

## CHAPTER II

### THEORETICAL BACKGROUND

#### 2.1 Fourier transform infrared (FT-IR) spectroscopy

Infrared (IR) spectroscopy is a popular method for characterizing matters.<sup>1,2</sup> This technique is based on the vibrations of atoms of a molecule. When IR radiation interacts with matter, the chemical bonds in the material consequently vibrate. In particular, molecular vibrations in a material are the requirement for IR absorption to be present in forms of spectrum. Each functional group composed in a molecule tends to independently absorb IR radiation in the same frequency range regardless of the molecular structure. There are a number of reasons for the success of IR techniques. The IR method is rapid and sensitive with easy-to-use sampling techniques.

In addition, an IR spectrum can provide quantitative information as well, such as the concentration of a molecule in a particular sample. The principle of quantitative analysis is based on Beer's law, which relates concentration to absorbance, as expressed below.<sup>3</sup>

$$A = \log \frac{I_o}{I} = \varepsilon cl \quad (2.1)$$

where  $A$  is the absorbance of the sample;  $I_o$  is the intensity of the incident IR beam;  $I$  is the intensity of the IR beam transmitted through the sample;  $\varepsilon$  is the molar absorptivity;  $c$  is the concentration of the sample; and  $l$  is the cell pathlength.

The absorbance of an IR spectrum can be measured in forms of peak height, peak-height ratio, peak area, or peak-area ratio. The absorptivity is the proportional constant between concentration and absorbance. However, the absorptivity is fundamentally a physical property of a molecule. The units of absorptivity are usually given in  $(\text{concentration} \times \text{pathlength})^{-1}$ , so the absorptivity cancels the units of the other two variables in Beer's law. This is necessary because absorbance is a unitless

quantity. The absorbance is linearly proportional to concentration but transmittance is not linearly proportional to concentration.

The transmittance ( $T$ ) is defined by the following equation.<sup>2</sup>

$$T = \frac{I}{I_0} \quad (2.2)$$

Substituting equation 2.2 into equation 2.1 and raising both sides of the equation with the power of 10 yield.<sup>2</sup>

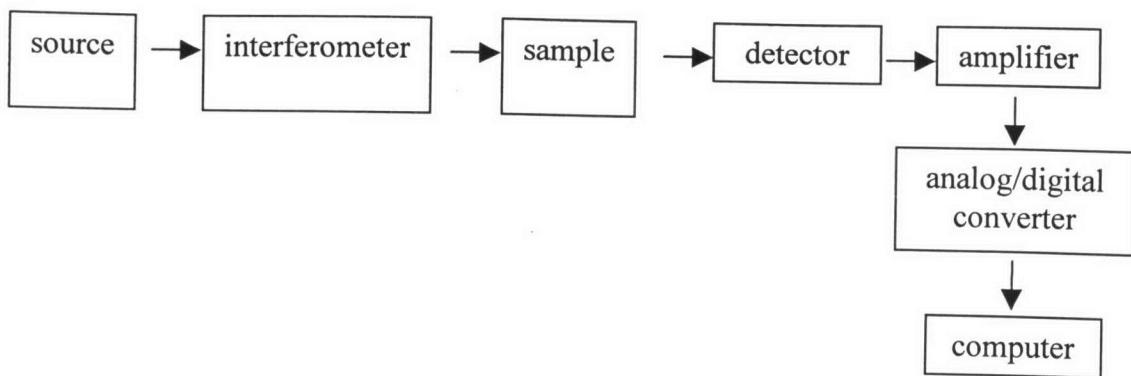
$$10^{\varepsilon cl} = \frac{1}{T} \quad (2.3)$$

and rearranging:<sup>2</sup>

$$T = 10^{-\varepsilon cl} \quad (2.4)$$

Usually, IR spectra have been plotted with the  $Y$  axis units as in terms of absorbance or transmittance,  $X$  axis as a function of wavenumber ( $\text{cm}^{-1}$ ).

Nowadays, IR measurements are carried out on *Fourier-transform Infrared* (FT-IR) spectrometers. In theory, Fourier transformation is based on the interference of radiation between two beams to yield an interferogram, *i.e.*, a signal produced as a function of the changing pathlength between the two beams. The two domains of distance and frequency are interconvertible by the Fourier-transform method. The basic components of FT-IR spectrometer are shown schematically in Figure 2.1.



