NANOBIOSENSOR FOR GLUCOSE BASED ON CHEMICALLY MODIFIED GLUCOSE OXIDASE LABELLED TO MAGNETIC NANOPARTICLES.

Natalia Martínez de Baroja[1,2], Pilar Faustino[1,2], Valeria Grazú[2], Susana de Marcos[1,2],Jesús M. de la Fuente[2] and Javier Galbán[1,2]

[1)]GBA (Analytical Biosensors Group), Analytical Chemistry Department, Faculty of Sciences, University of Zaragoza (Spain). [2)]INA (Institute of Nanoscience of Aragon) University of Zaragoza (Spain).

natmtz@unizar.es

In recent years our research group has used the spectroscopic properties of chemically modified enzymes with fluorophores (figure 1), in order to develop analytical methodology for the design and construction of autotransducer biosensors that could be used *in vivo.* The best results were obtained with the fluorescein derivative fluorescein-5(6)-carboxamido-caproic acid N-hydroxy-succinimide ester (FS), which produces changes in fluorescence, at wavelengths of the FS, which are proportional to the concentration of the corresponding substrate (analyte) and the system is reversible. In previous work, the research group has developed an enzymatic biosensors of chemically modified Glucose Oxidase (GOx-FS) immobilised on polyacrylamide [1].

Nanoparticles, can be channelled in a biological fluid, directed by the action of an external magnet to low tissue thickness and be maintained in these areas, without causing any deterioration in the body, so they could be used as a basis of a subcutaneous support for non-invasive nanobiosensors. In this work we are presenting the first results obtained using GOx-FS linked to magnetic nanoparticles (figure 2).

Magnetite magnetic nanoparticles were synthesized according to the Tago [2] protocol. Previous to enzyme immobilisation, the magnetic nanoparticles were functionalised with DMSA (dimercapto succinic acid) in order to keep them in solution.

The immobilisation of GOx-FS to nanoparticles (Np) was conducting by the –COOH terminal groups of the DMSA and $–NH_2$ groups of the enzyme. There were studied two different alternatives: a) protecting the –SH terminal groups of DMSA with 2-PDS in order to avoid competitive reaction, and b) without protection of the –SH groups. Best results were obtained by protecting the –SH groups and direct reaction of Np and GOx-FS at pH 5. After 5 minutes of reaction about 100% of GOx-FS was labelled to Np and the enzyme activity remaining at about 100%. It was also observed a change on fluorescence signal of GOx-FS-Np according to glucose concentration. A theory justifying the two different GOx-FS-Np immobilization procedures have been developed and it is being experimentally demonstrated. Simulation studies for “in vivo” determination of glucose are being carried out.

Acknowledgements. This work was supported by the Ministry of Education and Science (MEC) of Spain within the project CONSOLIDER-INGENIO 2010 **NANOBIOMED**, and by the University of Zaragoza within the project UZ2007-CIE-11 which is gratefully acknowledged. JMF thanks ARAID for financial support.

References:

[1] V Sanz, J Galbán, S de Marcos and JR Castillo, Talanta, **60** (2003), 415.
[2] T. Tago, et al, J. Am. Chem. Soc., **85**, (2002) 2188.

Figure 1: Change on fluorescence signal of GOX-FS (6.5 UI / ml) according to glucose concentration. Blue line: 1000 µg / ml and red line: 250 µg / ml.

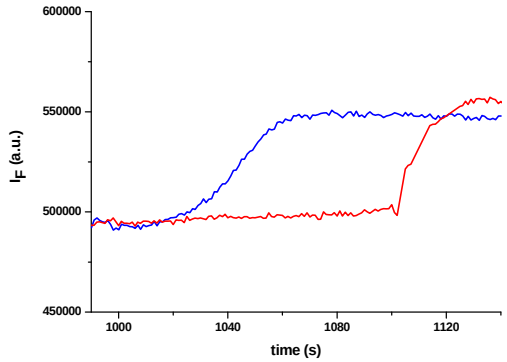

Figure 2: Change on fluorescence signal of GOX-FS-Np (6.5 UI / ml) according to glucose concentration. Blue line: 1000 µg / ml and red line: 250 µg / ml.