CHAPTER II

THEORETICAL AND LITERATURE REVIEWS

2.1 Surfactants

2.1.1 General structural features[4]

A surfactant (a contraction of the term *surface-active agent*) is a substance the, when present at low concentration in a system, has the property of adsorbing onto the surfaces or interfaces of the system and of altering to a marked degree the surface or interfacial free energies of those surfaces (or interfaces). The term *interface* indicates a boundary between any two immiscible phases; the term *surface* denotes an interface where one phase is a gas usually air.
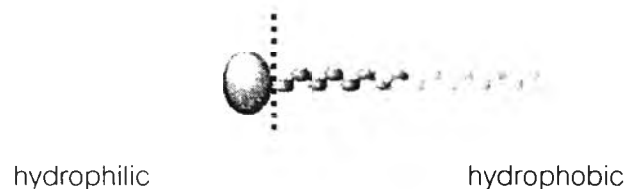
hydrophilic   hydrophobic

Figure 2.1 Surfactant.

Surface-active agent have a characteristic molecule structure consist of a structure group that has very little attraction for the solvent, know as a *lyophobic group*, together with a group that has strong attraction for the solvent, called the *lyophilic group*, this is known as an *amphipathic* structure (Figure 2.1). When a surface-active agent is dissolved in a solvent, the presence of the lyophobic group in the interior of the solvent may cause distortion of the solvent liquid structure, increasing the free energy of the system. In a aqueous solution of the surfactant this distortion of the water by the lyophobic (hydrophobic) group of the surfactant, and the resulting increase in the free

energy of the system when it is dissolved, means that less work is needed to bring a surfactant molecule than a water molecule to the surface. The surfactant therefore concentrates at the surface. Since less work is now needed to bring molecules to the surface, the presence of the surfactant decrease the work needed to create unit area of surface (the surface free energy per unit area, or surface tension). On the other hand, the presence of the lyophilic (hydrophilic) group prevents the surfactant from being expelled completely from the solvent as a separate phase since that would require dehydration of hydrophilic group. The amphipathic structure of the surfactant therefore cause not only concentration of the surfactant at the surface and reduction of the surface tension of the water, but also orientation of the molecule at the surface with its hydrophilic group in the aqueous phase and its hydrophobic group oriented away from it.[5]

2.1.2 Classification of surfactant

The chemical structures of groupings suitable as the lyophobic and lyophilic portions of the surfactant molecule vary with nature of the solvent and the conditions of use. In a highly polar solvent such as water, the lyophobic group may be a hydrocarbon or fluorocarbon or siloxane chain of proper length, whereas in the less polar solvent only some of these may be suitable. In the polar solvent such as water, ionic or highly polar groups may act as lyophilic groups, whereas in a nonpolar solvent such as heptane they may act as lyophobic groups. As the temperature and use conditions (e.g., presence of electrolyte or organic additives) vary, modifications in the structure of the lyophobic and lyophilic groups may become necessary to maintain surface activity at suitable level. Thus, for surface activity in a particular system the surfactant molecule must have a chemical structure that is amphipathic in that solvent under the conditions of use.

The hydrophobic group is usually a long-chain hydrocarbon residue, and less often a halogenated or oxygenated hydrocarbon or siloxane; the hydrophilic group is an ionic or highly polar group. Depending on the nature of the hydrophilic group, surfactants are classified as:

Anionics

An anionic surface-active agent is the reaction product of an organic compound such as a high molecular weight acid or alcohol with an inorganic compound such as sodium hydroxide or sulfuric acid, yielding a product wherein the organic part of the molecule, or the water-insoluble part of the molecule, has a negative charge and the water-soluble part of the molecule wherein the sodium ion has the positive charge. For example, soap is an anionic and has the following structure:

$$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COO^-Na^+$$

Also, the reaction product of a long-chain alcohol and sulfuric acid and thus neutralized with sodium hydroxide has the following structure:

$$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COSO_4^-Na^+$$

The anions have the advantage of being high and stable foaming agent; however, they do have the disadvantage of being sensitive to minerals and presence of minerals in water (water hardness) or pH changes.

Cationics

Cationics are formed in reactions where alkyl halides react with primary, secondary, or tertiary fatty amine. Here the water-insoluble part of the molecule has a positive charge and water-soluble part of the molecule is negatively charged, thus giving it the name of a cationic surface-active agent.

Cationic surface-active agents reduce surface tension and are used as wetting agents in acid media. However, a disadvantage of a cationic surface-active agent is that they have no detergent action when formulated into an alkaline solution.

$$C_{12}H_{25}NH_2^+(CH_3)_3Cl^-$$

Nonionics

Nonionic surface-active agents have a hydrophobic/hydrophilic balance wherein there is neither a negative nor a positive charge in either part of the molecule, thus giving it the nonionic terminology. These surface-active agents have the advantage that they are not affected by water hardness or pH changes as the anionic and cationic surfactant are, and in many case it is an advantage that they are considered medium to

low foaming agents. It is especially advantageous when a very low foaming surface-active agent is required. An example of the chemical structure of a nonionic surface-active agent is shown below in the reaction product of lauryl alcohol and ethylene oxide.

Zwitterionics

A surfactant in which has the hydrophilic part contains both positive and negative charges. Both positive and negative charges may be present in the surface-active portion. For example, $RN^-H_2CH_2COO^-$ (long-chain amino acid), $RN^+(CH_3)_2CH_2CH_2SO_3^-$ (sulfobetaine). It is possible for surfactants to have more than one charge of either sign, or to lose one charge by charge by addition or removal of a portion.

2.1.3 Properties of surfactant

Surface tension

The tension acting in the surface of a phase directed towards the interior of the phase is cause by intermolecular attractions between the molecules at the surface and those located the surface. But the interfacial tension is the tension at the interface between two phases.

Reduction of surface or interfacial tension is one of the most commonly measured properties of surfactants in solution. Since, it depends directly on the replacement of molecules of solvent at the interface by molecules of surfactant.

The molecules at the surface of a liquid have potential energies greater than those of similar molecule in the interior of the liquid. This is because attractive interactions of molecules at the surface with those in the interior of the liquid are greater than those with the widely separated molecules in the gas phase. Because the potential energies of molecules at the surface are greater than those in the interior of the phase, an amount of work equal to this difference in potential energy must be expended to bring a molecule from the interior to the surface. The surface energy per unit are, or surface tension; it is the minimum amount of work required to bring sufficient molecules to the surface from the interior to expand it by unit area. Although more correctly thought of as a surface free energy per unit area, surface tension is often

conceptualized as a force per unit length at a right angle to the force required to pull apart the surface molecules in order to permit expansion of the surface by movement into it of molecules from the phase underneath it.

