"Lab on Chip"- The development of Nanoporous Membrane for Bioanalytical Chemistry

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"Lab on a chip" (LOC) technology is exciting the interest of scientists in many areas. This technology can be used not only to synthesize chemicals efficiently and economically but also to carry out biological and clinical analyses, to perform combinatorial chemistry, and to carry out full-scale analyses from sample introduction to chemical separation and detection, on a single, miniaturized device.

Today, miniaturization methods are often applied to biotechnology problems. With the growth of the Biological Micro-Electro-Mechanical System (BIOMEMS) field, techniques for depositing organic materials for chemical and biological sensors, often arranged in some type of an array configuration, are gaining importance [1]. The use of miniaturised detection systems is now well established and recognised as the trend of the future for biomedical and biotechnical applications. Biochip, defined as arrays of selected biomolecules immobilised on a surface, is a broad term indicating the use of microchip technology in molecular biology whereas microarray is a rapid method of sequencing and analysing protein complexes [2].

There is a need for bioanalytical techniques that are capable of identifying extremely low molecular weight of protein complexes in gases and liquids. This project seeks to conduct research on the use of membrane technologies for protein complexes identification (Figure 1). The overall approach will be to develop membranes that have a

very high density of nanometer size pores. The goal is to produce a miniature biological identifier based on nanoporous membrane methodologies, which is able to identify very low molecular weight protein complexes in air and liquid samples with the minimal consumption of reagents.

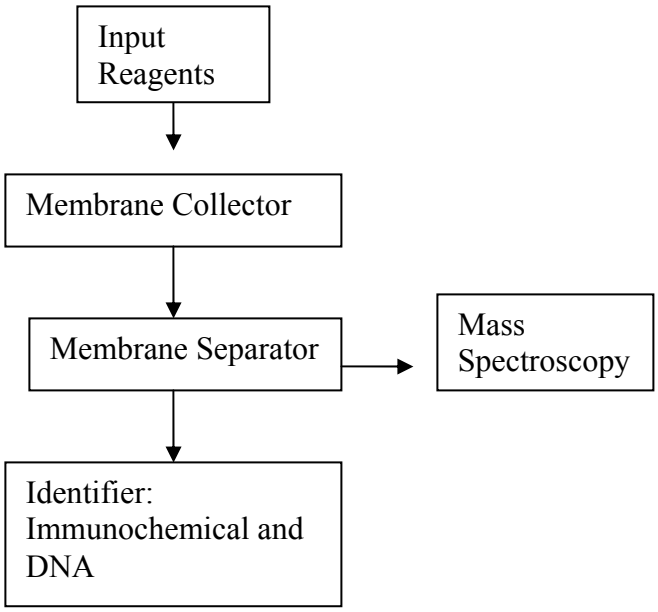


Figure 1. Nanoporous membrane identification system for ultrafiltration.

The novel nanoporous membrane for ultrafiltration (UV), which is integrated on LOC, has many important applications on clinical diagnostic, environment and health care industry. Many features of the LOC make it well suited for high-throughput analyses. Its small dimension reduces both processing times and the amount of reagents necessary for an assay, substantially reducing costs.

The nanoporous membrane is fabricated to have a pore size of 1 ~ 2 nm in diameter with molecular weight cut off (MWCO) of 1000 Dalton (Da). Since the methodologies are promised to be patented, ultrafiltration (UF) technique has been

chosen over nanofiltration (NF) in this project in order to come out with a new methodology of synthesizing a thin nanoporous membrane. This new methodology comprises spin coating a monolayer of thin of polymeric membrane film on the Anodisc alumina membrane.

Among inorganic membranes, the Anodisc alumina membranes have become a growing interest since improvements in production techniques have resulted in membranes of high quality in terms of pore size uniformity (Figure 2.), high pore size distribution (Figure 3), high flow rate and good thermal/mechanical properties [3 – 4]. Coating a polymeric membrane on the top of Anodisc alumina membrane will give the whole nanoporous membrane high pressure sustainability.

