CHAPTER II

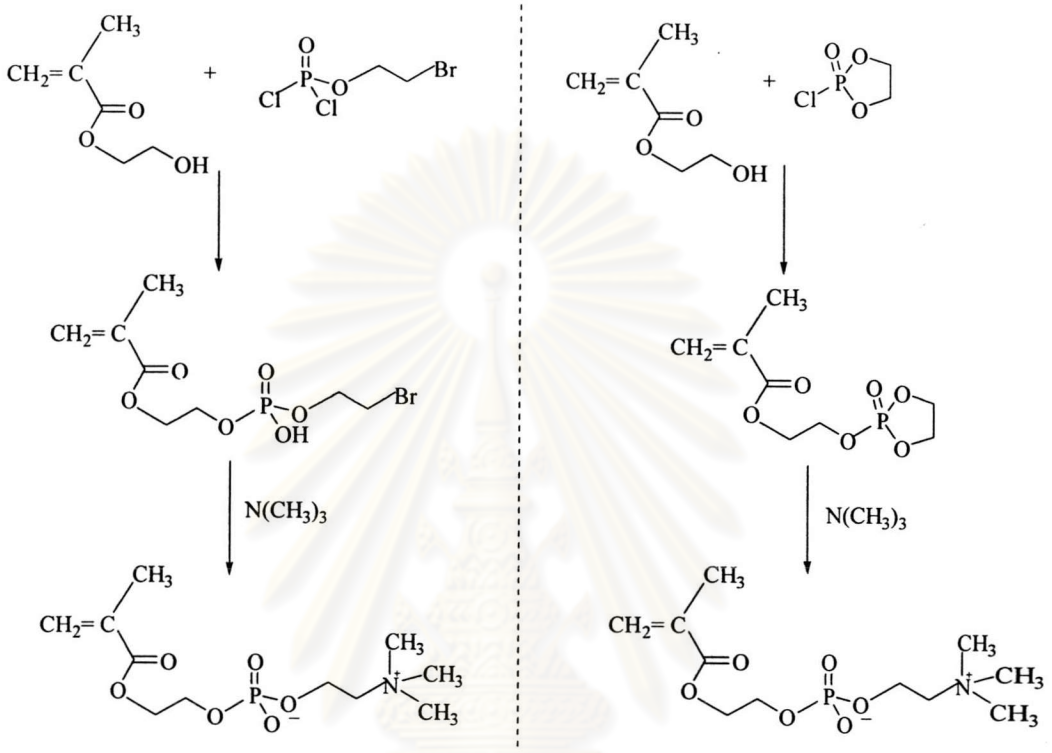
THEORY AND LITERATURE REVIEW

2.1 Development of Phosphorylcholine-containing Monomer

A biomembrane is a hybrid that consists of two chemical units, phospholipids and proteins. There is no covalent bonding between these, thus, the surface of a biomembrane is heterogeneous and dynamic. New biomaterials were proposed whose activity was based upon the mimicking of a simple component present on the extracellular surfaces of the phospholipid bilayer that forms the matrix of the cell membranes of blood cells, namely, the phosphorylcholine group of phosphatidylcholine and sphingomyelin. Phosphorylcholine, an electrically neutral, zwitterionic headgroup, is one of the phospholipid headgroups present on the external surface of blood cells, and it is inert in coagulation assays. The blood compatibility of a polymer surface coated with phospholipids was confirmed by several researchers [1-2]. For example, the interactions between polyamide microcapsules coated with the phospholipid-bilayer membrane and platelets were investigated [2]. It was found that platelet adhesion onto the microcapsules was significantly suppressed by the phospholipid coating. The mobility of the phospholipid layer coated on the surface affected the platelet adhesion. When the phospholipids were in a liquid crystalline state, platelet adhesion was smaller than that observed when the phospholipids on the surface were in a gel state [3].

New concepts for making blood-compatible polymer materials that utilize the characteristics of natural phospholipid molecules in plasma have been proposed [4]. It was thought that if a polymer surface possesses a phospholipid-like structure, a significant amount of natural phospholipids in plasma could be adsorbed onto the surface through self-assembly [4]. Based on this idea, a methacrylate monomer with a phospholipid polar group, 2-methacryloyloxyethyl phosphorylcholine (MPC) was designed and synthesized [5-6] (Scheme 2.1). The excellent blood compatibility of

the MPC-containing materials is proven to originate from the capability to induce self-assembly of natural phospholipids.



Scheme 2.1 The synthetic route of MPC: (a) Nakabayashi et al. [5]; (b) Ishihara et al. [6].

Since hydrophilic MPC homopolymer possesses inferior mechanical properties, MPC is generally used in the form of copolymers, polymer coating and grafting.

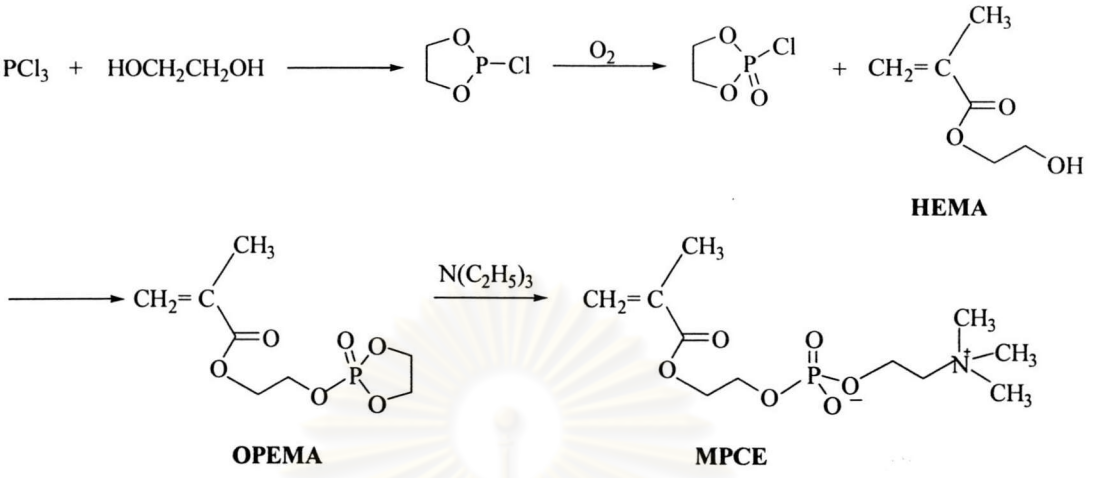
In 2001, Hasegawa's group prepared polymer blends composed of polysulfone and 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer (PSf/MPC polymer) to obtain protein-adsorption-resistant membrane for hemodialysis. The content of the MPC polymer in the blends was 7 and 15 wt%. The asymmetric porous membrane was obtained by dry/wet membrane processing

method. The mechanical strength of the PSf/MPC polymer membrane did not change compared with that of the PSf membrane. On the other hand, the permeability of solute having molecular weight below 2.0 x 10^4 through the PSf membrane increased with the addition of the MPC polymer, which is considered to be an effect of the hydrophilic character of the MPC polymer. The amount of protein adsorbed on the PSf membrane from plasma was reduced by the addition of the MPC polymer. Platelet adhesion was also effectively suppressed on the PSf/MPC polymer membrane. Based on these results, the MPC polymer could serve as a doubly functional polymeric additive, that is, to generate a protein-adsorption-resistant characteristic and to render the membrane hydrophilic [7]. In the same year, Konno and coworkers prepared the poly(L-lactic acid) nanoparticles immobilized with 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer, which has excellent blood compatibility by a solvent evaporation technique using the water-soluble amphiphilic MPC polymer as an emulsifier and a surface modifier. The diameter and ζ-potential of the obtained nanoparticles strongly depended on the concentration of the MPC polymer. Furthermore, various hydrophobic fluorescence probes could permeate through the MPC polymer layer and adsorb on the PLA surface. The amount of bovine serum albumin adsorbed on the nanoparticles was significantly smaller compared with that on the conventional polystyrene nanoparticles [8].

In 2002, new segmented polyurethanes (SPUs) grafted with phospholipid analogous vinyl monomer, 2-methacryloyloxyethyl phosphorylcholine (MPC) on surface were synthesized by Korematsu and coworkers. They found that fewer platelets adhered to the MPC-grafted surfaces [9]. In the same year, Uchiyama and coworkers prepared a novel polymer alloy membrane with both biocompatibility and permeability for fabrication of an implantable artificial pancreas. The polymer alloy was composed of a segmented polyurethane (SPU) and phospholipid polymer with 2-methacryloyloxyethyl phosphorylcholine (MPC) units. The MPC polymer was poly(MPC-*co*-2-ethylhexyl methacrylate) (PMEH), which can be dissolved in the same solvent for SPU. The SPU/PMEH alloy membrane was prepared by a solvent evaporation method. They found that the SPU/PMEH alloy membrane had

excellent mechanical properties. The glucose and insulin could permeate through the SPU/PMEH alloy membrane. The reduction of the number of fibroblasts that adhered to the surface of the SPU/PMEH alloy membrane was observed. That is, it has a good biocompatible surface compared with the original SPU. It was suggested that excellent functions, such as good insulin permeability, and reduction of fibroblast adhesion were promoted by the PMEH which dispersed both on the surface and inside the membrane [10].