At the interface between two condensed phases, the dissimilar molecules in the adjacent layers facing each other across the interface also have potential energies different from those in their respective phases. Each molecule at the interface has a potential energy greater than that of a similar molecule in the interior of its bulk phase by an amount equal to its interaction energy with the molecules in the interior of its bulk phase minus its interaction energy with the molecules in the bulk phase across the interface.

If we now add to a system of two immiscible phases (e.g., heptane and water, a surface-active agent that is adsorbed at the interface between them, it will orient itself there, mainly with the hydrophilic group toward the water and the hydrophobic group toward the heptane. When the surfactant molecules replace water and/or haptane molecules of the original interface, the interaction across the interface is now of the between the hydrophilic group of the surfactant a water molecules on one side of the interface and between the hydrophobic group of the surfactant and heptane on the other side on the interface. Since, these interactions are now much stronger than the original interaction between the highly dissimilar heptane and water molecules, the tension across the interface is significantly reduced by the presence thereof the surfactant. Since air consists of molecules that are mainly nonpolar, surface tension reduction by surfactants at the air aqueous solution interface is similar in many respects to interfacial tension reduction at the heptane-aqueous solution interface.

Measurement of the surface or interfacial tension of liquid system is accomplished readily by a number of methods of which the most useful and precise for solutions of surfactants are probably the drop-weight and Wilhelmy plate methods. An excellent discussion of the various methods for determining surface and interfacial tension is included in the monograph on emulsions.[6]

Critical micelle concentration (C.M.C.)

A property of surfactants that may be as fundamental, and certainly is as important, as their property of being adsorbed at interfaces. This property is micelle formation – the property that surface-active solutes have of forming colloidal-sized clusters in solution. Micelle formation, or micellization, is an important phenomenon not only because a number of important interfacial phenomena, such as detergency and solubilization, depend on the existence of micelles in solution, but because it affects other interfacial phenomena, such as surface or interfacial tension reduction, that do not directly involve micelles.

Almost from the very beginning of the study of properties of surfactant solutions (actually, soap solutions), it was recognized that their bulk properties were unusual and indicated the presence if colloidal particles in the solution.

When the equivalent conductivity (specific conductance per gram-equivalent of solute) of an anionic surfactant of type Na^+R^- in water and plotted against the square root of the normality of the solution, the curve obtained, instead of being the smoothly decreasing curve characteristic of ionic electrolytes of this types, has a sharp break in it, at low concentrations (Figure 2.2). This a break in the curve, with its sharp reduction in the conductivity of the solution indicating a sharp increase in the mass per unit charge of the material in solution, is interpreted as evidence of the formation at that point of micelles from the unassociated molecules of surfactant with part charge of the micelle neutralized by associate counterions.
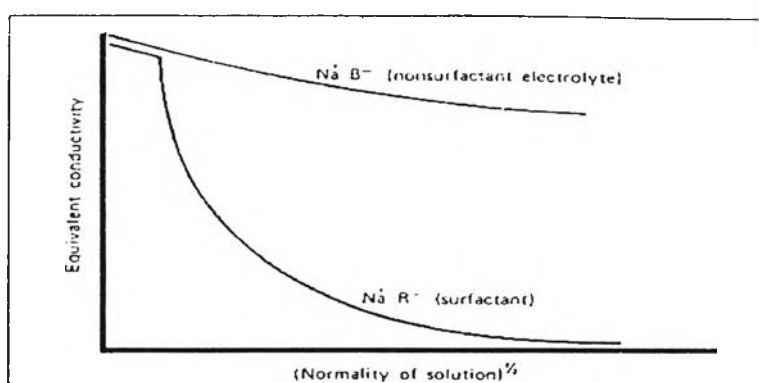
Figure 2.2 Plot of equivalent conductivity versus (normality of solution)$^{1/2}$ for an aqueous of surfactant of type Na^+R^-.

The concentration at which this phenomenon occurs is called the critical micelle concentration (C.M.C.). Similar breaks in almost every measurable physical property that depends on size or number of particles in solution are shown by all types of surfactants-nonionic, anionic, cationic, and zwitterionic in aqueous media.

The determination of the value of C.M.C. can be made by use of any of these properties, but most commonly the breaks in the electrical conductivity, surface tension, light scattering, or refractive index concentration curves have been used for this purpose. Critical micelle concentrations have also very frequently been determined from the change in the spectral characteristics of some dyestuff added to the surfactant solution when the C.M.C. of the latter is reached. However, this method is open to the serious objection that the presence of the dyestuff may affect the value of the C.M.C.

Detergency

The term detergency, as used to describe a property of surface-active agents, has a special meaning. As a general term, it means cleaning power, but no surfactant by itself can clean a surface. The term detergency, when applied to a surface-active agent, means the special property it has of enhancing the cleaning power of a liquid. This it accomplishes by a combination of effects involving adsorption at interfaces, alteration of interfacial tensions, solubilization, emulsification, and formation and dissipation of surface charges.

Solubilization

One of the important properties of surfactants that are directly related to micelle formation is solubilization. Solubilization may be defined as the spontaneous dissolving of a substance (solid, liquid, or gas) by reversible interaction with the micelles of a surfactant in a solvent to form a thermodynamically stable isotropic solution with reduced thermodynamic activity of the solubilized material. Although both solvent-soluble and solvent-insoluble material may be dissolved by the solubilization mechanism, the importance of the phenomenon from the practical point of view is that and makes possible the dissolving of substance in solvents in which they are normally insoluble.

Solubilization into aqueous media is of major practical importance in such areas as the formulation of products containing water-insoluble ingredients, where it can replace the use of organic solvents or cosovents; in detergency, where solubilization is believed to be one of the major mechanisms involved in the removal of oily soil; in micellar catalysis of organic reactions; in emulsion polymerization, where it appears to be an important factor in the initiation step; in the separation of materials for manufacturing or analytical purposes; and in enhanced oil recovery, where solubilization produces the ultra low interfacial tension required for mobilization of the oil.

