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Glucose biosensor based on disposable electrochemical paper-based transducers fully fabricated by screen-printing

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ABSTRACT

This paper describes a new approach for the massive production of electrochemical paper-based analytical devices (ePADs). These devices are fully fabricated by screen-printing technology and consist of a lineal microfluidic channel delimited by hydrophobic walls (patterned with diluted ultraviolet screen-printing ink in chromatographic paper grade 4) and a three-electrode system (printed with carbon and/or Ag/AgCl conductive inks). The printing process was characterised and optimized for pattern each layer with only one squeeze sweep. These ePADs were used as transducers to develop a glucose biosensor. Ionic strength/pH buffering salts, electrochemical mediator (ferricyanide) and enzyme (glucose dehydrogenase FAD-dependent) were separately stored along the microfluidic channel in order to be successively dissolved and mixed after the sample dropping at the entrance. The analyses required only 10 µl and the biosensors showed good reproducibility (RSD = 6.2%, n = 10) and sensitivity (0.426 C/M cm^2), wide linear range (0.5–50 mM; r^2 = 0.999) and low limit of detection (0.33 mM). Furthermore, the new biosensor was applied for glucose determination in five commercial soft-drinks without any sample treatment before the analysis. These samples were also analysed with a commercial enzymatic-kit assay. The results indicated that both methods provide accurate results.

1. Introduction

Paper has been used as substrate in chemical analysis for a long time. However, the concept of microfluidic paper-based analytical devices (PADs) was recently introduced in 2007 (Martinez et al., 2007). In this work, the authors described a method to create well-defined, millimeter-sized channels in paper, comprising hydrophilic areas delimited by hydrophobic polymer. Two years later, Dungchai et al., introduced the electrochemical detection in PADs, beginning a new way to get analytical quantification in these devices (Dungchai et al., 2009). Since then, the research for electrochemical PADs (ePADs) has been extensive (Adkins et al., 2015; Desmet et al., 2016; Mettakoonpitak et al., 2016; Nery and Kubota, 2013).

There are several patterning methods for the effective, simple and low cost fabrication of PADs (Yong et al., 2015). However, even though screen-printing has been successfully used for massive production of disposable (bio)sensors (Fanjul-Bolado et al., 2007), there are few reports about the fabrication of (e)PADs by this technique. W. Dungchai et al. used a screen-printing machine for rubbing solid wax through a patterned screen onto filter paper. These papers were heated afterwards so that the wax melted into the substrate to form hydrophobic barriers (Dungchai et al., 2011). Other approaches dealt with polymer solutions, which were used as conventional screen-printing inks. In those cases, the solvents provided the penetration of the polymer through the paper (Sameenoi et al., 2014; Sun et al., 2015). Banks et al. also demonstrated that ePADs can be printed by serigraphy on different kinds of conventional papers (Metters et al., 2013). In that work, they addressed some problems with thick and high porous papers, such as filter papers, because the dielectric paste used for patterning the hydrophobic walls was ineffective to prevent the capillary wicking of the solution from the hydrophilic areas. Recently, our group has reported a novel method for the fully fabrication of ePADs by screen-printing (Lamas-Ardisana et al., 2017). A mixture of commercial UV-curable inkjet and screen-printing inks was used for patterning hydrophobic barriers into chromatography paper grade 1. Afterwards, a three-electrode system was screen-printed over the hydrophilic areas.

Determination of glucose is very important, especially in the fields of healthcare, clinical, food and beverage industries. In food and beverage industries, glucose quantification is required for quality controls and fermentation processes (Galant et al., 2015). Conventional methods require many time consuming steps, reagents and samples, so simple, fast and low cost methods are still needed for the determination of glucose. In this context, (e)PADs present a powerful alternative to conventional methods (Busa et al., 2016) and several ePADs for glucose

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detection have been reported in the last years (Dungchai et al., 2009, 2011; Liu et al., 2016; Nie et al., 2010a, 2010b; Rungsawang et al., 2016; Yao and Zhang, 2016).

In this work, a new approach for fabricating ePADs is presented. The dilution of a commercial UV screen-printing ink with a mixture of monomers was optimized for patterning hydrophobic walls into chromatographic paper grade 4. Subsequently, the three-electrode system was printed with carbon and Ag/AgCl conductive inks. The resulting ePADs were used as electrochemical platforms for a glucose biosensor. The microfluidic channels of the ePADs were modified at three separate points with different solutions i.e. pH/ionic strength buffering salts, ferricyanide and glucose-dehydrogenase FAD-dependent solutions. The critical parameters of the glucose biosensor were optimized and the analytical performance was determined. Finally, the ePAD biosensors were used for analysing commercial soft-drinks, without sample treatment, and the results were compared with those obtained by a commercial enzymatic-kit.

