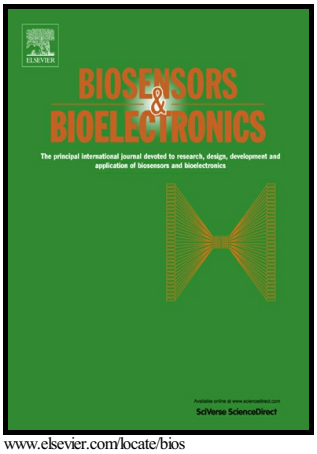

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Fluorescence Enhancement Aided by Metal Ion Displacement

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Abstract

Immunosensor are one of the most common platform used in clinical laboratories, in particular the class based on Enzyme Linked Fluorescent Assays (ELFA) that take advantage of the amplification step of the enzyme, usually the alkaline phosphatase, that catalyzes the hydrolysis of a fluorescent substrate leading it to fluoresce. Anyway, they suffer in sensitivity if compared to molecular diagnostic or more modern *in vitro* diagnostic devices. In our work, a simple and effective mechanism to enhance the fluorescent signal, and hence the sensitivity of the system, is presented. It is based on the metal ion displacement principle in which a second fluorophore, in our case Calcein Blue, quenched by a cobalt ion is add to the first one (4-MUP), and, in presence of inorganic phosphate, it will be progressively activated by the inorganic phosphate itself leading to the metal displacement. In this way Calcein Blue, newly free to fluoresce, contributes to global fluorescent signal generated by 4-MU. We have tested our proof of principle on a currently used immunoanalyzer, that is VIDAS® system (bioMérieux, Marcy l'Etoile, France) obtaining a fluorescence enhancement of about 50% for each concentration of hydrolyzed 4-MUP tested.

Keywords Fluorescence enhancement; metal ion displacement; immunosensors

1. Introduction

Immunoassays based on antibodies have been used over thirty years and they are still among one of the most important analytical tool since they are sensitive, simple and cost effectively (Saerens *et al.*, 2008).

Immunoassays use the high specificity of antibodies versus their antigens to detect them in samples, and they could be applied in a wide range of fields from in vitro diagnostic to food safety. With the growing literature describing research on new high sensitive assays (Agasti *et al.*, 2010 and Gu *et al.*, 2010), manufacturers are