2.2 Adjuvant

An adjuvant is any compound that can be added to herbicide formulation to facilitate the mixing, application, or effectiveness of that herbicide. Adjuvants are already included in the formulations of some herbicides available for sale (e.g. RoundUp®), or they may be purchased separately and added into a tank mix prior to use.[7] Herbicides must overcome a variety of barriers to their entry into plants in order to be effective. For example, herbicides applied to foliage must remain on the leaf instead of beading up and rolling off, then get past the leaf hairs and waxes on the leaf surface, then finally penetrate through the cell walls and cell membranes.[8,9] Some adjuvants alter the formulation so that they more completely and evenly cover plant surfaces thereby keeping the herbicide in contact with plant tissues rather than beading up and rolling off. Others increase the formulation's penetration through the cuticular wax, cell walls and/or stomatal openings. In some situations, an adjuvant may enhance the formulation's ability to kill the targeted species without harming other plants.[11] Adjuvants may also improve a herbicide's efficacy so that the concentration or total amount of herbicide required to achieve a given effect is reduced, sometimes as much as five- or ten-fold. In this way adding an appropriate adjuvant can decrease the amount of herbicide applied and lower total costs for weed control.[12] Adjuvants are chemically and biologically active (NOT chemically inert) compounds. They produce pronounced effects in plants and animals, and some adjuvants have the potential to be mobile and pollute surface or groundwater sources. Be especially aware of the use of adjuvants near water, as adverse effects may occur in some aquatic species. The Material Safety Data Sheets

(MSDSs) of most adjuvants will list materials that are incompatible with the adjuvant, conditions in which they should not be used, and some toxicological information (LC50 or LD50s), but this information is usually not as complete as that found on herbicide labels and MSDSs. Unfortunately, there is no good system available to help you assess which types of adjuvants (if any) to select for different situations, much less which brand will best meet your needs. Most herbicide labels specify the *type* of adjuvant to use for best control, but there are many different brands of most types of adjuvants to select from and few sources of good information regarding their relative performance under different conditions. The best source of information is most likely your local agriculture or university extension agent, local county weed coordinator or herbicide company representative. Local herbicide dealers may also offer suggestions, but be sure that the dealer is qualified to make recommendations.[13] Adjuvants may be classified in a variety of ways, such as by their function (activator or utility), chemistry (such as organosilicones), or source (vegetable or petroleum oils). This adds to confusion about which adjuvant to select in different situations. In this chapter, we group adjuvants by their function, as either activator adjuvants or utility adjuvants. Activator adjuvants work to enhance the activity of the herbicide, often by increasing rates of absorption of the herbicide into the target plant(s). Utility adjuvants, sometimes called spray modifiers, work by altering the physical or chemical characteristics of the spray mixture to improve its ease of application, its ability to remain on the plant surface rather than rolling off, or its persistence in the environment. There is much disagreement regarding how certain adjuvants should be categorized, and to complicate matters further some adjuvants perform more than one function and thus really do fit in more than one category.

Adjuvant Types*

Activator Adjuvants	Utility Adjuvants (including Spray Modifiers)
Surfactants	- Wetting agents (spreaders)
- Nonionic (incl. organosilicones)	- Dyes
- Ionic	- Drift control & foaming agents
- Amphoteric	- Thickening agents
	- Deposition agents (stickers)

Oil adjuvants (incl. crop oil concentrates)	- Water conditioners
- Petroleum oil concentrates	- Compatibility agents
- Vegetable oils	- pH buffers
	- Humectants
Ammonium (nitrogen) fertilizers	- Defoaming & antifoam agents
	- UV absorbents

Surfactants are the most widely used and probably the most important of all adjuvants. The name is derived from **surface active agents** and these compounds facilitate or enhance the emulsifying, dispersing, spreading, sticking or wetting properties of the herbicide tank mix (includes spray modifiers). Surfactants reduce surface tension in the spray droplet, which ensures that the formulation spreads out and covers plants with a thin film rather than beading up. This facilitates herbicide absorption into the plant. Surfactants can also directly influence the absorption of herbicides by changing the viscosity and crystalline structure of waxes on leaf and stem surfaces, so that they are more easily penetrated by the herbicide.[14,15] Some herbicide formulations come with a surfactant already added, but most require the addition of a surfactant for good control results. Surfactants are generally not added to pre-emergent herbicides that are applied directly to soil.

Surfactants work by improving contact between spray droplets and plant surfaces, and enhance absorption by:

1. Making the spray solution spread more uniformly on the plant

2. Increasing retention (or 'sticking') of spray droplets on the plant

3. Increasing penetration through hairs, scales, or other leaf surface structures

4. Preventing crystallization of spray deposits

5. Slowing drying and increasing water retention in the spray droplets

The effectiveness of a surfactant is determined by environmental conditions, characteristics of the target plant, and by interactions between the surfactant and the herbicide. Surfactants contain varying amounts of fatty acids, which are compounds capable of binding to two types of surfaces, such as oil and water. It is important that the degree of solubility of the surfactant in oil or water match the solubility of the herbicide. The Hydrophilic –Lipophilic Balance (HLB) is a measure of the balance

between hydrophilic (water-soluble) and lipophilic (oil-soluble) components in fatty acids. A surfactant's HLB can therefore indicate the conditions under which the surfactant will perform best.

Hydrophilic-Lipophilic Balance (HLB)

Surfactants contain both hydrophilic and lipophilic components (this is called amphiphatic). The hydrophilic lipophilic balance (HLB) is a measure of the molecular balance of the hydrophilic and lipophilic portions of the compound. Many herbicides have an optimum surfactant HLB, and surfactants that most closely match a particular herbicide's optimum HLB will optimize the formulation's spread on and penetration into plants. Unfortunately, information about the HLB of most surfactant products is not available or hard to find, and so matching them appropriately is difficult. For nonionic surfactants, the optimum surfactant HLB for a herbicide can be predicted based on the solubility of the herbicide in water. For ionic surfactants, the HLB can be estimated by observing their dispersablity in water (with no dispersion = 1 to 3; poor dispersion = 3 to 6; unstable milky dispersion = 6 to 8; stable milky dispersion = 8 to 10; translucent to clear dispersion = 10 to 13; and clear solution = 13+). Typically, low HLB surfactants work best with water insoluble herbicides, while high (>12) HLB surfactants work best for water-soluble herbicides. For example, surfactants with a high HLB are more active with the hydrophilic herbicide glyphosate, while more lipophilic, low HLB surfactants are more active with the lipophilic quizalofop-P ester. Surfactants with intermediate HLB values are the most active with intermediately soluble nicosulfuron. Additionally, low HLB surfactants permit the formation of invert emulsions (water-in-oil). Mid and upper-range HLB may be wetting agents or for oil-in-water emulsions, and high HLB surfactants are often used as detergents or solubilizers. One the other hand, a surfactant that is incorrectly matched may even.

