STUDY ON EFFECTS OF SURFACE MODIFICATION ON POLY(METHYL METHACRYLATE) (PMMA) SURFACE

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Name of Candidate: *Wan Nurlina binti Wan Yahya*

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ABSTRACT

This report presents study on photochemical and wet chemical surface modifications of poly(methy methacrylate) (PMMA) by means of UV (ultraviolet) irradiation and NaOH (sodium hydroxide), respectively. The PMMA solid substrate was prepared by PMMA solution casting on glass wafer resulting in PMMA thin films and commercial PMMA sheets. Both kinds of solid substrate were subjected to two different surface modifications techniques so as to yield carboxylic acid groups that enhanced PMMA surface hydrophilicity. The changes of surface properties of PMMA upon modifications were studied by non-spectral analyses which were contact angle measurement and Toiludine Blue (TB) assay. The findings reveal that both modifications techniques had increased the contact angle value by showing more hydrophobic water drop. On top of that, no carboxyl functional group was produced as confirmed by chemical surface analysis, TB. Considering the various factors, the increasing hydrophobicity is primarily due to surface roughness either pre- or post-treatment. Hence, spectral analysis is strongly recommended to verify each step of modifications in a more precise and accurate manner.

ABSTRAK

Laporan ini mengenai pengubahsuaian permukaan fotokimia dan cairan kimia pada poli (meti metakrilat) (PMMA), masing-masing secara dengan cara penyinaran UV (ultraungu) dan NaOH (sodium hidroksida), telah diterangkan. Substrat pepejal PMMA boleh disediakan dengan salutan PMMA pada wafer kaca untuk menghasilkan filem nipis PMMA dan helaian komersil PMMA. Kedua-dua jenis substrat pepejal ini telah dikenekan dua teknik pengubahsuaian permukaan untuk menghasilkan kumpulan asid karboksilik yang mempertingkatkan kehidrofilian permukaan PMMA. Apabila pengubahsuaian telah dilakukan, perubahan sifat-sifat permukaan PMMA telah dikaji melalui analisis bukan spektrum yang merupakan pengukuran sudut permukaan dan asai Toiludine Blue (TB). Daripada kajian ini, didapati bahawa kedua-dua teknik pengubahsuaian telah meningkatkan nilai sudut sentuhan dengan menunjukkan penurunan sudut air yang lebih hidrofobik. Selain itu, tiada kumpulan berfungsi karboksil telah dihasilkan sebagaimana yang telah disahkan melalui analisis permukaan kimia TB. Dengan mengambil kira pelbagai faktor yang berkaitan, kehidrofobian meningkat terutamanya disebabkan oleh kekasaran permukaan sama ada pra atau pasca-rawatan. Oleh itu, analisis spektrum adalah sangat disyorkan untuk mengesahkan setiap langkah pengubahsuaian dengan cara yang lebih jitu dan yang lebih tepat.

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List of Symbols and Abbreviations

AFM	Atomic force microscopy
ATR-FT-IR	Fourier transforms infra-red spectroscopy
$CHCL_3$	Chloroform
CNC	Computer numerical control
ELISA	Enzyme-linked immunosorbent assay
NaOH	Sodium hydroxide
N_2	Nitrogen
PC	Polycarbonate
PMMA	Poly(methyl methacrylate)
PS	Polystyrene
SEM	Scanning electron microscopy
SFM	Scanning force microscopy
TB	Toiludine blue
UV	Ultraviolet
XPS	X-ray photoelectron spectroscopy
ZnO	Zinc oxide

CHAPTER I: Introduction

1.1 Overview

ELISA (enzyme-linked immunosorbent assay) is one of the well known detection scheme to determine the presence of either antibodies or antigens in a sample which works by quantifiying enzyme that is linked to the antibody-antigen complex. The ELISA technology has been extensively used as a diagnostic tool in medicine, quality control in food, and environmental industry as well as discovery of biological and chemical substances in the biotechnology field.

Conventionally, ELISA was usually performed on a 96-well microtiter plate as the solid substrate for the immobilization of antigen or antibody. Following that is a series of mixing, incubation, washing, and blocking steps, which are not just time consuming but also require an expert and trained individual to deal with them. Hence, the intensive labor need had caught the attention of researchers to look for an alternative system with the same principle, more reliable result, and higher sensitivity and throughput.

Since the last two decades of the introduction of microfluidic, many attempts had been made to overcome complexity of the conventional assays applied in clinical diagnosis such as for cell-based analysis, drug discovery (Kang *et al*. 2008), biological and chemical studies (2003), as well as disease examination (Einav *et al*., 2008). The advantages of the microfluidic technologies include the capability to miniaturize assays by only small sample size needed and less reagent used. Other advantages would be the possibility to be point-of-care diagnostic tool since it is miniature and portable and perhaps it could be made a fully automated and integrated microfluidic system. For instance, various microfluidic

approaches had been implemented for ELISA immunoassays to overcome the laborious techniques, cut down the assays cost, and offer rapid diagnosis.

However, many factors should be considered, including the material of the microfluidic devices since there are tons of materials that has been introduced as a base for microfluidic device. In this study, poly(methyl methacrylate) (PMMA) had been chosen as the solid substrate due to its good quality in terms of both the physical and chemical properties. For protein immobilization which is required in the ELISA technique, surface properties of the material is significant to accelerate the immobilization by reducing incubation time and producing more stable attachment. Therefore, two different surface modifications had been carried out in this study, followed by surface characterization in order to enhance PMMA surface properties for better protein immobilization.

1.2 Objectives of the Study

The objectives of this study is:

1. To determine the effects of surface modification using Ultraviolet (UV) irradiation and Sodium Hydroxide (NaOH) treatments on poly(methyl methacrylate) (PMMA) substrate.

1.3 Significance of the Study

PMMA is one the best polymer used as alternative to glass and silicone substrate due to its low cost. On top of that, the good mechanical and optical properties have made it an excellent choice as a microfluidic device material. In order to implement it for ELISA immunoassay, surface modifications are required for a better immobilization of protein which lead to high throughput and high sensitivity. Both surface modifications performed are mainly to avoid protein denaturation or protein leakage upon immobilization by introducing carboxyl acid group on the PMMA surfaces which increase the hydrophilicity and highly stable surfaces.

1.4 Scope of the Study

In this study, surface modification was conducted on a polymeric material, which was poly(methyl methacrylate) (PMMA). There were two forms of samples used: i) Spin-coated PMMA thin films, and ii) PMMA sheets. As for surface modification, photochemical and wet chemical techniques were performed by using UV-irradiation and sodium hydroxide (NaOH), respectively. In order to analyze the results, non-spectral methods were carried out by means of contact angle measurement and Toiludine Blue (TB) assay. This study was specifically done for further protein immobilization in ELISA microfluidic device.

1.5 Hypothesis

Hydrophilic surface properties are required in order to obtain a good immobilization of proteins for ELISA application. Both surface modifications conducted in this study are expected to introduce carboxyl acid group on the PMMA surfaces which increase the hydrophilicity that favorable to covalent attachment of proteins. Therefore, analysis by contact angle measurement will show decreasing in water contact angle upon modifications and the presence of carboxyl acid group can be confirmed by TB assay. This hypothesis is strongly supported by several literatures (Hozumi *et al*, 2002; Welle and Gottwald, 2002; Wei *et al*, 2005; Kaczmarek and Chaberska, 2006; Liu *et al*, 2009).

1.6 Outline of the report

Follows are the structure of this dissertation:

a) Chapter 2 describes a summarizing of literature reviews focus on recent development in microfluidic device, material properties and its application on ELISA, immobilization mechanisms of protein for ELISA and also several of surface modification techniques for polymer.
b) Chapter 3 mention about the materials, reagents and equipment required for experiment. Besides, details on experimental setup and every procedure that has been done were illustrated on this chapter.
c) Chapter 4 shows the subsequent results achieved from the experiment including extensive discussion regarding the results
d) Chapter 5 draws out the overall conclusion of this study and some of its limitations. A brief summary of future work for improvement and recommendation are also included.

CHAPTER II: Literature Review

2.1 Introduction

This chapter reviews the previous and more recent work on the microfluidic devices and its application primarily for ELISA immunoassay. The material of interest and available immobilization mechanism will also be described briefly in this chapter. Moreover, this chapter also consists of numerous surface modifications technique and two surface characterization methods which will be performed.

2.2 Microfluidic Device

Recent expansive development in microfluidic technologies has given bright chance to enhance the medical diagnostics and biosensors field by means of throughput of samples, increase accuracy and lesser analysis cost. Such devices may also integrate numerous microscale detection schemes including optical detection that use fluorescence label (Powe *et al.*, 2010), chemiluminescence (Kim *et al*., 2009), nanoparticles and barcodes. Label-free methods include mass spectrometry and SPR (surface plasmon resonance) as well as mechanical and electrical methods include use of mass sensors, nanowires and dielectric spectroscopy also has been widely study.

Enzyme-linked immunosorbent assay (ELISA) is one of the practical biochemical techniques utilizing the principle of protein-protein interaction which has been used extensively as a diagnostic tool primarily in medicine (Voller *et al*., 1976; Conneely *et al*., 2007), biotechnological (Lipton *et al*., 2000) and environmental(Knopp et al., 1999; Cho *et al*., 2003; Sakamoto *et al*., 2005). However, conventional immunoassay deal with laborious sample usage, high operating expense of expensive reagents and analysis tools and long incubation and analytical time, which limit its further appliance.