In 2003, Yamasaki and coworkers studied the effects of the surface properties of phospholipid polymers having various bridging units between the phosphorylcholine and the backbone on their blood compatibility. The copolymers between 2-methacryloyloxyethyl phosphorylcholine (MPC), 2-methacryloyloxy ethoxyethyl phosphorylcholine (MEO2PC), and 6-methacryloyloxyethoxyhexyl phosphorylcholine (MHPC) with n-butyl methacrylate (BMA) were prepared. It was found that the surface density of adsorbed proteins and adherent platelets on the poly(MPC-*co*-BMA) was similar to that on poly(BMA). On the other hand, poly(MEO2PC-*co*-BMA) and poly(MHPC-*co*-BMA) effectively reduced biofouling compared with poly(MPC-*co*-BMA) They clarified that the copolymer surfaces having long bridging units, which could easily form a phosphorylcholine-enriched surface when the blood was in contact with the surface, showed excellent blood compatibility without prehydration [11]. Furthermore, in the same year, Zhu and coworkers synthesized 2-methacryloyloxyethyl phosphorylcholine (MPCE) using phosphorous trichloride, ethylene glycol, 2-hydroxyethyl methacrylate (HEMA) and triethylamine (Scheme 2.2), and then used in the preparation of *O*-butyrylchitosan-bonded MPCE (MPCE-BCS) by Michael addition of MPCE to amino groups of *O*-butyrylchitosan. The blood-compatibility of MPCE-BCS was evaluated by means of blood clotting and platelet adhesion assays. The blood-clotting assay indicated that *O*-butyrylchitosan was haemo-compatible. Both the blood-clotting assay and platelet adhesion assay confirmed that MPCE-BCS had excellent antithrombogenicity [12].



Scheme 2.2 The synthetic route of MPCE [12].

2.2 Living Polymerization

Synthetic polymers are long-chain molecules possessing uniform repeat units (mers). The chains are not all the same length. These giant molecules are of interest because of their physical properties, in contrast to low molecular weight molecules, which are of interest due to their chemical properties. Possibly the most useful physical property of polymers is their low density versus strength.

When synthetic polymers were first introduced, they were made by free radical initiation of single vinyl monomers or by chemical condensation of small difunctional molecules. The range of their properties was understandably merger. Random copolymers next refered the picture, greatly expanding the range of useful physical properties such as toughness, hardness, elasticity, compressibility, and strength. However, polymer chemists realized that their materials could not compare with the properties of natural polymers, such as wool, silk, cotton, rubber, tendons, and spider webbing. The natural polymers are generally condensation polymers made by addition of monomer units one at a time to the ends of growing polymer chains. Polymerization of all chains stops at identical molecular weights. For some time polymer chemists have realized that to approach nature's degree of sophistication, new synthetic techniques would be needed.

Conventional chain-growth polymerizations, for example, free radical synthesis, consist of four elementary steps: initiation, propagation, chain transfer, and termination. As early as 1936, Ziegler proposed that anionic polymerization of styrene and butadiene consecutive addition of monomer to an alkyl lithium initiator occured without chain transfer or termination. During transferless polymerization, the number of polymer molecules remains contant. Since there is no termination, active anionic chain ends remain after of the monomer has been polymerized. When fresh monomer is added, polymerization resumes. The name "living polymerization" was coined for the method by Szwarc [13]. Because the chain ends remain active until killed. (The term has nothing to do with living in the biological sense.) Before Szwarc's classic work, Flory [14] had described the properties associated with living polymerization of ethylene oxide initiated with alkoxides. Flory noted that since all of the chain ends grow at the same rate, the molecular weight is determined by the amount of initiator used versus monomer (Eq.1)

$$\text{Degree of polymerization} = [\text{monomer}]/[\text{initiator}] \qquad (1)$$

Another property of polymers produced by living polymerization is the very narrow molecular weight distribution. The polydispersity (D) has a Poisson distribution, $D = \overline{M}_w / \overline{M}_n = 1 + (1/dp)$; $\overline{M}_w$ is the average molecular weight determined by light scattering, $\overline{M}_n$ is the average molecular weight determined by osmometry, and dp is the degree of polymerization (the number of monomer units per chain). The values of $\overline{M}_w$ and $\overline{M}_n$ can also be determined by gel permeation chromatography (GPC). A living polymerization can be distinguished from free radical polymerization or from a condensation polymerization by plotting the molecular weight of the polymer versus conversion. In a living polymerization, the molecular weight is directly proportional to conversion (Figure 2.1, line A). In a free radical or other nonliving polymerization, high molecular weight polymer is formed in the initial stages (line B), and in a condensation polymerization, high molecular weight polymer is formed only as the conversion approaches 100% (line C).

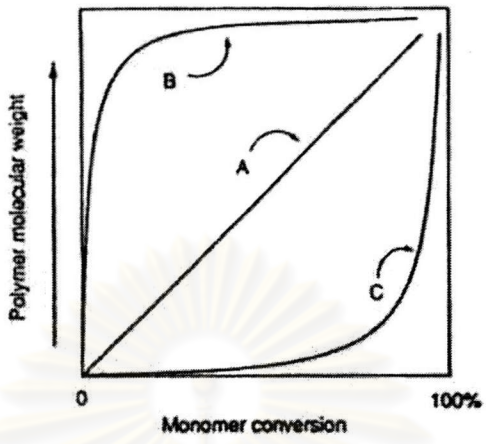
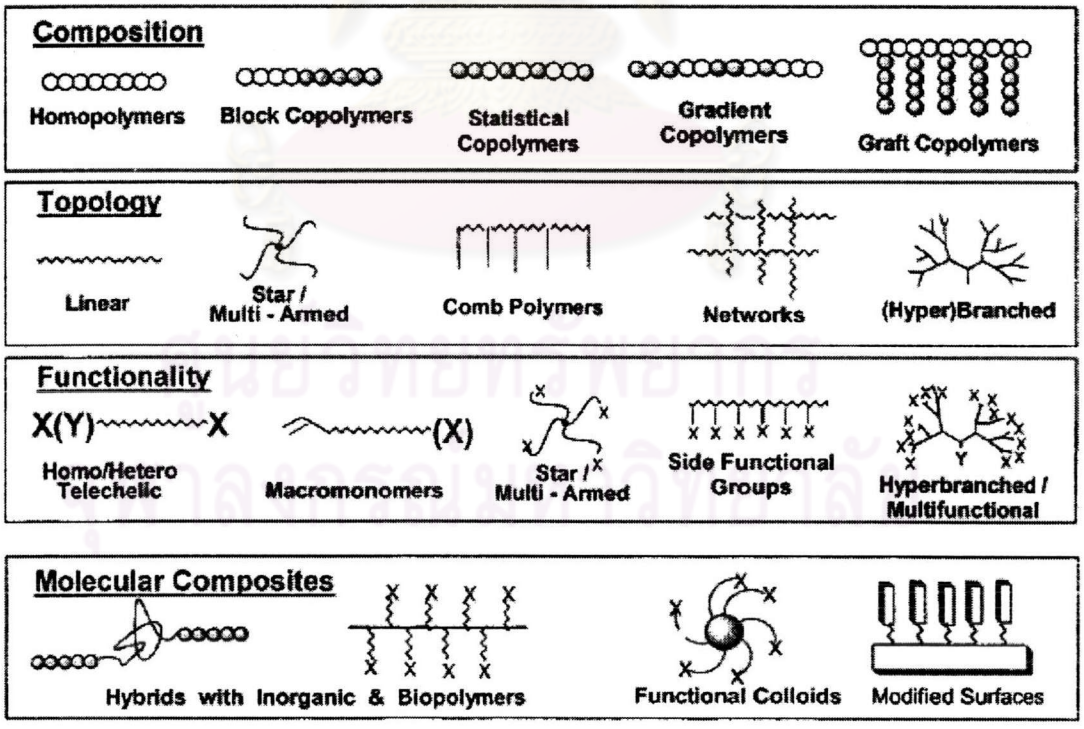


Figure 2.1 Molecular weight conversion curves for various kinds of polymerization methods: (A) living polymerization; (B) free radical polymerization; and (C) condensation polymerization [14].

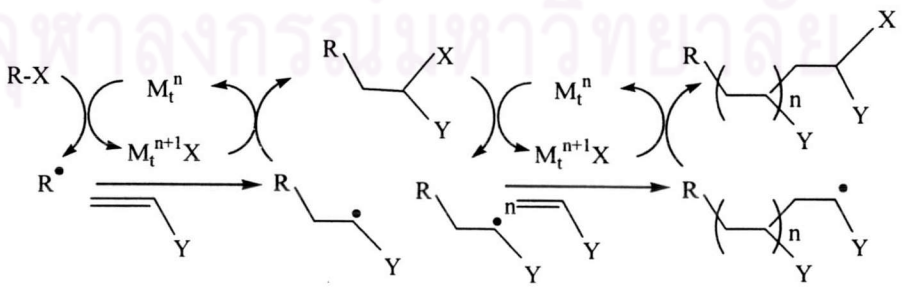
Living polymerization techniques give the synthetic chemist two particularly powerful tools for polymer chain design: the synthesis of block copolymers by sequential addition of monomers and the synthesis of functional-ended polymers by selective termination of living ends with appropriate reagents. The main architectural features available starting with these two basic themes are listed in Figure 2.2 along with applications for the various polymer types. Although living polymerization of only a few monomers is nearly perfect, a large number of other systems fit theory close enough to be useful for synthesis of the wide variety of different polymer chain structures In general, the well-behaved living systems need only an initiator and monomer, as occurs in the anionic polymerization of styrene, dienes, and ethylene oxide. For an increasing number of monomers, more complex processes are needed to retard chain transfer and termination. These systems use initiators, catalysts, and sometimes chain-end stabilizers. The initiator begins chain growth and in all systems are attached (or part of it, at least) to the nongrowing chain end. The catalyst is necessary for initiation and propagation but is not consumed. The chain-end stabilizer usually decreases the polymerization rate. When the catalyst is a Lewis

acid (electron-pair acceptor), the stabilizer will likely be a Lewis base (electron-pair donor), and vice versa. In all systems, the initiation step must be faster than or the same rate as chain propagation to obtain molecular weight control. If the initiation rate is slower than the propagation rate, the first chains formed will be longer than formed the last chains. If an initiator with a structure similar to that of the growing chain is chosen, the initiation rate is assured of being comparable to the propagation rate. A number of living systems operate better if excess monomer is present. A possible explanation is that the living end is stabilized by complexation with monomer [15]. Large counterions tend to be more effective than small counterions in living polymerization systems even when the ionic center is only indirectly involved.