Alkylamine ethoxylate[16]

$$CH_3(CH_2)_{m-1}N \begin{cases} (CH_2CH_2O)_xH \\ (CH_2CH_2O)_yH \end{cases}$$

$x+y = 2, 3, 4...$
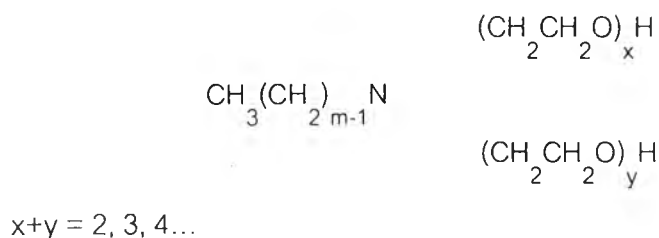
Polyethoxylated fatty amine surfactants are mindly cationic. They tend to approach nonionic character with increasing degree of ethoxylation. Members of this series of surfactants find application in leather and textile processing acids, paints, ink, cosmetics, manufacture and production of paper and plastic products as emulsifiers, dispersants, wetting agents, antistats and dye leveling. Especially, they were used as adjuvant in herbicide which was referred on above titles.

2.3 Mass spectrometry (MS)[17-21]

Mass spectrometry probable is the most versatile and comprehensive analytical technique currently at the disposal of chemists and biochemists. Since the early 1900s, it has enjoyed prominence in several areas of physics, chemistry, geology, cosmochemistry, nuclear science, material science, archeology, petroleum industry, forensic science, and environment science. The ultrahigh detection sensitivity and high molecular specificity are the hallmarks of this technique. Molecular mass determination, structure elucidation, quantification at trace level, and mixture analysis are some of the major applications of mass spectrometry. In addition, the technique has been used to study ion chemistry and ion-molecule reaction dynamics; to provide data on physical properties such as ionizing energy, appearance energy, enthalpy of a reaction, and proton affinities; and to verify theoretical predictions that are bases on molecular orbital calculations.

2.3.1 Basic concepts of mass spectrometry

Mass spectrometry is an analytic technique that measures the masses of individual molecules and atoms. As conceptualized in Figure 2.3, the first essential step in mass spectrometry analysis is to convert the analyte molecules into gas-phase ionic

species because one can experimentally manipulate the motion of ions, and to detect them (which is not possible with neutral species). The excess energy transferred to the molecule during the ionization event leads to fragmentation. Next, a mass analyzer separates these molecular ions and their charged fragments according to their m/z (mass/charge) ratio. The ion current due to these mass-separated ions is detected by a suitable detector and displayed in the form of a mass spectrum. To enable the ions to move freely in space without colliding or interacting with other species, each of these steps is carried out under high vacuum (10^{-4}-10^{-8}).
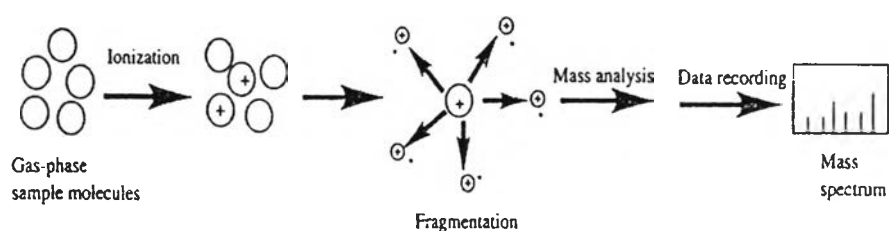
Figure 2.3 Basic concept of mass spectrometry analysis.

Thus, a mass spectrometry consists of several essential functional unit; they are depicted in Scheme 2.1 in the form of the block diagram. These units are
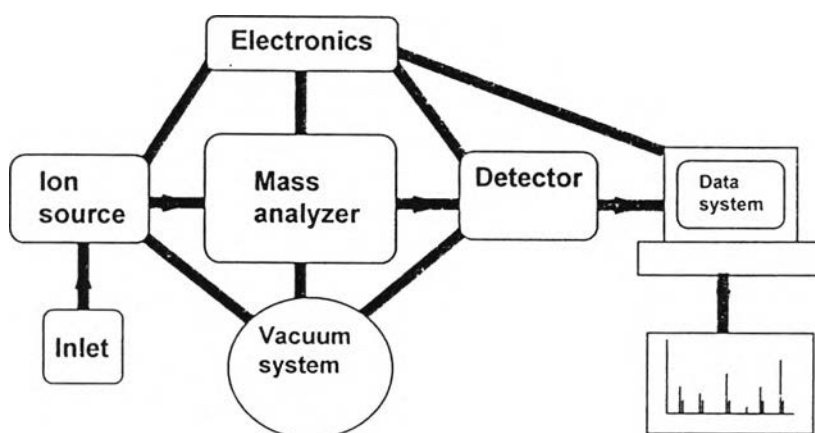
Scheme 2.1 Basic component of a mass spectrometer.

- An inlet system to transfer a sample to the ion source

- A vacuum system to maintain a very low pressure in the mass spectrometer
- An ion source to convert the neutral sample molecules into gas-phase ions
- A mass analyzer to separate and mass-analyze ionic species
- A detector to measure the relative abundance of the mass-resolved ions
- Electronics to control the operation of the various units
- A data system to record, process, store, and display the data

The overall analytic capability of a mass spectrometry system depends on the combined performance of these individual units. Several ionization techniques and mass analyzers have emerged, each with special purpose.

Ionization techniques

Example;

- Electron Impact (EI)
- Chemical Ionization (CI)
- Fast Ion or Atom Bombardment Ionization (FAB)
- Thermospray Ionization (TSP)
- Electrospray Ionization (ESI)
- Matrix-Assisted Laser Desorption/Ionization (MALDI) etc.

Mass analyzers

Example;

- Quadrupolar Analyzers
- The Quadrupole Ion Trap or Quistor
- Time-of-Flight Analyzers
- Magnetic and Electromagnetic Analyzers

Detectors

Example;

- Photographic Plates and Faraday Cylinders
- Electron Multipliers
- Array Detectors
- Photon Multipliers

For this work, the ionization technique which was chosen for analysis of alkylamine ethoxylate is Matrix-Assisted Laser Desorption/Ionization and the mass analyzer is Time-of-flight. The principles of these techniques were explained, respectively.