**Figure 2.1** Schematic of a typical FT-IR spectrometer

An IR spectrum can be divided into three regions, namely the far-IR ( $< 400 \text{ cm}^{-1}$ ), the mid-IR (4000-400  $\text{cm}^{-1}$ ) and the near-IR (4000-13000  $\text{cm}^{-1}$ ). Mostly the mid-IR region is applied for many fields of application because it contains fundamental mode of molecular vibration. However the near- and far-IR regions can additionally provide specific information of materials. The near-IR region consists of overtones and/or combination bands of those fundamental modes appearing in the mid-IR region. The far-IR region, on the other hand, can provide details regarding lattice vibrations.

### 2.1.1. Spectral subtraction

Spectral subtraction is one of the main tools used to solve the problem of mixture spectra by removing whether solvent bands or any bands due to an unwanted component (e.g., carbon dioxide and water vapor bands) out of the observed spectrum. In practice, the mixture spectrum is subtracted by that containing only bands of unwanted species, so-called the reference spectrum. The unit of both spectra used for spectral subtraction must be in “absorbance”, which is linearly proportional to the concentration, because spectral subtraction assumes the validity of Beer’s law for the spectra involved.<sup>2,4</sup>

The principle of spectral subtraction is straightforward: the absorbance values of the reference spectrum are subtracted point-by-point from those of the sample. For example, if the absorbance of the sample at 3400  $\text{cm}^{-1}$  is 0.7 and the absorbance of reference at the same wavenumber is 0.4, the subtraction result at 3400  $\text{cm}^{-1}$  would consequently be 0.3 absorbance unit. In order to get rid of an unwanted

band, the subtracting spectrum of an unmandatory species should have the same intensity as that present on the sample spectrum.

To perform a subtraction in such a direct manner, the fact that the concentration of the unwanted material may be different in the sample and in reference spectra should be ignored. For example, the concentration of water in its pure form is higher than in a soap solution. This is because the water is actually diluted when the soap is dissolved in it. In this case, if the subtraction without taking into account the concentration differences of water between the two spectra were performed, there would be water bands left on the resultant spectrum. In an attempt to solve the problem, the reference spectrum would then be multiplied by a subtraction factor. Adjustment of the subtraction factor allows users to match the absorbance values on the reference to those on the sample spectrum in order to minimize an existence of an unwanted species on the resultant subtraction. The general equation used to perform a subtraction is as follows<sup>4</sup>:

$$\text{(Sample)} - \{(\text{Subtraction Factor}) \times (\text{Reference})\} = \text{Result} \quad (2.5)$$

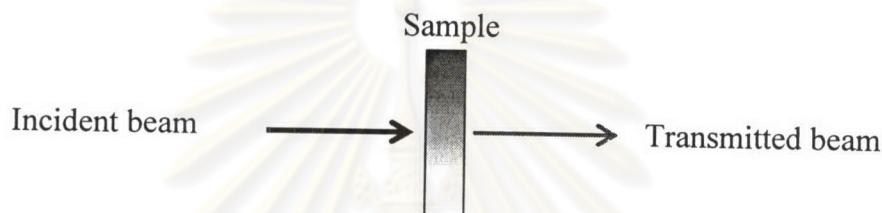
An impurity-free result can be achieved when absorbances of the sample and the reference are about the same.

Before performing a subtraction, a visual comparison of the sample and reference spectra should be kept in mind. Bands present on both spectra are due to the reference species, while bands appeared only in the sample are of interest and should be remained on the subtraction result. The subtraction factor is the only adjustable parameter in the process. Two steps to optimize values of the subtraction factor are:

1. Choose a spectral feature or features common to both the sample and reference spectra. Make sure that these features are less than 0.8 absorbance units. Bands which have higher intensities may not be effectively subtracted.
2. Interactively, adjust the value of subtraction factors until the features chosen in step (1) appear to be visually flat and become a part of the baseline.