Figure 2.2 Architectural forms of polymers avaiable by living polymerization techniques [15].



In this research, free radical process for living polymerization is selected and described. The concept of using stable free radicals, such as nitroxides, to reversibly react with the growing polymer radical chain end can be traced back to the pioneering work of Mozd and coworkers [16]. After further refinement by Georges [17], the basic blueprint for all subsequent work in the area of "living" free radical polymerization was developed. Subsequently, the groups of Sawamoto [18], Matyjaszewski [19], and Percec [20] and others [21-22] have replaced the stable nitroxide free radical with transition metal species to obtain a variety of copper-, nickel-, or ruthenium-mediated "living" free radical systems. These systems were called atom transfer radical polymerization (ATRP). This mechanism is an efficient method for carbon-carbon bond formation in organic synthesis. In some of these reactions, a transition-metal catalyst acts as a carrier of the halogen atom in a reversible redox process (Scheme 2.3). Initially, the transition-metal species, M_t^n, abstracts halogen atom X from the organic halide, RX, to form the oxidized species, $M_t^{n+1}X$, and the carbon-centered radical $R^{\bullet}$. In the subsequent step, the radical $R^{\bullet}$ participates in an inter- or intramolecular radical addition to alkene, Y, with the formation of the intermediate radical species, $RY^{\bullet}$. The reaction between $M_t^{n+1}X$ and $RY^{\bullet}$ results in a target product, RYX, and regenerates the reduced transition-metal species, M_t^n, which further promotes a new redox process. The fast reaction between $RY^{\bullet}$ and $M_t^{n+1}X$ apparently suppresses biomolecular termination between alkyl radicals and efficiently introduces a halogen functional group X into the final product in good to excellent yields.



Scheme 2.3 The mechanism of ATRP [19].

The ATRP system relies on one equilibrium reaction in addition to the classical free-radical polymerization scheme (Scheme 2.4). In this equilibrium, a dormant species, RX, reacts with the activator, M_t^n, to form a radical $R^\bullet$ and deactivating species, $M_t^{n+1}X$. The activation and deactivation rate parameters are k_{act} and k_{deact}, respectively. Since deactivation of growing radicals is reversible, control over the molecular weight distribution and, in the case of copolymers, over chemical composition can be obtained if the equilibrium meets several requirements [23-24].

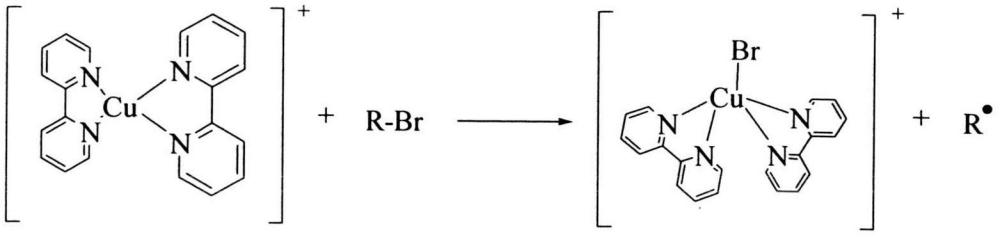
1. The equilibrium constant, k_{act}/k_{deact}, must be low in order to maintain a low stationary concentration of radicals. A high value would result in a high stationary radical concentration, and as a result, termination would prevail over reversible deactivation.

2. The dynamics of the equilibrium must be fast; i.e. deactivation must be fast compared to propagation in order to ensure fast interchange of radicals so as to maintain a narrow molecular weight distribution.

$$R\text{-}X + M_t^n \underset{k_{deact}}{\overset{k_{act}}{\rightleftharpoons}} R^\bullet + M_t^{n+1}X$$

Scheme 2.4 Equilibrium reaction in ATRP [25].

Futhermore, in 1995, Matyjaszewski has described the use of Cu^IX (X = Br, Cl) with 2,2'-bipyridine (bpy) as a "solubilizing" ligand. The active species has been described as "CuBr·bpy". This system is active toward styrene, acrylates, and methacrylates under the appropriate condition [19]. Percec has also described the role of bpy as partially solubilizing the Cu(I)/Cu(II) catalyst [26]. The role of the bpy is to co-ordinate to Cu(I) to give a *pseudo*-tetrahedral Cu(I) center in solution (Scheme 2.5).

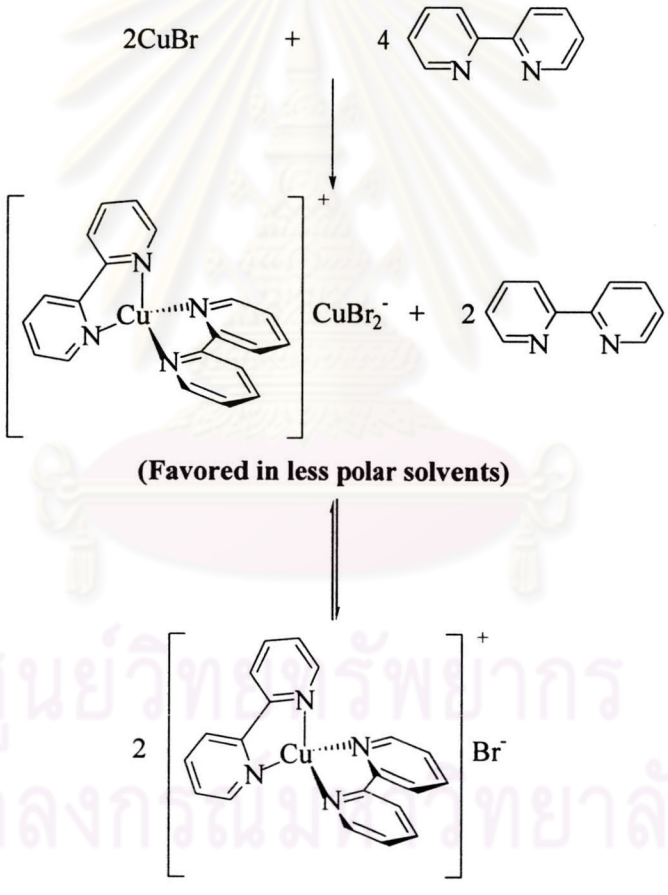


Scheme 2.5 The rotation of the bpy ligands from the tetrahedral and co-ordination of halide at the Cu center [26].

When uncoordinated, bpy exists predominately in the s-*trans* conformation in the solid state, in solution there is free rotation with the *trans* conformation and orthogonal rings are preferred over the *cis* conformation. Metal complexes contain bpy in a cisoid conformation, forming a planar 5-membered chelating ring. The ligand π^* orbitals can accept electron density from the metal thus stabilizing low oxidation states, in particular Cu(I). Abstraction by the $Cu(bpy)_2^+$ cation of halogen atoms from alkyl halides results in oxidation to Cu(II). The pentaco-ordinated species were shown in Scheme 2.4 which involves rotation of the bpy ligands from the tetrahedral and co-ordination of halide at the Cu center. This has also been proposed by Matyjaszewski [27]. Thus, if this proposed mechanism is correct, the two main roles of the ligand are (i) stabilization of Cu(I) by removal of electron density from the metal and (ii) the ability to interchange between tetrahedral Cu(I) and distorted square based pyramidal Cu(II). Copper(I) halides are very insoluble in organic solvents and monomers, and therefore the concept of solubilizing copper(I) indeed valid. However, the use of either Cu^IBr or Cu^ICl wirh bpy under atom transfer radical polymerization conditions results in a very heterogeneous reaction medium with a deep red Cu-$(bpy)_2$ complex in solution and insoluble copper halide visible as a pale green solid. Under these conditions the actual concentration of active catalystis impossible to determine. The system has been modified to give a homogeneous system by the use of bipyridines with alkyl substituents in the 4^{th} position e.g. *tert*-butyl [28-29]. The use of these homogeneous copper(I) complexes

results in a marked lowering of the polydispersity index (PDI) to approximately 1.05.

In 2003, Matyjaszewski's group studied the effect of [bpy]/[Cu(I)] ratio, polarity of the medium, and nature of alkyl bromides on the activation rate constants (k_{act}) in ATRP. The highest values of k_{act} for Cu(I)Br were obtained at [bpy]/[Cu(I)Br] ~2/1 and 1/1 in more polar and less polar solvents, respectively. This was ascribed to different structures of the complex, $Cu(bpy)_2^+Br^-$ and $Cu(bpy)_2^+CuBr_2^-$, correspondingly (Scheme 2.6) [30].