2.3.2 Matrix-Assisted Laser Desorption/Ionization

The irradiation by an intense laser beam is another suitable mode of depositing a large amount of energy into sample molecules for their desorption into the gas phase. In practice, the sample (guest) is admixed with an excess of the host matrix material, and is irradiated with a laser beam of short pulses of 10-20 ns duration and $\sim 10^{-6}$ W/cm^2 irradiance power. The main criterion of choosing a host matrix is that it absorbs energy at the wavelength of the laser radiation. This matrix-wavelength combination permits a large amount of energy to be absorbed efficiently by the matrix, and subsequently transferred to the sample in a controlled manner. Absorption of energy from the laser beam causes evaporation of the matrix. The analyte molecules are entrained in the resultant gas-phase plume and become ionized via gas-phase proton-transfer reactions. An astonishing feature of this process is that very large molecules are desorbed into gas phase without undergoing thermal degradation as is shown for a monoclonal antibody in Figure 2.4.
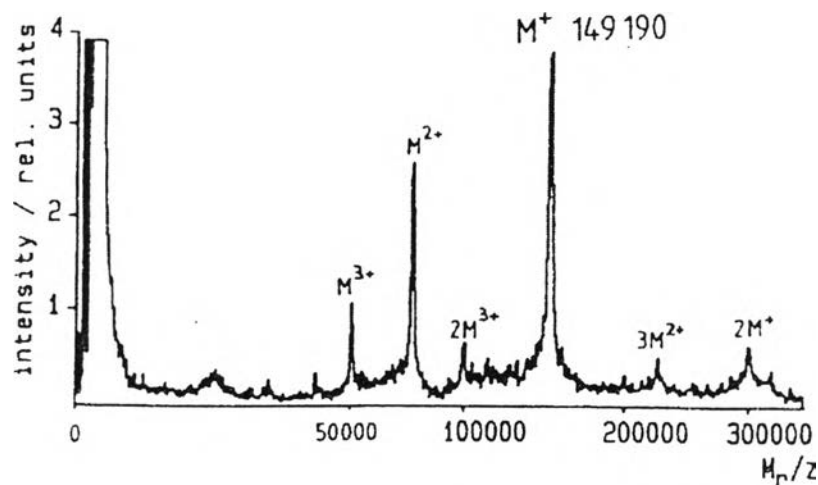
Figure 2.4 MALDI mass spectrum of a monoclonal antibody (IgG of mouse against a specific human lymphokine)

Positive- and negative-ion analyses can both be performed with MALDI. The MALDI mass spectra of proteins and peptides typically contain signals due to singly protonated target molecules and their oligomeric ions (e.g., $[M+H]^+$, $2M+H)^+$). In some cases, doubly and triply charged protonated ions of low abundance are also formed. With increasing mass of the analytes, multiply charged ions increase in abundance. In addition, the Na^+ and K^- adducts are also a common feature of MALDI spectra of biological extracts.

Mechanism of Desorption and Ion Formation

The process by which large molecules are desorbed and ionized by absorption of photons is not clearly understood. Three different models have been proposed to explain desorption of the matrix-sample material from the crystal surface : (1) quasithermal evaporation as a result of increased molecular motion, (2) expulsion of upper lattice layers, and (3) an increase in the hydrodynamic pressure dut to rapidly expanding molecules in the crystal lattice. The analyte species are entrained in a dense plume of the desorbed matrix molecules. The initial velocity is considered an important value in characterizing the desorption process. However, there is no consensus yet as to how the sample molecules are ionized. The widely accepted view is that which was shown in Figure 2.5, following their desorption as neutrals, the sample molecules are

ionized by acid-base proton transfer reactions with the protonated matrix ions in a dense phase just above the surface of the matrix. This view has been supported by the fact that the spectra of proteins obtained with UV- or IRMALDI are similar. The protonated matrix molecules are generated by a series of photochemical reactions. An alternate view is that the singly excited matrix molecules and not the frequently invoked photoionized matrix molecules are common precursors for all subsequent ionization events. According to this model, two excited matrix molecules are required for the generation of free gaseous ions from the sample molecules.
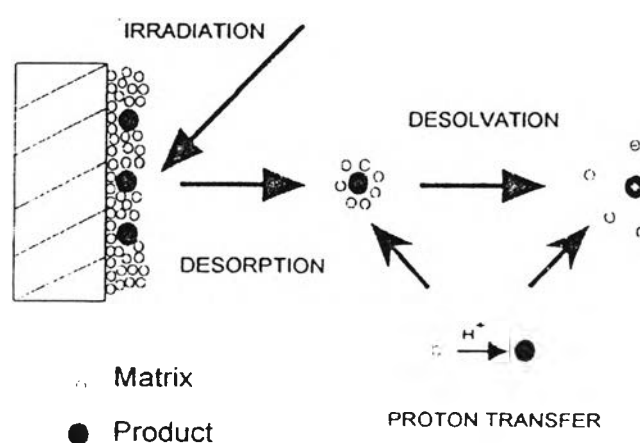
Figure 2.5 Diagram of principle of the MALDI.

A 1998 study by Beavis and colleages has advocated that the ionization of the sample molecules occurs in a warm polar fluid that is formed from the matrix-sample crystals by absorption of laser energy. The ionization of acidic matrices in this warm fluid creates a population of free protons. The acceptance or loss of a proton in this fluid results in the formation of the sample ions. This proposal, however, does not overrule the possibility of gas-phase ionization. The unified model presented by Karas et al. assumes the formation of initial clusters that comprise of matrix, protonated or deprotonated sample molecules, and counter ions and desolvation of the desorbed clusters to free ionic species via proton transfer and evaporation of neutrals. Highly charged clusters are neutralized to produce singly charged ions by capture of the electrons that are generated during photoionization of the matrix. The electron capture process is also assumed to be the cause of fragmentation reactions.

Instrumentation

Current applications of MALDI overwhelmingly use TOF instruments because the pulsed nature of a laser beam matches well with the pulsed scanning mode of TOFMS. In addition, the unlimited mass range, short duty cycle, high ion transmission, and multichannel detection features of TOFMS are also highly desirable for MALDIMS experiments. A schematic diagram of MALDI-TOFMS is presented in Figure 2.6. Linear and reflection TOF instruments have both been used for MALDIMS. The combinations of MALDI with magnetic sector, quadrupole ion trap, and FTICR instruments have also emerged. Especially FTICR has made a significant contribution to the accurate mass measurements of proteins.
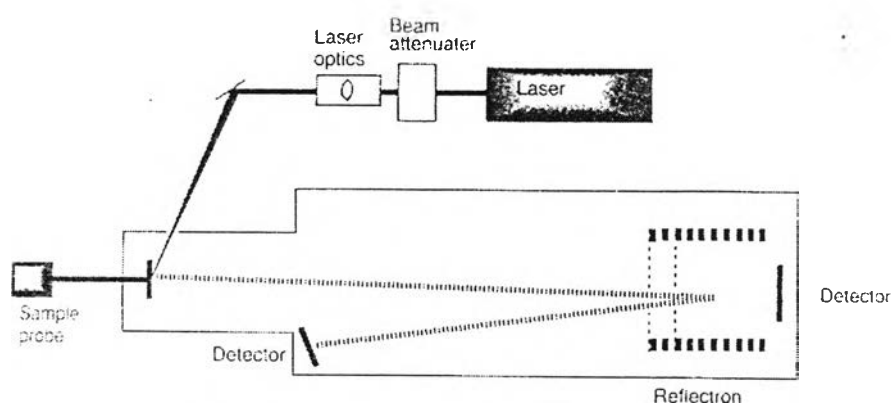
Figure 2.6 A schematic of MALDI-TOFMS.

