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A novel sensor for a food dye erythrosine at glucose modified electrode

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ABSTRACT

A simple and highly sensitive method for the determination of erythrosine using glucose modified carbon paste sensor was presented. The electrochemical measurements were studied using cyclic voltammetry, differential pulse voltammetry and square wave voltammetry. The modified sensor exhibited excellent catalytic activity towards electrochemical oxidation of erythrosine in highly basic phosphate buffer, i.e. pH 11.2. The modified sensor facilitated the determination of erythrosine in the linearity range 1.0×10^{-4} M to 1.0×10^{-7} M with a detection limit of 21.6 nM. Good recovery from biological sample such as spiked urine indicates the applicability of the proposed method for clinical trials. The proposed method was successfully employed to detect erythrosine in pharmaceutical as well as food samples. The method exhibits tremendous reproducibility and has proved to be highly reliable for the analysis of erythrosine in biological samples.

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1. Introduction

Synthetic colorants, a vital class of food additives, are added to products of food to make them more appealing, supplanting their characteristic shading that can be lost during the mechanical procedures, or to avoid variations in the color of the final product. They are imperative contents in many food materials, such as confectionery products, gelatin treats, snacks and drinks, since many of these would be colorless and would thus appear undesirable without the inclusion of colorants [1]. However, some of these substances pose potential dangers to human wellbeing and when in contact with a few medications can bring about allergic irritations, especially if they are excessively consumed and also induce the development of cancer and other diseases [2]. Thus, the utilization of manufactured colorants in food items is entirely controlled by different national legislations like World Health Organization (WHO). The regulation concentration range of erythrosine for food by WHO is 0.01 mg/kg [3].

Erythrosine (ERT) belongs to the class of xanthene dyes, chemically known as 2-(6-hydroxy-2,4,5,7-tetraiodo-3-oxo-xanthen-9-yl) benzoic acid (Scheme 1). Primarily, it is widely used as a food coloring agent and a host of other applications such as printing inks, a dental plaque disclosing agent, a biological stain and cosmetics. It is highly toxic, causes various types of allergies, thyroid problems, carcinogenicity, anemia, DNA damage behavior, neurotoxicity and xenoestrogen nature in humans and animals. Thus, due to the hazardous nature and harmful effects of erythrosine, it was considered valuable to make efforts to develop a simple method for the detection of erythrosine in different samples.

Owing to the importance, few methods were reported for the determination of erythrosine. These include spectroscopy, electro kinetic capillary chromatography, high-pressure liquid chromatography (HPLC) and voltammetric methods [4–10]. Electrochemical methods due to its accuracy, better sensitivity, and selectivity compared to other technique, time saving, and simplicity of solutions has attained a significant place. With use of these methods, information related to redox behavior of the analyte can be acquired easily [11,12]. In addition, new and interesting properties forming the base for new applications and novel devices can be obtained by the controlled modification of electrode.

In the present work, the complex oxidation mechanism of glucose at the surface of carbon paste electrode was used for the detection of erythrosine. It seems that the use of carbon paste electrode simplify the immobilization procedure. Carbon paste electrode has the advantages of the diversity of paste modification and the convenience in manipulation, providing a reliable and universal method for immobilization of metal particles, non enzymes, nanoparticles and active enzymes [13]. Glucose has various desirable properties, e.g. biocompatibility, low toxicity,

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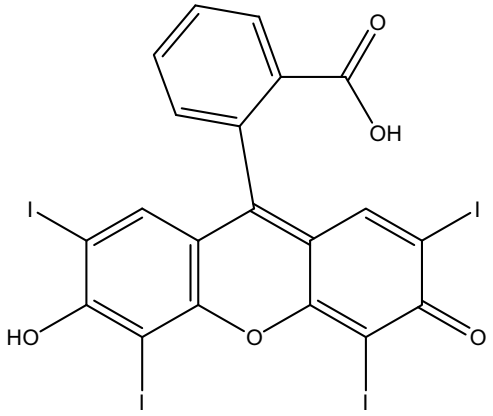

Scheme 1. Chemical structure of Erythrosine (ERT).

chemically inertness and high mechanical strength. The interesting chemical behavior of the glucose leads to understand the non-enzymatic oxidation mechanism of glucose [14–18]. Most of the work proposed, involved the glucose oxidation to gluconate. In the intermediate of the reaction, adsorption of the R-COO^- on the electrode surface is been demonstrated [19]. The negatively charged species can enhance the electrode analyte interaction to increase the selectivity and sensitivity of the electrode. Glucose having good adsorption property with carbon powder and has high affinity for oxidation of organic molecules. It is quite surprising that electrocatalytic activity of glucose is still not utilized for other analyte determination.

