

Reproducibility and Biofouling in Graphene Foam Electrodes Assessed by Direct ELISA

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Summary:

Graphene foam is an emerging electrode material in the field of biosensors. Here, we evaluate graphene foam electrodes in terms of reproducibility and biofouling using electrochemical direct ELISA. The electrodes exhibited significant inter-batch variability and elevated nonspecific blank signals in comparison to conventional screen-printed carbon electrodes. Overall, surface heterogeneity and nonspecific adsorption in graphene foam limit its analytical reliability, highlighting challenges for practical biosensing applications despite its high sensitivity potential.

Keywords: graphene foam, electrochemical immunosensor, biofouling, reproducibility, direct ELISA

Introduction

Single-use electrodes are widely employed in electrochemical biosensing due to their sensitivity, portability, and simplicity for rapid in situ analysis. Three-dimensional (3D) graphene foam electrodes, with their porous networks, offer high surface area, biocompatibility, and efficient biomolecule immobilization, enhancing antibody–antigen interactions [1]–[3]. However, their complex architecture can introduce interfacial heterogeneity and nonspecific adsorption, limiting reproducibility and analytical performance. Additionally, performance is often assessed under idealized conditions that overlook these constraints. Biofouling and batch-to-batch variability remain underexplored, and both represent key challenges affecting reliability. This study systematically evaluates the reproducibility and antifouling performance of commercial graphene foam electrodes using a direct electrochemical enzyme-linked immunosorbent assay (ELISA) as a controlled model system.

Materials and Methods

Gii-Sens Integrated Graphene electrodes were purchased from iGii (Stirling, UK). DropSens 110 carbon electrodes were obtained from Metrohm (Spain) for comparison. In an electrochemical direct ELISA, an HRP-labeled antibody reacts with the substrate 3,3',5,5'-tetramethylbenzidine

(TMB) to generate a current response, measured using chronoamperometry. Covalent immobilization anchored the target (IgG) to the graphene surface, using pyrene carboxylic acid to introduce carboxyl groups through π – π interactions and EDC/NHS coupling chemistry to anchor the antibody to the surface.

Results

Graphene foam electrodes owing to their porous structure (Fig. 1a), show promising electroanalytical performance due to their high surface area resulting in 3–4 times higher cathodic and anodic peak currents compared to planar carbon electrodes (Fig. 1c).

However, important challenges around reproducibility and biofouling were found when performing a direct electrochemical ELISA (Fig. 2a) on graphene foam electrodes. Chronoamperograms shown in Fig. 2b reflect up to 61% of inter-batch variation in the recorded signals for graphene electrodes, in contrast to 12% inter-batch variation for carbon electrodes. Overall, CoV values across multiple experiments on the same batch of graphene electrodes spanned from 9 to 149%, whereas for carbon it was systematically below 12%.

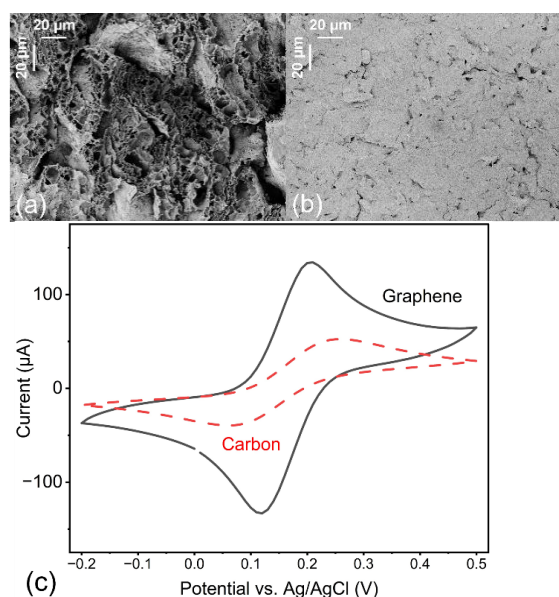


Fig. 1. SEM images of the bare working electrode of (a) graphene foam and (b) carbon. (c) Cyclic voltammograms of the graphene foam and carbon electrodes recorded in 5 mM ferro/ferricyanide in 0.1 M KCl aqueous solution at a scan rate of 100 mV s⁻¹.

Furthermore, graphene exhibited a 9 times higher blank signal compared to that recorded with carbon, indicating poor antifouling properties despite surface passivation using ethanolamine. Consequently, the signal-to-blank ratio for graphene was approximately threefold lower compared to carbon. Alternative blocking strategies were also evaluated, but all resulted in a 1.2-1.4-fold increase in the average current response for blank measurements. These findings demonstrate that surface heterogeneity and nonspecific interactions present on these electrodes critically impact the analytical performance of the proposed electrochemical immunosensor assay.

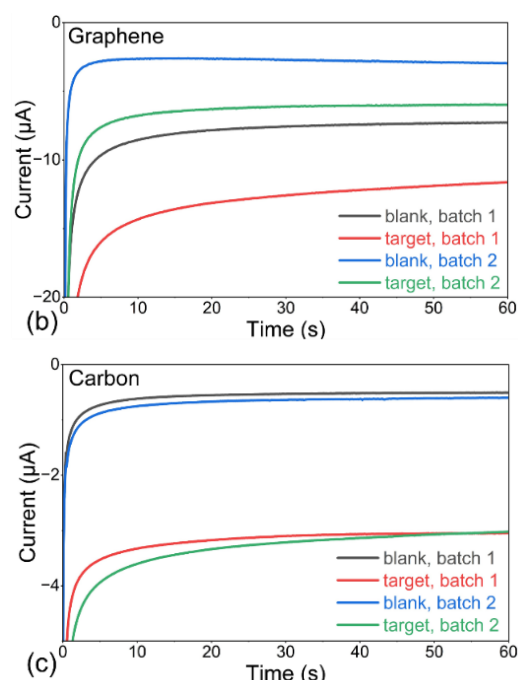
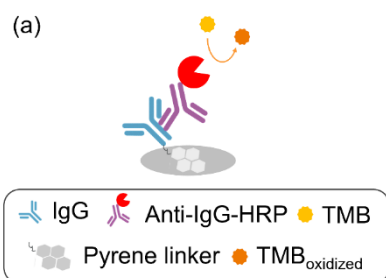


Fig. 2. (a) Schematic illustration of direct ELISA on the electrode surface. Surface passivation was done using 3 M (pH 9) ethanolamine. (b) Chronoamperometric responses (current vs. time) recorded for graphene foam electrodes, comparing blank and target conditions across two independent electrode batches. (c) Chronoamperometric responses for carbon electrodes under analogous conditions.

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