Various laser systems have been used to rapidly deposit energy into the matrix-sample combination. Most applications have used UV lasers, such as the N2 laser (337nm), the frequency-tripled (355nm) and frequency-quadrupled (266nm) Nd: YAG laser, and the ArF excimer laser (193 rim). IR lasers have also been used to produce the MALDI effect. The TEA C02 laser (10.6 µm), the Q-switched Er: YAG laser (2.94 µm), and the Cr: LiSAF or Nd: YAG pumped optical parametric oscillators (OPO) laser (3.28 µm) are the most common IR lasers. UV and IR lasers both yield similar spectra for proteins, although a better resolution has been obtained for some proteins with an IR laser.

The Role of a Matrix

The matrix performs two important functions: (1) it absorbs photon energy from the laser beam and transfers it into excitation energy of the solid system; and (2) it serves as a solvent for the analyte, so that the intermolecular forces are reduced and aggregation of the analyte molecules is held to a minimum. Some desirable characteristics of a typical MALDI matrix are

- A strong light absorption property at the wavelength of the laser flux.

- The ability to form microcrystals with the sample.

- A low sublimation temperature, which facilitates the formation of an instantaneous high-pressure plume of matrix-sample material during the laser pulse duration.

- The participation in some kind of a photochemical reaction so that the sample molecules can be ionized with high yields.

Several matrix-laser combinations have been tested successfully. Some commonly used matrices, along with the wavelengths at which they are used, the solvents in which they can be dissolved, and fields of their applications, are listed in Table 2.1. For peptides and, small-molecular-mass proteins (< 10,000 Da), good results are obtained with α-cyano-4-hydroxycinnamic acid, whereas high-mass proteins are analyzed with sinapinic acid. The use of 3-amino-4-hydroxybenzoic acid and 2,5-dihydroxybenzoic acid has been recommended for the analysis of oligosaccharides. Ice has been used as a matrix for IRMALDI of proteins. 3-Hydroxypicolinic acid (HPA) has gained a wide acceptance for the analysis of oligonucleotides.

Table 2.1. The matrices used in MALDIMS

Matrix	Mass (Da)	Solvents	Applications
3-Amino-4-hydroxybenzoic acid	153	ACN, water	Oligosaccharides
2,5-Dihydroxy benzoic acid (DHB)	154	ACN, water, methanol, acetone, chloroform	Oligosaccharides, peptides, nucleotides, polymers
5- Hydroxy-2-mthoxybenzoic acid	168	ACN, water	Lipids
2[4-hydroxyphenylazo]benzoic acid (HABA)	242	ACN, water, methanol	Proteins, lipids, polymers
Cinnamic acid	148	ACN, water	General
α-Cyano-4-hydeoxycinnamic acid	189	ACN, water, ethanol, acetone	Peptides, lipids, nucleotides, polymers
Dithranol	226	THF	Polymers
4-Methoxycinnamic acid	178	ACN, water	Proteins
Indole acrylic acid (IAA)	187	Acetone	Polymers
All-trans retinoic acid (RTA)	300	THF	Polymers

Sample Preparation

As with the other desorption ionization methods, the preparation of the sample for MALDI analysis requires utmost care. The homogeneity of the sample-matrix mixture is a critical factor to obtain good sample ion yields. Fortunately, MALDIMS is somewhat more tolerant of impurities, buffers, salts, and mixtures. Several techniques have emerged for the sample preparation. These techniques include the

- Dried-droplet technique
- Fast evaporation technique

- Sandwich matrix technique
- Spin-dry technique
- Seed-layer technique

A saturated solution (or at least a mmol/mL concentration) of the matrix is first prepared in the deionized water. Other solvents, such as acetonitrile or methanol, may be added to increase its solubility. The solvent used to dissolve the matrix must also be compatible with the sample to be analyzed. For example, for protein analysis, a solvent mixture of water-acetonitrile that contains 0.1% TFA is recommended. A few microliters of a μmol/liter sample solution in 0.1% TFA is mixed with equal volume of the matrix solution to give a matrix: sample molar ratio of 1,000-10,000: 1. In the *dried-droplet technique*, a 1-μL portion of this mixture is applied to a stainless-steel or gold-coated sample well. In order to obtain a fine-grained morphology of the crystal formation, the sample spot is evaporated slowly in the ambient air or by a gentle stream of cold air. Other researchers have advocated a *fast evaporation* of the matrix-sample mixture. In this procedure, a 0.5-pL drop of the matrix solution in acetone that contains 1-2% water is rapidly deposited on the probe. The acetone rapidly evaporates to leave behind a homogeneous surface of small crystals. This procedure yields enhanced resolution, sensitivity, and mass accuracy. In the sandwich matrix technique, a *thin layer* of the matrix is applied first, followed by 0.1% aqueous TFA, the sample solution, and an additional layer of the matrix. This mixture is allowed to dry. In the *spin-dry technique*, a solution that contains equal volumes of a nitrocellulose (NC) membrane and a matrix is applied to the rotating target. The solution is immediately spin-dried and yields a uniform NC-matrix layer. In the *seed-layer technique*, a seed layer of the matrix is prepared by depositing a droplet (0.5 μL) of the matrix solution on a sample target. The drop is allowed to spread and dry in ambient air. A 0.5-PL drop of 1:1 (vv) analyte-matrix mixture is deposited on the seed layer, and allowed to dry in ambient air. This technique allows rapid crystallization with a high degree of sample homogeneity.

2.3.3 Time-of-flight Mass Spectrometer

A time-of-flight (TOF) mass spectrometer is one of the simplest mass-analyzing devices. Since the 1990s, it has reestablished itself as a mainstream technique and is becoming increasingly useful in meeting the demands of contemporary research in biomedical sciences. The recent successes of TOFMS can be attributed to the development of MALDI, high-speed data processing devices, and focal plane detectors. A few reviews and books can be referred to as additional useful reading material on this subject.

