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Electrochemical sensing platforms based on the different carbon derivative incorporated interface

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ABSTRACT

In this work we present a comparative study of carbon derivative applications in amperometric biosensors and their effects on the properties of these biosensors. Biosensors were prepared by Horseradish peroxidase (HRP) immobilization on the composite electrodes composed of carbon black, carbon nanofiber (CNF), extended graphite, multiwalled carbon nanotube (MWCNT), reduced graphene oxide (REGO) and poly(glycidyl methacrylate-co-vinylferrocene) (P(GMA-co-VFc)) as mediator, covalent linker, and host matrix for carbon derivatives. The modified pencil graphite electrode (PGE) was used for the detection of hydrogen peroxide and to follow electrochemical behavior of different carbon derivatives which were recorded. The electrochemical characterization was investigated by cyclic voltammetry and electrochemical impedance spectroscopy methods. Amperometric measurements showed that the REGO and MWCNT modified electrodes have excellent performance in comparison with other carbon derivatives studied.

1. Introduction

Biosensors are generally defined as analytical devices — or units incorporating a biological or biologically derived sensing element, integrated or associated with a physio-chemical transducer [1–3] which converts a biological response into a quantifiable and processable signal [4,5]. Whereby biosensor is composed of three main parts: (1) the biological recognition element that recognizes the target molecule in the presence of various chemicals, (2) a transducer which converts the bio-recognition event into a measurable signal, and (3) a signal processing system that converts the signal into readable form [3,6–8]. Biosensors have two basic principles which differentiate them from chemical sensors: (i) sensing elements, which are biological structures such as cells, enzymes, or nucleic acids, and (ii) the sensor's usage for measurements of biological processes or physical changes [9]. Owing to the constant desire for developing a biosensor which fulfills the contemporary necessities at the time of unceasing technological development, we can produce small compact devices of higher sensitivity, greater analytic determination, and lower operating costs.

Nowadays, there are different methods used to detect biological and environmental chemicals, including high performance liquid chromatography (HPLC) [10,11], gas chromatography [12], chemiluminescence [13], and spectrophotometry [14]. Biosensors especially based on electrochemical methods are mostly used and have increasing trend to the analysis of biological and environmental substances. Due to the onsite detection, low cost, fast response, high sensitivity, simple instrumentation, and low energy requirement electrochemical techniques for determination of these substances have been studied. [15–19].

Amperometric biosensors are one of the most studied sensor types because of their real-sample application ability, high sensitivity, wide range of analyte detection, and lower operating costs. One of the studied amperometric biosensors is based on Horseradish peroxidase (HRP) for hydrogen peroxide (H_2O_2) detection [25] being a byproduct of several highly selective oxidases and also an essential mediator in food, biology, industry, environmental analysis, and medicine [20,21,26]. H_2O_2 is also characterized as non-radical reactive oxygen species (ROS), and ROS is accounted as one of the responsible species for mutagenic DNA damage in mammalian cells [22]. Many techniques for H_2O_2 determination have been employed such as spectroscopy [23], chemiluminescence [24], and titrimetry [25]. Because these techniques have some drawbacks such as interferences, long analysis time, and involvement of expensive reagents, they hardly fit into technological demands that continuously expand. One of the ways of achieving good electron transfer ability in amperometric biosensors is to introduce a mediator such as ferrocene between enzyme and electrode. Ferrocene (Fc) polymeric mediators are widely used to construct mediated amperometric biosensors due to the properties of having excellent electron transfer capability and allowing the incorporation of reagents to the sensing system that may be a requirement for further sensor developments [25]. Some redox copolymers widely used

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for the covalent attachment of ferrocene are ferrocene-containing polythiophene derivatives [27], poly(vinylferrocene-co-hydroxyethyl methacrylate) [18], acryl amide copolymers [29], poly(glycidyl methacrylate-co-vinylferrocene) [16], and poly(N-acryloylpyrrolidine-co-vinylferrocene) [30]. The electrochemical behavior of Fc (Fc → Fc+) attributed to the redox couple has been proved to be highly effective for electrode modification and functionalization [31]. Various ferrocene bioelectrodes have been developed to show the effectiveness of Fc derivatives such as in the study reported by Şenel et al. [36] named "amperometric hydrogen peroxide biosensor based on covalent immobilization of horseradish peroxidase on ferrocene containing polymeric mediator", another research has been reported by the Erden et al. [32] named "amperometric enzyme electrode for xanthine determination with 1,4 benzoquinone and poly (vinylferrocene) as a mediators", and by Tanushree et al. [31] in which uric acid biosensor is developed based on uricase electro-activated with ferrocene on a Nafion coated glassy carbon electrode. In general, construction of biosensor has to be designed with the emphasis on tight fixation of the mediator (and all other sensor components needed) to the electrode surface to create so-called "reagentless" biosensors [33].

Nanomaterials, particularly carbon based nanomaterials, have been the subject of intense research over the past twenty years [34,35], and they have a significant role to play in new developments in each of the biosensor size domains as they can help address some of the key issues in the development of all biosensors [36,37]. Those main issues include: i) constructing and developing of the biosensing interface so that the analyte can easily interacts with the biosensing surface [38,39]; ii) achievement of efficient transduction of the biorecognition event [36,40,41]; iii) increment in the sensitivity and selectivity of the biosensor [42,43]; and iv) improvement of response time in very sensitive systems [44]. There has been an explosion of interest in application of nanomaterials such as carbon black (C.Black), carbon nanotubes (CNTs), carbon nanofiber (CNF), expanded graphite (E.Graphite), and reduced expanded graphite oxide—(it is called as graphene in the text) (REGO) for the development of amperometric biosensors. Carbon derivative important properties and application in amperometric biosensor selected examples have been summarized in Table 1.

