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Amperometric detection of salivary α-amylase on screen-printed carbon electrodes as a simple and inexpensive alternative for point-of-care testing

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ABSTRACT

This study describes the amperometric detection of salivary α-amylase (sAA) in human saliva samples using screen-printed carbon electrodes (SPCE). The proposed method was based on the indirect determination of sAA in a sequence of two chemical reactions. Basically, the first reaction is the hydrolysis of starch by sAA to produce maltose. Then, the generated reducing sugar promotes the conversion of $[Fe(CN)_6]^{3-}$ into $[Fe(CN)_6]^{4-}$ in a second reaction. Different parameters, such as pH, reaction time, sAA volume and starch concentration were thoroughly optimised. The best electrochemical response was found using 5 mmol L^{-1} NaOH (pH = 12), reaction time of 20 min, sAA volume of 15 μL and 0.5% (w/v) starch. The analytical performance revealed good linear correlation for sAA concentration levels between 100 and 1200 U mL^{-1}. The achieved limit of detection (LOD) and sensitivity values were 1.1 U mL^{-1} and 10.7 μA/(log U mL^{-1}), respectively. The proposed electrochemical sensor exhibited great selectivity. The amperometric response for sAA was recorded in the presence of different glucose levels (0.01—0.10 mmol L^{-1}) and no noticeable change was observed. The feasibility of the proposed method was investigated through the determination of sAA levels in five human saliva samples. The concentration levels found ranged between 182.8 and 1117.1 U mL^{-1} and presented accuracy between 90 and 97%. The method reported herein emerges as a simple, inexpensive, portable, reliable and powerful tool for rapid point-of-care testing (POCT).

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1. Introduction

The detection of biomarkers aiming the monitoring of health conditions has become an essential part of human life [1]. Currently, different authors have reported noticeable advances in the development of novel analytical platforms or chemical sensors for detecting potential biomarkers associated with early clinical diagnosis [2–7]. Among the various target biomarkers, the activity of α-amylase represents an area of great importance for study, since its regular monitoring in different body fluids can be helpful to accomplish the clinical stage of pancreatitis, for example [8,9]. The presence of this enzyme at high concentration levels may be indicative of pancreatic cancer, acute pancreatitis, bile duct blockage, salivary gland infection or gastroenteritis. On the other hand, low levels of α-amylase can be associated with pancreatic or kidney failure and toxaemia of pregnancy [8].

Salivary α-amylase (sAA) is a digestive enzyme that catalyses the hydrolysis of α-1,4 glucan linkages present in starch, producing maltose and dextrins [10]. In recent years, the determination of sAA concentration levels has received growing attention for being considered one of the most sensitive targets of autonomic nervous system activity [11], and therefore, a potential biomarker for neurological disorders [12], as the change of sAA levels is correlated with stress [13], emotions [14] and fatigue [15]. Furthermore, the use of saliva as biological fluid is quite advantageous when compared to other body fluids. The sample collection method is non-invasive, less stressful and does not require specialised equipment or personnel for sampling [16,17].

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Various methods have been explored to measure sAA levels, including spectrophotometry [18], colorimetry [19], isoelectric focusing [20], chromatography [21], fluorescence [22], enzyme-linked immunosorbent assay (ELISA) [23], quartz crystal microbalance [24] and electroanalytical approaches [25–27]. Recently, Zhang and co-workers developed a smartphone-based potentiometric sensor to determine sAA levels [27]. In their report, the authors proposed a sensing chip composed of two electrodes (working and pseudo-reference) assembled in three layers of transparent films. Saliva was introduced by pressing the fingers on a detection zone pre-loaded with required reagents. The readout was performed by a potentiometric reader connected to a smartphone via USB port. The saliva volume introduced by pressuring is a critical point which may compromise the reproducibility of the clinical tests. In the same year, Wang and co-workers proposed a novel method to determine sAA levels using a personal glucose meter (PGM) [28]. The authors used maltopentose as substrate to be hydrolysed by sAA as well as α-glucosidase to convert maltose into glucose. The use of α-glucosidase enzyme as well as heating (37 °C) are some disadvantages that may hinder the implementation of the proposed method for on-site studies. In general, most of the recently reported methods present various disadvantages that discourage their use in remote areas, such as high-cost equipment, limited instrumental portability, requirement for trained and skilled users, long incubation periods, endogenous glucose interference and unstable colorimetric reactions [25–30].

Among the electroanalytical approaches explored to measure sAA levels, amperometric detection is simple and offers great sensitivity, adjustable selectivity and compatibility to be integrated in miniaturised analytical platforms, thus resulting in portable tools for on-site applications [31]. Electroanalytical sensors based on screen-printed electrodes (SPE) have often been used in different studies due to their simplicity, low cost, robustness as well as the possibility of chemically modifying the electrode surface for multiple purposes [32,33]. Many reports have successfully demonstrated the wide range of applications using SPEs, including the detection of heavy metals [34–39], pesticides [40,41], catechol [42], cholesterol [43], glucose [44,45], hormones [46,47], proteins [48], tumour markers [49] and pH measurements [50]. The advantages offered by miniaturised electroanalytical sensors have enabled their use in biomedical applications [51].

