, the inclusion of all the outer nanolayers resulted in improvement of the sulfite response beyond that obtained with the single (mono-) layer. This is due, in part, to the benefit of the outer layer in retaining the enzyme in the inner layer and enabling better containment of the enzymatic product for more improved detection. It is particularly interesting to note that the sulfite response with the use of PPy-Cl and PPy-NO<sub>3</sub> outer nanolayers were less than half of that obtained with the outer PPy-SOx nanolayer. It is therefore apparent that the presence of the required enzyme in two or more subsequent nanolayers enabled maximum catalytic reaction and, hence, generated the most catalytic product for amperometric detection at the platinum electrode. It was also found that the influence of the outer PPy-SOx nanolayer is dependent on the galvanostatic polymerization period and, hence, on the thickness of the layer. A film thickness of 50-60 nm for the outer PPy-SOx nanolayer gave optimum amperometric response for sulfite.

The galvanostatic assembly, based on layer-by-layer, of various combination of triple layers also resulted in the improvement of the sulfite amperometric response beyond that obtained with the single (mono-) layer. However, the addition of the third nanolayer did not result in more improvement in sulfite response beyond those obtained with the bilayers.

Both the bilayer and trilayer assemblies limited the interference of oxalic acid and tartaric acid on the amperometric response of sulfite. In fact, at the concentrations usually present in wine, < 20 mg/l, no interference was observed for sulfite determination with both types of layer-by-layer assemblies. The bilayer assembly was successfully applied to a range of beverages, with a recovery efficiency of 101±1 %.

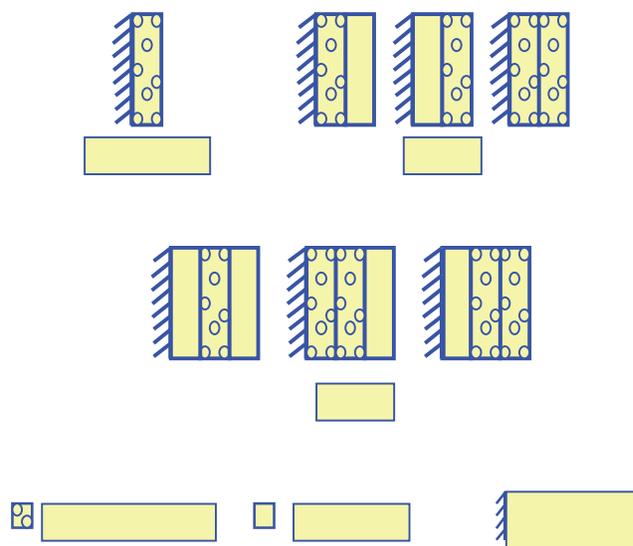


Figure 1: Different configurations for galvanostatic assembly of nanolayers based on layer-by-layer.

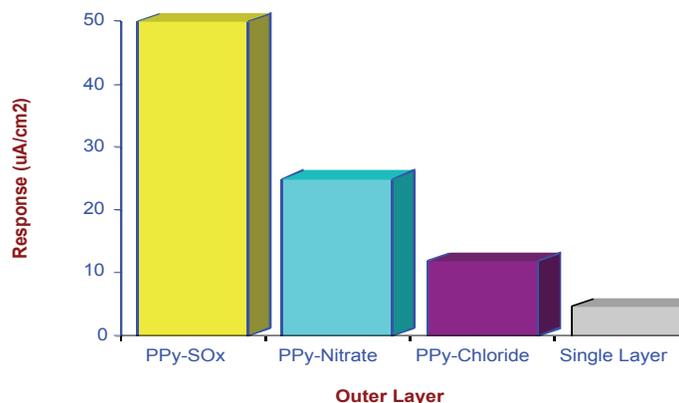


Figure 2: Influence of outer nanolayer composition on amperometric response of sulfite on a bilayer electrode. Inner layer is PPy-Dex-SOx. E<sub>app</sub> is -0.1 V.

### 4 CONCLUSION

Galvanostatic polymerisation, based on layer-by-layer assembly, of bilayers and trilayers has been successfully used to fabricate an ultra-sensitive biosensor for sulfite, and to enable its reliable determination in wine and beer. Oxalic and tartaric acids did not interfere with the biosensor.

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