



UNIVERSITI PUTRA MALAYSIA

IMMOBILIZATION OF BOVINE SERUM ALBUMIN (BSA) AND HEPATITIS B CORE ANTIGEN (HBcAg) ON OPTICAL FIBER CORE SURFACE

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**DEPARTMENT OF MICROBIOLOGY
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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “Immobilization of Bovine Serum Albumin (BSA) and Hepatitis B core Antigen (HBcAg) on optical fiber core surface” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Ameliawati binti Basri (163684) sebagai syarat untuk kursus BMY 4999 projek3.

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ABSTRACT

Hepatitis B virus (HBV) infections are one of the major worldwide health problems. Since the conventional laboratory diagnostics tool can be time consuming and laborious, the development of a biosensor with higher sensitivity and specificity in detecting the disease is crucial. For our future development of a new HBV biosensor, the hepatitis B core antigen (HBcAg) was selected to be immobilized on the optical fiber core surface. Techniques of immobilization involved in this project were physical adsorption of HBcAg on the core surface of the optical fiber and chemical modification of the core surface with the application of glutaraldehyde (GTA) as a crosslinker. Apart from that, optimization work were also carried out by using a model protein bovine serum albumin (BSA) with several parameters namely incubation time, amount of protein, concentration and type of washing agents. The coating on the core surface of the optical fiber will induce a light signal which will be analyzed by the Optical Spectrum Analyser (OSA). The wavelength and the optical power of each coating reagent at each stage of immobilization will determine the light transmission signal. Transmission signal is the determinant factor of the immobilization rate of the protein on the optical fiber core surface. Based on the OSA measurement and microscopic observation that were carried out in this project, BSA and HBcAg were successfully immobilized on the tapered optical fiber core surface by using chemical modification method. The immobilization rate of both proteins using chemical modification is about 98% and this is due to the application of strong and stable crosslinker, glutaraldehyde which provided a covalent link of the proteins to the optical fiber core surface. It was also determined that uniform coating should be maintained through the experiment to avoid light losses that will affect the transmission signal. This early development of the optical fiber-based biosensor gave a promising future and able to improve the current laboratory diagnostic tools. Further and continuous improvement shall be carried out to test its sensitivity and stability of the biosensor.

ABSTRAK

Jangkitan Hepatitis virus B (HBV) adalah salah satu masalah kesihatan utama di kebanyakkan negara di seluruh dunia. Disebabkan oleh alat diagnostik makmal konvensional memakan masa dan sukar, pembangunan biosensor dengan kepekaan yang lebih tinggi dan kekhususan dalam mengesan penyakit ini adalah amat penting. Untuk pembangunan biosensor HBV baru, teras hepatitis B antigen (HBcAg) telah dipilih untuk disalut dan disekat pada permukaan teras gentian optik. Teknik yang terlibat dalam projek ini ialah penyerapan HBcAg secara fizikal pada permukaan teras gentian optik dan pengubahsuaian kimia permukaan teras dengan penggunaan glutaraldehyde (GTA) sebagai crosslinker. Selain itu, kerja pengoptimuman juga telah dijalankan dengan menggunakan model protein bovine serum albumin (BSA) dengan beberapa parameter iaitu masa inkubasi, jumlah protein, tumpuan dan jenis reagen pembasuh. Lapisan pada permukaan teras gentian optik akan menyebabkan isyarat cahaya dianalisis oleh optik Spectrum Analyser (OSA). Panjang gelombang dan kuasa optik setiap reagen salutan akan menentukan isyarat penghantaran cahaya. Isyarat penghantaran adalah faktor penentu kadar bergerak protein pada permukaan teras gentian optik. Berdasarkan pengukuran OSA dan pemerhatian mikroskopik yang telah dijalankan dalam projek ini, BSA dan HBcAg telah berjaya disalut dan disekat pergerakan di atas gentian optik permukaan teras tirus dengan menggunakan kaedah pengubahsuaian kimia. Kadar bergerak kedua-dua protein menggunakan pengubahsuaian kimia adalah kira-kira 98% dan ini adalah disebabkan oleh pemakaian crosslinker yg kukuh dan stabil iaitu glutaraldehyde yang menyediakan pautan kovalen antara protein dan permukaan teras gentian optik. Selain itu, salutan lapisan reagen yang seragam perlu dikekalkan sepanjang eksperimen untuk mengelakkan kerugian cahaya yang akan memberi kesan kepada isyarat penghantaran. Perkembangan awal biosensor yang berasaskan gentian optik dapat memberikan masa depan yang cerah dan dapat meningkatkan keupayaan alat diagnostik makmal semasa. Walau bagaimapun, penambahbaikan secara berterusan dan konsisten seharusnya dijalankan bagi menguji dan meningkatkan kepekaan dan kestabilan biosensor.

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CHAPTER 1

INTRODUCTION

Hepatitis B disease is the disease caused by hepatitis B virus (HBV). HBV infection is one of infections that is responsible for about more than one million death worldwide annually (Yap et al., 2010). This infection can cause acute and chronic liver infection of human for example liver failure, liver cancer or even death. According to Aspinall et al. (2015), the chronic hepatitis B infections are associated with an increased risk of hepatocellular carcinoma and cirrhosis. There are several main mode of transmission of HBV including from mother to neonate, childhood infections, parenteral, sexual, and intravenous drug use (Rehermann and Nascimbeni, 2005). Therefore, it is essential to develop an effective diagnostics tool to detect the viral infection.

The current diagnosis tool of the hepatitis virus is still a challenging issue and exhibit many disadvantages. The current diagnosis of HBV is accomplished by a series of test using serological markers of HBV and involved additional testing to exclude possible alternative etiological agents such as Hepatitis A and C viruses (Krajden et al., 2015). Some of the disadvantages of the current diagnostic systems are laborious, require large proportion of blood and does not allow continuous monitoring. Apart from that, one of the biggest challenges is that it is time consuming which required 2 until 26 days to get the result.

In recent years there has been a rapid increase in the number of diagnostic applications for HBV based on biosensors and various technologies have been used and improved for this purpose (Perdikaris et al., 2009). The applications of the biosensor in detecting HBV can be considered outstanding as it has the ability to

overcome the weaknesses of the conventional diagnostic tool for the HBV by improving and increasing speeds and its sensitivity.

To minimize the limitation of the current diagnostic tools, nanotechnology field may play a major role to explore new possibilities in which protein or antigen immobilized on a nano-material such as optical fiber core surface can be developed as a biosensor. Walt (2006) stated that optical fibers are comprised of individual plastics or glass fibers that manufactured and undergo series of iterative process to create arrays of fiber. Optical fibers offer excellent advantages including simple, flexible, low cost, lightweight, allow multichannel and remote sensing (Ojeda and Rojas, 2013). On the other hand, fiber optics biosensor serves medical sciences in several ways which includes diagnostic routine tests, surgery, patient home care and intensive care.

The objectives of this project were:

- 1) To successfully immobilize a model protein, Bovine Serum Albumine (BSA) on optical fiber core surface.
- 2) To optimize the immobilization of BSA on optical fibre core surface.
- 3) To successfully immobilize HBcAg on optical fiber core surface by using the best method of immobilization.
- 4) To measure and compare the immobilization signal of BSA and HBcAg using microscopic observation and Optical Spectrum Analyser (OSA).

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