In view of that, microfluidic devices present the possibility of decreasing intact laboratory operations onto single platform with the advantage of lesser reagent and sample volumes, shorter incubation time and parallel process. Therefore, several efforts have been made to transfer ELISA to the microscale format using conservative microarrays and microfluidic system (Liu *et al*, 2009). Angenendt and et al. (2003) has proposed a typical immunoassay carries out on the standards microscope slide with the absence of wells.

2.3 Material properties for microfluidic device

One of the most important keys of the microfluidic device that need to be considered is the material they are made of. As for ELISA which requires the immobilization of an antigen or antibody onto a substrate surface, the choice of material is significantly essential since it provide a great concern on the sensitivity and specificity of the assays. Basically, the required functional properties of the optimal substrates are:

i. Must immobilize the protein in a way that sustain its ability to attach to its partner
ii. Must bind an adequate quantity in each well to be detectable
iii. Must bind the same amount consistently (well to well) for the assay to have satisfactory accuracy.

Glass and silicon are good quality of substrate material because of low auto-fluorescence for the optical detection and relatively low nonspecific binding than polymers. Besides the good surface properties, this material also well fabricated owing to high mechanical resistance as proved by many semiconductor industry which make it more attractive material for use in microfluidic system (Castano-Alvarez *et al*., 2008). However the machining and fabrication processes are time-consuming and cost of mass produce is

high making it quite pricey for use as disposable devices. Therefore in the late 1990s, many researchers and commercial manufacturers were seeking out for other materials.

As an alternative to conventional one, despite a widespread in physical and chemical properties, polymers offer a great potential as substrate material due to the likely low manufacturing costs which increase their commercial viability and let them to be disposable. Polymers compare to other materials offer great functional groups for high density protein immobilization which can be used for covalent attachment. Besides, by selecting suitable polymer with known function groups, it is easy to control the desire surface properties thus reducing nonspecific protein immobilization. The high accessibility to the specific analytes also render by the unique long chain of polymers which act as spacer between attach proteins and solid substrate. Consequently, all of the advantages of polymer material provide numerous surface modification strategies in order to alter or improve their adhesion feature for ELISA immunoassay application. Thus, numerous polymers, including polystyrene (PS), poly(methyl methacrylate) (PMMA) (Wang *et al*., 2008), polycarbonate (PC) (Liu *et al*., 2001), polyester and poly(dimethylsiloxane) (PDMS) have been employed as solid support for immunoassays.

2.3.1 Polystyrene (PS)

One particular polymer that has been used widely for conventional ELISA is polystyrene (PS) (Voller *et al*., 1976). PS is commonly employed due to its optically transparent, good mechanical properties, biocompatibility and modifiable surface. Naturally, the surface of PS is hydrophobic but it can be simply modified by chemical or physical techniques. A lot of researches have been done by using direct coupling reagents and physical techniques

including plasma treatment, UV/ozone treatment, electron beam irradiation and grafting of the polymer surface. For example, the performance of polystyrene microplate treated by electron beam irradiation has been evaluated and it shows improved adsorption and stronger attachment of protein on the substrate with the stability more than two years when kept at room temperature (Safrany and Deelder, 1999).

2.3.2 Poly(methyl methacrylate) (PMMA)

Another promising polymer for microanalytical device is poly(methyl methacrylate) (PMMA) which we are concern of in this study. The high transparency and low fluorescent background are very useful for optical density detection method. Recently, Ge et al., (2010), in their research has proved that a nonocomposite material contained PS-PMMA/ZnO show even high transparency and UV absorption which also suitable for optical composite films. However further study should be conduct on the mechanical properties whether it has good machineable properties similar to PMMA or PS itself.

Still, there is a disadvantage of this polymer as solid substrate for analytical assay due to its low wettability resulting to low attachment efficiencies for proteins. Therefore, the fact that it has ester side-chains have favorable to numerous of surface modification techniques which will be discuss in detail in Section 2.4.

2.4 Immobilization mechanisms

Immobilization can be described as the binding of molecules on a surface ensuing stationary phase. In ELISA, the proteins are required to immobilize on the solid substrate and stable enough to attach to the specific protein. In order to have high biological

recognition and activity, the protein should be attached onto surface with optimum concentration, addressable orientation, ease of access to analytes and less nonspecific protein binding.

Generally, there are wide varieties of immobilization mechanism which are categorized on the subsequent three techniques: physical adsorption, covalent binding and bioaffinity. However, there is no ideal immobilization strategy for all proteins, it varies according to physical and chemical properties of both protein and surface.

2.4.1 Physical adsorption

Conventionally, ELISA procedure was commonly performed using polystyrene microplate with protein passively immobilized onto the hydrophobic surface. The physical or passive adsorption is the simplest mechanism with no surface modification needed and only involves interactions between protein and solid substrate as shown in Figure 2.1. Also known as electrostatic interaction since it is the electrical ions on both protein and surface of substrate that support the immobilize mechanism.

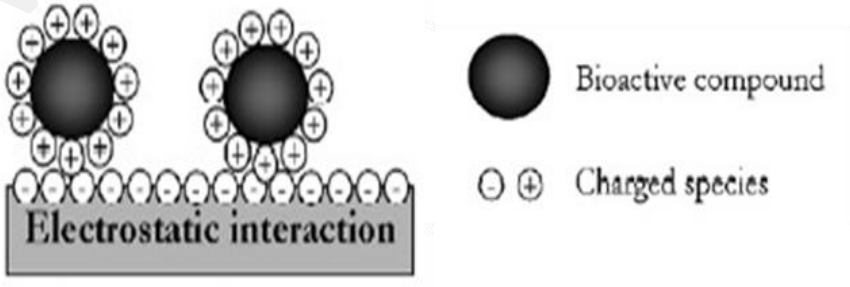

Figure 2.1: Physical adsorption of protein (bioactive compound) onto solid substrate (adapted from Goddard and Hotchkiss, 2007).

The process of physical adsorption comprise of adding together protein and an absorbable substrate under appropriate conditions of pH, temperature and ionic strength of the solution for a period of incubation, followed by a series of washing steps to remove the unbound proteins. However, immobilized protein via electrostatic interactions are regularly adsorbed on the surface of solid substrate in a random orientation which made the functional sites unfavorable to enzymatic reactions and tend to conformational change. The protein functional sites may vary in hydrophobic/hydrophilic avidity, thus the orientation of the protein may be affected by surface properties of the substrate. If the surface is too hydrophobic, the surface is likely to attract hydrophobic site of protein so as to induced denaturation and destabilize of adsorbed protein which gradually decrease the detection sensitivity. In contrast, the protein attachments are higher and more ideal orientation on the hydrophilic surface. Hence, physical adsorption can be optimized by combining proteins to the most appropriate surface to gain protein immobilization with ideal orientation and high sensitivity for further protein binding and enzyme detection (Qian *et al*., 2000).

2.4.2 Covalent Binding

Another option for protein immobilization in ELISA immunoassay is covalent binding meant to overcome the disadvantages of physical adsorption. This mechanism of immobilization involves formation of covalent bond between protein of interest and solid substrate via reactive group as shown in Figure 2.2. There are a lot of reaction procedures for immobilize protein using the covalent technique which required surface modifications that will be discussed in the Section 2.5.

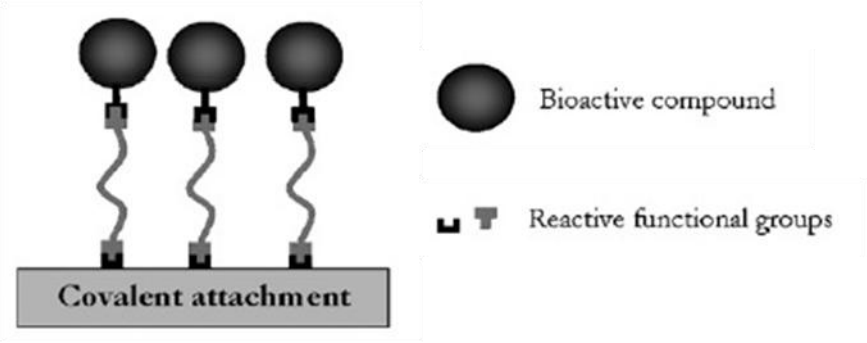

Figure 2.2: Covalent binding of protein (bioactive compound) on solid substrate via reactive functional groups (adapted from Goddard and Hotchkiss, 2007).

Covalent bound typically result in greatly stable and strongest attachment between protein and substrate compare to conventional method, physical adsorption as described before. Therefore, protein leakage or conformational changes is regularly minimized with covalently bound immobilized protein through generated reactive group and enhanced the immunoassay sensitivity by provide a good accessibility of attached protein to the subsequent analytes. As an extra bonus, the reactive group also approving immunoassay in minimizing and preventing non-specific binding of protein onto the substrate. Covalent binding is also controllable by changing the pH of mixing buffer that react with the reactive group. This means the desired concentration and orientation of immobilized protein can be simply control due to the dynamic of reactive group.

