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Amperometric biosensor based on prussian blue and nafion modified screen-printed electrode for screening of potential xanthine oxidase inhibitors from medicinal plants

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ABSTRACT

A simple and sensitive amperometric biosensor was developed for the screening of potential xanthine oxidase inhibitors from medicinal plants. This biosensor was prepared by immobilization of xanthine oxidase on the surface of prussian blue modified screen-printed electrodes using nafion and glutaraldehyde.

The developed biosensor showed a linear amperometric response at an applied potential of +0.05 V toward the detection of hypoxanthine from 5 μM to 45 μM with a detection limit of 0.4 μM (S/N = 3) and its sensitivity was found to be 600 mA M^{-1} cm^{-2}. In addition, the biosensor exhibited a good storage stability.

The inhibition of xanthine oxidase by allopurinol was studied under the optimized conditions. The linear range of allopurinol concentration is obtained up to 2.5 μM with an estimated 50% of inhibition I_{50} = 1.8 μM.

The developed biosensor was successfully applied to the screening of xanthine oxidase inhibitors from 13 medicinal plants belonging to different families. Indeed, Moroccan people traditionally use these plants as infusion for the treatment of gout and its related symptoms. For this purpose, water extracts obtained from the infusion of these plants were used for the experiments. In this work, 13 extracts were assayed and several of them demonstrated xanthine oxidase inhibitory effect, with an inhibition greater than 50% compared to spectrophotometry measurements that only few extracts showed an inhibition greater than 50%.

1. Introduction

Xanthine oxidase (XO) (EC 1.2.3.2) catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid and H_2O_2, the final reactions of the metabolism of purine bases. The overproduction and/or underexcretion of this acid lead to the incidence of hyperuricemia such as gout [1,2]. Accordingly, the use of XO inhibitors is one of the therapeutic approaches to treat gout by blocking the production of uric acid. Allopurinol is one of the XO inhibitors under the clinical application used to treat gout, but this drug suffers from many side effects such as hepatitis, nephropathy, allergic reactions, etc [3–5]. Therefore, there is an urgent need to search for new XO inhibitors with increased therapeutic activity and less side effects.

Natural products are excellent sources of new compounds in search for new medicines for the treatment of diseases. Morocco, a country possessing a long history of traditional medicinal system, has a number of medicinal plants used for gout, but no systematic investigations have been reported until now. In the present study, thirteen medicinal plants were selected to screen their XO inhibitory activity based on their ethnomedical use in the treatment of gout.

Nowadays, accurate, rapid, cheap and selective methods are needed for use in clinical diagnostic and food safety. The electrochemical biosensors have shown advantages like simplicity, rapid, high sensitivity, specificity, low cost and being economically viable.

Until now, a variety of amperometric biosensors for xanthine/hypoxanthine detection, based on the electro oxidation of hydrogen peroxide formed [6–10] have been reported. However, direct hydrogen peroxide amperometric detection at conventional electrodes is only possible at a potential of +0.6 V versus Ag/AgCl. At this high potential, the presence of easily oxidisable compounds such as ascorbate, phenols, urate, etc. can interfere in the measurement, being oxidized at the electrode together with the hydrogen peroxide [11]. To overcome this problem, the use of mediators such

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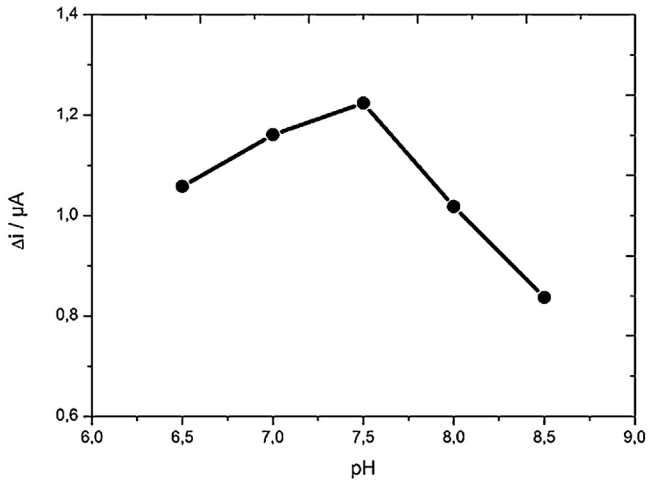

Fig. 1. Effect of pH on the response to 40 μM hypoxanthine in 50 mM phosphate buffer solution, 0.1 M KCl at 25 °C. The operating potential is +0.05 V vs Ag/AgCl.