In this work, we aim to determine the performance effect of the carbon derivatives on amperometric detection that is important for the consistency of the sensor's system fabrication. Although almost all of the carbon derivatives were studied for biosensor development to obtain highly efficient amperometric biosensors, these studies were not effectively conducted together under the same experimental setup. For the purpose of obtaining good electrochemical signals the hydrogen peroxide biosensor was constructed as model biosensor. This study indicates that REGO and MWCNT have an important role in terms of providing enhanced electrochemical sensing properties to biosensors when compared with other carbon materials studied.

2. Experimental part

2.1. Materials

Horseradish peroxidase (HRP) was purchased from Sigma-Aldrich (USA), vinylferrocene (VFc) purchased from Aldrich (Japan) and Glycidyl methacrylate and hydrochloric acid (37% HCl) were purchased from Sigma-Aldrich (Germany), Iron (III) nitrate nonahydrate, $Fe(NO_3)_3 \cdot 9H_2O$, iron (II) chloride tetra hydrate ($FeCl_2 \cdot 4H_2O$), sulfuric acid (H_2SO_4 fuming), hydrogen peroxide (30% H_2O_2), potassium permanganate ($KMnO_4$), ammonia solution (28% NH_3), sodium nitrate ($NaNO_3$), and hydrazine hydrate ($N_2H_4 \cdot H_2O$) were purchased from Merck (Germany). All the chemicals employed in the study were analytical grade and used as-received without purification. The EG was a commercial grade, thermally expanded product (TIMREX® BNB90), kindly provided by TIMCAL (Switzerland). The density, surface area, average particle size (d_{90}) and the oil adsorption number (OAN) values of EG were reported as 2.24 g/cm^3, 28 m^2/g, 85.2 µm and 150 ml/100 mg, respectively by the producer.

2.2. Preparation of poly (GMA-co-VFc)

The redox polymer, containing different compositions, was prepared according to our previous study [91]. A mixture of vinylferrocene (VFc) and glycidyl methacrylate (GMA) at a known molar ratio was injected into a Pyrex tube, AIBN (1 mol% based on the total monomer concentration was identical for all samples, 5 mol/dm^3), after which the mixture was degassed with Argon gas and vacuum sealed. In the next step, the tubes were placed in constant temperature baths, with constant temperature of 70 °C for 2 days. After, the reaction mixture was added drop wise, while rapidly stirring the diethyl ether to precipitate the copolymer. In the end, precipitated copolymer was washed with diethyl ether and reprecipitated in this manner, twice and finally vacuum dried.

Table 1
Comparison of different carbon derivatives properties and applications in different amperometric biosensors.

Carbon derivatives	Properties	Biosensor application
C.Black	–particles with diameter ranging from 15 to 100 nm [45], hard to disperse and easy to flocculate [46] –hard to disperse and easy to flocculate and strong cohesion between particles [46] –many polar radicals from the surface of particles and has large specific surface area in form of its congeries [46] –not widely used in biosensor applications [46].	Laccase [45] Glucose [46] Nerve agents [48] Bisphenol A [95]
E.Graphite	–widely used as gasket, fire resistant composite, thermal insulator [49–51], –has an important degree of crystallinity, long-range disorder, and high degree of porosity [52] –high compressibility and elastic recovery, chemical resistance and good thermal and electrical conductivity [52] –lamellar structure of graphite and can exhibit high porosity up to 99% [49] –good dispersion in a polymer matrix. [53]	Urea [55] Nitrate and nitrite [56] Phenol [57]
REGO	–high crystal quality and quite unusual electronic properties [59], excellent conductor [60] –enhanced electrochemically active site [62,63], and good biocompatibility –large number of electroactive sites in big area of its structure [61], high surface to volume ratio –excellent thermal conductivity, mechanical flexibility, chemical tolerance	H_2O_2 [69,67] Proteins [68] Pesticides [100] Glucose [70]
CNF	–submicron diameter sizes (typically below 100 nm) [71], they have been used as catalyst and catalyst support [75] –mechanical and thermal properties, high tensile strength and elastic modulus [74,75] –contain more edge sites on the outer wall [74], high electric conductivity [73] –they can facilitate the electron transfer of electroactive analytes [77], larger functionalized surface area [78] –one-dimensional macrostructures [81,72]	Dopamine and serotonin [79] H_2O_2 [80] Acetylcholine [101]
MWCNT	–have huge impact on amperometric biosensors due to the ability to blend into a number of formulations in order to improve current densities and overall performance of enzyme electrodes [82,83]. –huge importance for the production of renewable energy and construction of electrochemical sensors [83,86] –subtle structural changes that switch tubes from metallic to semiconducting having various band gaps [87] –electron transfer reaction with wide range biosensor applications [88] –incorporation of carbon nanotubes leads to fast electron transfer, lower limit detection, and high sensitivity [89]	H_2O_2 [90,84] Xanthine [96] Pesticides [98] Dopamine [99] Urea [97]