2.4.3 Bioaffinity binding

Further studies on protein immobilization have shown that more specifics interaction with protein of interest can be achieve via affinity attachment (Nareoja *et al*., 2009). This non-adsorption and non-covalent provide oriented and homogeneous attachment by high affinity of avidin and streptavidin for biotin as shown in Figure 2.3.

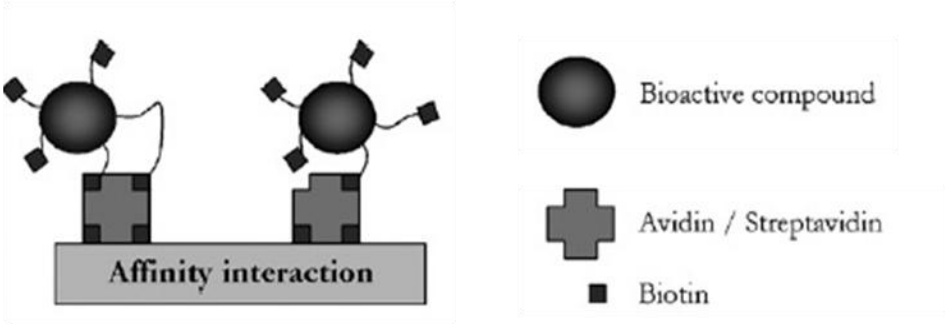

Figure 2.3: Bioaffinity binding of proteins (bioactive compound) onto solid substrate via avidin/streptavidin-biotin (adapted from Goddard and Hotchkiss, 2007).

The avidin/strepavidin-biotin interaction system may be bind to varieties of solid substrate through direct adsorption of avidin or covalent bound of biotin to the surface. This system offers a numbers of advantages: high availability for the attachment proteins of interest, detachable protein which make reusable solid surface and controllable surface density of interest binding sites. On the other hand, bioaffinity binding method utilizing peptide tags appears to be another promising binding strategy to gain well orientation and conformation of the attach proteins. Up till now, numerous peptide tags have been developed specifically to certain polymeric substrate and some of that have been commercialized (Kumada *et al*., 2006; Kumada *et al*., 2009).

2.5 Modification of polymer substrates

Surface properties of microfluidic devices are key features on their application especially for protein immobilization in immunoassay such as ELISA. Originally, PMMA surface property is hydrophobic but there are numerous methods of surface modification that successfully present in literature to improve the surface adhesion, wettability, topography and charge density and distribution. Up to now, there are wide variety of surface

modifications techniques range from the classical wet chemical method, organosilanes, ionized gas treatments including, plasma, corona discharge, flame treatment and UV irradiation.

2.5.1 Wet chemical

Chemical modification techniques are involved the use liquid chemical reagents to form reactive functional group on the solid surface. This conventional approach were usually preferred because it is required no specialized and expensive equipment and thus can be performed in most laboratories. Moreover, compare to other treatment methods, this treatment capable to penetrate into porous three-dimensional (3D) solid substrates. Fixe *et al.,* (2004), in their study shows the new method to chemically modify PMMA substrates with reaction who hexamethylene diamine to generate an aminated surface for DNA microarrays. Hydrolysis of PMMA by sodium hydroxide (NaOH) also have been shown to formed carboxylic acid group on the surface which can be further modified with poly(ethyleneimine) (PEI) to generate amine, NH_2 group for covalent attachment of α-fetoprotein (AFP) antibody (Bai *et al*., 2006; Liu *et al*., 2009).

2.5.5 Ionized gas

Surface modifications by means of ionized gas utilize three types of gaseous that include plasma, corona discharge and flame treatment. Plasma is the most popular among them with varieties of plasma gaseous including Ar, N_2, O_2, CO_2, NH_3 and H_2O and dynamic operating parameters such as time, power and pressure. A gas will somewhat ionized into other states such as electrons, charged or neuron particles in the highly-

energized environment. Main advantage of this treatment is it offers uniformly modification on the polymer surface without using any chemical or hazardous reagents with minimum surface roughness and defects upon modification. Low-temperature air-plasma has been used to enhanced the poly(vinyl chloride) wettability by generating oxygen on the polymer surface (Kaczmarek *et al*., 2002). Low-pressure argon discharged system has been used to generate oxygen surface on polystyrene group (Dhayal *et al*., 2006). More recently, a high-sensitivity polycarbonate (PC) DNA microarray has been introduced with the presence of carboxylic acid groups activated by oxygen plasma (Tamarit-Lopez *et al*., 2011). However, plasma treatment required vacuum treatment, thus lift up the cost of operation which limit its wide application. Besides, it is not a simple process that needs a lot of optimization such as gas flow rate and composition, pressure, temperature, power and some other critical parameters to control the desire functional groups.

2.5.2 UV-irradiation

The radiation induced modifications are other promising techniques by means of UV or gamma irradiation. Upon a period of time of exposure, it yields reactive functional groups on the irradiated polymer surfaces which can be further used for graft polymerization. Welle and Gottwald (2002) in their study show strong adhesion of hepatocytes cells on irradiated polymers coupled with graft acrylic monomers for tissue engineering application. Functional carboxyl acid group has been introduced upon simple UV photochemical process which then allows covalent attachment of amined protein (Situma *et al*., 2005). On top of that, a lot of studies done by a group on the effects of UV irradiation on different solid support of spin-coated PMMA thin films and well characterized by spectral analysis such as XPS (X-ray photoelectron spectroscopy) and

AFM (atomic force microscopy) (Kaczmarek *et al*., 2006; Kaczmarek *et al*., 2008; Kaczmarek *et al*., 2009). Nonetheless, UV-irradriation do have several disadvantages such as alteration of optical transparency, induced polymer degaradation and change the termo-mechanical properties of polymer (Eve and Mohr, 2009).

2.6 Methods of Surface Characterization

Surface analysis is usually performed by means of spectral and non-spectral method or either one of them. The spectral methods involve the use of spectroscopy and microscopy equipments such as XPS (X-ray photoelectron spectroscopy), AFM (atomic force microscopy), SEM (scanning electron microscopy), and some other advance equipments. This method is able to provide very define and accurate changes in surface chemistry yet the equipment is quite pricey and much time may be needed for the analysis. Therefore, for this study, the non-spectral, which is a simpler and fast technique, is used and will be outlined below. It can be done in the laboratory.

2.6.1 Contact Angle

Contact angle is a quantitative measurement and the most often used method is sessile drop. By dropping a liquid drop on a solid surface, the tangent angle between the water drop and solid surface can be measured. The contact angle value provides the information of surface hydrophilicity as shown in Figure 2.4.

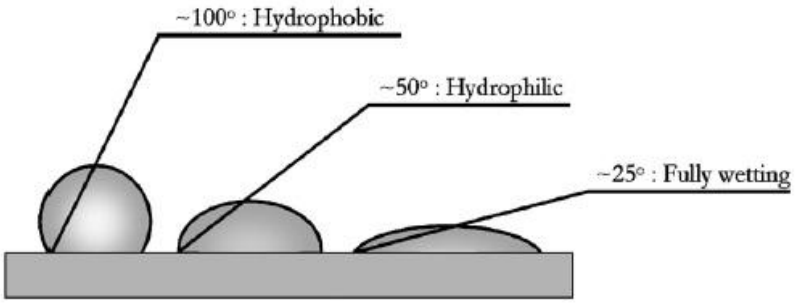

Figure 2.4: Schematic diagrame of water contact angle (adapted from Goddard and Hotchkiss, 2007).

Lower contact angle means the surface is more hydrophilic while high contact angle is hydrophobic. When the surface become further oxidized by functional groups such as carboxyl group, the hydrogen bonding between surface and water will be increased as indicated by a more spread droplet and lesser contact angle. In this study, side view image of the drop is captured and the contact angle is derived (Brugnara *et al*., 2006):

$$\theta = 2tan^{-1}\left(\frac{2h}{l}\right) \quad [1]$$

where, θ is angle, h is height of the drop, and l is the diameter of the drop. This mathematical equation is considering 2D (two-dimensional) situation with the view angle to the surface, α is zero and gravity effect is ignored.

2.6.2 Dye Assay

Another promising technique for surface analysis is by means of dye assay which can indicate the hydrophilic functional groups on the surface. The surface chemistry may be qualitatively defined by color changes in existence of specific functional group as is examined in this study.

Toiludine blue (TB), Figure 2.5 is a non-toxic basic dye which appears in blue color with absorb visible region at 635 nm. The mechanism is based on adsorbs/desorbs method which depends on ionic interaction between the specific functional group and the dye.

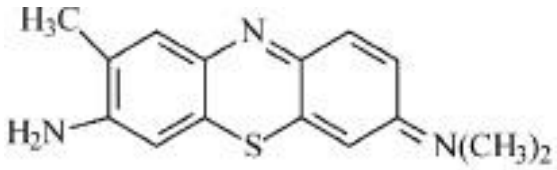

Figure 2.5: Toiludine Blue (TB) chemical structure (adapted from Jonnalagadda and Pare, 2010).

