CHAPTER I

INTRODUCTION

1.1 Rationale

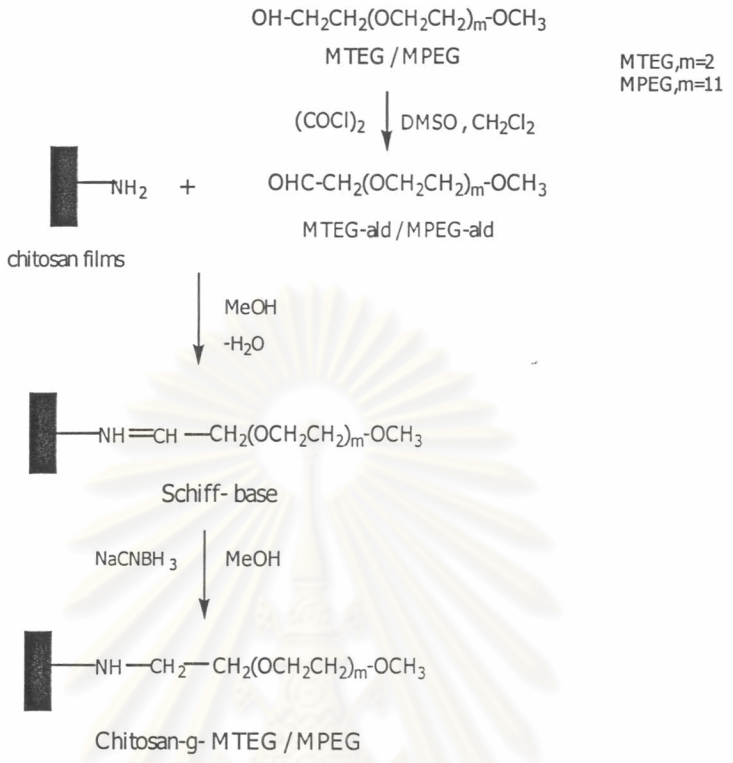
Chitosan is naturally originated and characterized as non-toxic biomaterials with good biocompatibility. This work has focused on modifying the outer surface of chitosan materials that are in the form of cast films. It was hypothesized that the reaction of chitosan films with chemical reagents was able to modify the film surface. The chemically modified films should, therefore, exhibit different surface properties from the original films. Consequently, the modified chitosan surface could be applied for use in specific applications, such as in biomedical fields.

1.2 Statement of the purpose

The purpose of this research is to modify hydrophilicity and protein adsorptivity of chitosan films. Monomethoxy poly(ethylene glycol) (MPEG) and Monomethoxy triethylene glycol (MTEG) were grafted onto chitosan films by reductive alkylation. The films before and after modification are characterized by ATR-IR for functional group, and by contact angle measurement and protein adsorption study for their surface properties.

1.3 Scope of this research

MTEG-ald and MPEG-ald were grafted on the films by a reductive alkylation to react with the amino groups in the chitosan films. MTEG and MPEG having the molecular weight of 550 were used in this study. In the research, chitosan films were allowed to react with MTEG-ald and MPEG-ald by reductive alkylation *via* Schiff-base formation (Scheme 1.1). Many factors were considered, including medium types, and the ratios of chitosan, aldehyde and $NaCNBH_3$ as 1:10:10 and 1:30:30.