Scheme 2.6 Complex formation equilibrium in polar and nonpolar solvents [30].

ATRP can be applied to a large variety of monomers [31-34] to produce polymers with well-defined microstructures [22]. The success of ATRP in

synthesizing hydrophilic polymer provides an addition advantage over the traditional living ionic polymerization. The first example of aqueous ATRP was from Matyjaszewski's group in 1998. They found that ATRP of 2-hydroxyethyl acrylate (HEA) can be carried out directly in water in the presence of CuX/bpy/R-X at 90°C. After polymerization for 12 h, 87% monomer conversion was achieved, the molecular weight of the final product was 14,700, and the final polydispersity was 1.34 [35]. Similar results were obtained by Armes's group for the controlled polymerization of sodium methacrylate in water at 90°C. Monomer conversions of 70-80% were achieved after 10 h, with polydispersity of 1.20-1.30 [36]. In 2000, Wang and Armes reported the facile ATRP of methoxy-capped oligo(ethylene glycol) methacrylate (OEGMA) in water at 20°C with various initiator. A remarkably fast rate of polymerization was observed, with unusually high monomer conversions (up to 99%), first-order monomer kinetics, and predetermined molecular weights with narrow molecular weight distributions, indicating good "living" character [37-38]. In 2001, the efficient, controlled polymerization of 2-hydroxyethyl methacrylate (HEMA) is achieved using ATRP in methanol/water mixtures or pure methanol at 20°C by Armes and coworkers [39]. In the same year, Armes's group polymerized to high conversions of the biocompatible polymers based on 2-methacryloyloxyethyl phosphorylcholine (MPC) in both aqueous and alcoholic media at ambient temperature via ATRP. Low polydispersities were obtained [40-41]. Furthermore, MPC-based diblock copolymers can also be synthesized via ATRP. For example, addition of 2-(diethylamino)ethyl methacrylate (DEA) to an MPC homopolymerization at high conversion in methanol resulted in further chain growth and the formation of an MPC-DEA diblock copolymer. This diblock copolymer dissolved molecularly in acidic solution due to protonation of the DEA residues but formed micelles at pH 8 on addition of NaOD [41]. Such micelles are expected to act as "stealthy" nanoparticles, since the MPC block should minimize protein adsorption and hence prevent phagocytosis. The MPC-DEA diblock copolymer coating gave a 76% reduction in fibrinogen binding compared to that with an uncoated PET substrate. Even greater fibrinogen reduction (up to 85%) was obtained using another MPC-DEA diblock copolymer. Thus, planar surfaces

coated with MPC-based copolymers prepared via ATRP are rendered highly biocompatible.

2.3 Polymer Brush

Polymer brushes refer to an assembly of polymer chains which are tethered by one end to a surface or an interface [42]. Tethering is sufficiently dense that the polymer chains are crowded and forced to stretch away from the surface or interface to avoid overlapping, sometimes much further than the typical unstretched size of a chain. These stretched configurations are found under equilibrium conditions; neither a confining geometry nor an external field is required. This situation, in which polymer chains stretch along the direction normal to the grafting surface, is quite different from the typical behavior of flexible polymer chains in solution where chains adopt a random-walk configuration. A series of discoveries show that the deformation of densely tethered chains affects many aspects of their behavior and results in many novel properties of polymer brushes [42].

Polymer brushes are a central model for many practical polymer systems such as polymer micelles, block copolymers at fluid–fluid interfaces (e.g. microemulsions and vesicles), grafted polymers on a solid surface, adsorbed diblock copolymers and graft copolymers at fluid–fluid interfaces. All of these systems, illustrated in Figure 2.3, have a common feature: the polymer chains exhibit deformed configurations. Solvent can be either present or absent in polymer brushes. In the presence of a good solvent, the polymer chains try to avoid contact with each other to maximize contact with solvent molecules. With solvent absence (melt conditions) polymer chains must stretch away from the interface to avoid overfilling incompressible space.

The interface to which polymer chains are tethered in the polymer brushes may be a solid substrate surface or an interface between two liquids, between a liquid and air, or between melts or solutions of homopolymers. Tethering of polymer chains on the surface or interface can be reversible or irreversible. For solid surfaces,

the polymer chains can be chemically bonded to the substrate or may be just adsorbed onto the surface. Physisorption on a solid surface is usually achieved by block copolymers with one block interacting strongly with the substrate and another block interacting weakly. For interfaces between fluids, the attachment may be achieved by similar adsorption mechanisms in which one part of the chain prefers one medium and the rest of the chain prefers the other.

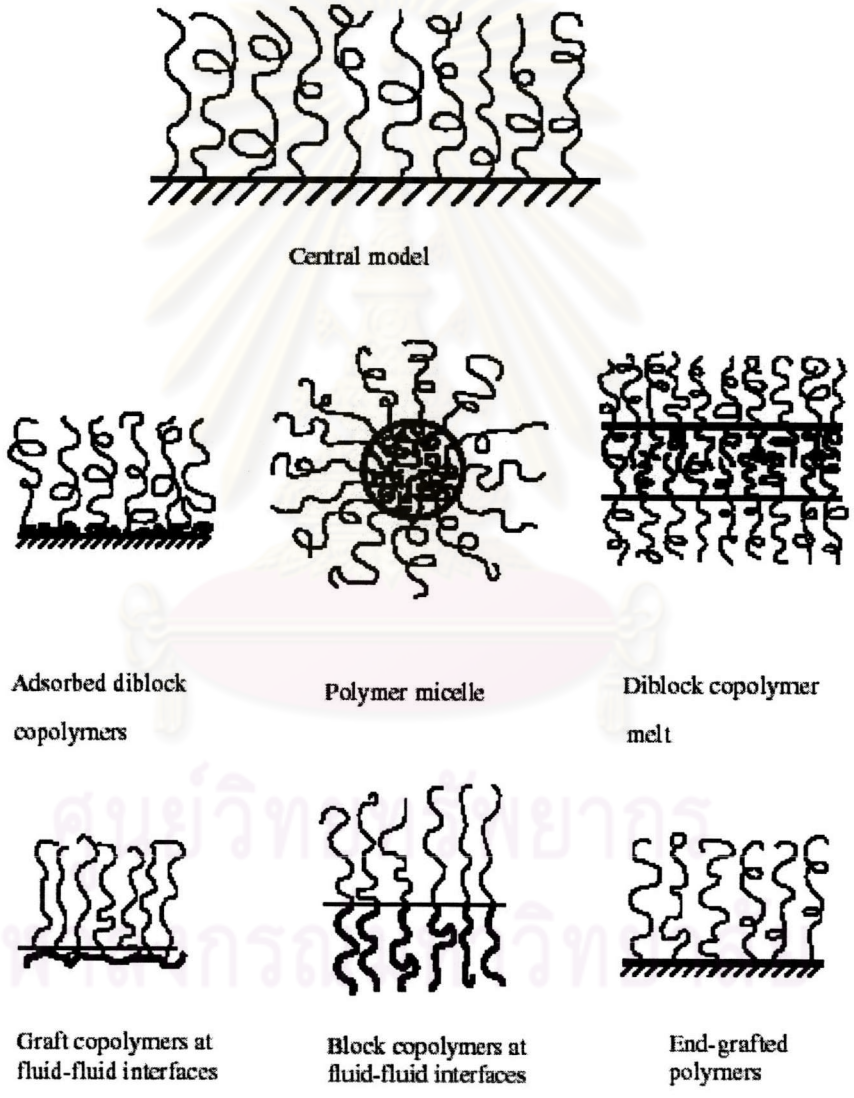


Figure 2.3 Examples of polymer systems comprising polymer brushes [42].

Polymer brushes (or tethered polymers) attracted attention in 1950s when it was found that grafting polymer molecules to colloidal particles was a very effective way to prevent flocculation [42]. In other words, one can attach polymer chains which prefer the suspension solvent to the colloidal particle surface; the brushes of two approaching particles resist overlapping and colloidal stabilization is achieved. The repulsive force between brushes arises ultimately from the high osmotic pressure inside the brushes. Subsequently it was found that polymer brushes can be useful in other applications such as new adhesive materials [43-44], protein-resistant biosurfaces [45], chromotographic devices [46], lubricants [47], polymer surfactants [42] and polymer compatibilizers [42]. Tethered polymers which possess low critical solution temperature (LCST) properties exhibit different wetting properties above and below LCST temperature [48]. A very promising field that has been extensively investigated is using polymer brushes as chemical gates. Ito and coworkers [49-51] have reported pH sensitive, photosensitive, oxidoreduction sensitive polymer brushes covalently tethered on porous membranes, which are used to regulate the liquid flowing rate through porous membranes. Suter and coworkers [52-53] have prepared polystyrene brushes on high surface area mica for the fabrication of organic–inorganic hybrids. Cation-bearing peroxide free-radical initiators were attached to mica surfaces via ion exchange and used to polymerize styrene. This process is important in the field of nanocomposites. Patterned thin organic films could be useful in microelectrics [54], cell growth control [55-56], biomimetic material fabrication [57], microreaction vessel and drug delivery [58].