2.7 Summary

In short, poly(methyl methacrylate) (PMMA) will be considered as the material of interest for microfluidic device due to its good properties and extensive application as has been discussed before. There is quite a number of surface modification techniques described but this study is restricted to the UV-irradiation technique and wet chemical technique considering the resourse availability. Moreover, the surface analysis will be conducted by non-spectral approach because of its simplicity and low-cost equipment or reagent required.

CHAPTER III: Methodology

3.1 Introduction

Generally, the methodology for this study is divided into the scheme shown in Figure 3.1:

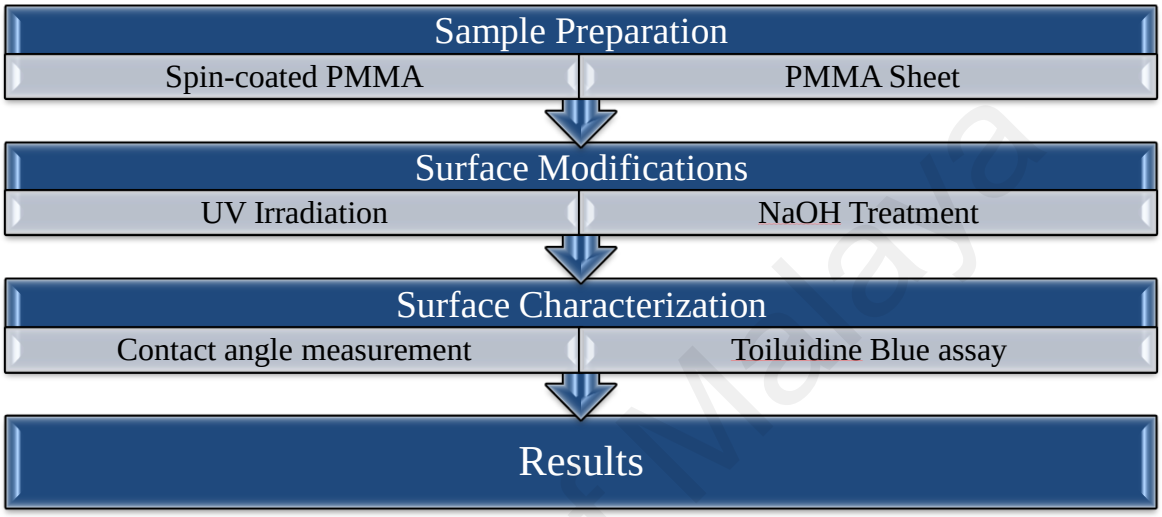

Figure 3.1: Methodology scheme.

1. Samples preparation: two types of samples
    a. 2 x 2 cm of PMMA thin layer were spin-coated on glass wafer and
    b. 2 x 2 cm of PMMA sheets were cut by using CNC machine.
2. Surface Modifications: two approaches of treatments
    a. UV treatment: samples were irradiated to UV light in a range of time.
    b. NaOH treatment: samples were treated with chemical reagents.
3. Surface Characterizations: two ways of non-spectral surface analysis
    a. Contact angle measurement: all samples were tested for water contact angle by using sessile method to check the changes of hydrophilicity.
    b. Toiludine blue assay: all samples were analyzed by toiludine blue dye to determine the presents of carboxyl group.
4. Results is analyzed and discussed in the next chapter.

3.2 PMMA preparation

There are two types of sample preparation used in this study. The following two sections describes each type of the samples preparation.

3.2.1 Spin-coated PMMA

The first substrates used in this study were PMMA thin films coating onto square glass wafer with dimensions of 2 x 2 cm. Before any type of experimental was carried out, each of the 60 glass wafers were cleaned by 70% alcohol with Kim Wipe and left 20 min for sonicated in ultrasonic cleaner (Lunas Edar) and finally dried them in an oven at 80°C for overnight. Special care must be taken to avoid scratching on the glass and all of them were covered with aluminium foil to avoid contamination.

Commercial poly(methyl methacylate), PMMA sheets were purchased from Enigma and were cut to small pieces. Approximately 2.7 g of PMMA were dissolved in 10 ml organic solvent, chloroform ($CHCl_3$) and left it on the magnetic stirrer overnight to get 20% (w/w) PMMA solution as can been shown in Figure 3.2.

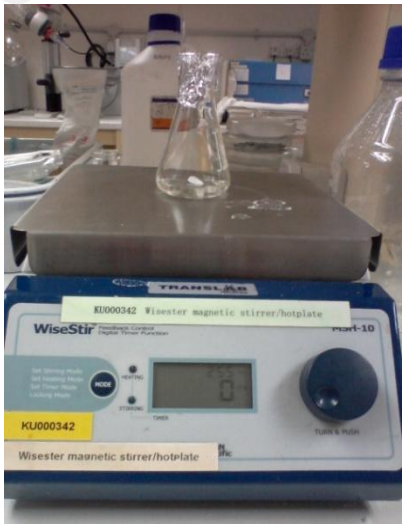

Figure 3.2: Dissolving PMMA pieces in chloroform on magnetic stirrer.

20% of PMMA solution was further diluted in order to obtain 0.5% concentrations. 60 thin films were then prepared by spin-coating PMMA solution onto 2 x 2 cm glass wafer. Extra care was taken to avoid shaking the beaker containing the PMMA solution from creating air bubbles. As shown in Figure 3.3 (a), spin coater from Nanorian Technologies was used which required 60-70psi of nitrogen, (N_2) gas to operate. The speed was set to 3000 rpm for 60 s and then press vacuum to hold glass wafer. Note that the glass substrate should totally cover the o-ring on the spinner chuck and well-centered as shown in Figure 3.3 (b). Next, close the lid and carefully drop 25 drops of the solution on the steady glass substrate, finally run the spin program. Same quantity of drops is needed to ensure that the PMMA solution completely cover the glass wafer and to obtain same thickness for all 60 samples. When the spin process is complete, press the vacuum button to release the vacuum from the substrate and carefully remove the substrate from the chuck with tweezers.

(a) (b)

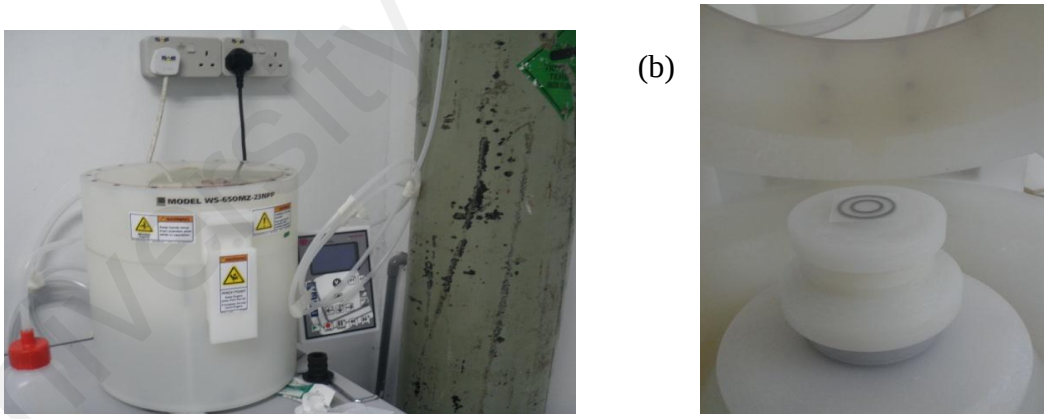

Figure 3.3: a) Spin coater with nitrogen gas cylinder, b) Glass wafer hold onto the chuck by vacuum

It is important that the spinner be cleaned after each use. The residue polymer left on the chuck may cause a vacuum leak and the substrate will not be held in place by the vacuum. After the spin process is complete, the spin coater must be cleaned, wiped out the lid, inside the spin coater and chuck adapter with alcohol.

3.2.2 PMMA sheets

The second types of sample used in this study were 2 x 2 cm PMMA sheets. It was prepared by cutting 2 mm thick of PMMA sheet using CNC (computer numerical control) machine as can be depicted in Figure 3.4.

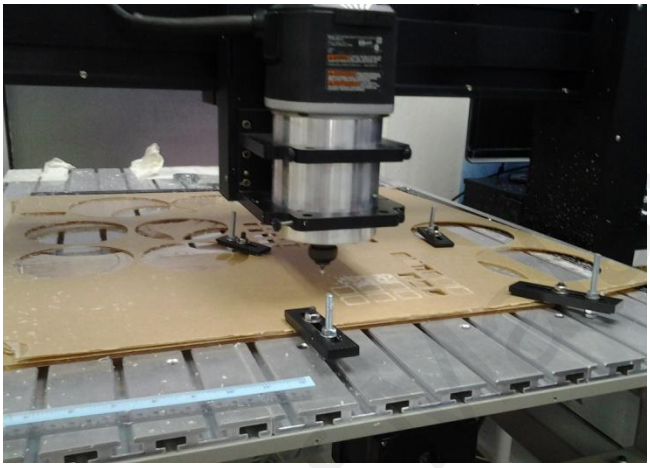

Figure 3.4: Cutting 2 mm thick of PMMA sheet using CNC machine.

Before the surface modification procedure, all samples were cleaned as outlined by Wei *et al*., (2005). Firstly, remove brown sticker that covered the plastic and gently wipe the plastic with 70% alcohol to get rid of the glue on plastic surface. Next, the PMMA pieces were sonicated in 2-propanol for 15 min and subsequently dried under gentle nitrogen flow. Place the samples on cleaned petri dish and cover with aluminum foil. The samples then ready for surface modifications.

