Sensing and Bio-Sensing Research 5 (2015) 105–111

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

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

Biocompatible laponite ionogels based non-enzymatic oxalic acid sensor

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A R T I C L E I N F O

Article history:
Received 25 March 2015
Revised 10 August 2015
Accepted 12 August 2015

Keywords:
Ionogels
Enzyme-free
Oxalic acid
Biosensor
Cyclic voltammetry

A B S T R A C T

An enzyme-free oxalic acid (OA) electrochemical sensor was assembled on indium tin oxide (ITO) plate on which a film of laponite ionogel was coated that resulted in an L/IL/ITO electrode. This ionogel electrode was characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and UV–Vis spectroscopy techniques. Electrochemical oxidation of OA on the electrode surface was investigated by cyclic voltammetry. Further this electrode exhibited high electrochemical activity that yielded well-defined peaks of OA oxidation, and a notably suppressed over-potential compared to the laponite–ITO (L/ITO) electrode. Under optimized conditions, a good linear response (anodic current) was observed for the OA concentration in the 1–20 mM range with a detection limit of 3 μM. Furthermore, this electrochemical strip sensor presented good characteristics in terms of stability, and reproducibility offering promise of applicability of this green sensor platform.

1. Introduction

Oxalic acid is a strong acid (pK_{a1} = 1.23 and pK_{a2} = 4.19) simple hydrophilic molecule [log KO/W = −0.7] having high solubility in water and is present in spinach, cabbage, broccoli, mushrooms and Brussels sprouts. Oxalic acid (OA) is widely distributed in various organisms, fungi, plants and animals. High levels of OA remove calcium from blood that can cause severe disturbance in the activity of heart and neural system. It has been found that OA may cause digestive tract irritation and kidney damage; hence there is an increasing demand for its determination in biofluids [1,2]. Excess of oxalic acid may result in the formation of oxalate stones in bladder and kidneys (calcium oxalate and calcium phosphate). Calcium oxalate stone formation may be caused by high level of calcium, and high oxalate excretion. Oxalic acid converts into oxalate in the body, and hence prevents the sedimentation of calcium in bones by sticking to it. In other words, it causes calcium excretion, and hence deficiency. Therefore, the need to design an electrochemical platform in the form of a strip sensor, for detection of OA can hardly be stressed.

Laponite, synthetic clay, known as 2:1 nanoclay consists of two tetrahedral silicate sheets sandwiching an octahedral sheet and separated from another unit via electrostatic interaction. The geometrical structure is disk-like with diameter of ∼30 nm and thickness of 1 nm. Clays are used in biosensors as immobilization matrices due to their large specific surface area, good absorbance ability and high cationic exchange capacity [3–6]. Due to the property of laponite to form non-ergodic soft solids in the aqueous dispersions, they have been used to cast films onto electrodes for the immobilization of proteins [7–11] Rawat et al. have studied the aspect ratio and concentration dependent cytotoxicity and antimicrobial activity of laponite and Montmorillonite nanoclay [12,13].

Ionic liquids (ILs) are class of compounds that are widely used in the development of electrochemical sensors [14,15]. ILs are defined as those which consist of an organic cation and an organic/inorganic anion which are molten salts at low temperature [16,17]. Maleki et al incorporated ILs into carbon paste electrode which exhibited excellent electrochemical behavior [18]. Safavi et al used carbon ionic liquid electrode where ionic liquid are basically used to improve the reversibility and kinetics of electrochemical reaction for the simultaneous determination of dopamine, ascorbic acid and uric acid [19].

A biosensor is usually defined as an analytical device through which a biological response is converted into signal that can be quantified and processed [20,21]. Clay colloid provides a favorable microenvironment for electron transfer and catalytic reactions on the electrode [22,23]. Electrodes modified with clay minerals have attracted the attention of many electrochemists because of their unique layered structure and their ion exchange properties [24].

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http://dx.doi.org/10.1016/j.sbsr.2015.08.001
2214-1804/

The entropic ionogels of laponite prepared in IL solutions causes the formation of self-assembled colloidal networks [25]. The aim of the present work was to make a biosensor using laponite (L), and [C2mim][Cl] ionic liquids (IL), and to study their sensing behavior towards different bio-analytes.

The determination of oxalic acid in food and urine is utmost importance as this toxic compound can cause kidney stones. Various instrumental techniques such as gas chromatography, liquid chromatography, spectroscopy, enzymatic method were developed but due to their high cost with low sensitivity, it is convenient to use electrochemical techniques. Thus, electrode materials are used for constructing oxalic acid sensor. Shimohigoshi and Karube [26] construct a multi-functional bio-thermo chip system for the determination of kidney calculus indices oxalic acid. Liu et al. [27] developed an HRP-immobilized RTIL based sol–gel matrix for constructing an electrochemical biosensor which exhibit excellent sensitivity as well as stability. Maiyalagam et al. [28] demonstrated the determination of oxalic acid in real samples, specifically in tomato extract using the tungsten carbide with tube like nanostructures supported platinum nanoparticles. A method for the determination of oxalic acid based on the use of oxalate oxidase where injection of oxalate oxidase in a buffer solution into an internal chamber of the SIRE biosensor by Hong et al. [29].