In terms of polymer chemical compositions, polymer brushes tethered on a solid substrate surface can be divided into homopolymer brushes, mixed homopolymer brushes, random copolymer brushes and block copolymer brushes. Homopolymer brushes refer to an assembly of tethered polymer chains consisting of one type of repeat unit. Mixed homopolymer brushes are composed of two or more types of homopolymer chains [59]. Random copolymer brushes refer to an assembly of tethered polymer chains consisting of two different repeat units which are randomly distributed along the polymer chain [60]. Block copolymer brushes refer to an assembly of tethered polymer chains consisting of two or more homopolymer

chains covalently connected to each other at one end [61]. Homopolymer brushes can be further divided into neutral polymer brushes and charged polymer brushes. They may also be classified in terms of rigidity of the polymer chain and would include flexible polymer brushes, semiflexible polymer brushes and liquid crystalline polymer brushes. These different polymer brushes are illustrated in Figure 2.4.

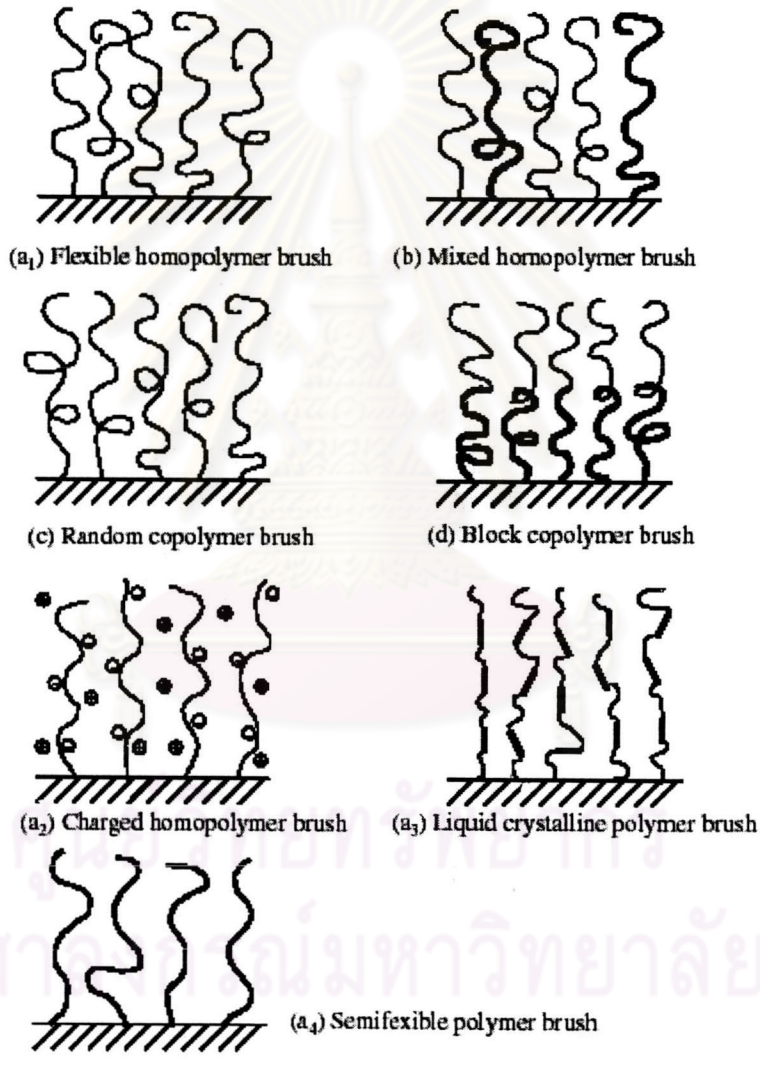


Figure 2.4 Classification of linear polymer brushes, (a_1–a_4) homopolymer brushes; (b) mixed homopolymer brush; (c) random copolymer brush; (d) block copolymer brush [42].

Generally, there are two ways to fabricate polymer brushes: physisorption and covalent attachment (Figure 2.5). For polymer physisorption, block copolymers adsorb onto a suitable substrate with one block interacting strongly with the surface and the other block interacting weakly with the substrate. The disadvantage of physisorption include thermal and solvolytic instabilities due to the non-covalent nature of the grafting, poor control over polymer chain density and complications in synthesis of suitable block copolymers. Tethering of the polymer chains to the surface is one way to surmount some of these disadvantages. Covalent attachment of polymer brushes can be accomplished by either "grafting to" or "grafting from" approaches. In a "grafting to" approach, preformed end-functionalized polymer molecules react with an appropriate substrate to form polymer brushes. This technique often leads to low grafting density and low film thickness, as the polymer molecules must diffuse through the existing polymer film to reach the reactive sites on the surface. The steric hindrance for surface attachment increase as the tethered polymer film thickness increases. To overcome this problem, the "grafting from" approach is a more promising method in the synthesis of polymer brushes with a high grafting density. "Grafting from" can be accomplished by treating a substrate with plasma or glow-discharge to generate immobilized initiators onto the substrate followed by in situ surface-initiated polymerization. However, "grafting from" well-defined self-assembled monolayers (SAMs) is more attractive due to a high density of initiators on the surface and a well-defined initiation mechanism. Also progress in polymer synthesis techniques makes it possible to produce polymer chains with controllable lengths. Polymerization methods that have been used to synthesize polymer brushes include cationic, anionic, TEMPO-mediated radical, atom transfer radical polymerization (ATRP) and ring opening polymerization.

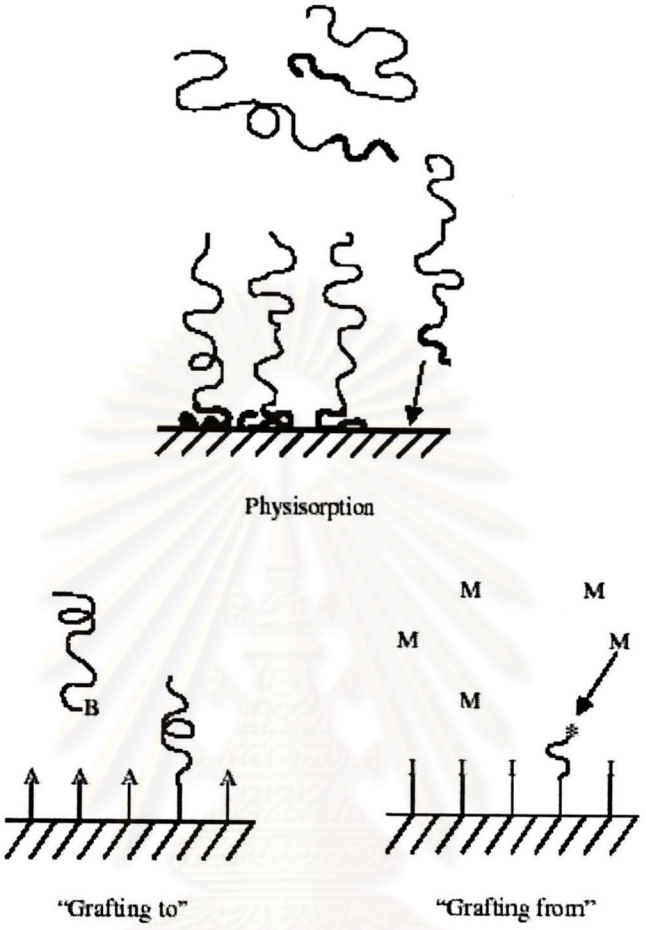


Figure 2.5 Preparation of polymer brushes by "physisorption", "grafting to" and "grafting from" [42].

In order to achieve a better control of molecular weight and molecular weight distribution and to obtain novel polymer brushes like block copolymer brushes, controlled radical polymerizations including ATRP, reverse ATRP, TEMPO-mediated and iniferter radical polymerizations have been used to synthesize tethered polymer brushes on solid substrate surfaces [62-67].

ATRP is a newly developed controlled radical polymerization [28]. It has attracted considerable attention due to its control of molecular weight, molecular weight distribution and synthesis of block copolymers. Husseman and coworkers [64] applied ATRP in the synthesis of tethered polymer brushes on silicon wafers and

achieved great success. They prepared SAMs of 5-trichlorosilylpentyl-2-bromo-2-methylpropionate on silicate substrates. The α-bromoester is a good initiator for ATRP. They have successfully synthesized PMMA brushes by the polymerization of MMA initiated from the SAMs. It has also been reported that tethered polyacrylamide has been obtained from surface initiated ATRP of acrylamide on a porous silica gel surface [65].

Recently, Matyjaszewski and coworkers [68] reported a detailed study of polymer brush synthesis using ATRP. Block copolymers of polystyrene-*b*-poly(*tert*-butyl acrylate) on silica wafer also have been prepared exclusively by ATRP. Modification of the hydrophilicity of the surface layer was achieved by hydrolysis of the *tert*-butyl ester to form polystyrene-*b*-poly(acrylic acid) and confirmed by a decrease in water contact angle from 86° to 18°. On the other hand, high contact angles were obtained when fluoroacrylates were polymerized from the surface (119°).

In 2001, Armes and coworkers synthesized polymer brush by ATRP of 2-hydroxyethyl methacrylate (HEMA) and methoxy-capped oligo(ethylene glycol) methacrylate (OEGMA) from silica modified with an initiator layer composed of 2-bromoisobutylrate. These polymer-grafted silica particles produced in this initial study are fascinating new "model" sterically stabilized colloids which are likely to prove attractive for both theoretical and experimental studies. The aqueous solution properties of the grafted polymer chains determine the colloid stability of the particles, as expected [69].