3.3 Methods in Surface Modification

In this study, two different methods were carried out to modify the surface properties PMMA substrate. Following two sections are the details on UV (ultraviolet) irradiation method and sodium hydroxide (NaOH) treatment method, respectively.

3.3.1 Ultraviolet (UV) Irradiation

UV irradiation was done in the safety hood to eliminate any kind of contamination and was purged with alcohol prior to irradiation. In order to have concentrated irradiation to the samples, the UV lamp was covered with aluminium foil as depicted on Figure 3.5. This lamp emits ultraviolet rays of 253.7 nm wavelength with operating power 30 Watt (Saynko Denki, G30T8).

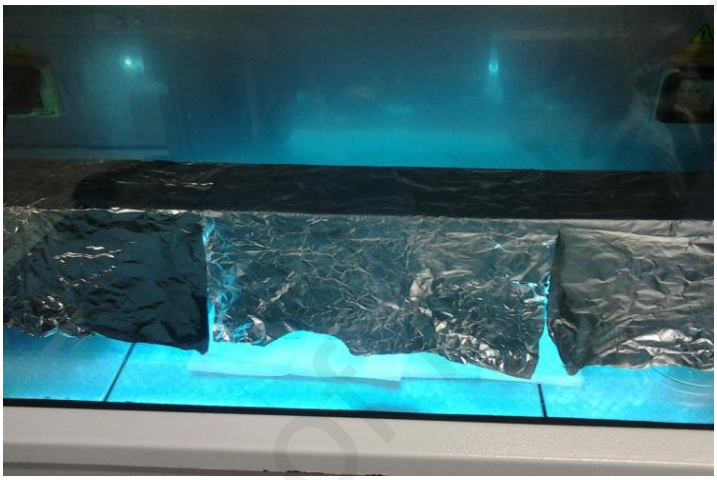

Figure 3.5: Experimental set-up for UV irradiation.

Spin-coated PMMA samples were placed under UV lamp at 12 cm distance and exposed to UV light at room temperature for different periods of time. For PMMA sheets, the cleaned PMMA samples were placed under UV light with the same condition and were exposed for 30 min. 30min was chosen as the optimum time according to study done by Wei *et al*., (2005). After UV irradiated, carefully move out the samples into cleaned petri dish using a tweezer. Make sure the UV-exposed surface is on top and always cover the sample with aluminium foil.

3.3.2 Sodium Hydroxide (NaOH) Treatment

Prior to modification, the sodium hydroxide (NaOH) standard solution must be prepared as shown in Figure 3.6. To make 1 liter of 1M NaOH aqueous solution, dissolve approximately 40 g of NaOH in some distilled water. Note that considerable heat will be generated when solid NaOH is dissolved in water. Carefully transferred the concentrated NaOH solution into volumetric flask and fill the flask up to the neck. Next, add drop by drop of distilled water until the meniscus is level with the line.

(a) (b) (c

Figure 3.6: Preparation of 1M NaOH standard solution. (a) Weighing 40g of solid NaOH, (b) dissolve solid NaOH in amount of distilled water and (c) 1 liter of 1M NaOH in a volumetric flask.

Following that, all 5 PMMA sheets were first cleaned as described early and properly wrapped with aluminium foil. Figure 3.7 shows the experiment setup for NaOH treatment.

Figure 3.7: Experimental setup for NaOH treatment.

Each of the samples was carefully hold by tweezers and immersed in the 1000 ml of NaOH. The beaker was placed on the magnetic stirrer to continuously stir the solution and gave a uniform treatment to each of the samples. The treatment goes for 30 min at 55°C and constantly monitored by a thermometer as shown in the Figure 3.8.

Figure 3.8: Five PMMA sheets immersed in stirring NaOH with temperature controlled at 55°C for 30 min.

Next, after removal from NaOH solution, rinsed the treated samples with deionized water (18 MΩ cm) and carefully transferred into uncontaminated petri dish. The samples were then dried under gentle nitrogen, N_2 stream and covered with aluminum foil.

3.4 Methods in Surface Analysis

3.4.1 Contact Angle Measurement

The simplified experimental setup for water contact angle measurement is shown in Figure 3.9 and it was carried out at room atmosphere. Based on sessile drop method, the static contact angle between a small volume of deionized water (18 MΩ cm) and PMMA substrate for both control and treated was examined. The image of water drop was captured by Nikon D90 DSLR.

Figure 3.9: Simplified experiment setup for water contact angle measurement.

The flat PMMA substrate was placed on a horizontal support and a micropipette cling to retort stand was located at the centre of substrate. The tip of micropipette was positioned as such a height that bottom of the drop will touch the surface and detach before it drop free of its own weight. The micro focus camera was adjusted parallel to the substrate while table lamp was put on top of the droplet and arranged so as to get the best illumination. In order to get rid of high droplet impact and minimum motion when the

water drop touch-off the surface, pump out the water slowly at rate 1µl/s or less. In the beginning, the droplet volume was chosen to be 20 µl, however it modified to 2 µl to eliminate the gravity effect.

Once the droplet has been released, image was then captured 30 s later to ensure it completely spread out and were repeated at least three times for each sample. The contact angle of the drop was then measured using the Java-based image processing program, *ImageJ* (National Institute of Health). Details on the steps using the software can be found in the Appendix.

Noticed that uploaded picture is upside down because the program of the software was written so. The first two points are for manual detection of baseline and the other three to identify the drop boundary. For this reason, a very well defined picture with clear baseline is required so as the best-fit analysis can automatically detect the drop profile. This plugin calculates the contact angle of the water drop using sphere approximation [1].

However, this simplified experimental setup has been confirmed by more accurate instrument as shown in Figure 3.10, Dataphysics OCA 15EC with SCA software provided by the manufacturer.

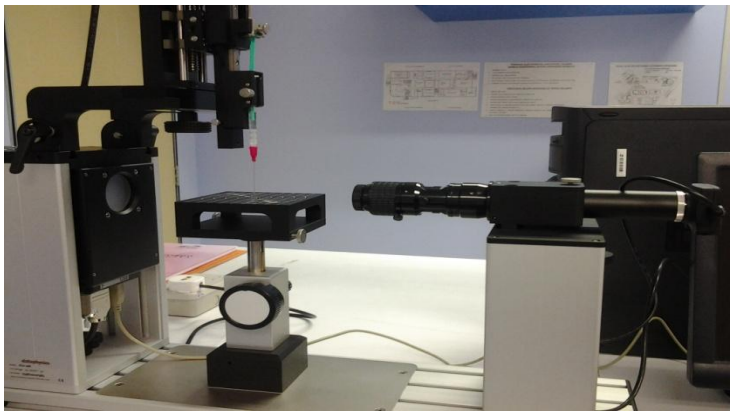

Figure 3.10: Dataphysics, OCA instrument for contact angle.

3.4.2 Toluidine Blue Assay

In this study, toluidine blue (TB) dye was used to confirm the presence of carboxylate group on the PMMA surfaces. Some reagents were required to be prepared prior to the assay process: i) 0.1 mM NaOH solution, ii) 0.5 mM alkaline solution of TB and iii) 50% acetic acid as shown in the Figure 3.11:

Figure 3.11: Reagents required for conducting TB assay.

The same samples used for contact angle measurement was then put in three different container and labeled for control, UV treatment and NaOH treatment. Soaked the samples in 20 ml of 0.5 mM alkaline solution of TB (in 0.1 mM NaOH) and agitated on a shaker (Multi Shaker, Labchem) for 5 h at room temperature as depicted in Figure 3.12.

Figure 3.12: Samples were immersed in TB on a continuously agitating shaker.

Following the TB adsorption, the samples were taken out and rinsed thoroughly with 0.1 mM NaOH solution to remove the non-complex dye. Next, the samples were immersed in 20 ml of 50% acetic acid for 20 min in order to desorbs complexed TB from PMMA surfaces.

The color changes of the resulting solution in Figure 3.13 were then observed. Note that, this adsorbs/desorbs method depends on the electrostatic interaction between the functional group on the PMMA surface and the TB dye, thus pH control is significant to minimize error.

Figure 3.13: Samples are incubating in 50% acetic acid.

CHAPTER IV: Results and Discussion

4.1 Introduction

This chapter shows the results of two different samples prepared from PMMA thin films and PMMA sheets. Besides, the resultant of each surface modification for each sample is also discussed in detail.

4.2 PMMA thin films

4.2.1 Surface morphology of films spin coated on glass substrates

For the purpose of surface analysis, a very featureless surface morphology of thin film with uniform thickness is required. Therefore, the spin coating method was carried out as described in Section 3.2.1 for obtaining 60 pieces of thin PMMA films used as solid substrate for surface modification study.

Figure 4.1 shows the PMMA thin films produced through spin coating PMMA solution on the glass wafer with the same setting for all samples. However, only half of them can be used due to the non-featureless surface obtain. As depicted in the figure, the diagram on the right side shows quite fine surfaces while that on the left site shows PMMA surface with surface defects. It is clearly shown that the defects comes from the detailed of two o-ring obtained from chuck of the spin coater. The region of the substrate in contact with the o-rings had less thickness maybe due to the nonuniform temperature distribution and evaporation rate that has been describe by Birnie et al (1992).

