POLYMER AND MECHANICAL-STRETCHING EFFECTS ON LATERAL FLOW MEMBRANE

BY

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List of Abbreviations

Abbreviations	Description
BSA	Bovine Serum Albumin
CA	Cellulose Acetate
DP	Degree of Polymerization
DI	Deionized Water
DNA	Deoxyribonucleic Acid
FET	Field Effect Transistor
LFA	Lateral Flow Assay
MA	Methyl Acetate
NC	Nitrocellulose
RNA	Ribonucleic Acid
PES	Polyethersulfone
PE	Polyethylene
PVDF	Polyvinylidene Fluoride
PEI	Polyethyleneimine

Kesan-Kesan Polimer dan Regangan Mekanikal Ke Atas Membran Aliran Sisi

Abstrak

Fungsi membran dalam pemegunan dan kadar pengangkutan analit banyak dipengaruhi oleh morfologi fizikal dan komposisi kimia. Kedua-dua faktor ini memainkan peranan penting untuk mendapatkan hasil yang cepat dan tepat, dan elemen-elemen ini dapat ditingkatkan dengan memanipulasi formulasi cecair membran seperti kepekatan polimer dan kelikatan polimer. Selain itu, struktur liang membran dapat diubah dengan regangan haba-mekanikal. Keputusan eksperimen menunjukkan bahawa pada kepekatan polimer yang lebih rendah iaitu 5% berat, masa aliran sisi adalah berkurangan tetapi keupayaan ikatan protein adalah rendah. Dari segi kelikatan polimer, pada kelikatan yang lebih rendah 25-50 kcP, larutan menjadi terlalu cair (membran tidak dapat dibentuk) manakala pada kelikatan tinggi 800-1000 kcP menyebabkan ikatan protein yang tinggi tetapi perlahan masa aliran sisi berbanding dengan kelikatan polimer medium 50-100 kcP. Sementara itu, membran dengan regangan mendatar memberikan masa aliran sisi yang lebih cepat berbanding dengan konfigurasi lain tetapi ini menyebabkan ikatan protein yang rendah. Walau bagaimanapun, regangan membran masih disyorkan, kerana pembaikan dari segi masa aliran sisi daripada 404 s kepada 281 s untuk jarak aliran 2 cm jalur dengan hanya sedikit penurunan pada ikatan protein daripada 5107 μg cm^{-3} kepada 5017 μg cm^{-3}. Kajian ini dapat memberikan kefahaman yang lebih mendalam berkaitan dengan proses manipulasi membran untuk memberikan prestasi membran yang lebih baik dalam aplikasi pengesanan bio. Pada ketika ini, kajian lebih mendalam perlu dilakukan untuk memperbaiki ikatan protein membran kerana prestasinya sentiasa tidak berkadar langsung dengan masa aliran sisi.

Polymer and Mechanical-Stretching Effects on Lateral Flow Membrane

Abstract

The function of membrane in immobilization and transport rate of analyte is greatly influenced by its physical morphology and chemical composition. These two factors play a vital role in order to get fast and accurate result, and these elements could be improve by exploring surface and internal layer of membrane. The improvement can be done by manipulating the membrane casting formulation such as polymer concentration and polymer viscosity. Besides, the membrane pore structure could be change by thermal-mechanical stretching. The result showed that the at lower polymer concentration of 5 wt%, the lateral wicking time was decreased but in expense of lower protein binding. In term of polymer viscosity, lower viscosity of 25-50 kcP resulted in too dilute solution (unable to form a piece of polymeric membrane) while high viscosity of 800-1000 kcP resulted in high protein binding but in sacrifice of slow lateral flow time compared to the medium polymer viscosity of 50-100 kcP. Meanwhile, uniaxial horizontal membrane stretching provides the faster wicking time compared to other configurations but as in concentration and viscosity studies, this lead to the low protein binding. However, the stretched membrane was still been recommended, since the wicking time improved from 404 s to 281 s for the water to migrate 2 cm height in the membrane strip at expense only showed slight dropping of the protein binding from 5107 $\mu g\ cm^{-3}$ to 5017 $\mu g\ cm^{-3}$. This study can give us the inside looks on the membrane manipulation process to provide better performance of membrane. At this point, more deep research needs to be done to improve membrane protein binding since its performance always not proportional to the lateral wicking time.

