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A non-enzymatic sensor based on the redox of ferrocene carboxylic acid on ionic liquid film-modified screen-printed graphite electrode for the analysis of hydrogen peroxide residues in milk

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ABSTRACT

Globally, the use of hydrogen peroxide (H_2O_2) for the preservation of raw milk has a long established history. However, in the EU, US and most parts of the world, where access to refrigeration facilities is widely available, the adulteration of milk with H_2O_2 is generally not permitted. An in-house hand-printed carbon electrode consisting of graphite printing ink modified with the room temperature ionic liquid (RTIL), 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]), ferrocene carboxylic acid (Fca) and cellulose acetate (CA) for the electrochemical sensing of H_2O_2 in commercially packaged aseptic milk is described. The developed electrode successfully enabled sensitive determination of H_2O_2, free from interference from some known electroactive species such as ascorbic acid (AA), dopamine (DA), glucose and uric acid (UA). The linear range for the determination of H_2O_2 was 1.0 μM–1.2 mM with a limit of detection of 0.35 μM and a sensitivity of 10.6 nA $\mu A^{-1}\ \mu M^{-1}\ cm^{-2}$. When used for the analysis of H_2O_2 residues in milk samples, the resulting precision ($n = 6$) and recovery were 0.53% and 97.8%, respectively.

1. Introduction

The determination of hydrogen peroxide (H_2O_2) is of great relevance in the food and beverage industry; thus, creating the need for the fabrication of easy-to-use, selective, sensitive, and single-use disposable H_2O_2 sensors [1–4]. H_2O_2 has inherent bactericidal and sporicidal properties and is often used to clean mixing, bottling, transporting and packing equipment in the food industry [2,3]; hence, H_2O_2 may become incorporated into these surfaces and require an additional processing step to remove or decompose it. Its bactericidal and sporicidal properties are based on the fact that fresh raw milk contains the enzyme lactoperoxidase (which has no antibacterial properties on its own) but has the ability to catalyse the oxidisation of inherent thiocyanate (SCN^-) in the presence of H_2O_2 (Eq. (1)) [2];

$$SCN^- + H_2O_2 \rightarrow OSCN^- + H_2O. \quad (1)$$

The resulting chemical compound ($OSCN^-$) has an antibacterial effect in fresh raw milk. Hence, H_2O_2 is often used to activate this inherent lactoperoxidase enzyme system (shown in Eq. (1)) to preserve raw milk particularly in areas where refrigeration is not widely available [3–5]. However, against some species of the normal flora of human gut including *streptococci* and *lactobacilli*, the compound $OSCN^-$ has a bacteriostatic effect (inhibition of bacterial growth). Consequently, the ingestion of milk containing residues of H_2O_2 can cause gastrointestinal problems [6]. H_2O_2 in milk can also cause the degradation of vitamins (e.g. folic acid) [7] and some essential amino acids such as methionine [8] resulting in a reduction of its nutritional value. Therefore, the use of H_2O_2 for the preservation of raw milk within the EU, United States, and most parts of the world, where refrigeration is widely available, is not permitted except in certain applications such as cheese-making and modified whey [1,5].

Currently, the measurement of H_2O_2 in the food and beverage industry includes spectroscopy, [9,10] and enzyme-based fluorescence or chemiluminescence assays [11,12]. However, these methods are relatively expensive, require special storage facilities to preserve the enzyme activities, cumbersome and often require that samples are pre-treated prior to their analysis thus, rendering them unsuitable for routine analysis of H_2O_2. Owing to the electroactive nature of H_2O_2, the use of electrochemical sensors is most attractive for H_2O_2 analysis in milk [13,14] because they are rapid, simple, relatively less expensive, selective and sensitive, and allows for direct real-time and online data analysis that excludes sample pre-treatment procedures [15,16]. But the main drawbacks associated with traditional electrode materials for electrochemical measurements are low reproducibility due to electrode fouling and the poor selectivity arising from common interfering species such as dopamine (DA), ascorbic acid (AA), and uric acid (UA). Electrodes modified with redox mediators [15] and perm-selective

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membranes [16–18] have been useful in solving problems from intereferences.