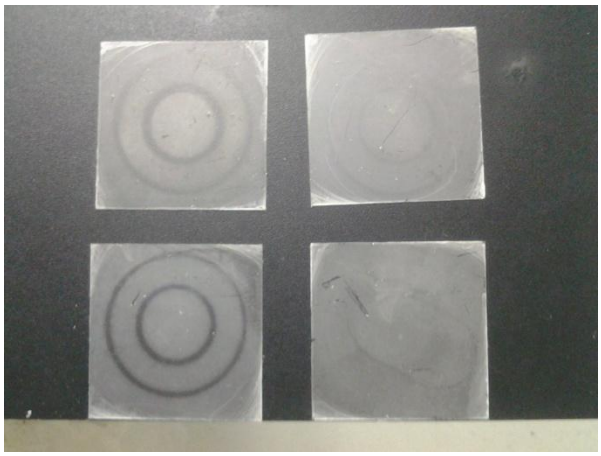

Figure 4.1: Spin-coated PMMA thin films on the glass wafer.

Study done by Kaczmarek and Chaberska (2006) proved that support type has no influence on surface properties of pristine PMMA. All samples were in good condition with no defect and strong attachment for both aluminum and glass support as has been examined by Fourier transforms infra-red spectroscopy (ATR-FT-IR). Hence, it is understood that the unsmooth surface is not because of glass substrate as support material for PMMA coating.

Organic solvent used in this study to prepare the 0.5% (w/w) PMMA solutions was chloroform ($CHCl_3$) as has been used and studied in many literatures (Abel *et al*, 1996; Bistact and Schultz, 1997; Briscoe *et al*, 2002). Chloroform, the acid-base solvent did exhibited a good interaction between acrylate group of PMMA and proved to produce smooth surface of PMMA films compare to other types of solvents. However, it was recommended in the literature that films morphology highly influence by the volatility of the solvents and solution concentration (Gennes, 2002; Semaltianos, 2007).

Other than that, the spin coating process parameters such as spinning rate and temperature also need to be considered which affect surface morphology, uniformity and thickness of the films (Mellbring *et al*, 2001). In broad, many factors manipulate the surface properties of films obtained: attachment of polymer to support, solvent volatility,

polymer concentration, and spinning parameters. Therefore, many optimizations are required to obtained high-quality PMMA thin films with featureless, smooth, and even thickness spin coated onto glass substrate. For that reason, as alternative, another type of sample was then used to investigate the effect of surface modification which has been described earlier in Section 3.2.2. Nevertheless, surface modification by UV irradiation has been carried out to the considerably fine samples from the spin coating process and the results is shown and discussed in the next Section 4.2.2.

4.2.2 Contact angle measurement of UV-irradiated PMMA thin films

The average contact angle for unmodified PMMA using sessile drop method of deionized water was found to be 77° ± 8° (3 replicates) which is in a range of literature value of 70° (Henry *et al*, 2000; Wei *et al*, 2005). The slightly different value compared to literature and the high standard deviation may be due to unsmooth surface of PMMA thin films which was discussed before.

After surface modification in air as described in Section 3.3.1, the water contact angle is increased to about 80° ± 5° at 30 min exposure time, see Figure 4.2. Increasing water contact angle means the surface become more hydrophobic than nature PMMA, consequently not favorable to protein adsorption required for ELISA immunoassay. This result clearly is not supporting the hypothesis stated earlier and opposite to most of the literature (Henry *et al*, 2000; Wei *et al*, 2005).

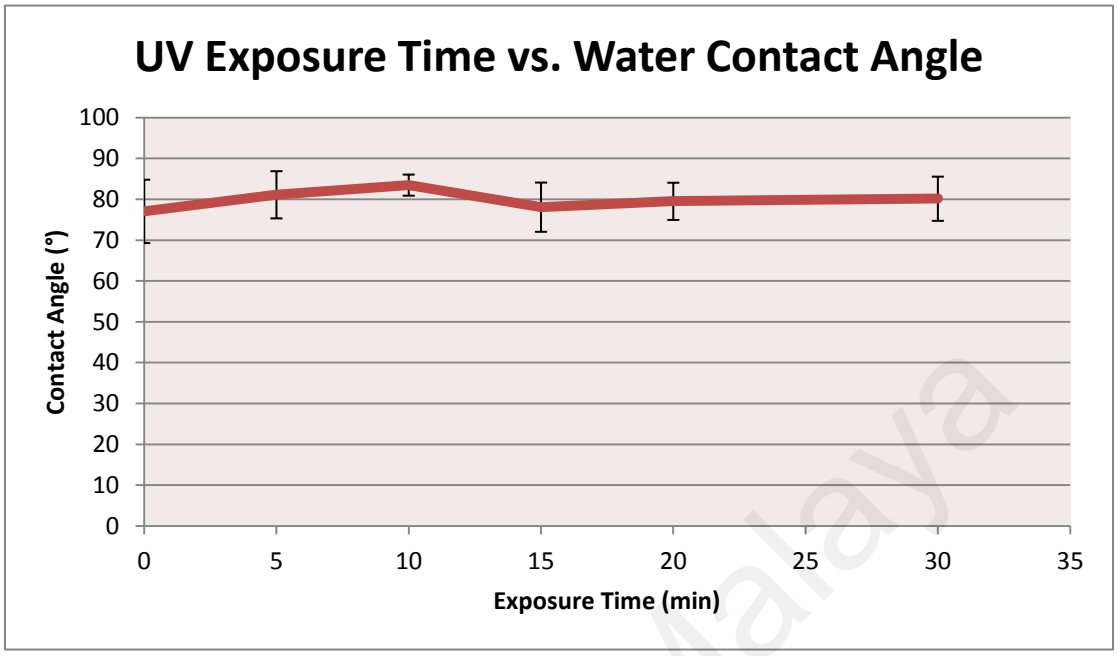

Figure 4.2: Effects of UV modification on the water contact angle on PMMA surfaces for different UV exposure time.

The water contact angle increased possibly as a result of increased surface roughness caused by the left over photoresist solvent in PMMA. The residue of chloroform probably due to non-optimal solution concentration or solvent instability, was forming free radical during the photochemical modification process which led to polymer degradation. Therefore, analysis of PMMA by using AFM (atomic force microscopy) are suggested to examine the influenced of solvent residue towards the surface roughness of the PMMA films pre- and post-UV modification as strongly recommended by Kaczmarek and Chaberska (2008).

4.3 PMMA Sheets

Figure 4.3(a) shows the PMMA sheets that were machined to 2 x 2 cm while Figure 4.3 (b) is the same samples after cleaning protocol which has been explained in Section 3.2.2. Wei *et al* (2005) reported that the cleaning protocol is independent on surface roughness as confirmed by SFM (scanning force microscopy) method.

(a) (b)

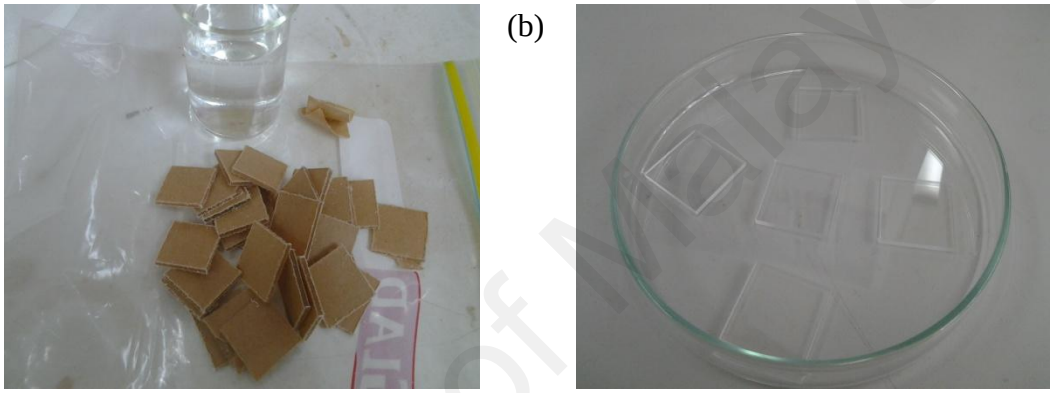

Figure 4.3: (a) PMMA sheets cut to 2 x 2 cm by CNC machine, (b) cleaned PMMA sheets.

4.3.1 Contact angle measurement of pristine and modified PMMA sheets

Figure 4.4 shows the effects of two surface modifications on the surface hydrophilicity of PMMA sheets. The average contact angle for control PMMA surfaces using deionized water was found out to be 56.82° ± 6.86° (5 replicates). This value is shown to be increasing upon the surface modifications but with lower standard deviations.