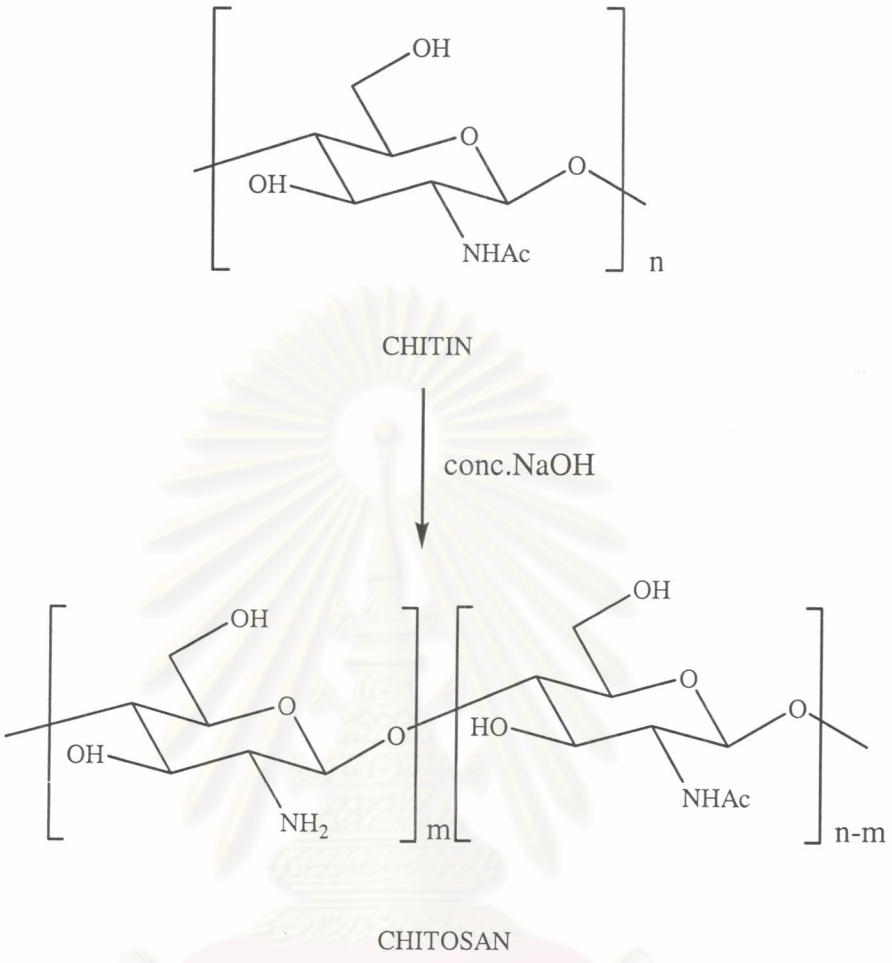


Scheme 1.1 Preparation of chitosan-g-MTEG / MPEG in heterogeneous state

1.4 Theory

1.4.1 Chitosan

Chitosan, an *N*-deacetylated[1] derivative of chitin, has a structure similar to cellulose. Chitosan mainly consists of 2-amino-2-deoxy-D-glucose (GlcN) repeating unit with a small amount of 2-acetyl-2-deoxy-D-glucose residues. The amount of GlcN unit in chitosan is generally referred to by percent degree of deacetylation or %DD. Various techniques were used for determination of %DD such as IR,[2] NMR,[3] and metachromatic titration.[4] The structures of chitosan and chitin are shown in Scheme 1.2.



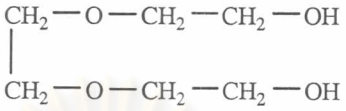
Scheme 1.2 Structures of chitin and chitosan

Chitosan is easily obtained by deacetylation of chitin, a polysaccharide widely distributed in nature (*e.g.* crustaceans, insect and certain fungi).[5] It is dissolved in inorganic acids such as HCl, HNO_3, and organic acids, preferably acetic acid and formic acid. Pure chitosan precipitates from solutions if the pH rises above 6.

The intriguing properties of chitosan have been known for many years and the polymer has been used in the fields of agriculture, industry and medicine. Chitosan has been noted for its application as a film-forming agent in cosmetics,[6] a dry-binder for textiles, a strengthening additive in paper.[7] It has been used extensively as a biomaterial owing to its immunostimulatory activities,[8] anticoagulant properties,[8] antibacterial and antifungal action[10] and for its action as a promoter of wound healing in the field of surgery.[11]

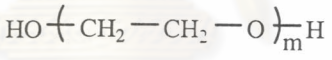
1.4.2 TEG and PEG

Formula of TEG:



TEG is an important non-volatile industrial solvent. It is also useful in the dehydration of gases, manufacture of insecticides and in the synthesis of some organic derivatives. Pure TEG is useful in the production of plasticizers for cellophane, glue, cork, powdered ceramics and some plastics. TEG is a component in the formulation of some pigments, printing dyes, inks and pastes. It is also used for air fumigation and distinguished by its antibacterial properties.