2.4 Blood Compatibility

The term "biocompatibility" encompasses many different properties of the materials, however, two important aspects of biomaterial screening refers to their in vitro cytotoxicity and blood compatibility behavior. Artificial surfaces in contact with blood trigger a number of biological systems through the adsorption of protein and cells. It is generally believed that the nature of adsorbed protein layer determines

all adverse events that impair the use of artificial materials in medical devices: thrombus formation as a result of platelet adhesion, platelet activation, initiation of coagulation and activation of the complement system that in turn results in leukocyte adhesion and activation.

2.4.1 Human Plasma

Human blood is a highly complex substance. Its major components are red blood cells, which carry oxygen from the lungs to the body; white blood cells, which have major roles in disease prevention and immunity; and platelets, which are key elements in the blood clotting process. These blood elements are suspended in blood plasma, a yellowish liquid that comprises about 55 % of human blood. When the blood was spin in a centrifuge, the red cells go to the bottom of the container, and the white cells and platelets to the middle, leaving the yellowish plasma at the top.

The plasma is the river in which the blood cells travel. It carries not only the blood cells but also nutrients (sugars, amino acids, fats, salts, minerals, etc.), waste products (CO_2, lattic acid, urea, etc.), antibodies, clotting proteins (called clotting factors), chemical messengers such as hormones, and proteins that help maintain the body's fluid balance.

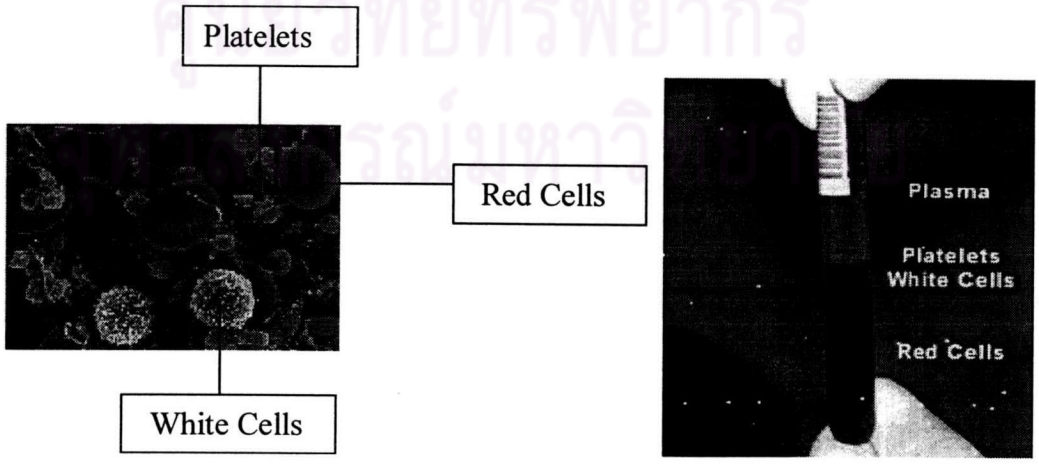


Figure 2.6 Pictorial representation of human blood [70].

Many specific functions of blood are carried out by proteins found in plasma. Human plasma contains a number of proteins such as albumin (Alb), immunoglubulins (Ig), complement factors, fibrinogen (Fg), Fibronectin (FN), coagulation factors (activators and down regulators), and lipoprotein (LP). These protein all have various biological role: Alb is considered a biological passivator ; immunoglobulin G (IgG) activates the complement system and, for example, binds lymphocytes; the complement factors (including C3) are a part of immune defense ending in the lysis of cells with the membrane attack complex repturing the cell types and bacteria have receptors for FN, α_2-macroglobulin (α_2M) is a down regulator of the coagulation cascade that ends in the formation of blood clot in which factors like high molecular weight kininogen (HMWK), factor XII (F XII), factor VII (F VII) and prekallikrein (PK) are component, anti thrombin III (ATh III) is another potent down regulator of coagulation, as it binds thrombin, LP can act as transport protein of such agents as cholesterol [70].

2.4.2 Mechanism of Thrombus Formation on Polymer Surface

When artificial materials contact a living organism, severe biological responses are induced. Particularly, thrombus is formed when blood encounters a foreign surface as shown in Figure 2.7. The mechanism of coagulation is very complicated, but for simplicity can be classified into three processes: (1) the coagulation system, (2) the platelet system, and (3) the complemental system. The coagulation system can be further classified into two processes. One is started by Factor VII when the tissue is damaged extrinsically (i.e. outside the body). The other is induced by Factor XII (Hageman Factor) which is activated via an inflammation originating within the body (i.e. intrinsic pathway).

It is well known that platelets also contribute to thrombus formation. A foreign substrate induces adhesion and activation of platelets with the adsorbed protein layer serving as a controlling factor of the platelet response. The adhesion of

platelets to a biomaterial surface is followed by the platelet release reaction taking place in the adhering platelets and then platelet aggregation on the surface.

The complement system can also be classified into two processes: (1) the classical pathway and (2) the alternative pathway. The classical pathway is started from the interaction between the immunocomplex contained within immunoglobulin G (IgG) or immunoglobulin M (IgM) and C1q in the C1 complex. The alternative pathway is started with C3a work for adhesion of leukocyte and activation of C5.

These three mechanisms of coagulation are not independent. Normally, thrombus formation on a foreign surface results in an interaction between platelets and intrinsic pathway. Initiation of the intrinsic coagulation cascade may be induced by thromboplastins liberated from platelets or by Factor XII activation caused by platelets stimulated by released adenosine diphosphate (ADP). Thrombin formation caused by activation of the intrinsic pathway induces the production of a fibrin monolayer on a biomaterial surface and the promotion of platelet adhesion and aggregation.

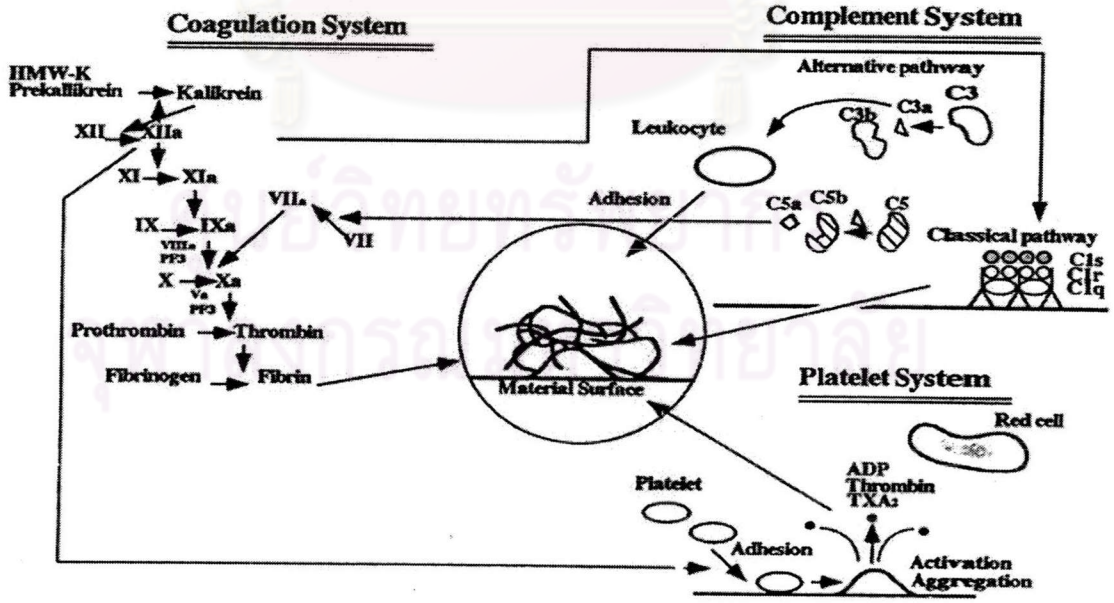


Figure 2.7 Schematic representation of blood coagulation system [70].

2.5 Characterization Techniques

2.5.1 Gel Permeation Chromatography

Gel permeation chromatography, more correctly termed *size exclusion chromatography,* is a separation method for high polymers, similar to but advanced in practice over *gel filtration* as carried out by biochemists, that has become a prominent and widely used method for estimating molecular-weight distributions since its discovery just over two decades ago in 1961. The separation takes place in a chromatographic column filled with beads of a rigid porous "gel"; highly cross-linked porous polystyrene and porous glass are preferred column-packing materials. The pores in these gels are of the same size as the dimensions of polymer molecules.

A sample of a dilute polymer solution is introduced into a solvent stream flowing through the column. As the dissolved polymer molecules flow past the porous beads, they can diffuse into the internal pore structure of the gel to an extent depending on their size and the pore-size distribution of the gel. Larger molecules can enter only a small fraction of the internal portion of the gel, or are completely excluded; smaller polymer molecules penetrate a larger fraction of the interior of the gel. The larger the molecule, therefore, the less time it spends inside the gel, and the sooner it flows through the column. The different molecular species are eluted from the column in order of their molecular size as distinguished from their molecular weight, the largest emerging first.

A complete theory predicting retention times or volumes as a function of molecular size has not been formulated for gel permeation chromatography. A specific column or set of columns (with gels of different pore sizes) is calibrated empirically to give such a relationship, by means of which a plot of amount of solute versus retention volume can be converted into a molecular-size-distribution curve.