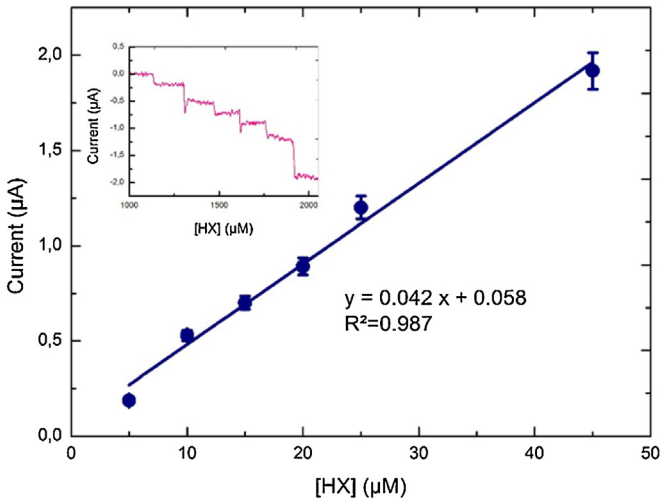

Fig. 2. Calibration curve obtained with SPE-PB/XO/nafion modified electrode in 50 mM phosphate buffer, 0.1 M KCl pH 7.5 at +0.05 V vs Ag/AgCl. Standard errors indicated by bars of three repeated measurements ($n = 3$). Inset shows a typical response to successive hypoxanthine injections.

as prussian blue (PB), which were able to reduce the enzymatic product, hydrogen peroxide, is proposed. This PB mediator may lead to a high selectivity owing to the low operating potential.

The screen-printed electrodes are frequently used in analytical applications because of their unique properties such as mass production, low cost, high reproducibility, small size, etc. [12]. Those electrodes modified with PB are an excellent substrate for the development of oxidase enzyme biosensor [11,13–15]. In fact, these biosensors can represent a good alternative method to spectrophotometry. Indeed, this is the first report of the use of biosensor to evaluate the enzyme inhibitors from medicinal plants.

In this paper, we report an amperometric biosensor of XO, which was immobilized onto the surface of the SPEs modified with PB. To prevent loss of the enzyme molecules and to improve the stability of the biosensor, nafion (polymer) was used.

Indeed, the purpose of this study was to develop a biosensor incorporating immobilized xanthine oxidase and prussian blue to evaluate XO inhibition from different medicinal plants so as to discover a natural substitute of plant origin that can be used as an alternative to allopurinol for the treatment of gout. The optimum working conditions as the substrate concentration, the method of immobilization and the pH were investigated.

2. Experimental

2.1. Materials and reagents

XOD (EC 1.17.3.2, 0.1 units/mg protein, Grade I from bovine milk) allopurinol and hypoxanthine were purchased from Sigma (USA). Glutaraldehyde was supplied by Shanghai Lingfeng Chemical Reagent Co., Ltd. All the other chemical reagents used were of analytical grade and without further purification. Phosphate buffer saline (PBS, 0.05 M) with various pH values was prepared by mixing K_2HPO_4 and KH_2PO_4, containing 0.1 M KCl. All the solutions were prepared with distillated water.

2.2. Apparatus and measurements

The amperometric and voltammetry measurements were carried out at room temperature using a PalmSens potentiostat interfaced to a computer. The measurements were performed by the three-electrode system. The polyester film was used as substrate for printing the graphite-working, graphite-counter and Ag reference electrodes [16]. In all measurements 0.05 M PBS + 0.1 M KCl was used as an electrolyte.

Screen-printed electrodes (SPEs) consisted of a working electrode in graphite, a reference electrode in silver/silver chloride, and a counter electrode in graphite (Ø = 3 mm). The electrodes were printed with a 245 DEK (Weymouth, UK) screen-printing machine. Graphite based ink (Electrodag 421, Acheson, Henkel, UK), silver/silver chloride ink (Electrodag 4038 SS) and insulating ink (Carboflex 25.101.S, Acheson, Henkel, UK) were used. SPEs were produced in the laboratory of University of Tor Vergata of Rome (Italy). For the spectrophotometric measurements, a JENWAY 6850 UV–vis spectrophotometer was used.

2.3. Plant materials

Thirteen medicinal plants were collected from Casablanca region in the west north of Morocco. Plant parts have been chosen in relation to Moroccan popular medicine use: The leaves and flowers of twelve plants: *Ailanthus altissima* (*Simaroubaceae*), *Artemisia herba-alba* (*Asteraceae*), *Caryophyllus aromaticus* (L.) (*Myrtaceae*), *Ginkgo biloba* (L.) (*Ginkgoaceae*), *Hyssopus officinalis* (L.) (*Lamiaceae*), *Lavandula angustifolia* (*Lamiaceae*), *Melissa officinalis* (L.) (*Lamiaceae*), *Mentha spicata* (L.) (*Lamiaceae*), *Rosmarinus officinalis* (L.) (*Lamiaceae*), *Solidago virgaurea* (*Asteraceae*), *Thymus vulgaris* (L.) (*Lamiaceae*) and *Urtica dioica* (L.), (*Urticaceae*), and the stigma of