Formula of PEG:



PEG is a linear polymer of ethylene oxide with a hydroxyl terminal. The longer chains are simply referred as poly(ethylene oxide); (PEO). It has been used most widely for surface modification because of its unique properties such as hydrophilicity, flexibility, high exclusion volume in water, nontoxicity, and nonimmunogenecity.[12] The synthesis of its derivatives is very important and is the first step for the application of PEG. When PEG is properly linked to a polypeptide, it modifies many of its features while the main biological functions may be maintained. The PEG-drug conjugates showed longer duration of activity, due to slow release. And insoluble drugs are solubilized by PEG conjugation and thus more easily administered. When PEG derivatives are crosslinked with a proper polymer network, the release of drug from the network can be controlled. This will lead to a longer duration of drug release and a higher efficiency.

PEG has been used extensively as a component in materials for biomedical application. When it is included as a comonomer in polymeric material, or when grafted to a surface, it usually increases biocompatibility. A self-assembled

monolayers (SAM) of poly(ethylenimine) and oligo(ethylene glycol) (Figure 1.1) was shown to resist the adsorption of protein and the adhesion of bacteria. This study demonstrate that some of the structural priciples useful for designing SAMs that resist the adsorption of protein can be extended to thin polymer films grafted on the surface of SAMs.[13]

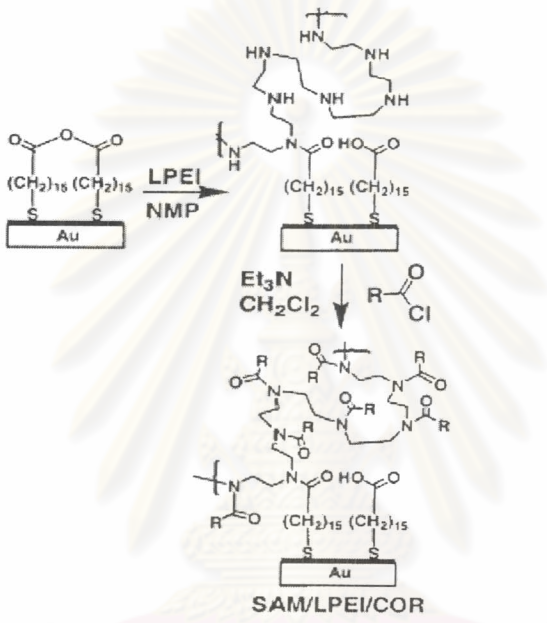


Figure 1.1 Reaction of interchain anhydrides with linear poly(ethylenimine) (LPEI), followed by reaction of the surface-grafted LPEI with an acyl chloride (ClCOR, R=CH_3,$CH_2(OCH_2CH_2)_2OCH_3$)

Surface-grafted PEG molecules are known to prevent protein adsorption to the surface.[14] The protein-repulsive property of PEG molecules are maximized by covalent grafting. Silanated MPEGs was synthesized for covalent grafting of PEG to glass surfaces. (Figure 1.2). The modified glass surface fibrinogen adsorption by more than 95% as compared with the control surface. Silanated PEG is an example of PEG derivatives that can be used to graft on certain material.