As in all chromatographic processes, the band of solute emerging from the column is broadened by a number of processes, including contributions from the apparatus, flow of the solution through the packed bed of gel particles, and the

permeation process itself. Corrections for this zone broadening can be made empirically; it usually becomes unimportant when the sample has $\overline{M}_w/\overline{M}_n > 2$.

Gel permeation chromatography is extremely valuable for both analytic and preparative work with a wide variety of systems ranging from low to very high molecular weights. The method can be applied to a wide variety of solvents and polymers, depending on the type of gel used. With polystyrene gels, relatively nonpolar polymers can be measured in solvents such as tetrahydrofuran, toluene, or (at high temperatures) *o*-dichlorobenzene; with porous glass gels, more polar systems, including aqueous solvents, can be used. A few milligrams of sample suffices for analytic work, and the determination is complete in as short a time as a few minutes using modern high-pressure, high-speed equipment.

The results of careful gel permeation chromatography experiments for molecular-weight distribution agree so well with results from other techniques that there is serious doubt as to which is correct when residual discrepancies occur.

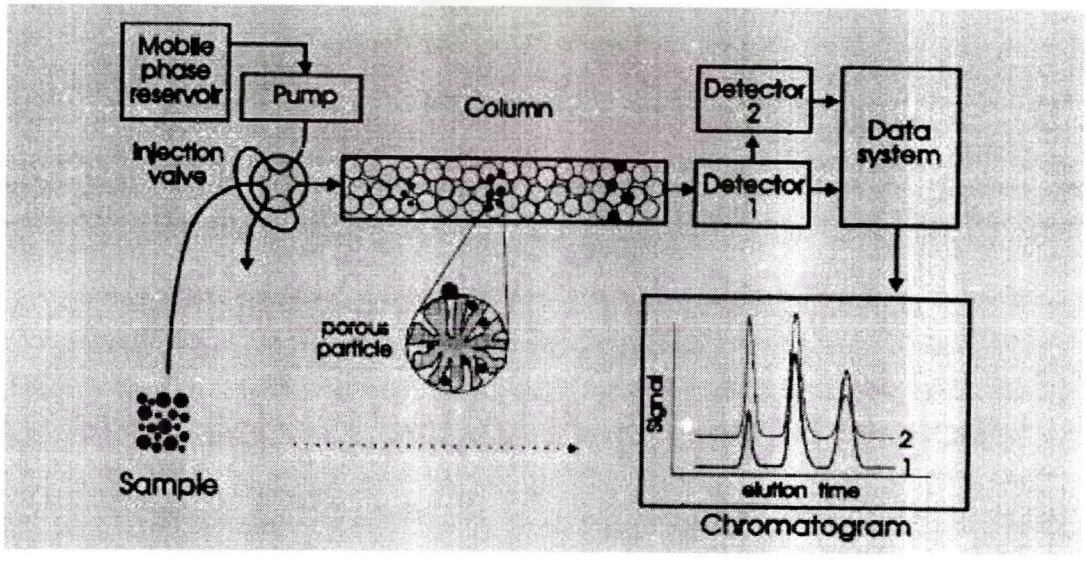


Figure 2.8 Schematic representation of the gel permeation chromatography [71].

2.5.2 Ellipsometry

Ellipsometry is a sensitive optical technique for determining properties of surfaces and thin films. If linearly polarized light of a known orientation is reflected at oblique incidence from a surface then the reflected light is elliptically polarized. The shape and orientation of the ellipse depend on the angle of incidence, the direction of the polarization of the incident light, and the reflection properties of the surface. Ellipsometry measures the polarization of the reflected light with a quarter-wave plate followed by an analyzer; the orientations of the quarter-wave plate and the analyzer are varied until no light passes though the analyzer. From these orientations and the direction of polarization of incident light are express as the relative phase change, Δ , and the relative amplitude change, Ψ, introduced by reflection from the surface. These values are related to the ratio of Fresnel reflection coefficients, R_p and R_s for p and s- polarized light, respectively.

$$\mathrm{Tan}(\Psi)\, e^{i\Delta} = \frac{R_p}{R_s} \qquad (2)$$

An ellipsomer measures the changes in the polarization state of light when it is reflected from a sample. If the sample undergoes a change, for example a thin film on the surface changes its thickness, then its reflection properties will also change. Measuring these changes in the reflection properties allow us to deduce the actual change in the film's thickness [72].

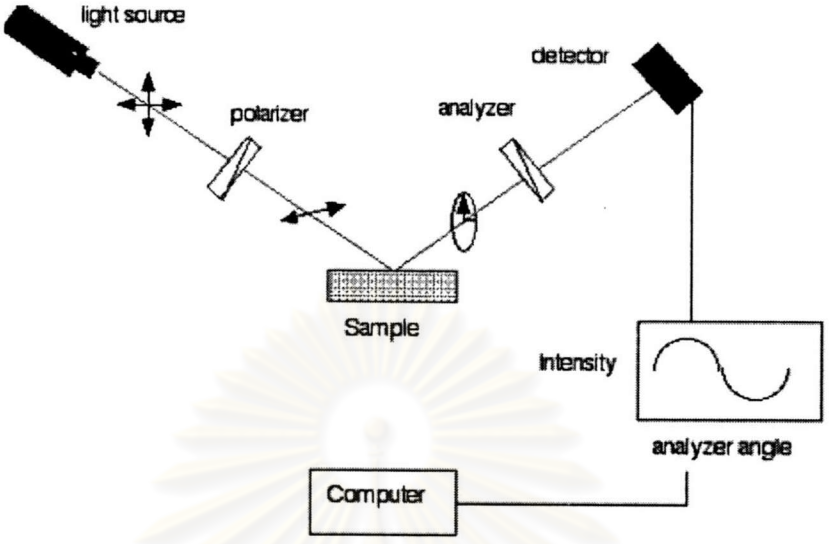


Figure 2.9 Schematic of the geometry of an ellipsometry experiment [72].

2.5.3 Contact Angle Measurement

Contact angle measurements are often used to assess changes in the wetting characteristics of a surface and hence indicate a change in surface energy. The technique is based on the three-phase boundary equilibrium described by Young's equation (Eq. 3).

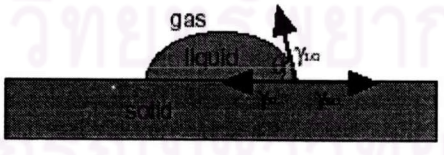


Figure 2.10 Schematic representation of the Young's equation [73].

$$\gamma_{LG}\cos\theta = \gamma_{SG} - \gamma_{SL} \quad (3)$$

where γ_{LG}, γ_{SG} and γ_{SL} are the interfacial tension between the phases with subscripts L, G, S corresponding to liquid, gas, and solid phase, respectively and θ refers to the

equilibrium contact angle. The Young's equation applies for a perfectly homogeneous atomically flat and rigid surface and therefore supposes many simplifications. In the case of real surfaces, the contact angle value is affected by surface roughness, heterogeneity, vapor spreading pressure, and chemical contamination of the wetting liquid. Although the technique to measure contact angles is easy, data interpretation is not straightforward and the nature of different contributions to the surface is a matter of discussion. Generally, we can define the complete wetting, wetting, partial wetting, and nonwetting according to Figure 2.11.

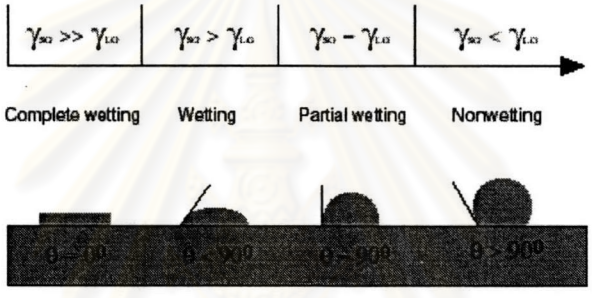


Figure 2.11 Schematic representation of wettability [73].

2.5.4 X-ray Photoelectron Spectroscopy (XPS)

XPS is an abbreviation for X-ray Photoelectron Spectroscopy. Another name is ESCA which is an acronym for Electron Spectroscopy for Chemical Analysis. In XPS or ESCA, a beam of (monochromatic) X-rays is first produced by electron bombardment of an anode material (Al, Mg, Si). When the X-rays interact with the sample under investigation, they can ionize electrons that are in core levels (such as 1s, 2s, etc.). If the binding energy of the electron in the core hole was EB, then the kinetic energy of the electron ejected from the surface can be given in the energy diagram (Figure 2.12).

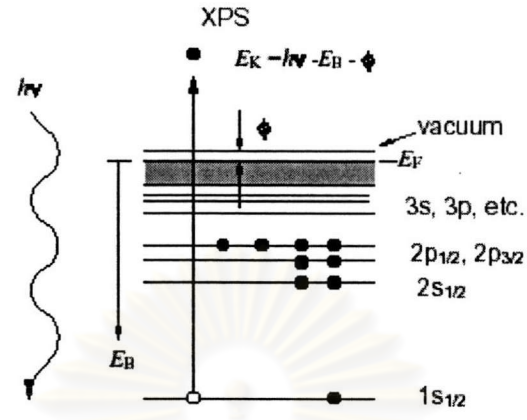


Figure 2.12 Schematic diagram of the X-ray photoelectron emission process [73].

$$E_K = h - E_B - \phi \qquad (4)$$

where E_K is the measured electron kinetic energy, $h\upsilon$ the energy of the exciting radiation, E_B the binding energy of the electron in the solid, and ϕ the spectrometer work function. Since $h\upsilon$ is a well-defined quantity, the electron binding energies can be calculated by measuring the kinetic energies of the electrons that are ejected from the sample, using the above equation. The electron energies are measured using an electrostatic energy analyzer such as a "hemispherical analyzer". The analyzer measures the kinetic energy distribution of the emitted electrons. A general schematic drawing of the main components of the XPS instrument is shown in Figure 2.13. The main components of the system include the vacuum system, the x-ray source, the sample stage, and the analyzer. The energy discrimination of the electrons is obtained by sweeping the potential(s) in the lens or by using a grid system in front of the analyzer. The sensitivity of the instrument is dependent on the X-ray source used, the analyzed area, geometrical factors and the efficiencies of the lens, the analyzer and the detector. The energy resolution is due to the inherent width of the X-ray radiation and the resolving power of the spectrometer.