### 2.1.2 Transmission technique

Transmission technique is the most popular of obtaining an IR spectrum. In transmission measurement, IR beam passes directly throughout a whole sample as shown in Figure 2.2. Therefore, the obtained result is the average information of both surface and bulk of the sample. The advantage of this technique is that the spectrum exhibits a high signal-to-noise ratio, comparatively the sample preparation is quick and low cost. In addition, it is the universal technique available for all solids, liquids, gases and polymers.<sup>2,3</sup>



**Figure 2.2** Experimental setup of transmission technique

The major disadvantage of the technique is the problem on sample thickness. Samples thicker than 20 microns generally absorb too much IR radiation, giving rise to over absorption. On the other hand, sample thinner than 1 micron usually have too weak absorbance to be detected. Ideally, the thickness of a sample should be between in 1 and 20 microns. In some cases, a time-consuming preparation may be required for either melting, squishing, or diluting a sample in order to achieve an appropriate amount of light. The following discussion of transmission technique is divided by types of samples.

#### 2.1.2.1 Sample preparation for solids

There are two methods of sample preparation for solids. The first method, called potassium bromide (KBr) pellet, is suitable for powders or samples that can be ground into powder. Briefly the process can be done by into powder, then diluting the sample powder in an inert matrix of KBr. On the other hand, in case of mull method, the mulling agent is added during grinding process.

#### 2.1.2.1.1 Pressed pellet

To press a pellet of sample powder mixed in an alkali-halide matrix is a widely used method for the IR measurement. KBr is most popular alkali-halide for making pellets, as well as cesium bromide (CsBr) and cesium iodide (CsI). Prior to step of making a pellet, the sample is first ground into uniformly fine particles ( $0.5 \mu m$  average particle size) and then dispersed into KBr powder. The powdered mixture is subsequently transferred into a die. A pressure is applied to the die until the alkali-halide particles coalesce into a clear, transparent and translucent disk. With this method, the fine sample particles are dispersed in a matrix of comparable refractive index in order to reduce scattering caused by the sample particulates.

However there are several problems occurring with this KBr pellet method. The opaque pellet gives rise to a poor spectrum because only a small amount of light can pass through. White spots in a pellet indicate the sample was not ground well enough, or was not dispersed properly in the KBr. To try grinding a little longer and mixing the sample and KBr more thoroughly are practically required. If only little sample powder is placed into the KBr pellet press, just little piles of powder would be obtained instead of a pellet. The applied pressure must be high enough to produce a thin transparent film. Because pellets are thin and brittle, They can fall out of the holder and crack, or sometimes it can be disintegrated with a slightest provocation. Care must be paid for in handling.

In addition, KBr is a hygroscopic material. Which means its in nature adsorbs water directly from the atmosphere. Therefore KBr powder should be dried, preferably in an oven at  $> 100^\circ C$  before uses. Cloudy area on a pellet indicated adsorbed moisture resulting bands around  $3500$  and  $1630 \text{ cm}^{-1}$ . According to this hygroscopic property, a freshly made pellet should not be left exposed to the air. Alternatively, the KBr pellet method can be performed for a quantitative measurement as well. This can be achieved by precisely weighting a certain amount of sample mixture used for every pellet. Since the pathlength of a pellet is unknown, ratio of band-area is thus useful to eliminate the variation of pathlength. Method of using internal standard is recommended as well.

### 2.1.2.1.2 Mull method

Mull is alternative way of obtaining a transmission spectrum of solid material. Based on this method, the sample is first ground in an agate mortar with a pestle to acquire substantially fine particles without a resultant scattering effect. A drop of mineral oil, called as *mulling agent*, is added, and the mixture is further ground a bit more to uniformly disperse the solid in the oil. The mineral oil used as mulling agent is normally known under the trade name Nujol. To obtain a spectrum with this method, a small amount of the sample/oil slurry is smeared onto a KBr window, and a second KBr window is squeezed against the first window to make a thin film. The windows are then placed against the IR beam.

The advantage of mull method over a KBr pellet is an easier and faster preparation because no time-consuming step of making a pellet is involved. Mulls are also preferred for a sample that may degrade upon exposure to water because the mulling oil is able to protect the sample from water vapor in the atmosphere by its hydrophobic property. In contrast, the hygroscopic KBr adsorbs water from the atmosphere, potentially causing a damage of water-sensitive samples.

On the contrary, the technique presents a main drawback of oil contaminant on the sample spectrum. Nujol is a mixture of long, straight-chain hydrocarbons which IR light strongly absorbs at around  $3000$  and  $1400\text{ cm}^{-1}$ . In theory, Nujol bands can be subtracted out of the sample spectrum. However, those oil bands are usually too intense to be completely clear after subtraction process.

