Sensors and Actuators B 264 (2018) 448–466

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

# Chemically modified carbon based electrodes for the detection of reduced glutathione

Noel Nesakumar, Sheela Berchmans, Subbiah Alwarappan*

*Electrodics and Electrocatalysis Division, CSIR-Central Electrochemical Research Institute, Karaikudi 630003, Tamilnadu, India*

ARTICLE INFO

*Article history:*
Received 17 September 2017
Received in revised form
12 December 2017
Accepted 30 January 2018
Available online 1 February 2018

*Keywords:*
Reduced glutathione
Carbon electrodes
Electrochemical sensor
Modified surface
Electrocatalyst

ABSTRACT

The early diagnosis of cataract formation, alcoholism, chronic renal failure, Parkinson's disease, Alzheimer's disease, malignant disorders, ageing, arthritis, epilepsy and diabetes plays a critical role in the clinical treatment. Therefore, there is an intensive research for the determination of reduced glutathione (GSH) in human blood serum. In order to protect human health from these pathophysiologic conditions, it is very important to design and develop simple, sensitive and rapid sensors for GSH detection and quantifying GSH concentrations in human blood serum. In this context, electrochemical GSH sensors appear promising among all GSH sensors in which electrode material and architecture play major roles in achieving stable and sensitive detection. This review is focussed on the use of various carbon electrodes like glassy carbon, carbon paste, carbon microfiber, basal plane pyrolytic graphite and screen-printed electrodes in the electrochemical detection of GSH. Finally, the analytical applications of carbon electrodes based electrochemical sensors for glutathione electroanalysis are discussed.



**Contents**



## 1. Introduction

L-Glutathione (GSH, L-γ-glutamyl-L-cysteinyl-glycine), an intracellular low molar mass thiol species, is an important antioxidant cofactor responsible for the metabolisms of living cells [1]. Reduced GSH is composed of three amino acids namely L-cysteine, L-glutamine and glycine. Reduced GSH has L-glutathione on the cysteinyl portion, which acts as a strong electron donor. Initially, reduced GSH loses electrons and becomes oxidized. As a result, disulphide bridge links two such reduced GSH biomolecules to form oxidized GSH, which is often called as glutathione disulfide (GSSG) [1]. The disulphide bridge linkage established between two reduced GSH biomolecules is reversible upon re-reduction. The usage of reduced and oxidized GSH in human's natural defence system is maintained by a dynamic balance between GSH synthesis and it's recycling from GSSG [2,3]. Two major steps are involved in GSH synthesis: (i) At first, gamma-glutamyl cysteinyl synthetase integrates glutamate and cysteine. (ii) Second, GSH synthetase associates glycine with gamma-glutamylcysteine to form GSH. In the process of GSH recycling, the oxidized GSH or GSSG is reduced to GSH by glutathione reductase using NADPH [1]. Reduced GSH plays an important role in living being systems

* Corresponding author.
*E-mail address:* alwarappan@cecri.res.in (S. Alwarappan).

https://doi.org/10.1016/j.snb.2018.01.224
0925-4005/

for enzyme activity maintenance, participation in bioreductive reactions, amino acid transport and detoxification of free radicals, hydrogen peroxide and toxins [2,3]. GSH is also responsible for programmed cell death, cell homeostasis and gene regulation [4,5]. When the concentration of GSH in biological fluids varies, the thiol-disulfide bond homeostasis gets disturbed and as a result, the living cells tend to increase its length to a greater extent to normalise the homeostasis [4,5]. High concentrations of GSH are found in mammalian and eukaryotic cells [4,6–8]. Under oxidative stress, GSH can be transformed into GSSG [2,7–10]. Thus, the ratio of GSH to GSSG determines whether a living cell is prone to oxidative stress or not.

The normal GSH level in both plant and mammalian tissue is between 1 and 10 mM [8]. However, elevated concentration of GSH in mammalian tissues is known to induce cataract formation, alcoholism, chronic renal failure, Parkinson's disease, Alzheimer's disease, malignant disorders, ageing, arthritis, epilepsy and diabetes [1,2,11]. Studies also show that reduction in GSH production in γ-glutamyl cycle may be due to the decrease in the amount of enzymes involved in the production of reduced GSH [12–15].

In order to protect human health from these pathophysiologic conditions, it is very important to design and develop a simple, sensitive and rapid sensor for GSH detection and quantifying GSH concentrations in human blood serum [2,16,17] and commercial products [1,18]. However, owing to the matrix complexity, quantification of GSH in human blood serum, plasma, urine, erythrocyte, orange, tomato, drug, eye drop solution and pharmaceutical products is a difficult task [2,16,17]. Spectrophotometry [2,16], high performance liquid chromatography (HPLC) [19], titrimetry [12,13], flow injection analysis [10,12], spectrofluorimetry [2,16], surface enhanced Raman scattering [20], proton nuclear magnetic resonance [13,14] and capillary zone electrophoresis [12,13] are some analytical method that are most commonly used to detect GSH in human blood serum and commercial products.

