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IMMOBILIZATION OF A MODEL PROTEIN, BSA ONTO GRAPHENE OXIDE

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk " IMMOBILIZATION OF A MODEL PROTEIN, BSA ONTO GRAPHENE OXIDE" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh C.R.A WAHIDA FAZLINA BINTI ABDUL HALIM bernombor matrik 162846 sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

This project was carried out to immobilize BSA protein onto nanomaterial, which is graphene oxide (GO). Two different methods have been used in order to immobilize this protein, the first method for immobilization is by physical adsorption and the second method is by chemical modification of GO using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride-N-hydroxysuccinimide (EDC-NHS) as the crosslinker. The aim is for potential development of a GO-based immunoassay and to choose the best method to be used to immobilize BSA protein onto GO. Several parameters have been selected for the optimization in order to develop effective GO-based immunoassay such as amount of BSA to be immobilized, concentration of GO, and incubation time for BSA immobilization. Suitable GO concentration for BSA immobilization is 1 mg/ml, and 1 hour incubation time has been selected. For the amount of BSA, 0.5 mg is the sufficient amount of BSA that give a good electrochemical signal in CV measurement. For immobilization method, the chemical modification method is the best method to be used to immobilize BSA protein onto GO.

ABSTRAK

Projek ini dijalankan untuk menetapkan protein BSA pada nanomaterial iaitu graphene oksida (GO). Dua kaedah yang berbeza telah digunakan untuk menetapkan protein ini pada GO iaitu dengan menggunakan penjerapan fizikal dan pengubahsuaian kimia keatas GO menggunakan EDC-NHS sebagai penjalin. Tujuan projek ini adalah untuk potensi perkembangan immunoassay berasaskan GO dan untuk memilih kaedah yang terbaik untuk menetapkan protein BSA pada GO. Beberapa parameter telah dipilih untuk pengoptimuman bagi membina immunoassay berdasarkan GO yang berkesan, seperti jumlah BSA untuk penetapan, kepekatan GO, dan masa inkubasi untuk penetapan BSA. Kepekatan GO yang sesuai untuk penetapan BSA pada GO ialah 1 mg / ml, dan 1 jam masa inkubasi telah dipilih. Bagi jumlah BSA, 0.5 mg adalah jumlah yang mencukupi dan memberikan isyarat elektrokimia baik dalam ukuran CV. Untuk kaedah penetapan, kaedah pengubahsuaian kimia adalah kaedah yang terbaik untuk digunakan untuk menetapkan protein BSA pada GO.

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CHAPTER 1

INTRODUCTION

Immobilization is defined as the attachment of molecules to a surface resulting in reduction or loss of mobility (Mosbach, 1988). In order to fully retain biological activity, proteins should be attached onto surfaces without affecting conformation and function. (Rusmini et al., 2007). Besides the application in industrial processes, the immobilization techniques are the basis for making a number of biotechnological products with applications in diagnostics, bioaffinity chromatography and biosensors (Guisan, 2013).

The major components in immobilization system are the molecule, for example protein, the matrix or surface, and the mode of attachment. Adsorption onto a support, entrapment within a membrane and covalent binding to the support are the methods used to immobilize protein (Scouten et al., 1995). These methods can be performed on materials as diverse as steel (Charles et al., 1975), cellulose (Emer et al., 1972), titanium (Nakabayashi et al., 1985), and polystyrene as the matrix or support material (Morrissey & Han, 1978).

Recently, along with the development of nanostructured materials, a range of nanomaterials with different sizes and shapes have been utilized as the matrix for protein and enzyme immobilization (Kim et al., 2009). Graphene oxide (GO), due to their small size, large specific surface area, desired aqueous suspending ability (Kim et al., 2009), and abundant functional groups, such as epoxide, hydroxyl and carboxylic groups, have exhibited advantages over traditional bulk materials and afford GO great promise for many more applications (Li et al., 2008).

Many researchers have succeeded to immobilized protein and enzyme on GO. For instance, immobilization of horseradish peroxidase enzyme on graphene oxide and has been employed in phenolic compound removal (Zhang et al., 2010). Not only that, a medicinal drug (10-hydroxy camptothecin (HCPT)) and bovine serum albumin has been immobilized on GO to develop serum albumin biosensor (Ni et al., 2013).

In immobilization process, protein-surface and protein-protein interaction are essential. However, limitation such as low sensitivity or selectivity in biosensor or immunoassay are often resulting a non-specific protein-surface and protein-protein interactions (Rankl et al., 2003). So, blocking reagents is employed in order to reduce such non-specific interactions. Many biological and chemical reagents have been used for blocking purposes. Bovine serum albumin (BSA) is one the blocking reagent that can be used to reduce non-specific interactions. So, the objectives of this project:

1. To immobilized BSA protein onto GO for potential development of a GO-based immunoassay.
2. To immobilize BSA onto GO using physical adsorption and chemical modification methods and compare which is the best method.
3. To physically immobilize modified GO-BSA onto the active site of glassy carbon electrode (GCE).
4. To investigate the electrocatalytic response of GO-BSA using potentiostat.

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