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An electrochemical in-vitro tool for study of in-vivo relevant biochemical oxidation/reduction of sulfide ion by human whole blood: Evidence for the biological detoxification of hydrogen sulfide

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ABSTRACT

Studies related to redox reaction of human whole blood with hydrogen sulfide is highly important in biological system (e.g., detoxification of sulfide ion) and there have been several indirect experimental evidences for their interaction. This is the first and direct report for electrochemical oxidation and reduction of sulfide ion on a human whole blood chemically modified electrode system in physiological solution. A specific diffusion controlled electrochemical oxidation signal at 0 V vs Ag/AgCl and reduction signal at −0.5 V vs Ag/AgCl corresponding to the electrochemical conversion of sulfide ion to sulfate and sulfide to sulfur atom respectively by the Blood-Hemoglobin-$Fe^{III/II}$ redox site were noticed. These reactions are similar to the red blood cell functional behaviours with hydrogen sulfide (exist as HS^- in a neutral solution) as detoxification mechanism in the human body. Control results of electrochemical sulfide oxidation/reduction reactions with commercial hemoglobin and heme derivative (hematin) modified electrodes support the observation. Calculated electrochemical sulfide oxidation parameters such as Tafel slope, transfer coefficient (α) and heterogeneous rate constant (Andrieux and Saveant model) values are 120 mV $decade^{-1}$, 0.5 and $4.77 \pm 1.51 \times 10^{-6}$ mol^{-1} cm^3 s^{-1} respectively. Selective flow injection analysis of sulfide ion (HS^-) using the blood chemically modified electrode was demonstrated as a proof of concept for the applicability of the working electrode to analytical application.

1. Introduction

Unlike the nitric oxide, hydrogen sulfide and sulfide ion role in physiological and toxicological systems [1] are not much explored. Only after the discovery of presence of H_2S in brain by Marcus et al. in 1989 [2], perception about it as just being malicious is changed. Since then, extensive research is being done to explore the importance, mechanism and toxemic effect of H_2S in human system. While the high level (>500 ppm) is fatal due to respiratory failure (it replaces the O_2 binding site in hemoglobin), lower levels are proven to be useful for immune, cardiovascular, central nervous system, modulating blood pressure, diabetics control, anti-aging, maintaining electrolyte equilibrium and apoptosis [3–5]. In general, H_2S is produced in-sitully in tissues and cells by the action of enzymes cystathionine-γ-lyase, cystathionine-β-synthetase and 3-mercaptopyruvate sulfurtransferase which utilizes sulfur containing amino acids (ex: L-cysteine, homocysteine and its derivatives) as substrate [6]. In aqueous phase, H_2S dissociates to form HS^- (hydrogen sulfide anion; about 80%) at $pK_a = 7.0$ and S^{2-} (sulfide anion) at $pK_a = 11$, as per the following equation: $H_2S \Leftrightarrow HS^- + H^+ \Leftrightarrow S^{2-} + 2H^+$ [7]. In case of unavoidable circumstances, if human body gets exposed to lethal concentrations of H_2S (~500 ppm) for a short period of time, body has an excellent defence mechanism to eradicate the excess H_2S. Two major kinetics are involved for the metabolism of surplus H_2S: (i) Accordingly to the physiological condition, it gets oxidized in liver mitochondria, blood hemoglobin (expected to be redox mechanism) and cytosol (part of the cytoplasm) to oxygenated sulfur products which are then excreted through urine [8], (ii) H_2S can also be rummaged by methemoglobin or by oxidized form of glutathione [9]. Upon long time exposure, these mechanisms fail and formation of large amount of sulfhemoglobin takes place (S-Hb) [10], wherein, reduced form of sulfide, sulfur (atom) incorporated hemoglobin protein generates and cause respiratory catastrophe and death. There are many questions unanswered and yet to be studied, for example, the mechanism of oxidation of H_2S in body and interaction of blood cells with H_2S in physiological processes etc. Note that yet there is no direct human blood (interaction) study reported in this regard. In this work, we introduce a new strategy for the in-vitro analysis of interaction of sulfide ion with human whole blood sample, wherein, fresh blood chemically modified electrode controllable by potential and current, is

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taken as measuring system in a nitrogen purged pH 7 PBS. Since, heme group containing iron redox site ($Fe^{III/II}$) is prone to reduce the dissolved oxygen [11], all the experiments were carried out in a deaerated physiological pH PBS.

To study the interaction of the sulfide with blood, analytical protocol developed by Winter et al. for the thio group specific derivatization coupled HPLC-fluorescence technique has been referred as a best method and widely adopted in the clinical systems [12]. In this procedure, blood sulfide is derivatized as a sulfide-dibimanne using monobromobimane (MBB) in slightly alkaline condition. The resultant fluorescent active sulfide-dibimanne was separated by column chromatographic method and further analysed by reverse phase-HPLC chromatographic techniques using a fluorescence detector. Meanwhile, methylene blue based biological sulfide detection and many amperometric/flow injection analysis based electrochemical sensors, where the working electrodes being, poly methylene blue modified glassy carbon electrode [13], ion selective electrodes [14], palladium particles modified glassy carbon electrode [15], hexadecylpyridiniumbis(chloranilato)-antimonyl(V) modified screen printed graphite electrodes [16], poly(*N*-vinyl-2-pyrrolidone) (PVP)-capped CdS quantum dots modified glassy carbon electrode [17], quercetin modified pencil graphite electrode [18] for detection of sulfide in blood serum have been reported in the literature.