Silanated PEG II

Silanated PEG II

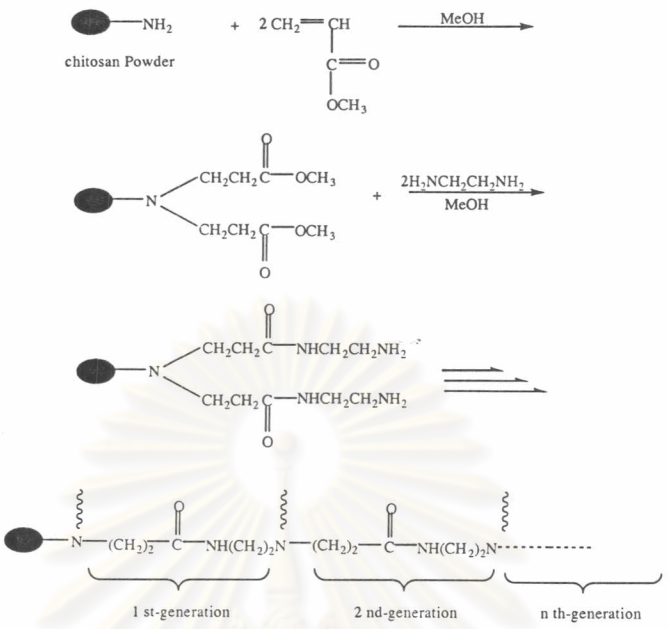
Figure 1.2 Two silanated PEG used to grafted on glass surface[14]

1.4.3 Chemical modification of chitosan

Chitosan carries a number of hydroxy (-OH) and amino ($-NH_2$) groups. This prompts many researchers to find ways of modifying its properties either by physical blending or chemical reactions.

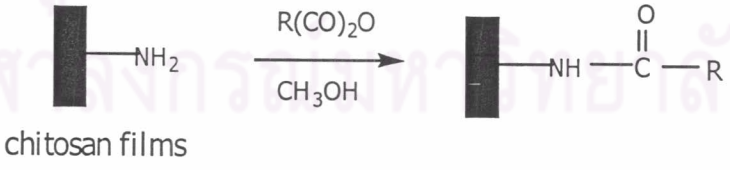
To improve the soluble property chitosan,[15] generally, *N*-Acylation partially deacetylated chitin derivatives were prepared *via* ring-opening reactions with various cyclic acid anhydrides in aqueous MeOH system. *N*-Alkylation of deacetylated chitin were also performed in aqueous MeOH with various aldehydes, monosaccharides, and disaccharide. The water solubility of *N*-acylated and *N*-alkylated chitosan derivatives at various pHs were studied.

To modify the surface chitosan powder, grafting of hyperbranched dendritic polyamidoamine, a new class of topological macromolecules, onto the surface was investigated.[16] It was found that hyperbranched dentritic polyamidoamine was propagated from chitosan powder surface by repeating two processes: (1) Michael addition and amidation.



Scheme 1.5 Grafting of dendritic polyamidoamine onto a chitosan powder surface .

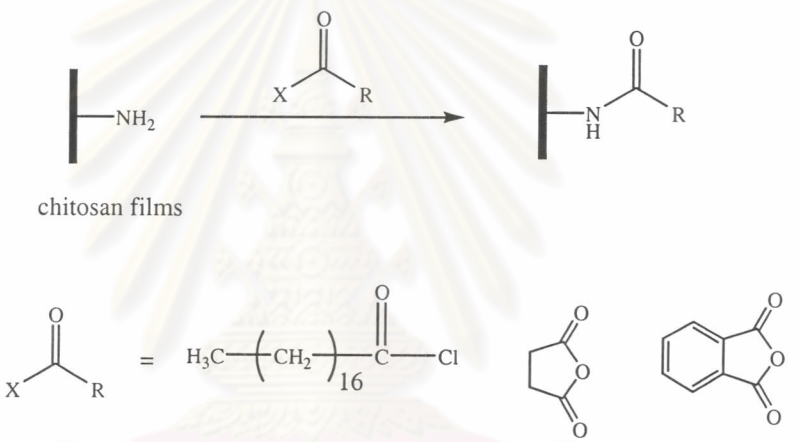
The chitosan films were acylated under hxeterogeneous condition in methanol with acetic and hexanoic anhydride[17] (Scheme 1.6). They were characterized by proton nuclear magnetic resonance, elemental analysis and multiple internal reflective Fourier transform infrared spectroscopy. Biodegradation studies of the acylated chitosan films carried out in laboratory-scale aerobic reactors revealed that the formation of chitin at the film surface enhanced the biodegradability of the films.



