IMMOBILIZATION OF LABELED GLUCOSE OXIDASE TO MAGNETIC NANOPARTICLES FOR DEVELOPMENT OF A GLUCOSE NANOBIOSENSOR

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In recent years our research group has developed new alternatives for fluorescence enzymatic determinations. The methods are based on the alteration of the enzyme fluorescence during its reaction with the substrate, which is proportional to the concentration of the corresponding analyte. Chemically modified glucose oxidase (GOx) with fluorophores has been used in order to work in spectral areas where organic interferences were minimized and to make possible *in vivo* determinations. In previous work the research group has proposed the use of labeled GOx with a fluorescein derivative for the direct determination of glucose in serum[1]. In this work we present first results obtained using GOx covalently linked to bis(2,2′-bipyridine)-4′-methyl-4-carboxybipyridine-ruthenium N-succinimidyl ester-bis(hexafluorophosphate) (Ru). Because of his long fluorescence lifetime this a very interesting fluorophore.

Magnetic nanoparticles could be used as a subcutaneous support for non-invasive nanobiosensor. They can be channelled in a biological fluid and directed by the action of an external magnet to low tissue thickness, without any deterioration in the body. Nanoparticles used in this work consist of a magnetite core which is covered with hydrophilic polymers containing amino terminal groups used for linking to biomolecules.

The immobilization of GOx-Ru to nanoparticles was done via reductive amination through the amino terminal groups of nanoparticles and aldehyde groups, previously generated by oxidation of polysaccharide chains of GOx with periodate[2]. Enzyme molecules retained their enzymatic activity after immobilization and the change of fluorescence during the reaction was observed. Fluorescence lifetimes measurements are being carried out in order to both, nanoparticles characterization and scattering problems correction.

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References:

[1] Sierra, JF; Galbán, J; de Marcos, S, et al. Analytica Chimica Acta 414 **1-2** (2000) 33-41
[2] Sun, YY; Yan, F; Yang, WW, et al. Analytical and Bioanalytical Chemistry 387 **4** (207) 1565-1572