Talanta 111 (2013) 8–12

Contents lists available at SciVerse ScienceDirect

Talanta

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

Screen-printed biosensor based on the inhibition of the acetylcholinesterase activity for the determination of codeine

Laura Asturias-Arribas, M. Asunción Alonso-Lomillo *, Olga Domínguez-Renedo, M. Julia Arcos-Martínez

Analytical Chemistry Department, Faculty of Sciences, University of Burgos, Plaza Misael Bañuelos s/n, 09001 Burgos, Spain

ARTICLE INFO

Article history:
Received 4 December 2012
Received in revised form
14 March 2013
Accepted 16 March 2013
Available online 22 March 2013

Keywords:
Codeine
Acetylcholinesterase
Screen-printed electrodes
Tetrathiafulvalene
Enzymatic biosensor

ABSTRACT

The current paper presents the chronoamperometric determination of codeine using screen-printed carbon electrodes that incorporate tetrathiafulvalene in the matrix of the working electrode, as mediator, and cross-linked acetylcholinesterase. Applying a potential of +250 mV, a 1 mM solution of acetylthiocholine in electrolyte solution pH 7 gives an oxidation signal due to the dimerization of its metabolite after the reaction with the enzyme. This electrochemical signal is decreased by consecutive additions of a solution of codeine, which allows the performance of curves of calibration for the validation of this electrochemical method, giving a reproducibility of 3.31% ($n=6$) and a capability of detection of 20 μM. This type of inhibition has been studied by means of a Lineweaver–Burk plot. Additionally, the developed biosensor was used for the determination of the quantity of codeine in pharmaceutical commercial tablets and urine samples.

1. Introduction

Codeine, or methylmorphine, is an alkaloid that is extracted from opium and one of the most common used antitussive drugs, being also used as analgesic and antidiarrheal [1,2]. Although it belongs to the same opium family as morphine and heroin, codeine is used for the treatment of moderated pain and induces less euphoria and sedation than the first two, but it can also produce addiction [3]. Even though codeine does not produce as strong effects as heroin, it is also considered as a drug of abuse.

This drug has been determined in diverse matrixes by different methodologies such as gas chromatography [4,5], liquid chromatography [5], electrophoresis [6], chemiluminescence [7] or electrochemistry [3,8–19]. Although electrochemical methods have given good results, rather high potentials must be applied for the direct oxidation of this analyte, which carries possible interferences. This problem can be sometimes solved by the incorporation of a biorecognition element, giving a specific biosensor. In this way, an enzymatic procedure based on the immobilization of morphine dehydrogenase and salicylate hydroxylase onto a Clark-type oxygen electrode [20] and an aptamer based method [21] were developed to quantify codeine.

In this work, the detection of codeine has been attempted using acetylcholinesterase (AChE) based screen-printed carbon electrodes (SPCEs), taking into account the reversible inhibition of the enzyme activity in the presence of this drug [22]. Acetylthiocholine iodide (ATI) acts as a substrate for AChE [23,24], generating thiocholine and acetic acid (Fig. 1). The enzymatically-generated thiocholine can be electrochemically detected at slightly high potentials using SPCEs [25,26], which could provoke the interference of other substances in the media or even the direct oxidation of the analyte [3]. In order to reduce this potential, chemical mediators such as cobalt phtalocyanine (CoPC) [27–32], Prussian Blue [33,34], 7,7,8,8-tetracyanoquinodimethane [35–37] or poly(3,4-ethylenedioxythiophene) (PEDOT) [38] have been added in solution. Moreover, the use of SPCEs allows incorporating the mediator into the working electrode in the screen-printed fabrication, which undoubtedly simplifies the chronoamperometric measurement procedure. In this way, CoPC [39] and PEDOT [40] have been screen-printed for the detection of the enzymatically-generated thiocholine. Tetrathiafulvalene (TTF) seems an optimum mediator in order to be screen-printed, since its non-solubility in water avoids risks of dissolution when working in aqueous solutions. Thus, TTF based SPCEs have been built by screen-printing a mixture of mediator and carbon paste in this work. In the next step, AChE have been cross-linked to TTF modified SPCEs (TTF-SPCEs) with glutaraldehyde (GA) and bovine serum albumin (BSA) to obtain a biosensor for codeine detection. According to Fig. 1, two molecules of the enzymatically-generated thiocholine dimerize thanks to the mediator which is converted in its reduced form. The mediator is then reoxidized on the electrode giving an oxidation signal, which can be related to the substrate. When codeine is added to the solution, the transformation of acetylthiocholine in thiocholine is avoided and consequently, the

* Corresponding author. Tel.: +34 947258818; fax: +34 947258831.
E-mail address: malomillo@ubu.es (M. Asunción Alonso-Lomillo).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.03.042

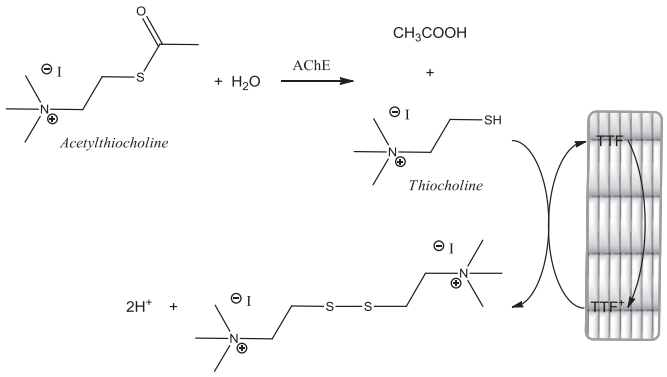

Fig. 1. Enzymatic reaction involved in the codeine chronoamperometric determination.

decreasing in the oxidation signal can be related to the concentration of the drug in solution. Immobilization and operational conditions that affect the chronoamperometric response of codeine have been optimized. The performance of the developed biosensor has been checked in terms of precision, capability of detection and application to pharmaceutical samples.