2. Experimental

2.1. Reagents, materials and apparatus

Glucose-dehydrogenase FAD-dependent (GDH) from microorganism, 500 U/mg lyophilized powder, was purchased from Sorachim S.A. Ortho-phosphoric acid 85% (w/w) and sulphuric acid 98% (w/w) were purchased from Scharlab. Other chemicals were obtained from Sigma-Aldrich. They were analytical grade and were used without any further purification. Glucose and GDH stock solutions were prepared in 0.01 M phosphate buffer pH 7.0 (PB). Glucose assay kit K-GLUHKR from Megazyme International was used for the spectrometric analyses of real samples. Whatman chromatography paper 4 from GE Healthcare Life Sciences, two conductive inks (BQ 242 carbon and 5874 silver/silver chloride, from Dupont), UV screen-printing ink (blue Ultraswitch UVSW from Maribu) and UVV6 thinner from Maribu were used for the ePAD fabrication. Other apparatus and materials are detailed in the Supplementary material.

2.2. Rheological characterization

The rheological tests were performed at 25 °C with parallel plate geometry (40 mm diameter stainless steel plane and 1 mm gap). A continuous ramp with an increasing shear rate from 1 to 1000 s^{-1} was applied during 5 min for each sample. The experimental data were fit to the power law model ($\tau = K \cdot \gamma^n$), where τ is the shear stress, γ is the shear rate, n is the flow behaviour index (or a shear-thinning index when $n < 1$) and K is the power law coefficient or consistency.

2.3. ePAD fabrication

75 ml Ultraswitch UVSW and 25 ml UVV6 were mixed and shaken manually. This mixture was rubbed onto the surface of the screen stencil with a squeegee. Only one sweep was enough for the total penetration of the ink across the chromatography paper. The patterned papers were immediately introduced into the UV oven to cure the ink. Afterwards, graphite and silver/silver chloride layers were successively printed and cured into the convection oven (120 °C, 5 min each). Graphite ink was used for the working and counter electrodes, while silver/silver chloride ink was used for the reference electrode and the conductive pads of each electrode. Fig. 1 shows the front and back sides of the ePAD. The hydrophobic walls delimited a rectangular microfluidic channel of 3 × 50 mm. The arrangement of the working electrode was perpendicular to this channel and its area just over the hydrophilic paper was 3 × 1 mm. The counter electrode was located at the end of the channel.

Fig. 1. Front and back sides of the ePAD.

2.4. ePAD characterization

The sealing tests were carried out using 10 µl of 25 mM phenol red in 0.1 M NaOH solution. For this, one single drop was carefully casted at the channel entrance (on the front printed side) and the solution was allowed to spread freely into the paper. The scanning electron micrographs were taken after sputtering 20 nm gold layers over the samples. The photos were obtained by the second electron signal at high vacuum, 20 kV accelerating voltage, 16 mm working distance and 36 spotsize.

2.5. Preparation of the glucose biosensors

The ePADs were cut to 25 mm channel length. The reagents were loaded through the back printed side. Three drops were deposited on different places. First, 1 µl of 0.5 M NaCl and 0.5 M phosphate buffer solution pH 7 was deposited at the channel entrance. Second, 1 µl of 0.5 M potassium ferricyanide was deposited at one centimetre from the channel entrance. Third, 1 µl of GDH-FAD 5 U/µl in PB was deposited at two centimetres from the channel entrance i.e. just at the back side of the working electrode. Finally, the biosensors were dried at 30 °C during 10 min.

2.6. Electrochemical measurements

The electrochemical measurements were started after 10 µl of the sample solution were dropped from a micropipette at the channel entrance. The measurement procedure included three sequential steps: 1) amperometric detection at + 0.3 V with a 100 nA current cut-off, 2) cell off during 20 s, 3) coulometric detection at + 0.3 V during 30 s. The analytical signals were the charges in the former step. The measurement of each point was repeated at least three times, using one biosensor for each repetition. The limit of detection was estimated from the IUPAC recommendation, using the $3S_b/m$ criteria (Mocak et al., 1997), where m is the slope of the linear range and S_b the standard deviation of the background (signal without glucose).

2.7. Real sample analysis

Five commercial soft drinks (Fanta zero orange, Kas zero orange, Sprite, Solan de Cabras multifruits and Pascual Bifrutas mediterraneo) were analysed in order to evaluate the biosensor applicability in real samples. The analyses performed with the biosensors were carried out without any sample treatment. The spectrophotometric analyses with the commercial enzymatic-kit required the samples dilution with PB solution, following the manufacturer instructions.