In an attempt to solve the problem with Nujol bands, a technique called *spilt-mull method*, becomes an alternative option base on using two different mulling agents. The first mull is prepared in Nujol, with Fluorolube-mixture of chlorofluorocarbons with latter mull is ground instead, long chain alkanes where the C-H bonds as in Nujol have been replaced by C-F and C-Cl bonds. Fluorolube exhibits no absorption bands at high wavenumbers, but some bands present below  $1300\text{ cm}^{-1}$  due to C-F and C-Cl stretching and bending vibrations. In case of Nujol, only one weak band appears below  $1300\text{ cm}^{-1}$ . As a result, Nujol and Fluorolube mask different region on the IR spectrum. With the spilt-mull technique, two spectra are obtained with the sample dispersed in either by an assistance of a mulling agent. The

spectra are then combined by using the computer program, or by literally cutting and pasting the spectra. Therefore the resultant spectrum contains no bands of either mulling agent, only bands due to the sample remain.

### **2.1.2.2 Sample preparation for liquids**

The simplest way to obtain transmission spectra of liquids is to drop or to smear the sample on a KBr window, then sandwiched with another window before placing against the IR beam. This is known as the capillary-thin-film method. The advantage of the capillary-thin-film technique is a good quality spectrum of liquids can be produced in a fast and easy manners. It takes only a few seconds for sample preparation with this method.

Nevertheless, there are some problems with the capillary-thin-film method. Volatile liquids usually evaporate during spectral collection. Moreover sample evaporation makes capillary-thin-films inappropriate for quantitative analysis. Because sample becomes disappeared with time, thereby the exact concentration of sample cannot be determined with this method. To avoid such an evaporation, a sealed liquid cell should be employed. In addition, the capillary-thin-film technique is inappropriate for analyzing liquids containing water because KBr can be dissolved in water whose bands usually cause difficulty in subtraction process. However, other window materials, such as zinc selenide (ZnSe) or silver chloride (AgCl), do not dissolve in water and thereby can be utilized for spectroscopic study of aqueous samples with capillary-thin-film method.

### **2.1.2.3 Sample preparation for gases**

Gas cell is usually used to obtain spectra of gases. Gases are practically introduced to the cell by using a vacuum manifold consisting of a vacuum pump and an appropriate plumbing. The sample is contained in a lecture bottle, a glass bulk, or a gas cylinder connected to the manifold. To introduce gas into the cell, the manifold and the cell are first evacuated. The vapor phase spectra of liquids and solids can be obtained by using a gas cell. The brief approach is to hook up a reservoir containing the solid or liquid of interest to a vacuum manifold, and then allowing the vapor above the solid or liquid inside the reservoir to pass through the gas cell. As a consequence FT-IR spectroscopy becomes an excellent method for determining

compositions of unknown gases. Nowadays FT-IR technique is being used extensively to determine air quality and to characterize air polluters.

## 2.2 Analysis of orientation in polymers using infrared spectroscopy

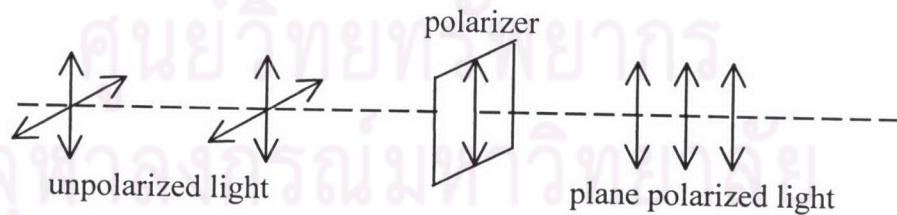
### 2.2.1 Polarization

Light is by nature not polarized (see Figure 2.3), such as that emitted by either an ordinary incandescent light bulb or the sun.



**Figure 2.3** Vibrations of electric field vector in unpolarized light

Unpolarized light consists of light with random electric field vector.<sup>5</sup> Each of the polarization directions can theoretically be resolved into components along two mutually perpendicular directions. Accordingly, an unpolarized beam can be thought as two plane polarized beams, with an equal magnitude, perpendicular to another. However a polarized can be produced from the unpolarized light by a polarizer as shown in Figure 2.4.