2. Experimental

2.1. Reagents

Screen-printed transducers were fabricated by sequential deposition of different commercial inks that define the different parts of the three-electrode system, namely carbon ink (C10903P14, Gwent Electronic Materials, Torfaen, UK), dielectric ink (D2071120D1, Gwent Electronic Materials, Torfaen, UK), Ag/AgCl ink (Electrodag 6037 SS, Acheson Colloiden, Scheemda, The Netherlands) and Ag ink (Electrodag 418, Acheson Colloiden, Scheemda, The Netherlands).

Analytical grade chemicals with no further purification were used. All solutions were prepared in Milli-Q water.

TTF was purchased from Acros Organics (Geel, Belgium).

GA and BSA were obtained from Sigma (Steinheim, Germany) as well as the enzyme AChE from *Electrophorus electricus* (Type IV-S, 410 units/mg protein).

100 mM NaH_2PO_4·(Merck, Darmstadt, Germany) and NaOH (J.T. Baker, Deventer, The Netherlands) buffer containing 100 mM of KCl (Merck, Darmstadt, Germany) was used as supporting electrolyte.

ATI was supplied by Fluka Analytical (Steinheim, Germany). Stock solutions were daily made in supporting electrolyte and stored at 4 °C when not in use.

Stock standard solution of codeine was prepared by dissolving the adequate amount of codeine hydrochloride (Alcaliber S.A., Madrid, Spain) in supporting electrolyte.

2.2. Apparatus

A DEK 248 printing machine (DEK, Weymouth, UK) was used for the manufacture of the SPEs.

A μAutolab electrochemical system with GPES software (Eco Chemie, Utrecht, The Netherlands) was utilized to carry out electrochemical measurements.

pH of the solutions was adjusted with a pH meter from HANNA instruments Model HI221 (USA).

3. Methods

3.1. Fabrication of TTF-SPCEs

Each different component from the electrochemical transducer, that is conductive silver tracks, Ag/AgCl reference electrode, carbon counter electrode and dielectric layer, was defined by sequential deposition of the different inks on polyester films (HiFi Industrial Film, Dardilly, France) and its subsequent curing, according to the manufacturer's specifications.

The working electrode ink was prepared by thoroughly mixing the carbon ink with TTF (5% w/w) and immediately screen-printed.

3.2. Immobilization of the enzyme AChE onto TTF-SPCEs (AChE-TTF-SPCEs)

Cross-linking immobilization through GA and BSA was used in order to attach the enzyme to the carbon surface of the working electrode. Volume and concentration of AChE, GA and BSA were optimized to obtain the highest analytical response for the inhibition with codeine (Table 1). The optimum conditions of immobilization obtained for the development of this biosensor were 2.5 μL of a 0.1%w/v AChE solution in 10 mM phosphate pH 6, 1.25 μL of a 6% w/v BSA solution in 10 mM phosphate pH 6 and 1.25 μL of a 2.5% v/v GA solution in water. These quantities were mixed and deposited onto the working electrode. After 120 min under 4 °C, the biosensor was ready to be used.

3.3. Measuring procedure

Cyclic voltammetric measurements were executed at room temperature in a 100 μL drop of KCl 100 mM, scanning the potential between 100 mV and 600 mV vs. screen-printed Ag/AgCl electrode, at a scan rate of 50 mV s^{-1}.

Chronoamperometric measurements were made at room temperature in a cell containing 5 mL of supporting electrolyte, of the desired pH, under constant mechanical stirring. Experimental measurements were performed applying a fixed potential of +250 mV vs. screen-printed Ag/AgCl electrode. After reaching a stable baseline, a volume of a solution of ATI was added into the electrochemical system to a final concentration of 1 mM, except for the optimization process, and after the stabilization of the signal, successive additions of 10 μL of a 10 mM codeine solution were made.

4. Results and discussion

The use of screen-printed technology allows the fabrication of electrodes with different characteristics. In this case, the screen-printed mediator leads to the reduction of the usual high applied potential needed in chronoamperometric measurements using AChE

Table 1
Influence of the amount of the cross-linking reagents used for AChE immobilization onto TTF-SPCEs in the chronoamperometric current registered for codeine (pH 7, ATI concentration = 1 mM, applied potential = 250 mV vs. screen-printed Ag/AgCl).

μL AChE 0.1%	μL BSA 6%	μL GA 2.5%	Current (nA)
2.5	0	2	59
2.5	0.5	1.5	86
2.5	1	1	89
2.5	1.5	1.5	105
2.5	1.5	0.5	81
2.5	2	0	46