In our work we are using laponite–IL electrode for determining oxalic acid which is simple to construct and most important is that it does not require any enzymes. The introduction of Ionic Liquid into laponite makes it more stable film and enhanced the conductivity as compared to pure laponite. A lot of work has been done in literature for oxalic acid sensor using enzymes as well as enzymes free some of which is shown in Table 2.

We have performed an extensive electrochemical surface characterization of the electrodes using cyclic voltammetry (CV), FTIR and scanning electron microscopy (SEM). To the best of our knowledge, we have found no report on the electrochemical study of laponite ionogels. This profiling conclusively determined the specific sensitivity of these electrodes towards different analytes.

2. Materials and method

The IL, 1-ethyl-3-methyl imidazolium chloride [C2mim][Cl], was purchased from Sigma–Aldrich and was used as received. Deionized water from Organo Biotech Laboratories, India, was used to prepare the solutions. laponite RD® clay was purchased from Southern Clay Products, USA. Clay dispersions (2% w/v) were prepared by dissolving laponite in deionized water with constant stirring for 2 h, to which a given amount of IL was added, and stirred for 10 min. The solutions of different analytes i.e. glucose, urea, citric acid, ascorbic acid, cholesterol and oxalic acid were prepared as stock solutions (20 mM) and stored in refrigerator at 4 °C. Indium-tin-oxide (ITO) coated glass plates obtained from Balzers, UK, (Baltracom 247 ITO, 1.1 mm thick) with a sheet resistance and transmittance of 25 Ω sq^{-1} and 90%, respectively were used as electrode substrates.

Electrochemical experiments (cyclic voltammetry (CV)) were performed using Autolab Potentiostat/Galvanostat (Eco Chemie, Netherlands) with three-electrode cell where L/IL/ITO (laponite/Ionic Liquid/ITO) was used as working electrode, platinum (Pt) wire as the auxiliary electrode and Ag/AgCl as reference electrode in 3.3 mM Zobell's solution (3.3 mM ferric and ferrous solution mM; pH 7.0) containing 0.9% (w/v) KCl.

Fourier transform infrared (FTIR) spectroscopy studies were carried out using PerkinElmer, Spectrum BX II instrument. Scanning electron microscopy (SEM) was done using SEM, EVO, 40 ZEISS Instrument. The UV-absorptivity was measured using Cecil, model CE-7200 (Cecil Instruments, UK) spectrophotometer through which the optimum binding constant was evaluated.

3. Results and discussion

The resulting films, after the preparation of L/IL dispersions, were fabricated by uniformly spreading 10 μl solution of L/IL sol on conducting side of ITO which had 0.25 cm^2 surface areas. The film formed was kept overnight for drying (~24 h) at room temperature. The electron transfer kinetics of electroactive species was studied using cyclic voltammetry (CV), a widely used technique to obtain information about electrochemical reactions. Before undertaking a thorough study, it was felt necessary to optimize the electrochemical behavior of L/IL/ITO electrode as a function of IL concentration in Zobell's solution at a given scan rate of (≈20 mV/s). The optimization studies were necessary to explicitly determine the dependence of various experimental parameters like concentration, scan rate, pH, pulse amplitude etc. to establish the best experimental working condition [12,13,30–32].

The concentration of IL can change the properties of the electrode surface; hence we first investigated the effect on peak currents by varying concentration of IL. The highest anodic and cathodic peak currents was obtained for 0.04% (w/v) IL (shown in Fig. 1) which indicated that this concentration of IL in L/IL/ITO had better electrochemical properties compared to others. Therefore, we have selected electrodes made with 0.04% (w/v) ILs for further studies.

The electrochemical response of L/IL/ITO electrode was monitored by a voltammetric sweep curve ranging from −0.1 to +0.4 V as a function of scan rate varying from 10 to 100 mV/s (Fig. 2(a)). From this figure we could observe well-defined peak shapes at different scan rates with magnitudes of both anodic (I_a) and cathodic (I_c) peak current increasing linearly. This linear dependence of peak current (Fig. 2(b)) indicated that the electrode provided sufficient accessibility to electron between electrolyte and electrode revealing surface controlled electrode process. The anodic (E_a) and cathodic (E_c) peak potentials did not show any shift with scan rate. The independent behavior of separation of peak potentials ($\Delta E = E_a - E_c$) with scan rate and linear dependence of peak current with scan rate suggested that redox reaction which was undergoing in L/IL/ITO electrode was reversible. The cyclic voltammetric behavior for only laponite electrode (L/ITO) is shown in Fig. S1 (Supplementary Information).

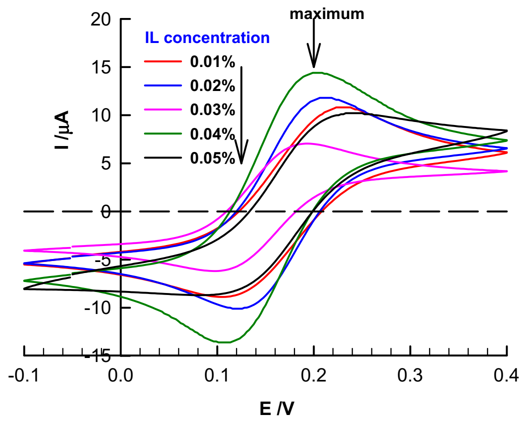

Fig. 1. Cyclic voltammetric response of laponite with different concentration of IL varying from 0.01% to 0.05% (w/v). The highest peak current was found for 0.04% (w/v) IL sample.