In this study, the development of a simple and inexpensive point-of-care testing (POCT) device based on the screen-printed carbon electrodes (SPCEs) for amperometric detection of sAA levels is described for the first time. To achieve the best electroanalytical response, different parameters, such as pH, reaction time, sAA volume and starch concentration were investigated and optimised. The feasibility of the proposed method was demonstrated through the quantification of sAA levels in five human saliva samples. The analytical accuracy of the proposed method was demonstrated by recovery experiments.

2. Materials and methods

2.1. Chemicals and materials

Potassium hexacyanoferrate(III), maltose, potassium chloride, starch, sodium hydroxide, α–amylase from human saliva (sAA), calcium chloride, anhydrous sodium monobasic phosphate and anhydrous sodium dibasic phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nylon-type syringe filters (0.22 μm pore size and 25 mm diameter) were acquired from Allcrom (São Paulo, SP, Brazil). All chemicals were used as received and prepared in ultrapure water (18 MΩ cm^{-1}, Millipore, Kansas City, MO, USA) without further purification.

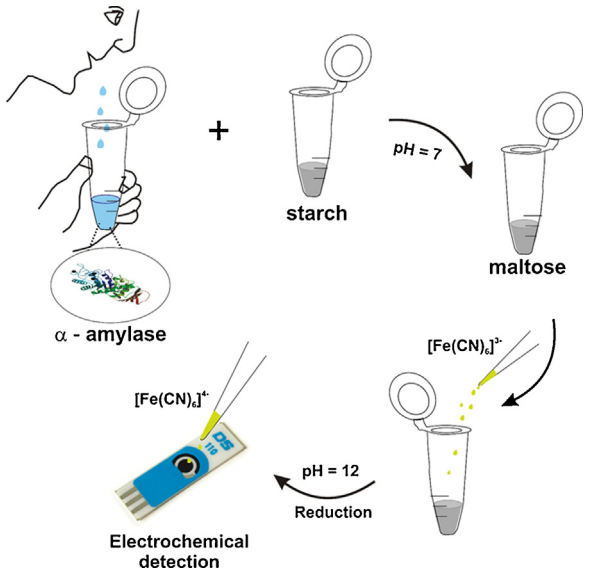

Fig. 1. Illustration showing the steps involved in the electrochemical measurements of sAA.

2.2. Instrumentation

Electrochemical measurements were performed using a bipotentiostat/galvanostat model μStat 400 (DropSens S.L., Oviedo, Spain) and monitored by DropView® software. SPCEs were provided by DropSens (model DRP 110) and consisted of a ceramic substrate (34 mm long × 10 mm width × 0.5 mm thick) containing a carbon working electrode (4 mm diameter), a carbon counter-electrode and a Ag/AgCl pseudoreference electrode.

2.3. Experimental optimisation

The effects of NaOH concentration, reaction time, sAA volume and starch concentration on the electrochemical measurements were thoroughly investigated and optimised. Initially, the NaOH concentration was studied because maltose acts as reducing sugar at optimum pH around 12 [27]. For this reason, the NaOH concentration was varied from 2.5 to 50 mmol L^{-1}. Afterwards, the reaction time ranged from 2 to 60 min to determine the kinetics of conversion of $[Fe(CN)_6]^{3-}$ into $[Fe(CN)_6]^{4-}$. In this same way, the sAA volume used in the reaction was optimised to achieve the best analytical signal, considering the behaviour of sAA in the hydrolysis of starch. For this purpose, the sAA volume varied from 5 to 35 μL and the amperometric signal was monitored versus the sAA volume. Lastly, the starch concentration was ranged from 0.5 to 2.0% (w/v) to evaluate its influence on the electrochemical response.

2.4. Enzymatic reaction and electrochemical detection

The hydrolysis products between starch and sAA were monitored by amperometric measurements using SPCEs. In this study, a sequence of two reactions was used where the first step involved the enzymatic reaction to produce maltose, which acts as a reducing sugar in the second reaction to convert $[Fe(CN)_6]^{3-}$, previously added to the reaction medium, into $[Fe(CN)_6]^{4-}$, as displayed in Fig. 1 and Scheme 1. It is important to highlight that the current signal resulting from the $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ conversion is proportional to the sAA levels, making it possible to perform an indirect detection of this important biomarker. In all cases, the electrochemical response was monitored for 60 s under the application of 0.24 V at the working electrode (versus Ag/AgCl).

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