Therefore in the present protocol, glucose is utilized to prepare an electrochemical sensor for the determination of erythrosine. The carbon paste electrode surface was used as a material for the modification, due to its wide potential range, high sensitivity and good conductivity [20]. The resulting electrochemical sensor exhibits excellent stability, high sensitivity and fast response toward electrochemical analysis of xanthene food dye, erythrosine. The results obtained indicated the glucose immobilized on carbon paste displayed a high affinity to oxidize erythrosine dye.

2. Experimental

2.1. Chemicals and reagents

Erythrosine from Sigma–Aldrich was used without further purification. Use of analytical grade reagent and doubled distilled water throughout the experiment was been included. Effect of pH ranging between pH 3.0–11.2 was studied using phosphate buffer solution prepared by mixing KH_2PO_4, Na_2HPO_4, and Na_3PO_4 purchased from Sigma–Aldrich as per the literature [21].

2.2. Instrumentation

With three electrode system and a 10 ml single compartment, experiments were carried out in CHI Company, USA (Model D 630) electrochemical analyzer at an ambient temperature of 25 ± 0.1 °C. The oxidation of ERT was carried on the surface of glucose modified carbon paste electrode (working electrode), Ag/AgCl (reference electrode) and platinum wire as a counter electrode. In all the measurements back ground subtraction were made. The pH measurements were made by Elico pH meter model LI120.

2.3. Preparation of electrode

The glucose modified carbon paste was prepared by mixing graphite powder and paraffin oil in an agate mortar in a ratio of 7:3 and the mixture was then homogenized. A portion of the resulting paste was packed firmly into a cavity of a poly tetra fluoro ethylene tube (PTFE). The surface of the electrode was smoothed against weighing paper and rinsed with water. Glucose solution was prepared (1.0 mM) and 100 μl was deposited on the electrode surface and dried. The resulting electrode was called as GCPE.

Randles–Sevcik equation was used to calculate the active area of the electrode using cyclic voltammetric technique and $K_3Fe(CN)_6$ 1.0 mM as a probe at different scan rates in 0.1 M KCl as supporting electrolyte [22]. At $T = 298$ K and for a reversible process the equation is as follows:

$$I_p = (2.69 \times 10^5) n^{3/2} A D_0^{1/2} \nu^{1/2} C_0^* \quad (1)$$

In Eq. (1) I_P refers to the anodic peak current, n is the number of electron transferred during the electrode reaction = 1. A is the surface area of the electrode, D_0 is the diffusion coefficient i.e. 7.6×10^{-6} cm^2 s^{-1}, ν is the scan rate and C_0^* is the concentration of $K_3Fe(CN)_6$. The surface area of the electrode (A) was calculated from the slope of the plot of I_p vs. $\nu^{1/2}$. For the bare electrode, the area was found to be 0.042 cm^2 and for the modified electrode, the surface area was found to be 0.13 cm^2.

2.4. Procedures for pharmaceutical preparations

Ten pieces ERT tablets were finely grounded using a mortar and weight corresponding to stock solution was transferred in 100 ml volumetric flask and dissolved and diluted with double distilled water upto the mark. After sonication for ten minutes for proper dissolution, suitable aliquots were diluted with buffer solution pH 11.2. Transferring each solution in 10 ml cell, analysis was carried out with standard addition methods. The differential pulse voltammogram were recorded and the oxidation peak current of ERT was measured. Accuracy of the proposed method was checked by recovery studies.

2.5. Procedures for ERT analysis in food samples

The method was applied to determine ERT in commercial soft drinks and in bakery products. Commercially available soft drinks (Sipon Guava) and erythrosine lake was taken and real samples have been prepared in pH buffer 11.2. The differential pulse voltammogram was subsequently recorded in optimized conditions. The content of the drug in food samples was determined referring to the calibration graph.

2.6. Analysis of human urine

Human urine was obtained from four healthy volunteers of similar sex and age. Aliquots were centrifuged at 7000 rpm for 5 min at room temperature (25 ± 0.1 °C). These urine samples were analyzed immediately or they were stored at low temperature until analysis.

3. Results and discussion

3.1. Cyclic voltammetric behavior of ERT

Voltammetric studies were carried out using cyclic voltammetric technique (pH 11.2). Voltammogram obtained at bare and modified electrode exhibited a distinct anodic peak with significant increase in sensitivity and selectivity at modified electrode. The results were shown in Fig. 1. Erythrosine exhibited two anodic peaks (A and B), one at 0.783 V with anodic current 1.8 μA and another at 0.986 V with anodic current 2.2 μA. First peak was generally recorded, since it was more intense than second one. Therefore

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