Application and testing of chitosan protective layers for screen printed carbon electrodes (SPCE)

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Bioelectrodes play a critical role in electrochemical sensing, with applications in areas such as environmental monitoring (Tsai *et al* (2021)), glucose detection (Mross *et al* (2015)), and more. Enzyme-based bioelectrodes are key components of biosensors, leveraging the specificity of enzymes (biorecognition elements) to detect and quantify target substances in complex fluids, such as wastewater and biological fluids (George *et al* (2022)). However, enzymes are highly sensitive and prone to inactivation after prolonged exposure to high temperatures (Wang *et al* (2016)) and their performance is often hampered by interferences (Campuzano *et al* (2019)).

Various coatings, including Nafion (Trouillon *et al* (2009)), chitosan (Hlavatá *et al* (2014)) and polyurethane (Yu *et al* (2006)) layers, have been utilized to protect biosensors from (bio)fouling. These coatings offer antifouling protection by preventing non-specific adsorption and the presence of low and high molecular weight substances that impede the active surface of the bioelectrode (Ambrózy *et al* (2013)). Despite their protective properties, they often reduce detection capability by interfering with the sensing process. To address this issue, we propose the development of transient protective coatings designed to completely detach from the bioelectrode during use. These coatings will protect biosensors during storage (increased self-time) and prior to detection, preventing (bio)fouling without compromising sensing performance.

Chitosan is a natural, linear polysaccharide that is obtained by the partial deacetylation of chitin, the second most abundant natural biopolymer after cellulose and it is composed of randomly distributed β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units. Chitosan is derived from chitin, a naturally occurring polysaccharide found abundantly in the exoskeletons of crustaceans such as shrimp, crabs, and lobsters, as well as in the cell walls of fungi and insects, where it serves as a structural component that provides mechanical strength and protection. Chitin is composed primarily of N-acetyl-D-glucosamine units, and its tightly packed, crystalline nature contributes to its limited solubility and reactivity. To convert chitin into chitosan, deacetylation is performed to remove the acetyl groups, thereby revealing the primary amine functional groups, as shown in Figure 1.

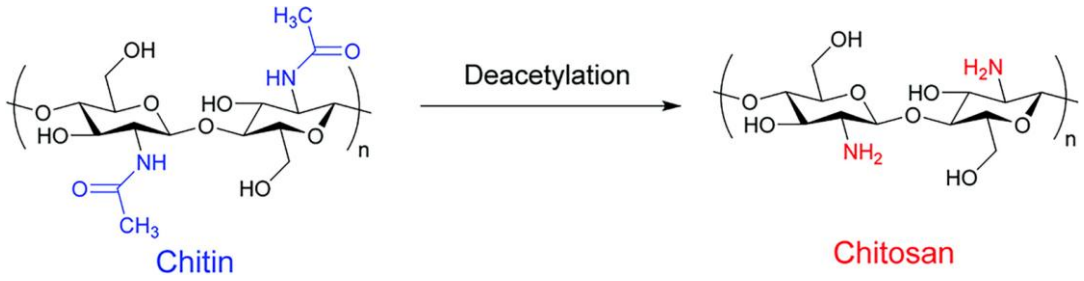

Figure 1. Deacetylation of chitin to chitosan (Ashrafizadeh *et al.*, 2023).

In our study, commercial screen-printed carbon electrodes were modified with chitosan using two distinct deposition techniques: drop-casting and electrodeposition and evaluated for their ability to be deposited and subsequently be removed upon immersion in water (or any other simple (electro)-chemical procedure) leaving no residue on the electrode surface. The dissolution capability was evaluated using ATR-FTIR, Confocal Laser Scanning Microscopy (CLSM) and Atomic Force Microscopy (AFM). In addition, the selected materials will be modified to increase their water solubility under specific pH conditions.

In the case of drop-casting, the presence of chitosan before and after rinsing was examined by ATR-FTIR. In the case of electrodeposition, SEM/EDS analysis confirmed the presence of chitosan after rinsing, despite no obvious morphological change seen compared to the bare electrode. In both modification approaches, CV and EIS measurements demonstrated that the chitosan remaining on the electrode surface enhanced the electrochemical response. Specifically, peak currents increased with higher drop-cast volumes and longer electrodeposition times, while in the case of drop-casting, greater chitosan solution loadings (15 μL and 20 μL) also affected the capacitive

current and electrical double-layer behavior, likely due to stronger electrostatic interactions between the protonated amino groups and the negatively charged $[Fe(CN)_6]^{3-/4-}$ ions.

This research aims to address the challenges of biosensor storage and biofouling through sustainable, cost-effective coating techniques that preserve and potentially enhance the sensing capabilities of bioelectrodes.

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