CHAPTER 1

INTRODUCTION

1.1 Immunoassay

The products of biomedical technology are getting smaller and more portable. Bio-microelectromechanical systems (bioMEMS) usually contain sensors, actuators, mechanical structures and electronics. These systems are being developed as diagnostic and analytical devices at an incredible rapid speed. Since the mid-1960s, immunoassays have been used in hospitals, laboratory medicine, and research. In industry, immunoassays are used to detect contaminants in food and water, and in quality control to monitor specific molecules used during product processing. Immunoassays are performed in laboratories, using a variety of instrument-based technologies or on-site via rapid test techniques (Rosen, 2008).

Rapid immunoassays test commonly come in two configurations. The first one is the lateral flow, a single-step technique where the sample is placed on a test device and the results are read in 5–30 min. Lateral flow devices can test for a single analyte or multiple analytes. The second is a flow-through system, which requires a number of steps - placing the sample on the device, a washing step, and then adding an analyte-colloidal gold conjugate that makes the test result visible to the eye. The results can then be read after a few minutes, but the whole process can take as long as 20 minutes. An Example is Enzyme-Linked Immunosorbent Assay (ELISA) that has been used for the detection of disease such as leptospirosis (Dey et al.,2007). However, this second system is less popular than lateral flow because it required multiple assay steps and the

greater skill that is required for operating these devices (Rosen, 2008). Table 1.1 below shows some comparison between the lateral flow (single step) and flow through (multi steps) system.

Table 1.1. Comparison between lateral low assay and flow through system (Rosen, 2008; Pang, 2010)

Lateral Flow Assay	**Flow Through System**
One step techniques	Requires a number of steps - placing the sample on the device, a washing step, and then adding an analyte–colloidal gold conjugate that makes the test result visible to the eye.
Required only sample for testing.	Other than sample containing interested analyte for testing, it is also required washing buffer, second antibody conjugate to the enzyme which gives the color of colorless solution.
Point to care (POC) testing which testing can be conducted anywhere.	Testing can only be conducted in laboratory.
Lateral flow assay (LFA)/biosensor such as pregnancy kit is produced for public usage. So, test can be conducted by anybody.	Require skill and trained person to perform testing.
However, most of LFA only provides qualitative (positive and negative) result, which means no quantitative measurement.	The intensity of color can be measured. This Color intensity proportional to bound enzyme-antibodies.

1.2 Immunoassay for Biomedical Application

In the recent year, the lateral flow immunoassay tests which also known as immunochromatographic strip tests have experienced a remarkable growth in the biomedical industry. A large number of tests available are currently used for qualitative, semi quantitative and to some extent quantitative monitoring in resource-poor or non-laboratory environments. Applications include tests on pathogens, drugs, hormones and metabolites in biomedical (Depierreux et al., 2000), phytosanitary, veterinary, feed/food and environmental settings. They are especially designed for single use at point of care/need, i.e. outside the laboratory. Applications are often designed where an on/off signal is sufficient. The best-known application is the pregnancy test and results usually come within 10–20 min.

