Sensors and Actuators B 258 (2018) 331–341

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Solid glucose biosensor integrated in a multi-well microplate coupled to a camera-based detector: Application to the multiple analysis of human serum samples

Javier Pla-Tolós, Yolanda Moliner-Martínez, Carmen Molins-Legua*, Pilar Campíns-Falcó*

Department of Analytical Chemistry, Faculty of Chemistry, University of Valencia, Doctor Moliner 50, Burjassot, Valencia, Spain

ARTICLE INFO

Article history:
Received 5 July 2017
Received in revised form 10 October 2017
Accepted 15 November 2017
Available online 21 November 2017

Keywords:
Colorimetric biosensor
Glucose
Smartphone-based photometer
Multi-well microplate

ABSTRACT

In the present work, a biosensor adapted in a 96-well microplate has been coupled with a smartphone-based photometer in order to develop a low-cost colorimetric multi-sample dispositive. The strength of this biosensing system is based on the integration of the biosensor into the 96-well microplate and the use of a smartphone and free image analysis software as a microplate reader. The performance of the proposed biosensor has been demonstrated to determine glucose in several human serum samples. This method is simple, cost-effective, sensitive and selective for the determination of glucose in serum, with detection limits of 1.8 mg/dL and a good linearity over the range 6–88 mg/dL. Precision was also satisfactory (relative standard deviation, %RSD<5). The behaviour of the biosensing system has also been compared with the conventional derivatization method, adding all the reagents in solution and measuring the absorption at 653 nm. Results obtained indicated that this approach simplifies significantly the analytical measurements, avoids the need to prepare derivatization reagents, is portable and allows multiple in-situ measurements simultaneously. Thus, the simplicity of the test has been improved not only in the reaction step but also in the response measurement.

Contents

Abbreviations: PDMS, Polydimethylsiloxane; TEOS, tetraethyl orthosilicate; TMB, 3,3′,5,5′- tetramethylbenzidine; HRP, horseradish peroxidase; GOx, glucose oxidase; NPs, nanoparticles.

* Corresponding author.
E-mail addresses: c.molins@uv.es (C. Molins-Legua), p.campins@uv.es (P. Campíns-Falcó).

https://doi.org/10.1016/j.snb.2017.11.069
0925-4005/

1. Introduction

Currently, trends in the development of new analytical methodologies are focused in the simplicity. Productivity-related properties (rapidity and inexpensiveness) are prioritized in this type of procedures, although quality parameters, such as the accuracy, robustness, sensitivity and selectivity are also taken into account.

These new methodologies are specially designed to provide analytical information in a simple, rapid way, when the number of samples to analyze is high [1]. Furthermore, one interesting aspect of these devices is the portability, which allows the possibility to carry out in-situ analysis, avoiding the need of transporting the samples into the laboratory.

In the clinical field, where lots of different samples have to be treated daily, methods using multi-well plates combined with microplate readers are still one of the most used methodologies applied to perform multiple analyses. Nevertheless, although microplate readers are widely used in various readout tests, these instruments are expensive and non-portable, limiting their use in rural areas or developing countries. Therefore, a simple, cost effective, easy-to-use and portable alternative to microplate reader would be welcomed to mitigate these problems [2,3].

In recent years, smartphones have become an interesting tool in the analytical field due their high resolution cameras, easy-operation, portability, programmability and high storage capacity. All of these features can be applied in the areas of monitoring and mobile diagnostics [4–7]. Additionally, free image-editor applications can also be a very useful alternative to scientific equipment [8,9].

On the other hand, reliable and accurate saccharide sensors are continually being developed in order to analyze glucose and/or their derivatives in physiological media (approximately the 85% of the current world biosensor market are for glucose determination) [10,11]. The main analytical characteristics of some techniques have been summarized in Table 1. Additionally, diabetes was declared as a global epidemic by the World Health Organization owing to its growth worldwide [7]. Therefore, the challenge of providing accurate control of diabetes has remained the driving force in numerous research efforts [10,12,13].

In this sense, a dispositive capable of combine both methodologies (glucose sensor and smartphone-based photometer) would take advantage of their benefits, providing a new interesting approach. In previous works, Pla-Tolós et al. [14] developed a biosensor applicable to determine H_2O_2, based on the immobilization of the reagents in a polymeric support (PDMS-TEOS-SiO_2NPs).

This work goes further; a new biosensor integrated in a multi-well plate has been developed to analyse glucose in human serum samples. Moreover, a smartphone has been employed as a microplate reader, avoiding the need of sophisticated instrumentation. The biosensor is based on the immobilization of glucose oxidase (GOx), horseradish peroxidase (HRP) and 3,3′,5,5′-tetramethylbenzidine (TMB) in a polymeric support. Two configurations of the sensor have been developed: First of all, single discs were developed as a prototype and, in a second stage, the biosensor was adapted in a multi-well microplate in order to allow multiple measurements simultaneously (Fig. 1). This new approach improves the portability, simplicity and can be a promising tool to determine analytes which requires the use of biomolecules and organic reagents as substrate.

2. Materials and methods

2.1. Instruments

UV-vis measurements were recorded by a HP-8453 UV–vis spectrophotometer from Hewlett Packard (USA) furnished with 1 cm path length quartz microcell. Absorption spectra were registered from 190 to 900 nm. The LG Optimus L5 II smartphone (LG, South Korea) was used to take photos of the sensors. The images were analyzed by the open-source software ImageJ. For preparing the composite, ultrasonic bath (300W) from Sonitech and magnetic stirrer (45W) from Stuart Scientific (United Kingdom) was used. Plastic well-plates (Sharlau, Spain) and 96-well microplates (Termo Scientific, Denmark) were used as template to fabricate the sensor. An iPAD mini (Apple, USA), with the free app MyLight installed, was employed as a source of uniform illumination. BGStar® blood glucose meter was used to measure the level of glucose in blood (Sanofi, France).

2.2. Reagents and solutions

Nanopure water obtained using Nanopure II system (Barnstead, USA) was used for preparation and dilution of all solutions. Sylgard® 184 silicon elastomer base and Sylgard® 184 silicon elastomer curing agent were purchased from Dow Corning (United States). Silicon dioxide was provided from Sigma-Aldrich (China). D(+)-Glucose 1-hydrate was purchased from Panreac (Spain). Glucose oxidase was obtained from Sigma-Aldrich (United Kingdom). Tetraethyl orthosilicate was got from Sigma-Aldrich (Germany). 3,3′,5,5′-Tetramethylbenzidine was bought from Sigma-Aldrich (Switzerland). Horseradish peroxidase was obtained from Sigma (United States). Sodium acetate was provided from Panreac (Spain). Acetic acid was purchased from Sharlau (Spain).

2.3. Preparation of the PDMS-TEOS-SiO_2NPs-TMB-HRP-GOx biosensor

Two different modalities of sensors were assayed in this work: firstly, a dispositive to carry out single analysis was developed as a prototype. Secondly, this sensor was adapted in a 96-well microplate to allow the measurement of several samples simultaneously.

2.3.1. PDMS-TEOS-SiO_2NPs-TMB-HRP-GOx single analysis biosensor

The prototype sensor used to perform single analysis was developed by mixing 8 mg of TMB, 5 mg of silicon dioxide, 1 mg of GOx