R = CH_3, $CH_2(CH_2)_3CH_3$

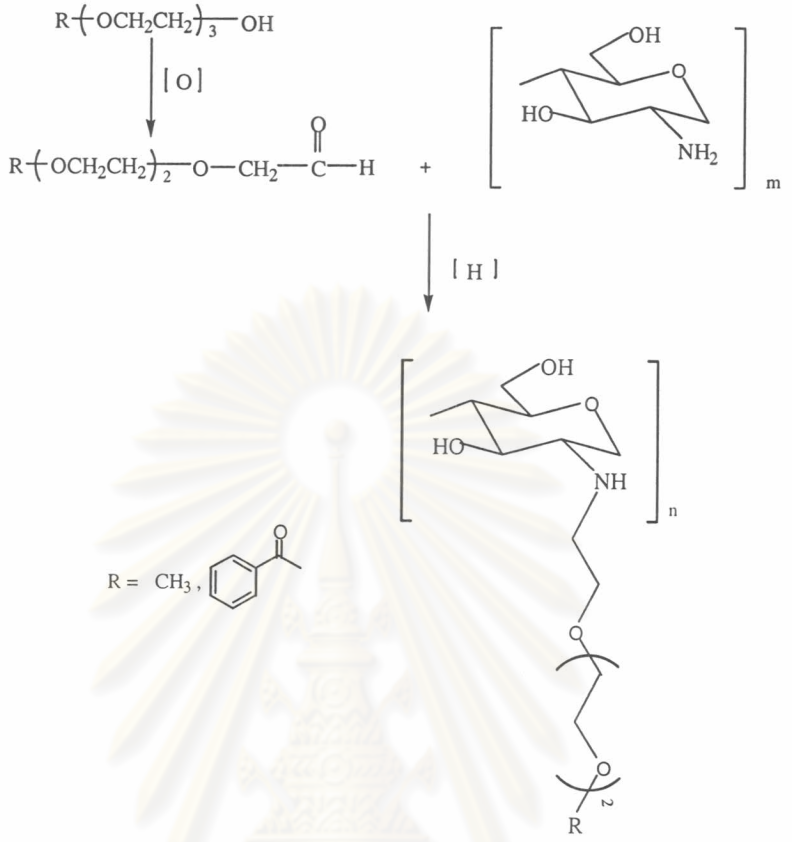
Scheme 1.6 Acylation of chitosan films under heterogeneous state

Surface modification using hydrophobic polymers has been shown to effect of protein adsorption.[18] The surface of chitosan films was modified using acid chloride and acid anhydride (Scheme 1.7). The improved surface hydrophobicity affected by the stearoyl groups promoted protein adsorption. In contrast, selective adsorption behavior was observed in the case of the chitosan films modified with anhydride derivatives. Lysozyme adsorption was enhanced by H-bonding and charge attraction with the hydrophilic surface. While the amount of albumin adsored was decreased possibly due to negative charge that gave rise to repulsion between the modified surface and albumin.

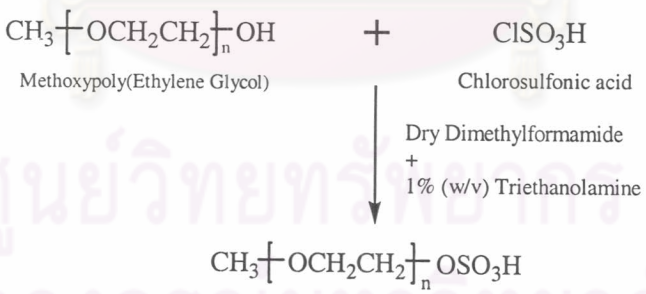


Scheme 1.7 Attachment of carboxylic acid derivatives on to the surface of chitosan film *via* amide linkages

