Smart-phone read optical biosensor for glucose determination using polymer-assisted cascade biocatalysis

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Abstract.

Diabetes mellitus, commonly known as diabetes, is an urgent global health crisis. These motivated the widespread development of non-invasive point-of-care (POCT) technologies for continuous, real-time glucose monitoring in biofluids such as urine. The sensitive colorimetric method is the easiest and fastest method that produces a distinctive color change to the target analyte and is observed by the naked eye, excluding the requirement of sophisticated or expensive equipment.

The classic multienzyme optical detection process uses glucose oxidase (GOx) and horseradish peroxidase (HRP) enzymes but regardless of widespread use, it has been demonstrated that their cascade reaction catalytic efficiency is significantly reduced due to low diffusion ability and unstable intermediates [1]. In addition, enzymes are sensitive to external factors affecting their stability and promoting their reduced activity or inactivation, which can ultimately compromise the enzyme-based colorimetric assay.

To overcome these limitations, the GOx/HRP enzyme dyad can be co-immobilized on the same support. The spatial organization or compartmentalization of the multienzyme complex is crucial for its overall catalytic performance and efficiency, thus new enzyme organization and supports are being developed [2]. Polyamides are structural analogs of enzymes permitting effective hydrogen bonding in the polymer-biomolecule complexes, which allows efficient non-covalent immobilization. Furthermore, stable and robust polymeric micro-/nanostructured materials such as porous polyamide microparticles (PA MP) have also proven to be effective as protein/enzyme carriers for biosensors production [3].

In this work, we present the first report on the construction and testing of a smartphone-read polyamide-supported optical disposable biosensor for glucose detection in urine. The biosensor integrates: (i) PA6 microparticles (PA6 MP) synthesized by activated anionic ring-opening polymerization in solution; (ii) co-localization of GOx and HRP onto PA6 MP coated by (iii) a water permeable hydrophilic polymer that contains the optical transducer 3,3',5,5'-tetramethylbenzidine (TMB). This coating allows the diffusion of glucose and, at the same time, provides a good confinement of all the elements of the PA6/GOx/HRP/TMB complex keeping them in close proximity to one another. A smartphone camera was used for color images acquisition, the data handling was performed by a specially developed image processing algorithm.

The sensor displayed relative activity being slightly higher than the free GOx/HRP/TMB complex, the zone of linearity being in the 0.01-10 mM (0.18-180 mg/dL) range, detection time of 30 s, and LOD of 0.012 mM (0.216 mg/dL). The sensor was tested for the detection of glucose in fresh urine showing good low-concentration glucose detection, as well as good pH and storage stability.

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