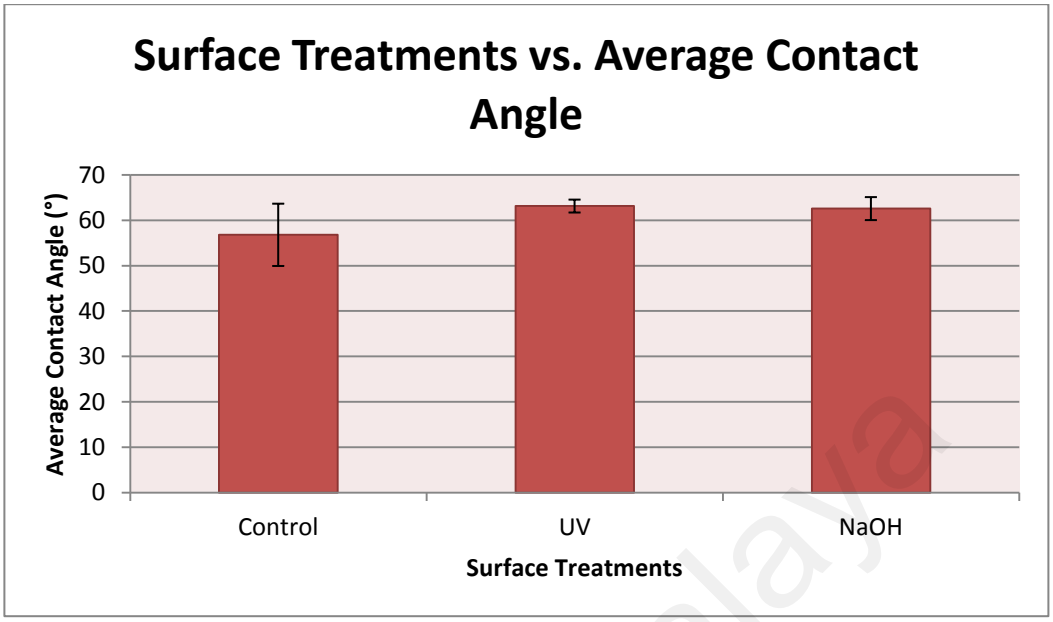

Figure 4.4: Effects of UV modification and NaOH treatment on the average water contact angle on PMMA surfaces.

The value of average water contact angle after UV irradiation is shown to increase to 63.13° ± 1.42°. This pattern is in good agreement with the previous result with UV-treated on the PMMA thin films. This is quite surprising because it has been assumed that there are no surface defect on the pristine PMMA sheets surface. Therefore, another assumption has been made to find the reasons of unexpected result: one more possible reason may be due to the experimental setup itself. The UV treatment process was done according to the literature reviews, mainly the study done by Wei *et al*, (2005). However, the literatures usually do not give a very detailed description and justification of the procedures. Hence, some modifications have been made to the experiment setup, as shown in Figure 3.4 based on the available equipments.

For that reason, an attempt has been made to come out with another method of surface modification. Wet chemical modification, the classical approach, has been chosen because no specialized equipment is needed and it can be performed easily in the

laboratory. Thus, modification by concentrated sodium hydroxide (NaOH) has been conducted as described in Section 3.3.2 according to Liu *et al* (2009). This chemical process is expected to generate carboxylic acid group by hydrolysis of ester side chains on PMMA. Similar to the UV-modification, the introduced carboxylic acid group is needed to make the surface hydrophilic thus improve protein immobilization.

Unfortunately, the same negative pattern was found for the NaOH treatment. The average water contact angle increased to 62.60° ± 2.53°. Hence, increasing hydrophobic is suggested to be due to the surface roughness either before or after treatment. Cheng *et al* (2004), in their SEM (scanning electron microscopy) pictures, shows a very rough surface obtained after sample preparation by using laser machining and an improvement has been made prior to surface modification by means of thermal annealing. In addition, in Goddard and Hotchkiss (2007) extensive reviews, they did mention about the irregular surface obtained from the wet chemical modifications and also subjected to the orientation of side chains on the PMMA surface. Therefore, again it is shown how important is spectral image analysis such as SEM and AFM to be performed before and after treatment to further confirm the results and the possible reasons of unexpected outcomes.

Figure 4.5 (red bar) shows the result of contact angle calculated by SCA software utilizing Dataphysics instrument. Upon NaOH treatment, the contact angle seems to increase from 62.60° ± 0.94° to 70.40° ± 1.20°. This result shows that the surface becomes more hydrophobic after treatment which is consistent with result gained from simplified the experimental setup. Hence, this indicates that the fault is not from the simplified contact angle measurement. However, it is noted that lower standard deviation can be obtained when using the appropriate instrument.

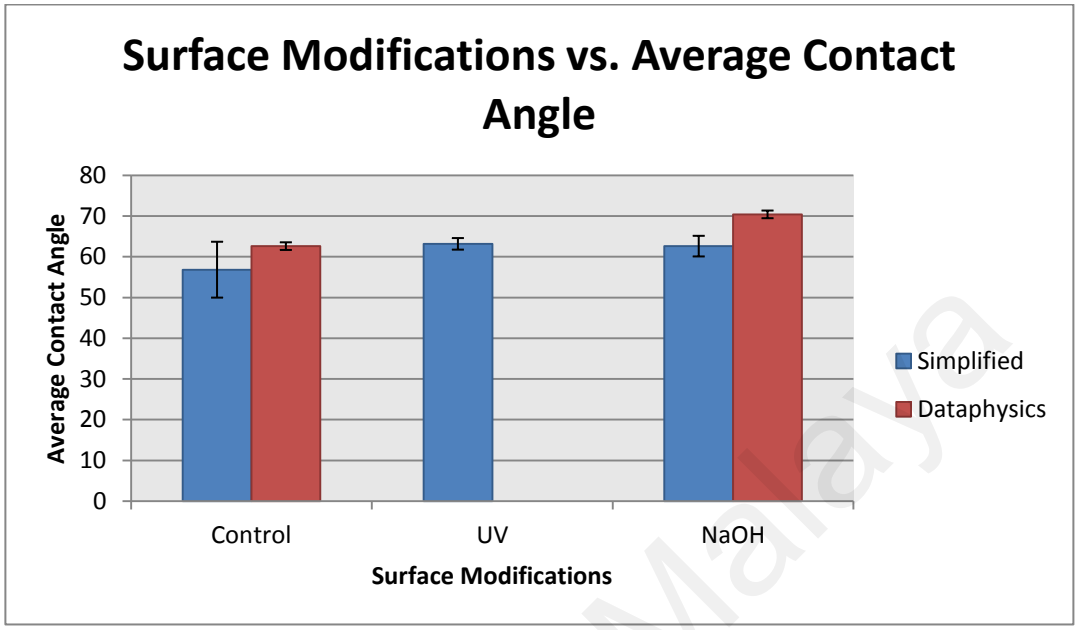

Figure 4.5: Comparison of contact angle measurement performed by simplified experiment setup (blue) and dataphysics instrument (red).

4.3.2 Toiludine Blue assay of pristine and modified PMMA sheets

Another convenient method for surface characterization is by means of chemical structure through the use of dye assays. In this study, Toiludine Blue (TB) dye was used to qualitatively determine the presence of carboxylic acid group by observing color changes of assay solution. Surface density of carboxylic group can be further quantitatively analyzed by measuring the absorbance of TB using UV-Vis spectrophotometer at 635 nm with the calibration curve. However, this study only focuses on qualitative analysis and the detail procedure described in Section 3.4.2 is based on study done by Djordjevic *et al* (2010).

Principally, the behavior of TB is that it adsorbs on a substrate surface in alkaline and desorbs in acidic solution. As shown in previous work (Li *et al*, 2005 (a, b); Djordjevic *et al*, 2010), during incubation of samples in TB for 5 h, the positive charge of TB can be

attached to the negative charge of carboxyl group on the polymeric surface. Subsequently, the positive charge of TB adsorbed on the surface will be exchanged with positive charge in acidic solution. As results of this sequence interaction, the resulting solution appears in blue color.

Figure 4.6 (a) shows the sample, from left, NaOH treated, control, and UV-treated after being soaked in TB for 5 h and washed with 0.1mM NaOH to remove unbound TB from the surface. As can be seen in the figure, there is no difference between the control and treated samples. Thus the same outcome is expected when immersed in 50% acetic solution as shown in Figure 4.6 (b). These results indicate that there is no carboxyl groups generated on the PMMA surface for all samples since no color change is shown. No electrostatic interaction occurred during incubation time. Consequently, this result is also not supporting the hypothesis, same as surface characterization by water contact angle.

(a) (b)

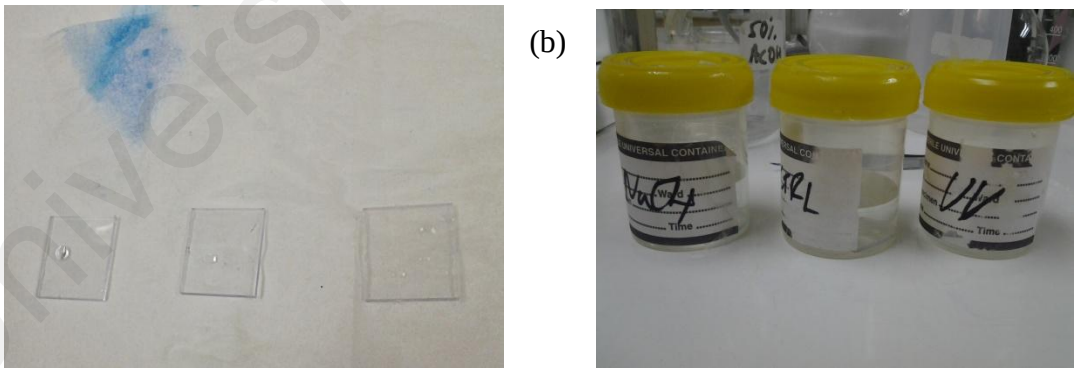

Figure 4.6: (a) Samples after taken out from TB solution, (b) samples immersed in 50% acetic acid.

