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Influence of porous dimension on template assisted Prussian Blue analogues platforms for choline biosensors.

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Abstract – Choline oxidase enzymes based biosensors have been studied because choline can be associated with different kind of brain disorders, such as Parkinson`s and Alzheimer`s disease. Once H_2O_2 is a secondary product of the interaction between enzyme and analyte, Prussian Blue analogues can be employed as transducers in this kind of systems [1]. In this work is presented template techniques to synthesize macroporous and mesoporous Prussian Blue analogues and the results, considering H_2O_2 detection and choline oxidase immobilization, are discussed in function of pore size effects.

It is known that Prussian blue does not show good electroactivity in electrolytes containing only Na^+. With this propose, Prussian blue analogues such as copper hexacyanoferrate have been synthesized and applied in oxidase based biosensors. However, this compound exhibits very low sensitivity towards H_2O_2 detection (0.14 μA mmol^{-1} L cm^{-2}). In order to improve this value, copper hexacyanoferrate was introduced in a polypyrrole network creating a hybrid material which sensitivity achieved 193 μA mmol^{-1} L cm^{-2}. It was possible to synthesize a macroporous CuHCNFe/Ppy mediator (460nm pore diameter) using polystyrene spheres as template and the sensitivity achieved was 158 μA mmol^{-1} L cm^{-2}, which is lower than the bulk analogue [2]. However, normalizing the sensitivity by the amount of mediator deposited over the electrode, it is possible to observe that the performance achieved by the macroporous transducer (850 μA mmol^{-1} L cm^{-2} mC^{-1}) is 4.5 times higher than that one obtained with the bulk CuHCNFe/Ppy in mediums containing Na^+.

We have also tried to synthesize the CuHCNFe/Ppy mesoporous mediator using lyotropic liquid crystalline phases. However, due to the interactions existed between pyrrole monomers and surfactants micelles, it has not been possible to synthesize the hybrid inorganic/organic transducer over the electrode. Once it was not possible to synthesize a nanostructured mediator, an alternative was to confine metallic hexacyanoferrates transducers into a mesoporous TiO_2 substrate (20nm pore diameter) synthesized through this template technique [3]. This confinement has been realized through layer-by-layer techniques and the sensitivity has increased until 5 bilayers, where it was possible to achieve a value of 637 μA mmol^{-1} L cm^{-2} when copper hexacyanoferrate was immobilized and 982 μA mmol^{-1} L cm^{-2} when iron hexacyanoferrate was immobilized. After the porous are completely occupied by mediators, there is a drastic decrease in the sensitivity values. For 6 bilayers, for example, the performance achieved was 407 μA mmol^{-1} L cm^{-2} for copper systems and 637 μA mmol^{-1} L cm^{-2} for iron systems, which is attributed to the loss of the confinement effect with lose of the nanostructures. The results presented above were performed in electrolytes containing K^+ and it was very interest to observe that self assembled Prussian blue transducer built up with this technique presented a sensitivity of 972 μA mmol^{-1} L cm^{-2} in mediums containing only Prussian Blue blocking cations, suggesting a different structure material related to the electrodeposited bulk analogue.

Finally, macroporous CuHCNFe/Ppy and mesoporous Prussian blue mediators were selected to act as transducers in choline biosensors. The choline oxidase was immobilized with glutaraldehyde in both cases and the sensitivities achieved through choline determination were 23 μA mmol^{-1} L cm^{-2} for the sensor with the macroporous platform and 28 μA mmol^{-1} L cm^{-2} for the biosensor with the mesoporous one. This result is very interest too, once de performance of the mesoporous Prussian blue through H_2O_2 detection is significantly better than the macroporous CuHCNFe/Ppy. We believe that this fact is also related with confinement effect, once with the macroporous mediator there is the possibility to immobilize the enzymes inside the porous.

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