Electrochimica Acta 282 (2018) 147–154

Contents lists available at ScienceDirect

Electrochimica Acta

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

Immunoassay of C-reactive protein by hot electron-induced electrochemiluminescence at polystyrene-carbon black composite electrodes

Kalle Salminen [a, *], Päivi Grönroos [a], Jarkko Eskola [b], Eemi Nieminen [a], Harri Härmä [c], Sakari Kulmala [a]

[a] *Department of Chemistry and Materials Science, Aalto University, FI-000076, Aalto, Finland*
[b] *Department of Pathology and Forensic Medicine, University of Turku, FI-20520, Turku, Finland*
[c] *Department of Chemistry, University of Turku, FI-20014, Turku, Finland*

ARTICLE INFO

Article history:
Received 4 May 2018
Accepted 3 June 2018
Available online 4 June 2018

Keywords:
Hot electron electrochemistry
Electrochemiluminescence
C-reactive protein
Carbon paste electrodes
Immunoassay

ABSTRACT

Aromatic Tb(III) chelates can be detected down to subnanomolar concentrations at polystyrene-carbon black composite electrodes on the basis of hot electron-induced electrochemiluminescence, and used as electrochemiluminescent labels in bioaffinity assays. The excitation mechanism is based on chemiluminescent reactions that are initiated by the field emission/tunnel emission of hot electrons from the composite electrode. The composition and the properties of the novel composite electrodes were studied in detail. Hot electron-induced electrochemiluminescence intensities obtainable with the present composite electrodes are comparable to the previously used metal/insulator-type electrodes without exhibiting considerable long-lived solid state electroluminescence background emission, unlike e.g. oxide-coated silicon and aluminum electrodes.

C-reactive protein was finally determined in a heterogeneous sandwich-assay by using the present composite electrodes as a solid support material for the capture antibodies. The detection antibody was labeled with a commercially available Tb(II) chelate. Calibration curve for the determination of C-reactive protein was linear over two orders of magnitude and the detection limit was well below the clinical reference value, approx. 1 $\mu g\,l^{-1}$. Disposable polystyrene-carbon black composite electrodes are superior alternatives for e.g. silicon-based electrodes due to the low cost, easier manufacturing process and a very good performance e.g. in immunoassays.

1. Introduction

Electrogenerated chemiluminescence (ECL) is a process in which species generated at electrode(s) undergo electron-transfer reactions leading to the emission of light. ECL can be further divided in two broad categories, anodic ECL and cathodic ECL, depending on whether the luminescence-generating intermediates or mediators are generated at the anode or at the cathode. Hot electron-induced electrochemiluminescence (HECL) is a subset of the latter that involves the injection of energetic hot electrons into the electrolyte solutions. Hot electrons (i.e. electrons having higher energy than the Fermi level of the surrounding phase) and the subsequently formed hydrated electrons offer the means of overcoming the narrow potential window of water that normally limits the energy obtainable for electrochemical reactions in fully aqueous solutions at active electrodes. Unlike many other types of chemiluminescence, HECL does not typically induce any irreversible changes to the structure of the luminescent molecule. This gives the possibility of increasing the S/N-ratio by integrating the measured emission intensity over multitude of excitation pulses/cycles. In addition, the hydrated electrons transform the reduction reactions from heterogeneous surface electrode reactions into a somewhat “bulk type” reduction reactions that occur at the close vicinity of the electrode surface, typically near diffusion controlled rates [1].

Various organic luminophores (e.g. xanthene dyes, luminol), metal ions (e.g. Sn, In, Hg) and metal-complexes (e.g. $Ru(bpy)_3^{2+}$, lanthanide complexes) can be excited by chemiluminescent

---

* Corresponding author.
E-mail address: kalle.salminen@aalto.fi (K. Salminen).

https://doi.org/10.1016/j.electacta.2018.06.016
0013-4686/© 2018 Elsevier Ltd. All rights reserved.

reactions involving primary reducing and oxidizing radicals in HECL [2–6]. Analytes of interest can be labeled with luminophores that allows their detection via HECL, past examples include e.g. β2-microglobulin, human thyroid stimulating hormone and C-reactive protein [6–8]. There have also been reports of label-free immunoassays where a decrease in the intrinsic solid-state electroluminescence background emission is used to determine the amount of biological analytes attached to the electrode surface [9,10]. It is also possible to quantity analytes based on their interference in the light generating pathways (e.g. catechol and quercetin) [11,12].

Past HECL studies have used the following electrode types: electrodes covered by a thin insulating/dielectric films (e.g. Al/Al_2O_3, Si/SiO_2, C/C_xO_{1-x} and Ta/Ta_2O_5), microelectrodes with very sharp tip areas on the electrode surface that concentrate the macroscopic electric field to sufficient values (e.g. Pt wires) and metal/insulator/metal electrodes (e.g. Ta/Ta_2O_5/Pt) [8,9,13–16]. The present working electrode type, i.e. conducting particles embedded in insulating or dielectric matrix, is mostly used in vacuum conditions, in e.g. field emitting displays, where they are normally referred as Lantham emitters [17]. We have previously shown that polystyrene-graphite composite electrodes can be used to generate hydrated electrons during both cathodic pulse and direct voltage polarization [18]. The insulating polystyrene layer on the working electrode enables electron tunnel emission (field assisted direct tunneling) from the graphite flakes that lack sufficient field enhancing geometrical protrusions at low-macroscopic electric fields due to internal field enhancement [19]. The polystyrene surface can additionally be utilized as a binding medium for e.g. biomolecules under study. This makes polystyrene one of the most attractive insulating electrode materials available for real world HECL applications as polystyrene is among the most commonly used supportive substrate surfaces in various bioanalytical protocols, such as, in enzyme-linked immunosorbent assays and fluoroimmunoassays. The low material cost of polystyrene-based composite electrodes combined with the possibility of manufacturing them in mass with low-technology methods, e.g. screen or inkjet printing, makes them an ideal disposable electrode choice for e.g. point-of-care testing applications. The issue with earlier polystyrene-graphite composite electrodes was low HECL emission intensity and relatively high background emission due to the impurities in the graphite flake powder [18]. One of the aims of the present study is to alleviate those problems by using carbon black as the conductive filler material. Field emission/tunnel emission currents obtainable with carbon black composite films are comparable to values observed with carbon nanotubes, but at a fraction of the cost [20]. Field emission is taken here as a process that occurs in high-field conditions e.g. at sharply pointed cathodes and tunnel emission is regarded as a process that requires a thin insulation film upon the cathode to facilitate the tunneling of hot electrons through the insulating barrier [21].