CHAPTER V: Conclusions and Future Work Recommendations

5.1 Conclusions

This study highlights the effects of surface hydrophilicity upon surface modifications on PMMA substrates. The effects were characterized by means of water contact angle measurement and Toiludine blue assay. It was found that the PMMA surfaces became more hydrophobic upon UV irradiation for 30 minutes for both type of samples, spin-coated PMMA thin films and PMMA sheets. The water contact angle increase from 77° ± 8° to 80° ± 5° for PMMA thin films and from 56.82° ± 6.86° to 63.13° ± 1.42° for PMMA sheets. The increasing of water contact angle values may be due to increasing of surface roughness after the photochemical process. However, this results would be better if the standard variation value for control samples are low.

Similarly, same pattern was found when the PMMA sheets were modified with sodium hydroxide (NaOH). Following 30 minutes of treatment, the contact angle was increased to 62.60° ± 2.53° point toward higher hydrophobicity. On top of that, another method of analyzing was carried out by using TB assay in order to determine the presence of carboxyl acid group on the PMMA surfaces. The resulting solution showed no change of color for pristine PMMA, UV-modified, and NaOH-treated PMMA, indicating no carboxyl group was generated upon both modifications.

These results are not in good agreement with the hypothesis stated in Section 1.5. As discussed earlier, there were many factors that led to this discrepancy, such as unsmooth samples, improper UV-treatment chamber, error during contact angle measurement, and lack of surface characterization before and after modifications. Hence, based on the

literatures, several works are recommended for improving sample preparation, methods in sample modifications, and characterizations.

5.2 Limitations

The following are two major limitations to the design and methods of the study:

i. UV-irradiation process was done in the safety hood meant for sterilization; the inappropriate chamber might have affected the photochemical process.
ii. The contact angle measurements was done using simplified experimental setup which possibly slightly affected the contact angle value obtained.

5.3 Recommendations for future works

The analyses discussed in this report described the difficulties encountered throughout this study. In view of that, several recommendations for improvement and future work are outlined for each method as follows:

a) Sample preparations
    i. Lots of optimizations are required for preparing spin-coated PMMA thin films including spinning speed and duration, solution concentration, surrounding temperature and the organic solvent.
    ii. Cleaning protocol must be carried out with extra caution because it may affect the surface properties. Thus spectral method such as SEM is recommended to observe the surface morphology prior to modifications.

b) Surface modifications

i. UV treatment should be performed in a proper system since such photochemical process is very sensitive to environmental conditions. Besides, sufficient period and dose of exposure are recommended to be optimized for efficient improvement.

ii. The classic approach of treatment by wet chemical is easier to be conducted in a laboratory. Though, this method is non-specific and highly dependent on polymers distinctive properties. However, the NaOH treatment performed in this study can be further improved by varying the conditions such as the use of higher period of time, lower temperature, or high concentration of NaOH.

c) Surface Analyses

i. Contact angle measurement is a very sensitive technique, especially for early detection of surface properties. However, one of the major limitations in this study is the experimental setup for water contact angle measurement. Minimal error is encountered with more sophisticated instrument such as the Dataphysics OCA 15EC or more appropriate simplified experiment setup as been done by Lamour *et al*, 2010.

ii. To further improve the surface analysis method, the surface physical and chemical properties must be verified prior and after each step of modifications in order to validate the proposed mechanism. Therefore, spectral methods are strongly recommended such as AFM, SEM, ATR-FT-IR and XPS to obtain more reliable and accurate results.

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Appendix

Detail steps using contact angle plugin for *ImageJ* software:

1. Since this image analysis works offline, upload a picture and drop profile is then detected, Figure A1.

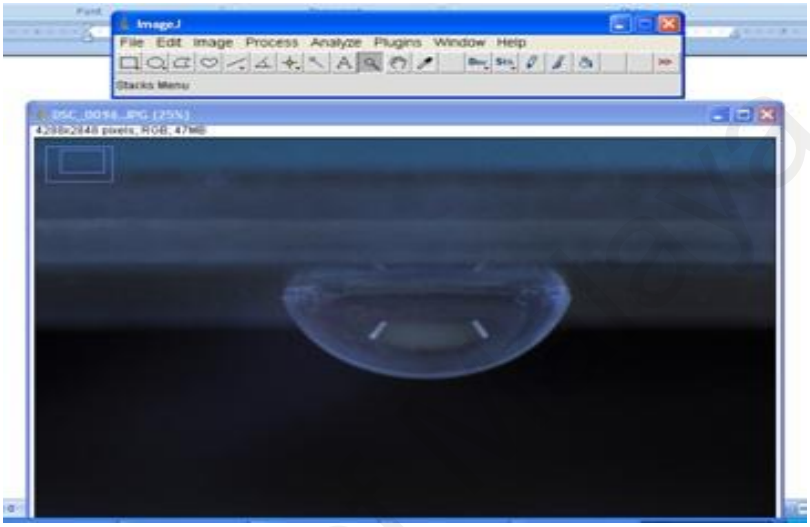

Figure A1: Drop profile.

2. In the plugin tab, choose contact angle. Select the first two points on the baseline and other three points along the drop profile, Figure A2.

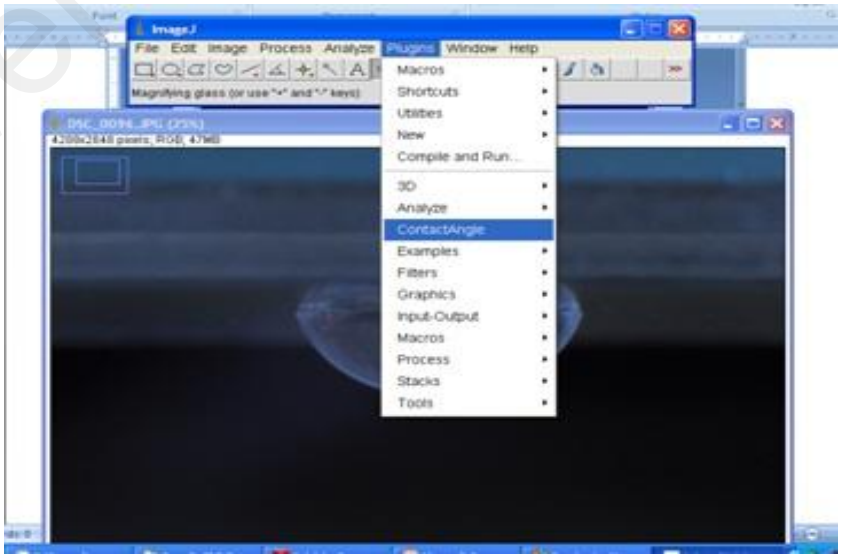

Figure A2: Selecting baseline of the drop.

3. To calculate the contact angle, choose measurement by using circle best-fit, Figure A3.

Figure A3: Measuring the drop profile using circle best-fit.

4. The gray scale image is then analyzed and the result is shown as in Figure A4.

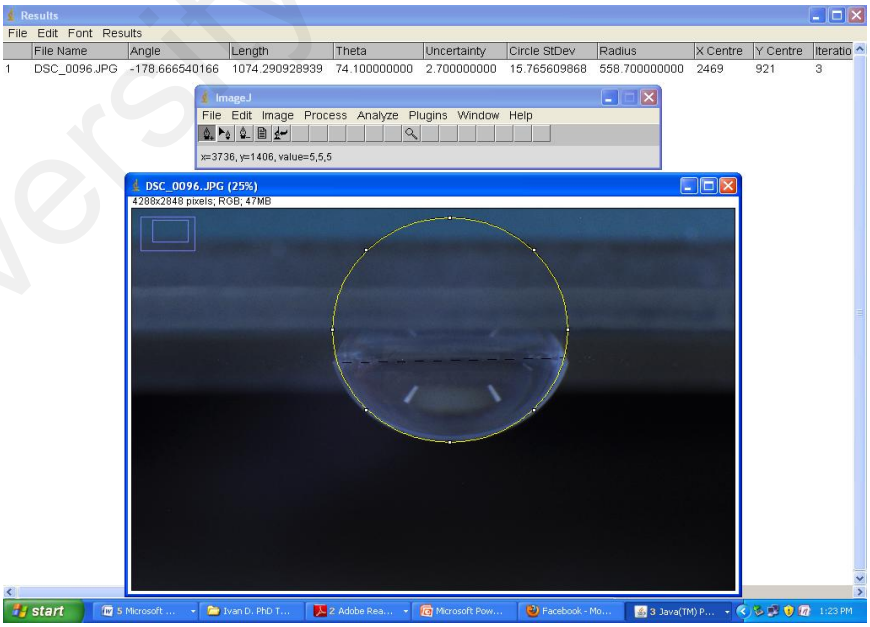

Figure A4: Contact angle result of the drop.