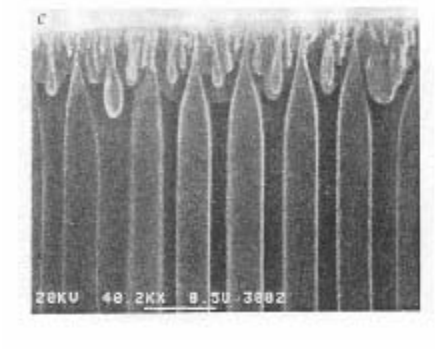


Figure 2. Cross sectional SEM image of Anodisc alumina membrane

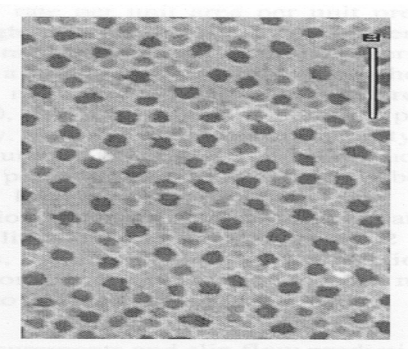


Figure 3. SEM of the pore distribution of Anodisc alumina membrane

Selecting an appropriate polymer for the nanoporous membrane is very crucial. Hydrogels have been selected due to their high efficiency separating protein in Electrophoresis. Extensive studies have been done on two types of hydrogels: Agarose and Polyacrylamide gel. Polyacrylamide gel (PAG) is chosen due to its ability to separate smaller protein or protein complexes than Agarose [5]. Plus, PAG has the advantages of being chemically inert and mechanical stable [5].

The pore size of PAG can be controlled through two factors: T and C, which are the total acrylamide concentration and degree of polymer cross-linking, respectively.

$$T = \frac{(a+b)*100}{V} \quad [\%] \quad ; \quad C = \frac{b*100}{(a+b)} \quad [\%], \text{ where}$$

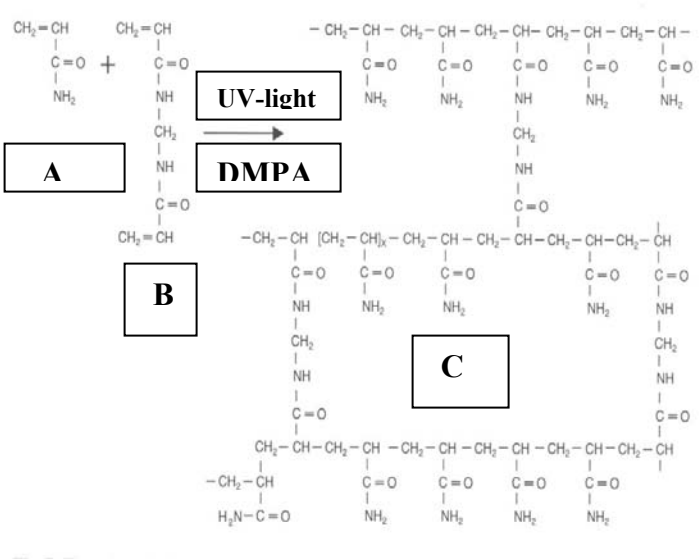
a: the mass of acrylamide in grams,

b: the mass of methylenebisacrylamide in grams,

V: volume of gelling solution in mL.

According to recent research, when C remains constant and T increases, the pore size decreases. When T remains constant and C increases, the pore size follows a parabolic function: at high and low values of C the pores are large, the minimum being at C = 4 ~ 5 % [6]. Based on these reasons, 30 % PAG with 4.75 % degree of cross-linking has been made up in order to have an average pore size of about 2 nm.

After having chosen the suitable polymer, UV-initiator free radical polymerization methodology (Figure 4) has been adopted over Thermal-initiator free radical polymerization since it is more suitable for simultaneous occurrence of the spin coating and polymerization processes [7 – 8]. Problems such as the calibration of spin speed and spin time in the spin- coating process are reduced as well. In this project, UV-initiator 2, 2-Dimethoxy-2-phenyl Acetophenone (DMPA) (Figure 5) is generally being used due to its reasonable pricing and higher radical fragments.



A : Acrylamide B: N,N'-Methylenebisacrylamide C: PAG

Figure 4. Mechanism of UV-Initiator free radical polymerization for PAG. (Source: R. Westermeier. Electrophoresis in Practice, 2nd Edition. pp. 11)

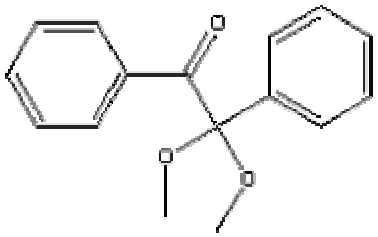


Figure 5. Molecular Structure of 2, 2-Dimethoxy-2-phenyl Acetophenone (DMPA) (Source: www.chemfinder.com)

Since a monolayer of thin film is required, 30 % Polyacrylamide gel is spin-coated on Anodisc alumina membrane by photoresist spin-coater for 3000 rpm for 30 seconds. The sample was exposed to UV light with an intensity of 20 mW/cm^2 for a minute and then allowed to soak in deionized distilled water (dd-H_2O) for 24 hours to remove any unreacted monomer.

The physical and chemical properties of the nanoporous membrane will be characterized by using Atomic Force Microscope (AFM), Ellipsometry, Scanning Electron Microscopy (SEM) and Liquid/Gas Flow-rate meter. Finally the performance of the nanoporous membrane will be examined as well.

References

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