Principle of Operation

A TOF mass spectrometer behaves as a velocity spectrometer, in which ions are separated on the basis of their velocity differences. A short pulse of ions, after exiting the source, is dispersed in time by allowing it to drift in an FFR of a long flight tube. The principle behind the mass analysis is that after acceleration to a constant kinetic energy (equal to zV, where z is the charge on the ion and V the accelerating potential), ions travel at velocities, v, that are an inverse function of the square root of their m/z values:

$$v = (\frac{2zV}{m})^{1/2}$$
(2.1)

The lighter ions travel faster and reach the detector placed at the end of the flight tube (of length L) earlier than do the heavier ones. Thus, a short pulse of ions is dispersed into packets of isomass ions (Figure 2.7). Therefore, mass analysis of ions that enter the flight tube can be accomplished by determining their time of arrival given by

$$t = \frac{L}{v} = L(\frac{m}{2zV})^{1/2}$$
(2.2)

In order to convert the time spectrum into a mass spectrum, the instrument is mass-calibrated by measuring the flight times of two different known mass ions.
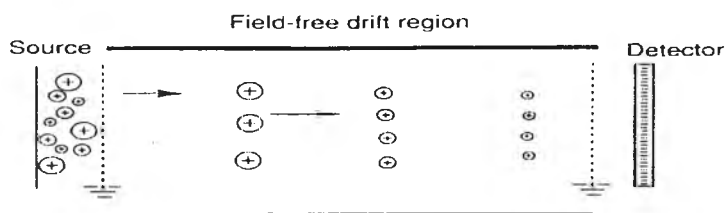
Figure 2.7. Principle of the mass separation by a time-of-flight mass analyzer. Ions are separated on the basic of their size; high-ions (big circles) travel more slowly than the lighter ions

A primary requirement in the operation of a TOFMS is that all ions enter the flight tube precisely at the same time. Generating ions in short bursts fulfills this condition. In this respect, TOF instruments are well matched to ^{252}Cf-plasma desorption (PD) and MALDI ion sources. The continuous ion beam sources (e.g., electron ionization and ESI), however, can be coupled with a TOF mass spectrometer, but only after conversion of the generated ions into discrete packets. Pulsing of the accelerating potential converts a continuous ion beam into discrete ion packets.

Mass Resolution

In the past, poor mass resolution was one of the major limitations of TOFMS. The mass resolution of simple linear TOF mass spectrometers is usually less than 500. In TOFMS, this term is given by Eq. 2.3, where Δt is commonly measured in terms of FWHM. The spatial, temporal, and velocity dispersions of ions are the limiting factors in achieving higher resolution in TOF instruments. A higher resolution is obtained only when all ions are formed in the source at the same time and the same location (i.e., their temporal and spatial distributions are minimum), and all have the same kinetic energy. The temporal distribution, which is a combined effect of uncertainties in the time of ion formation as well as limitations of ion detection and time-recording devices, can be minimized with the use of very short ionization pulses and also by increasing the difference in arrival times of two adjacent ions. Increasing the flight path of ions or reducing their velocities (i.e., with lower accelerating potentials) has the effect of increasing the arrival times of different mass ions. The use of lower accelerating

potentials is counterproductive because of the concomitant loss of transmission, and of the greater impact of the energy spread on resolution at lower accelerating potentials. The pulsed ion extraction with very fast rise time can also be used to correct the temporal distribution. The spatial distribution degrades resolution because ions formed in different regions of the ion source are accelerated to different kinetic energies. Ions that are formed to the left of the central line are accelerated to a higher velocity than are ions formed to the right of the central line, resulting in loss of mass resolution. The use of ionization methods that produce ions from a surface, such as ^{252}Cf-M and MALDI, eliminates the spatial width to some extent because in these ionization techniques the plane of ion formation is well defined. Another way to correct for the spatial distribution is to use dual-stage ion extraction optics:

$$R = \frac{m}{\Delta m} = \frac{t}{\Delta t} \tag{2.3}$$

The dominating factor that restricts the resolution in TOF instruments, especially with desorption ionization methods, is the kinetic energy inhomogeneity within the ion beam. The higher-initial-energy ions arrive at the detector sooner than do the same-mass lower initial energy ions. The acceleration region energy spread can be eliminated by the prompt ejection of ions from the source (i.e., by using high accelerating potentials, usually > 10 kV). A further reduction in the energy spread is achieved through an energy-correcting device, known as the reflectron (described below). Another factor that limits resolution is the turnaround time taken by the ions that are traveling initially away from the exit slit. These ions take extra time in exiting the source than ions that have the same initial velocity, but are facing the exit slit. Longer flight tubes and longer flight times can reduce the effect of turnaround time.

In TOF instruments, the time difference in the arrival of various ions at the detector is very short. As an example, m/z 2500 after acceleration through a potential of 6000 V will reach the end of the one-meter-long flight path 5.01 µs after the arrival of m/z 2000. The difference in the arrival times of ions differing by one dalton (say, 2000 and 2001) is even shorter (in nanoseconds). Therefore, the mass selectivity of TOF instruments is also limited by the accuracy with which short intervals of time can be measured.

Time-of-flight mass spectrometers have a number of attractive features, such as theoretically unlimited mass range, high ion transmission, very high spectrum acquisition rate, multiplex detection capability, simplicity in instrument design and operation, reasonable mass resolution, and low cost. The detection sensitivity of TOF instruments is much higher than in scanning instruments because they can record all the ions that reach the detector after each ionization event, and because they have high ion transmission efficiency. A major asset of TOF mass spectrometers is their ability to record a complete mass spectrum in time intervals as short as 25. These attributes make TOFMS an attractive research instrument as well as a valuable analytical tool.

Reflectron

A reflectron is a new development that corrects for any initial position and energy dispersions in the accelerator region of a TOF instrument. This elegant device is in fact an electrostatic mirror that consists of a series of electrical lenses, each with progressively increasing repelling potential (Figure 2.8). The initial spatial spread is translated into a velocity spread, which can be readily corrected by the mirror. A reflectron works on a principle that the ions that enter this device after traversing the first FFR (L_1) are slowed down until they come to rest, and then their direction of motion is reversed, and finally, they are reaccelerated into a second FFR (L_2). Qualitatively, the faster-moving ions (i.e., ions with excess energy $zV + U_0$) spend less time in the drift
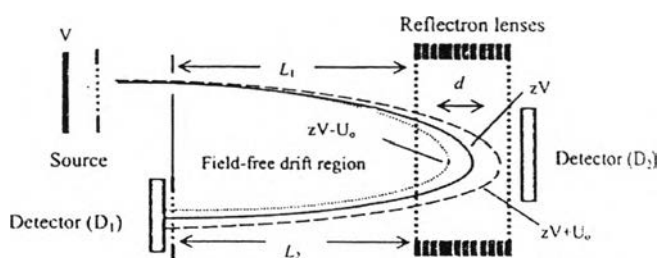

Figure 2.8 A sketch of a reflectron time-of-flight mass analyzer. All ions of the same mass, but that differ in kinetic energy, are made to arrive at the same time at a detector (D_1) that is located at the end of second field region (L_2).