GSH is optically inactive due to the absence of spectroscopically active functional groups and thereby, GSH detection system based on spectrofluorimetric and spectrophotometric methods requires derivatization, which makes the quantification of GSH in real samples slow and tedious [9,12,14,16]. Moreover, the GSH detection based on spectrofluorimetric method needs external light sources and fluorescent materials. Furthermore, the colorimetric mode of GSH detection has low sensitivity [5,21,22]. The HPLC method suffers from high cost, the need for sample preparation and low output sensor response [2,13,17,20]. Since these equipments are expensive and require highly trained personnel, their use is limited to highly specialised laboratories. However, electrochemical GSH sensor overcomes these limitations. Compared with other analytical techniques used for the quantification of GSH in commercial products, electrochemical sensors offer advantages such as high sensitivity, specificity, low detection limit (LOD), low-cost, user-friendly operation, rapidity and simplicity [9,16,17,19].

Direct electrochemical oxidation of GSH at the surface of conventional working electrodes is difficult due to the requirement of high overpotential for GSH detection and slow electron transfer rate [23]. The resultant sensors based on traditional electrodes have high LOD. Such GSH sensing devices are often limited by the existence of comparatively large non-Faradaic current associated with the Faradaic current due to the oxidation of GSH at the electrode surface [24]. In an attempt to resolve this problem, working electrodes are chemically modified with electrocatalysts to reduce the overpotential required for GSH oxidation and to enhance the electron transfer rate. Zaidi et al. [25] reviewed the application of various nanostructured materials modified bare electrodes (gold disc, indium tin oxide, platinum and glassy carbon) in electrochemical GSH sensors. Despite this great review article [25], a comprehensive overview on the chemically modified carbon electrodes (glassy carbon, carbon paste, basal plane pyrolytic graphite, carbon microdisk and screen-printed graphite) for fabricating electrochemical GSH sensors is still absent. In this review, we provide an overview of electrochemical GSH sensors, since their introduction in 1985, that used chemically modified carbon based electrodes for the detection of GSH in human blood serum and commercial products. Finally, the current challenges and perspective of chemically modified carbon based electrodes for GSH detection are outlined.

## 2. Electrochemical GSH sensor

Many electroanalytical techniques based on the oxidation of GSH or reduction of GSSG on various working electrodes have been reported [26–29]. The commonly employed electroanalytical techniques for the detection of GSH include amperometry [30], chronoamperometry [31], square-wave voltammetry [32], differential pulse voltammetry [33], cyclic voltammetry [3], stripping voltammetry [34] and hydrodynamic voltammetry [35]. However, the main issue with the detection of GSH employing these voltammetry techniques is that the oxidation peak arising from the electrochemical transformation of GSH to GSSG may overlap with the redox peaks of electroactive interferents such as cysteine, dopamine, uric acid and ascorbic acid present in the commercial products [8]. When amperometric- or chronoamperometric- mode of GSH detection is used, the applied potential essential for the oxidation of GSH to GSSG is extremely lower than these coexisting electroactive species in order to prevent their interference during the electroanalysis [8].

Platinum, gold, glassy carbon, carbon paste and mercury are the commonly preferred electrodes used for the detection of GSH in the commercial products [25]. Electrochemical GSH sensors based on mercury electrode detect GSH indirectly by oxidizing the surface of mercury to form a mercury-thiol complex, which requires less positive potential (+0.1 V *vs. Ag/AgCl*) [36]. Such a detection at a lower potential is expected to improve the selectivity of GSH in human blood serum and commercial products by avoiding or greatly reducing the contribution from potential interfering biomolecules eg. alanine, methanol, sucrose, ascorbic acid, urea, thiourea, starch, glycine, L-threonine and phenylalanine [2,16,17,37]. However, it has been reported that the electroanalysis based on mercury electrode induces toxicity, instability and deteriorate the sensor response rapidly [15,34,38].

On the other hand, the anodic oxidation of disulfides facilitated by the surface oxides formed on the platinum and gold working electrodes in the same applied potential range makes the analytical application very complex. Furthermore, at carbon electrodes, GSH oxidizes to GSSG at +1.0 V *vs. Ag/AgCl* [39]. Owing to the high working potential, low signal to noise ratio and low heterogenous electron transfer rate, the use of bare carbon based working electrode in the design of electrochemical GSH sensor faces drawbacks in rapid detection of GSH in human blood serum samples. In order to enhance the electron transfer reaction of GSH at the surface of the electrode and to minimize the working potential for the determination of GSH, chemically modified carbon based electrodes have been recently employed for the design of electrochemical GSH sensors [20,40]. By immobilizing a wide variety of redox active mediators on the surface of carbon based electrodes, the shuttling of electrons between GSH and carbon based electrodes can be enhanced which in-turn decreases electrode surface fouling and working potential. Wang et al. [16] deployed cobalt phthalocyaninetetrasulfonate intercalated Zn–Al layered double hydroxide (ZnAl–CoTsPc–LDH) as an electrocatalyst in order to reduce the working potential for the determination of GSH. The developed ZnAl–CoTsPc–LDH modified glassy carbon electrode exhibited high electrocatalytic activity with a lowered working potential by more