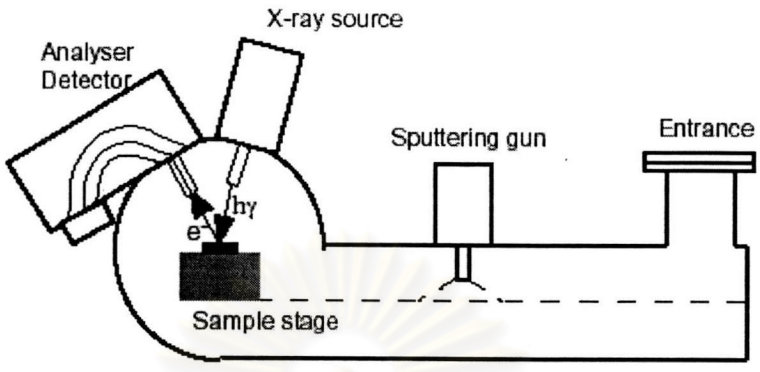


Figure 2.13 General schematic drawing of the XPS instrument [73].

XPS can provide the following information:

1. Elemental identification. Because the number of protons increases as we progress through the periodic table, the electron binding energies for a fixed core level (such as the 1s level) will increase monotonically; thus, measuring the electron kinetic energy is equivalent to determining which elements are present on the surface.

2. Oxidation states for any given elements. There will be small shifts in the binding energies due to changes in oxidation states; higher oxidation states generally have higher binding energies, and emit electrons with lower kinetic energies.

3. Quantitative analyses through curve fitting and calculation of atomic concentrations because the photoelectron intensity is directly related to the atomic concentrations of the photoemitting atoms.

4. Depth profiling when combined with ion etching (sputtering) techniques.

5. Images or maps showing the distribution of the elements or their chemical states over the surface. Modern instruments can have a spatial resolution down to a few microns.

2.5.5 Atomic force Microscopy (AFM)

The Atomic Force Microscope (AFM) is being used to solve processing and materials problems in a wide range of technologies affecting the electronics, telecommunications, biological, chemical, automotive, aerospace, and energy industries. The materials being investigated include thin and thick film coatings, ceramics, composites, glasses, synthetic and biological membranes, metals, polymers, and semiconductors. The AFM is being applied to studies of phenomena such as abrasion, adhesion, cleaning, corrosion, etching, friction, lubrication, plating, and polishing. By using AFM one can not only image the surface in atomic resolution but also measure the force at nano-newton scale. The publications related to the AFM are growing speedily since its birth.

The first AFM was made by meticulously gluing a tiny shard of diamond onto one end of a tiny strip of gold foil. In 1985, Binnig and Gerber used the cantilever to examine insulating surfaces. A small hook at the end of the cantilever was pressed against the surface while the sample was scanned beneath the tip. The force between tip and sample was measured by tracking the deflection of the cantilever. This was done by monitoring the tunneling current tot a second tip positioned above the cantilever. They could delineate lateral features as small as 300 Å. The force microscope emerged in this way. In fact, without the breakthrough in tip manufacture, the AFM probably would have remained a curiosity in many research groups. It was Albrecht, a fresh graduate student, who fabricated the first silicon microcantilever and measured the atomic structure of boron nitride. Today the tip-cantilever assembly typically is microfabricated from Si or Si_3N_4. The era of AFM came finally when the Zurich group released the image of a silicon (111) pattern. The world of surface science knew that a new tool for surface microscope was at hand. After several years the microcantilevers have been perfected, and the instrument has been embraced by scientists and technologists.

The force between the tip and the sample surface is very small, usually less than 10^{-9} N. The detection system does not measure force directly. It senses the

deflection of the microcantilever. The detecting systems for monitoring the deflection fall into several categories. The first device introduced by Binnig was a tunneling tip placed above the metallized surface of the cantilever. This is a sensitive system where a change in spacing of 1 Å between tip and cantilever changes the tunneling current by an order of magnitude. It is straightforward to measure deflections smaller than 0.01 Å. Subsequent systems were based on the optical techniques. The interferometer is the most sensitive of the optical methods, but it is somewhat more complicated than the beam-bounce method which was introduced by Meyer and Amer. The beam-bounce method is now widely used as a result of the excellent work by Alexander and colleagues. In this system an optical beam is reflected from the mirrored surface on the back side of the cantilever onto a position-sensitive photodetector. In this arrangement a small deflection of the cantilever will tilt the reflected beam and change the position of beam on the photodetector. A third optical system introduced by Sarid uses the cantilever as one of the mirrors in the cavity of a diode laser. Motion of the cantilever has a strong effect on the laser output, and this is exploited as a motion detector

The principles on how the AFM works are very simple. An atomically sharp tip is scanned over a surface with feedback mechanisms that enable the piezo-electric scanners to maintain the tip at a constant force (to obtain height information), or height (to obtain force information) above the sample surface. Tips are typically made from Si_3N_4 or Si, and extended down from the end of a cantilever. The nanoscope AFM head employs an optical detection system in which the tip is attached to the underside of a reflective cantilever. A diode laser is focused onto the back of a reflective cantilever. As the tip scans the surface of the sample, moving up and down with the contour of the surface, the laser beam is deflected off the attached cantilever into a dual element photodiode. The photodetector measures the difference in light intensities between the upper and lower photodetectors, and then converts to voltage. Feedback from the photodiode difference signal, through software control from the computer, enables the tip to maintain either a constant force or constant height above the sample. In the constant force mode the piezo-electric transducer

monitors real time height deviation. In the constant height mode the deflection force on the sample is recorded. The latter mode of operation requires calibration parameters of the scanning tip to be inserted in the sensitivity of the AFM head during force calibration of the microscope.

Some AFM's can accept full 200 mm wafers. The primary purpose of these instruments is to quantitatively measure surface roughness with a nominal 5 nm lateral and 0.01nm vertical resolution on all types of samples. Depending on the AFM design, scanners are used to translate either the sample under the cantilever or the cantilever over the sample. By scanning in either way, the local height of the sample is measured. Three dimensional topographical maps of the surface are then constructed by plotting the local sample height versus horizontal probe tip position.

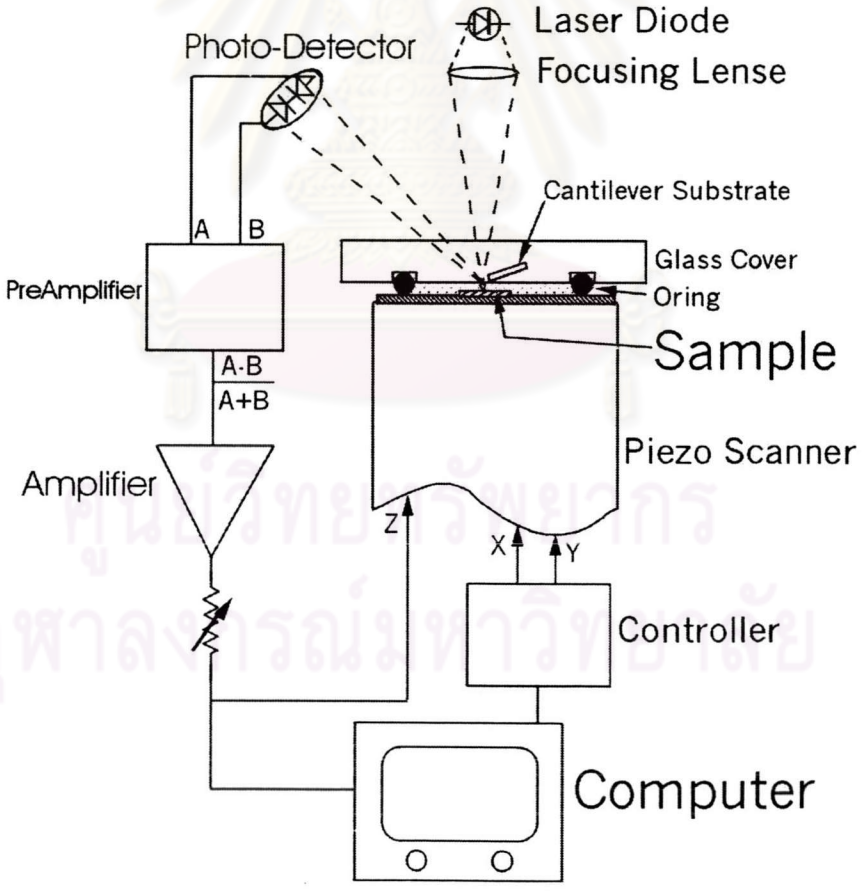


Figure 2.14 Schematic diagram of an atomic force microscope [74].