regions, but penetrate to a greater depth (d) into the reflecting field and consequently, spend more time there. This extra time in the mirror compensates for the shorter flight

times of faster ions in the drift regions, with the result that all isomass ions arrive simultaneously at the detector that is located at the end of the flight path L_2. Mass resolution is, thus, improved. An additional contributing factor in improving the mass resolution is the longer pathlength of the reflectron (i.e., $L_1 + L_2$). Amass resolution of >20,000 (FWHM) has been achieved with a grid-free reflectron. A considerable improvement in performance is realized over linear TOFMS instruments for <10-kDa molecular mass compounds. The total flight time of an ion in the reflectron is given by

$$t = (\frac{m}{2zV})^{1/2}(L_1 + L_2 + 4d) \tag{2.4}$$

The single-stage and dual-stage reflectrons are both in common use. A *single-stage reflectron* is a simple ion mirror in which a single retarding-reflecting field is used. It consists of an entrance grid electrode and a series of ring electrodes as shown in Figure 2.8. The single-stage reflectron provides a first-order correction for the kinetic energy distribution. The *dual-stage reflection* contains two linear retarding voltage regions that are separated by an additional grid. The first stage is smaller than the second stage, but with considerably higher field strength capable of reducing ion kinetic energies to about one-third of their initial values. The dualstage reflectrons provide higher mass resolution than do the single-stage devices. The gain in resolution, however, is at the expense of sensitivity because transmission losses occur when ions pass through the additional grid lenses. The reflecting TOF instruments are usually outfitted with an additional detector behind the reflectron. A conventional linear TOF mass spectrum is recorded with this detector when the reflectron voltage is turned off.

Usually, the detection region of a reflectron is separated from the ionization region by reflecting the ions at a small angle (Figure 2.8) with respect to the incoming ions. In an alternative design, called axial *symmetry reflectron*, the reflected ions travel back along the flight axis of the incoming ions, but with a slight angular divergence. The multichannel plate (MCP) detector used in this design is of annular shape, and is located just outside the ion source.

2.4 Applications of MALDI-TOF-MS to Synthetic Polymers

MALDI is the newest and most promising desorption method for synthetic macromolecules. The amount of structural and compositional information that can be gathered from MALDI analysis of synthetic polymeric materials is substantial. It includes repeat units and end-group identification, structural analysis of linear and cyclic oligomers, tracking of polymerization kinetics, and the estimate of composition and sequence for complex copolymer systems.

Another important application of MALDI-TOF mass spectrometry is the measurement of molar mass of synthetic polymeric materials up to 10^6 Da and beyond. The conventional MM characterization techniques (viscosity, SEC, light scattering) are indirect ways of measurement of molar masses, and the prospect of direct measurements of high MM and MMD has stirred much expectation among polymer scientists.

The MALDI matrices are usually organic compounds. In UV-MALDI, which is most widely used for synthetic polymers, the matrix is an aromatic organic compound carrying oxo, hydroxyl, and/or carboxyl groups; commonly selected matrices are 2,5-dihydroxybenzoic acid (DHB), 2-(4-hydroxyphenylazo)-benzoic acid (HABA), α-cyano-4-hydroxycinnamic acid (CCA), trans-3-indoleacrylic acid (IAA), dithranol, and all-trans retinoic acid.

Determination of average molecular weights of polydispersity polymer

1. Mass-average molecular weight; $\overline{M}w$

$$\overline{M}w = \frac{\sum (N_i M_i^2)}{\sum (N_i M_i)}$$

2. Number-average molecular weight; $\overline{M}n$

$$\overline{M}n = \frac{\sum (N_i M_i)}{\sum N_i}$$

Where N_i, M_i represent signal intensity in peak area and mass for the oigomer containing i monomer.

2.5 Literature Reviews

Surfactant of alkylamine ethoxylate is adjuvant in which increase diffusive rate of glyfosate into membrane of herbs. Two research groups published work on analysis of nonionic polyethoxylate in environment waters by liquid chromatography/electrospray mass spectrometry. The first group, Crescenzi, Corcia and Samperi[22] extracted aliphatic ethoxylate alcohols (AEOs) and nonylphenol polyethoxylates (NPEOs) in aqueous environment waters by graphitized carbon black (GCB). Then they isolated AEOs and NPEOs from both surfactants and biointermediates acidic in nature by differential elution. Liquid chromatography/electrospray mass spectrometry were used to analysis of these surfactants. Chromatography was adjusted for elution all the oligomers of NPEO and of the various AEO homologues as single peaks. They found that this chromatographic condition enhances detection levels and simplifies quantification of the analytes. The limit of detection (signal-to-noise ratio = 3) was estimated to be about 20 pg/ component injected into the column or 0.6, 0.02, 0.002 and 0.0002 µg/L of each analyte in the influents and effluents of sewage treatment plants, river water, and drinking water, respectively. This procedure was employed for assessing the concentrations of the analytes in seven influent and seven effluent samples of three mechanical-biological treatment plants. The analysis of a municipal water sample revealed the presence of the analytes at parts-per-trillion levels. Next, Krogh et.al anayted alcohol ethoxylates (AEs) and alkylamine ethoxylates (AMEs) in environmental aqueous samples by liquid chromatography-mas spectrometry[23]. They prepared samples by using solid-phase extraction with Porapak Rdx crartridges. They found that the detection limits and recoveries in ground water and surface water are, respectively, AEs: 16-60 ng/L, 35-93% and AMEs: 0.3-6 µg/L, 28-96%. The lower recoveries are obtained for the apolar surfactant. The procedure was employed on samples of ground water and soil interstitial water collected from farming areas. The individual AEs were detected at concentration levels ranging from 33 to 189 ng/L water.

MALDI is a new useful quantitative technique for biological and polymer. For polymers, there are two research groups. The first group, Yan et.al utilized MALDI mass spectrometry to quantitatively study mixtures of PDMS and PMMA technical polymer

systems. They found that the different length oligomers of PDMS displayed equal ionization/desorption probabilities over a range of 1000 Da to 10,000 Da, regardless of the presence of a third polymer PMMA and MALDI could provide quantitative information of systems with an error less than 2 standard deviation. Next, Murgasova and Hercules quantitatively study of a polystyrene/poly(α-methylstyrene) blend by MALDI mass spectrometry and size-exclusion chromatography[24]. They found that the combined SEC/MALDI MS technique was utilized to quantitative examine a PS/PAMS model blend. The most important finding of this study is that combined SEC/MALDI technique can provide valuable quantitative data for a certain class of polymer blends and this technique has been successfully used to analyze polymer blends of constituents with similar chemical structures and molecular weights, for which the difference are often too small to allow separation by liquid chromatography. In this case, MALDI MS can be used as a highly selective SEC detector for discrimination of individual blend homopolymer constituents. The proposed MALDI data treatment can provide, in one experimental set up, precise, quantitative information on the blend chemical composition distribution as well as on molecular weights and distributions of individual blend components.