C-reactive protein (CRP) was used as a model analyte in a heterogeneous sandwich-assay in the present study to demonstrate the feasibility of polystyrene-carbon black electrodes for electrochemiluminoimmunoassays. CRP is perhaps the most important nonspecific inflammatory biomarker and a strong independent predictor for cardiovascular heart disease (CHD) risk and events. Up to 50% of all CHD events occur in apparently healthy individuals who have few-to-none of the traditional risk factors such as dyslipidemia. In addition to being an inflammatory marker CRP also acts as a mediator for both atherosclerosis and CHD. CRP levels can be regarded as an inflammation fitness score of sorts that can provide external motivation for healthy lifestyle choices (e.g. weight loss and smoking) [22,23].

2. Experimental

2.1. Fabrication of the composite electrodes

Polystyrene ($M_w = 350\,000$, Sigma-Aldrich, abbreviated hereafter as PS) and carbon black (Vulcan XC72, Cabot, abbreviated hereafter as CB) were added to chloroform (≥99.0%) in varying mass ratios. The combined mass concentration of PS and CB was 50 g dm^{-3}. PS and the SB were mixed by Branson SFX150 sonicator to form a suitable PS-CB ink for spin coating. The PS-CB inks were spin coated to brass substrates ($\varnothing = 14$ mm) in normal laboratory atmosphere at 1200 RPM for 30 s. Spin coating was done immediately after the sonication process to avoid any potential problems related ink stability. A 1-mm thick ring-shaped Teflon tape ($\varnothing_{\text{outer}} = 14$ mm, $\varnothing_{\text{inner}} = 5$ mm) was attached to the ink coated electrode surface for HECL measurements.

2.2. Electrochemical measurements

The HECL performance of PS-CB-layered electrodes was probed with Terbium(III)-4-(Phenylethyl)(1-hydroxybenzene)-2,6diyl)bis(methylenenitrilo)tetrakis(acetic acid) (abbreviated hereafter as Tb(III)-L1, obtained from Turku University). All HECL measurements were performed in 0.05 M borate buffer solution (pH 9) with 0.1 sodium sulfate as the supporting electrolyte. Flat aluminum discs (Merck, 99.999% pure) were used as a reference material without any pretreatments.

HECL measurements were performed in Teflon cell ($\varnothing = 5$ mm) equipped with a vertical Pt wire counter electrode. The volume of sample solution was 150 µl in every measurement. The cathodic excitation pulses were generated with an in-laboratory-built coulostatic pulse generator set to deliver constant charge voltage pulses of −35.0 V with a pulse charge of 12.6 µC at a rate of 50 Hz. Optical detection was performed with a photomultiplier tube module (PerkinElmer MH1993, 1364-H-064) through an interference filter (550 nm, bandwidth of 40 nm) passing the $^5D_4 \rightarrow {}^7F_2$ spectral line of Tb(III). A photon counter (SR400, Stanford Research System) was connected to the photomultiplier tube module trough an amplifier (DC-300 MHz amplifier, Stanford Research System). With Tb(III)-L1 the photon counter delay and gate times were 200 µs and 4 ms, respectively. The amplified photon pulses were additionally recorded via Nucleus MCS-II multiscaler card. The emissive species in HECL was confirmed by luminescence lifetime and spectrum measured with Ocean Optics USB2000 + spectrometer.

Cyclic voltammetry of ferrocenemethanol (FcMeOH, Sigma-Aldrich) with 100 mM LiCl (Sigma-Aldrich) as the supporting electrolyte was used to characterize the electroactive surface of composite electrodes. Saturated calomel electrode and a Pt wire were used as the reference and as the auxiliary electrode, respectively. The top-down area of the characterized electrode had a diameter of 10 mm. The sheet resistivity of PS-CB −inks spun to soda glass substrates was measured with Jandel RM3000 four-point probe system. Resistivity values were measured from the middle of the samples. Long exposure time photographs (10 s, ISO 1600) of Tb(III)-L1 emission were taken with Canon EOS 7D digital camera equipped with Canon EF 100 mm f/2.8 L Macro IS USM lens.

2.3. Electrochemiluminoimmunoassay

PS-CB electrodes were coated with primary hCRP antibodies (anti-h CRP clone 6405, Medix Biochemica) via physical adsorption by dispensing 50 µl of 5 µg ml^{-1} coating solution containing 50 mM Trizma base, 0.9% NaCl and 0.05% NaN_3 (pH 7.7). The coating reaction was carried out overnight (>12 h) in a humidity chamber. After coating process the electrodes were dried with cotton swabs