Although, all these above mentioned ex-situ methods including the Wintners' protocol provide information of the sulfide in blood, exact mechanistic feature of the origin of the sulfur and its derivative could not be supported by these existing analytical techniques. A new electrochemical study introduced in this work not only provides electron-transfer reaction between sulfide and blood hemoglobin but also biomimics the natural detoxification mechanism of body against surplus sulfide ion in physiological condition (Scheme 1). In further, this study helps us to analyse changes in the whole blood's redox potential upon contamination with excess sulfide. A flow injection analysis coupled electrochemical technique was developed using human blood chemically modified electrode and sulfide interaction with respect to concentration vs current was studied.

2. Experimental section

2.1. Materials and reagents

Graphitized mesoporous carbon (GMC, purity assay≥99.95%, <500 nm pore size), Nafion (1%), hematin (8.4% iron content) and human hemoglobin (Hb) lyophilized were obtained from Sigma Aldrich (USA). Blood sample was collected from an adult 25 year old healthy female with the help of health care center of VIT University, after the consent from Institutional Ethical Clearance Committee for Human studies (Ref. No. VIT/IECH/020/Jan.24.2015). Sodium sulfide flakes (purity assay = 55–58%) was bought from Merck Chemicals (Germany). The supporting electrolyte used here is 20 min N_2 purged pH 7 phosphate buffer solution (PBS). Other basic chemicals of analytical grade were also used.

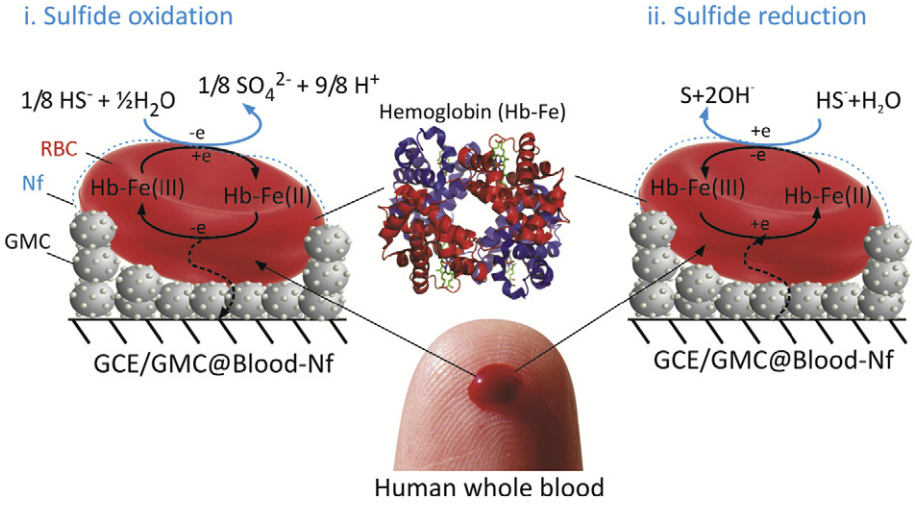
2.2. Apparatus

Cyclic voltammetry (CV) experiment was performed on CHI model 660C work-station, USA with 10 mL working volume. The three working electrode system consists of Ag/AgCl with 3 M KCl as reference electrode, platinum as a counter and blood modified glassy carbon electrode (GCE) as a working electrode with the surface area of 0.0707 cm^2. UV–Vis experiments were carried out by using UV–Vis NIR spectrophotometer, JASCO V-670, Germany. FTIR spectroscopy was performed by using JASCO FTIR-460 PLUS spectrometer. The flow injection analysis system consisted of Hitachi L-2130 pump delivery (Japan), a Rehodyne model 7125 sample injection valve (20 μL loop, Japan) with interconnection Teflon-tubes and a conventional electrochemical cell (BASi, USA) [19]. A FIA-GCE modified electrode was used as a detector in the FIA system.

2.3. Procedure

2.3.1. Preparation of blood chemically modified electrode

In first, glassy carbon electrode surface was cleaned by mechanical polishing (with 0.5 μm alumina powder) followed by electrochemical pretreatment. Blood chemically modified electrode was prepared as per our previous stated method [20], wherein, 5 μL of GMC (50 nm and 99.95% purity)-ethanol suspension (2 mg mL^{-1}) was drop casted on a clean GCE surface (air dried at room temperature (RT) for 5 ± 1 min) (GCE/GMC) followed by coating 5 μL of a mixture of 8 μL of human whole blood (with anticoagulant EDTA) and 2 μL of pH 7 PBS and air dried at room temperature. Then, 5 μL of 1% Nf-ethanolic solution was coated as an over-layer film and air-dried for 5 ± 1 min. 10 mM sodium sulfide solution in pH 7 PBS was prepared as a stock solution was used. As controls, commercial human hemoglobin (Hb) and hematin modified electrodes, GCE/GMC@Nf-Hb and GCE/GMC@Heme were prepared similar to the above procedure. Briefly, 5 μL of Hb (10 mg 500 μL^{-1} pH 7 PBS) or hematin (1 mg 500 μL^{-1} 0.01 N NaOH) was drop-casted on GCE/GMC, air dried at room temperature (RT) for 5 ± 1 min followed by dilute Nf or dilute chitosan-acetic solution (hematin) as an over-layer coating.



Scheme 1. Cartoon for the GCE/GMC@Blood-Nf and its electron-transfer reactions for sulfide oxidation (i) and reduction (ii) by redox mediation pathway.