Typically, the use of mediators can allow H_2O_2 to be detected at lower potentials, thus substantially reducing the interfering influence of other electroactive species [17]. However, the immobilisation of redox mediators can be problematic as it can be poorly adsorbed onto such electrodes [15]. One possible route to solve problems from interferences is to use perm-selective membranes in conjunction with a redox mediator [18], thereby excluding the access of many interfering species to the surface of the electrode.

Room temperature ionic liquids (RTILs), salts that are liquid at room temperature, are generally considered to be 'green solvents' and have been used as catalytic supports [19]. Consequently, in the current study, the RTIL, 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]) was used as a 'green support' for the immobilisation of a redox mediator, ferrocene carboxylic acid (Fca) onto the working area of a screen-printed graphite electrode (SPGE). Additionally, cellulose acetate (CA), serving as a perm-selective membrane, was incorporated into the sensor design to act as an exclusion barrier. Overall, the specific advantages of screen-printed platforms (miniaturisation and low-cost) coupled with the use of RTIL in conjunction with the perm-selective membrane, CA to immobilise the redox mediator, Fca, are assembled to fabricate a simple, selective and sensitive sensor for the quantification of H_2O_2 in commercially packaged aseptic milk; thus, providing an alternative in routine analytical sensing of milk adulteration with H_2O_2. Details of the sensor fabrication and characterisation are described and discussed.

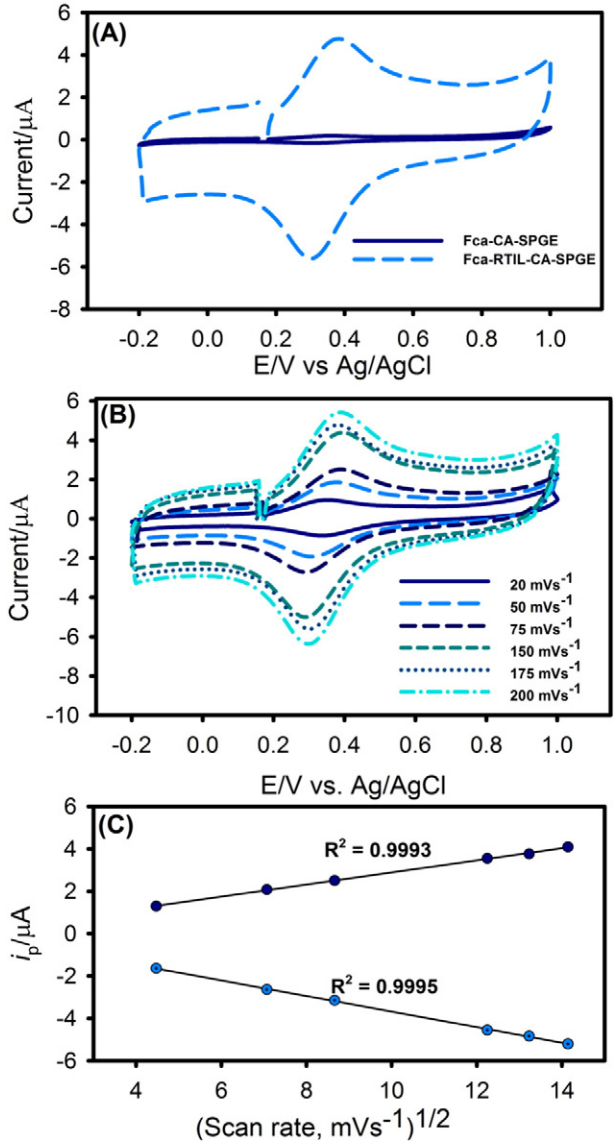

Fig. 1. Cyclic voltammograms obtained at (A) Fca-CA-SPGE and Fca-RTIL-CA-SPGE in Britton–Robinson (BR) buffer (pH 7.0) after overnight storage at 4 °C, 175 mVs^{-1} scan rate; (B) Fca-RTIL-CA-SPGE in BR buffer (pH 7.0) at scan rates of 20, 50, 75, 150, 175 and 200 mVs^{-1}. All buffer solutions contained 0.1 M KCl solution; (C) plot of I_p vs. $\sqrt{V}$.