The principal analysis of biosensors is based on the specific interactions between a substance of interest (target analyte) and the biological recognition elements (capture analyte such as enzymes, antibodies, nucleic acids, etc.) that are immobilized on the lateral flow membrane (Luppa et al., 2001). An important part in the lateral flow immunoassay is membrane strip that allow the sample analyte to diffuse to the immobilized biological recognition. Membrane strip is a strip made of flat and highly porous membrane. Firstly, a known antigen is immobilized at predetermined location (capture zone) along the porous membrane. Test sample containing target analyte is then mixed with the buffer solution and reporting agent such as colloidal gold. This solution is then introduced into the membrane by capillary forces. As the solution flows along the capture zone, the analyte and/or the reporting agent would bind onto the immobilized antigen (Qian and Bau, 2004). Biosensor reader device is responsible for

display the results in a user-friendly way, in either color response due to the biochemical reaction or voltage signal due to electrical response (Fischer, 1995).

1.3 Nitrocellulose Membrane (NC)

The membrane is parts of diagnostic kit which play a significant role in determining the sensitivity and effectiveness of the diagnostic kit. The unique separation principle governs by a membrane, acts as the detector surface or carrier for the target analyte and makes it an ideal candidate as the key functional element in a biosensor. The purpose of membrane is to bind the capture reagent at the test and control areas; and also to maintain lateral capillary flow for the target analyte to move within the porous structure. Without the membrane, the diagnostic test strip would never be able to function.

In recent years, the implementation of lateral flow diagnostics using adsorptive nitrocellulose (NC) membrane as chromatographic media is becoming more apparent (Mansfield, 2009; Yoon et al., 2003). NC membrane is widely used due it ability to bind with various antibodies, antigen and other biological components along the porous membrane (Qian and Bau, 2004). As mentioned earlier, NC membrane is commonly used as the platform in diagnostic kit which is essential in immobilization of biological recognition and transport rate of sample analyte to its binding partner on the membrane surface (Mulder, 2003). Besides of its widely usage as pregnancy kit in medical industry, now its application has been expanded to other areas such in detection of viruses (Depierreux et al., 2000), environmental monitoring (Nishi et al., 2003) as well as the detection of contamination in poultry feeds (Campbell et al., 2007). Comparing

to other type of membranes, NC is favorable to use in lateral flow mode due it ability to easily wick the solution and binding of any biomolecules. Both criteria are important as the analyte are carried through the pores parallel to the plane of the membrane as liquid moved from one end of the membrane to the other and bind to the immobilized receptor (Mansfield, 2009).

1.4 Problem Statement

The function of NC membrane in immobilization and transport rate of analyte is greatly influenced by its physical morphology and chemical composition (Pinnau and Koros, 1993; Ahmad et al., 2008). The performance of membrane such as the lateral flow wicking time and protein binding ability are very much important to generate consistent results for diagnostics purposes. Different diagnostic kit need different surface properties of membrane, structures and dimension. Hence, it is paramount to study the surface and internal layer of membrane such as pore size and pore alignment as it is the fundamental in developing diagnostic kit for medical and healthcare purposes. If the membrane surface and internal layer could be controled, various diagnostic kits could be effectively and accurately performed.

It is not a trivial task to synthesis lateral flow membrane to maintain both important performance of a biosensor such as lateral wicking speed and protein binding ability. Since polymer and its viscosity appeared to be the main factors in affecting the membrane morphology, this study intended to explore the effects in terms of the synthesized membrane chemical and physical properties, as well as its application in sensing-related applications.

The modification of the membrane morphology can be done on two different way; membrane casting formulation or modification of membrane physiochemical. The mechanical modification of membrane is more preferable since casting formulation is complex because many factors need to be taken care during the casting process (Ahmad et al., 2009b). In the membrane modification method, there are many challenging factors that can affect the membrane surface and internal layer.

The thermal mechanical stretching technique is a technique that applied the mechanical strength and heat treatment to membrane to yield desired morphology (Huang et al., 2004; Green et al., 2006; Li et al., 2007). The synthesized membrane is stretched under certain heat temperature. The purpose of stretching is to modify the membrane structures and with appropriate heat applied it will ensure the polymer is ductile enough to be stretched. It is expected that the modified membrane could provide a better performance for diagnostic application.