The uses of ethylene glycol (EG) moiety to modify the properties of chitosan were reported by a number of researchers. Monoaldehyde derivatives of tri- and tetra-(ethylene glycol) were grafted onto chitosan chains by reductive alkylation (Scheme 1.8) to form comb-shaped chitosan.[19] The modified polymer showed the increasing adsorption capacity toward metal ions. Moreover, PEG's with molecular weight up to 5,000 daltons were also grafted on the chitosan chain by using their aldehyde derivatives *via* reactive alkylation[20] or by using sulfonate derivatives.[21] (Scheme 1.8) Protein and platelet adhesion on these modified chitosan were reduced significantly, possibly due to steric repulsion caused by the PEG chain.



Scheme 1.8 Synthesis of chitosan form comb-shaped polysaccharides



Scheme 1.9 Synthesis of methoxypoly(ethylene glycol)sulfonate

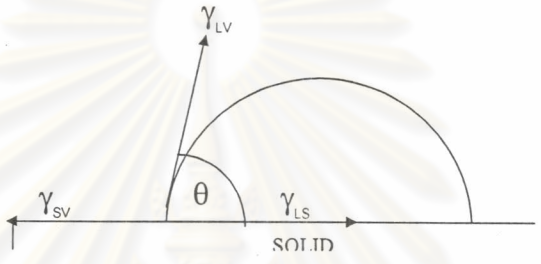
1.4.4 Surface characterization

Surface characterization is a method for analyzing chemical and physical properties of material surface. In this research, surface of chitosan films, before and after modification, were analyzed for functional groups using ATR-IR, hydrophilicity using air-water contact angle, and protein adsorptivity.

Air-water contact angle measurement

Contact angle measurement is probably the most common method of surface tension measurement of solids. The basis of the measurement of solid surface tension by contact angle is the equilibrium of the three-phase boundary, shown in Figure 1.3. As the surface becomes more hydrophobic, θ will be larger.

In this research, air-water contact angle was primarily used for determining the hydrophilicity of the chitosan surface.



$\gamma_{SV} - \gamma_{LS} = \gamma_{LV} \cos\theta$: 3-phase equation

γ_{LV}: interfacial tension between liquid and vapour phases

γ_{SV}: interfacial tension between solid and vapour phases

γ_{LS}: interfacial tension between liquid and solid phases

Figure 1.3 Equilibrium of the three-phase boundary on solid surface

Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectrometer is based on the interference of light between two beams, producing an interferogram. Time and frequency domains are interconvertible by the Fourier transform method. The basic components of FT-IR spectrometer are shown schematically in Figure 1.4. The radiation generated by the IR source passes through an interferometer, then being absorbed by a sample before reaching a detector. Upon the signal amplification, in which high-frequency contributions have been eliminated by a filter, the data are converted into a digital from by an analog-to-digital converter. Then they are transferred to the computer for of Fourier transformation. The finally

FT-IR spectrum is obtained from the ratio of a single beam of the sample against that of the reference.