2. Experimental

2.1. Apparatus and reagents

Electrochemical experiments were conducted using VSP-300 Multichannel Potentiostat/Galvanostat/EIS (Bio-Logic Science Instruments, France) with a standard three-electrode configuration. The SPGEs were hand-printed using a Stainless Steel Screen Mesh (DEK: 159784, ASM Assembly Systems). Valox substrate was purchased from Cardillac Plastics, UK. A Ag/AgCl (1.0 M KCl) reference electrode was used throughout. The working electrode was SPGE with a platinum wire as the counter electrode. The room temperature ionic liquid 1-ethyl-3-methyl imidazolium tetrafluoroborate ([EMIM][BF_4]), cellulose acetate, acetone, catalase, ethanol were obtained from Sigma Aldrich. Ascorbic acid, uric acid and ferrocene carboxylic acid were purchased from Alfa Aesar, UK. All other chemicals were of analytical grade and used without further purification. Commercially packaged aseptic milk samples were obtained from a local convenience store.

2.2. Preparation and analytical application of the sensor

The base unmodified SPGE transducer was prepared using graphite ink (GEM Product code: C205010697) and the sensors were screen-printed in groups of eight onto valox substrate and cured at 70 °C for 90 min. The SPGE was modified by drop-coating 5 μl of 1.5% CA-acetone solution directly onto the working area (3.5 × 3.5 mm) of the electrode to form CA-SPGE. The presence of CA was crucial for forming a stable RTIL film. Once dry, the electrode was exposed to 10% RTIL-ethanol solution for 1 h to form RTIL-CA-SPGE. Lastly, the RTIL-CA-SPGE was immersed in 5 mM ferrocene carboxylic acid (Fca) solution for about 24 h to form Fca-RTIL-CA-SPGE. In addition to this, Fca-CA-SPGE, Fca-RTIL-SPGE and Fca-SPGE sensors were fabricated in a similar fashion. Prior to each measurement, all solutions were degassed with N_2 for about 10 min. Once prepared, the sensors were placed in Britton–Robinson buffer (pH 7.0) and stored at 4 °C overnight.

The milk samples were prepared by mixing 5 ml of milk in 25 ml of Britton–Robinson (BR) buffer (pH 7.0) and spiked with 500 μM H_2O_2. Prior to this, the enzyme catalase was used to verify whether H_2O_2

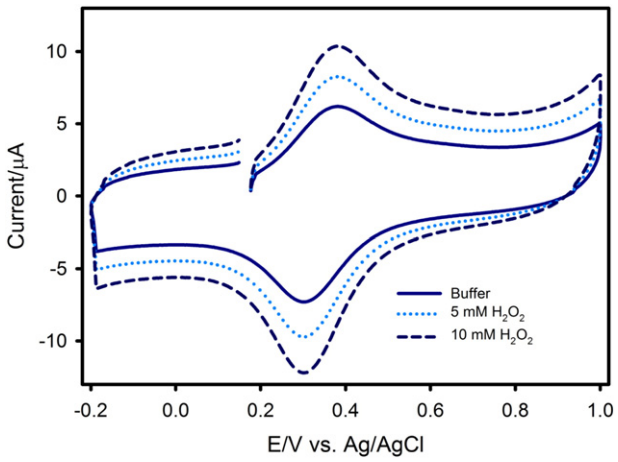

Fig. 2. Cyclic voltammograms obtained at Fca-RTIL-CA-SPGE electrode for 5 mM and 10 mM H_2O_2 in BR buffer (pH 7.0) containing 0.1 M KCl. Scan rate: 175 mVs^{-1}.