Although a few studies has been conducted on NC membrane thermal-mechanical stretching by researchers (Ahmad et al., 2009b; Morehouse et al., 2006), most of the studies were concentrated on the horizontal uniaxially membrane stretching and its performance. Furthermore, the membrane being studied were the different types of membranes such as Polytetrafluoroethylene (PTFE) (Kurumada et al., 1998), CaCO3/polyolefin composite membrane (Green et al., 2006), Polyvinylidene Fluoride (PVDF) (Morehouse et al., 2006) and Sulfonated Poly (phenylene oxide) (SPPO) membrane (Li et al., 2007). By such, there is no intensive study on the thermal mechanical stretching on NC membrane in three configurations; horizontal uniaxial, vertical uniaxial and biaxial. Technically speaking, if the membrane pore structure were

modified, it might eventually change the membrane performances. Capillary flow rate is related to the size of the pores oriented in parallel to the wicking direction of the membrane. By having the different stretching configurations, the outcome of the lateral wicking flow rate would be different based on the changes orientation of the membrane pore size. In addition, the pore shape would also affect the width of the capture reagent line, which in turn defines the width of the signal line when the strip is tested.

1.5 Objectives

The primarily objective of this study is to produce a highly performances NC membrane for biomedical application. The present study has the following objectives:

1. To synthesis lateral flow NC membrane consists of micro pores structure.
2. To investigate the effects of polymer concentration and viscosity in terms of membrane characteristics and bio-sensing performances.
3. To study the correlations between different stretching configurations: horizontal uniaxial, vertical uniaxial and biaxial stretching and evaluated the performance in a bio-sensing applications.

1.6 Scope of Study

In this research, the purposes were to study the effect of the polymer concentration, viscosity and mechanical-membrane stretching toward the membrane performance. The membranes were synthesized with varying polymer concentration; 5 wt%, 6 wt% and 7 wt% by using polymer with viscosity of 50-100 kcP and varying polymer viscosity; 18-25 kcP, 50-100 kcP and 800-1000 kcP with polymer

concentration of 5 wt%. For the mechanical membrane stretching part, the membrane used was with 7 wt% polymer concentration and with polymer viscosity of 50-100 kcP. The membrane characterization was done by using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) for the functional group, Scanning Electron Microscope (SEM) to determine the pore size and structure of the membrane, porosity for determination of void presence and porometer for membrane pore size. The evaluation of the membrane was done based on the protein binding capacity, lateral wicking time and dot staining-ponseu S.

For the study of mechanical-stretching, the membrane was first stretched with different configuration; horizontal and vertical uniaxial and biaxial before membrane characterization and performance evaluation was carried out. The stretching process was performed using thermal mechanical stretching machine which provide the heat for stretching process. The membrane characterization and performance was done for SEM, lateral wicking time and protein binding ability. The result obtained was compared to the unstretched membrane to see for any improvement of membrane structures and performances. In addition to that, the effect of varying membrane thickness to membrane maximum elongation also was studied.

1.7 Organization of Thesis

There are five chapters in this thesis and each chapter described the sequences of the research that were carried out in this study.

Chapter 1 introduced the application of biosensor in various field and the role of the membrane in bio-sensing application. This chapter also highlighted the problem statement, research objectives, scope of research and organization of thesis.

Chapter 2 covered the overview of the principle of biosensor, different types of biosensors and their applications. This chapter also stressed on the different types of membranes, synthesis processes and modification methods of membranes for various applications.

Chapter 3 highlighted on the material and method, describing the procedures for synthesize of membrane, membrane stretching as well as the method for membrane characterizations and performances.

Chapter 4 presented the result and discussion on the membrane characterizations and performances of the membrane with different polymer concentration, viscosity and stretching configurations.

Chapter 5 covered the overall conclusions that are based on the finding obtained in the results and discussions. Recommendations for future research are also given in this chapter.