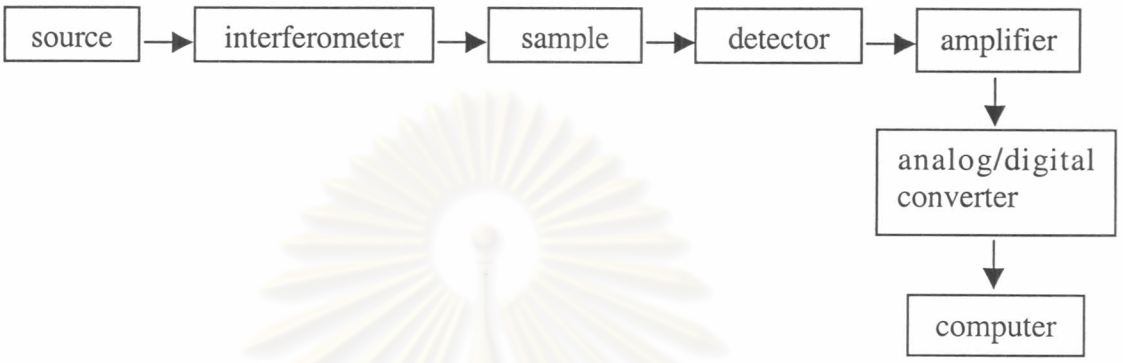


Figure 1.4 Schematic of a typical FT-IR converter spectrometer.

Attenuated total reflectance infrared spectroscopy (ATR-IR)

The infrared beam from the spectrometer is focused onto the beveled edge of an internal reflection element (IRE). The beam is then reflected, generally numerous times, through the IRE crystal, and directed to a detector (Figure 1.5).

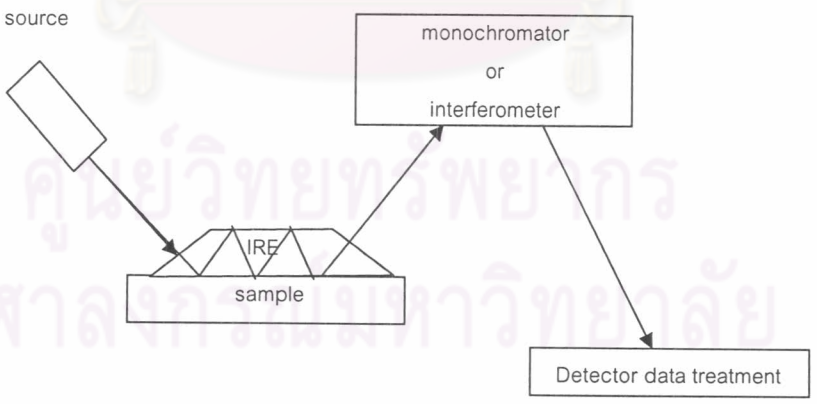


Figure 1.5 Diagram of ATR-IR

The radiation can penetrate a short distance into the sample, thus interact with any functionalities existed within that depth. The depth of penetration (d_p, defined as

the distance from the IRE-sample interface where the intensity of the evanescent wave decays to 1/e of its original value) can be calculated using the formula in Equation:

$$d_p = \frac{\lambda}{2\pi n_p(\sin^2\theta - n_{sp}^2)^{1/2}}$$

where λ = wavelength of the radiation in the IRE, θ = angle of incidence, n_{sp} = ratio of the refractive indices of the sample vs. IRE, and n_p = refractive index of the IRE. Practically, the sample is placed in close optical contact with one of the crystal. In this study, ATR-IR was used for identifying functional groups on the surface of chitosan films before and after chemical modifications. Sampling depth of characterization is 1-1.5 μm.

1.4.5 Protein adsorption on polymer surface

The study of protein adsorption has attracted considerable attention in the last few decades.[22,23] The adhesion of proteins to surfaces is particularly important in such fields like bioengineering and bioscience. The driving force for protein adsorption may include, van der Waals, hydrophobic interactions, hydrogen bonding, and electrostatic interactions between oppositely charged surface and protein domains.

In this work, protein adsorption on the modified chitosan films surfaces was studied using albumin (bovine serum albumin or BSA) and lysozyme (chicken egg white). BSA is one of the more abundant blood proteins. Its biological functions are transport and maintenance of colloid osmotic pressure.[24] Lysozyme is an antimicrobial protein that has a hydrophobic patch and an uneven charge distribution.[25] An isoelectric point (pI) of lysozyme is 11 and contains larger amount of –OH, $–NH_2$ than –COOH groups. Albumin has a pI of 4.8. It is therefore a –COOH rich protein. Adsorptivity of proteins on the chitosan surface before and after modification was compared. These results could be used to design a surface that can control the amount of protein adsorption.