**Figure 2.4** Schematic operation of a polarizer

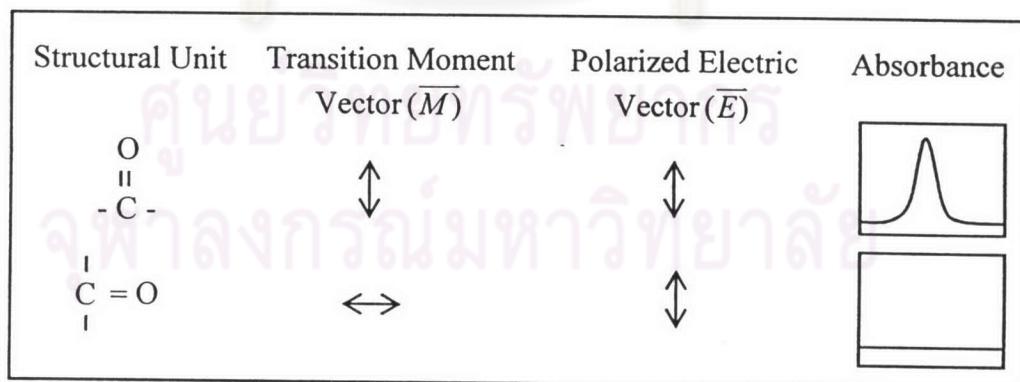
### 2.2.2 Infrared linear dichroism

By using linearly polarized IR radiation, the orientation of functional groups composed in a polymer system can be potentially determined<sup>3,6</sup>. The molecular orientation of chains in a polymer indicates its mechanical properties, such as strength, ductility, and glass transition temperature. Particular IR absorption bands are assigned to the crystallinity and amorphousness of the polymer. IR absorption is occurred by the interaction between the electric vector and the molecular dipole moments of a certain vibrational mode. Its intensity would be maximum when the electric vector is parallel to the dipole moment, and becomes totally zero if the angles of two vectors are 90° (see Figure 2.5).

Base on equation<sup>6</sup> (2.6) the absorption intensity of each vibrational mode ( $I$ ) is proportional to the square of dot product of the electric and transition-moment vectors.

$$I = C(\bar{E} \cdot \bar{M})^2 = (EM\cos\theta)^2 \quad (2.6)$$

where  $C$  is a proportionality constant,  $E$  is the magnitude of the electric field produced by incident beam,  $M$  is the magnitude of the transition moment representing a certain vibrational mode, and  $\theta$  is the angle between the two vectors.



**Figure 2.5** Linearly polarized IR absorbance of a functional group (i.e., carbonyl bond).

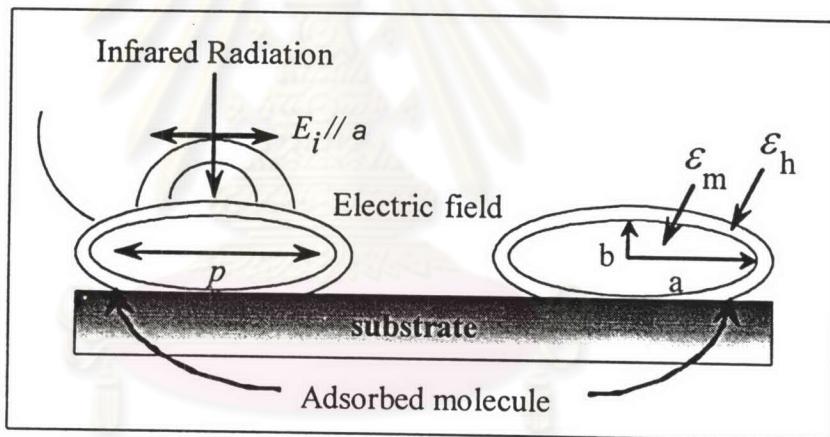
Based on the principle, the orientation of molecular components can be characterized by using the term dichroic ratio. Measurement of infrared linear

dichroism requires polarized light both in parallel and perpendicular to a fixed reference direction. For a parallel polarized light, the absorbance is generally represented as  $A_{\parallel}$ , and  $A_{\perp}$  is used for that of a perpendicular polarized light. Therefore the dichroic ratio,  $R$ , is defined as<sup>6</sup>:

$$R = (A_{\parallel})/(A_{\perp}) \quad (2.7)$$

For a random orientation,  $R$  is equal to 1. The observed absorption bands are basically classified as parallel or as perpendicular, orientation base on  $R$  value (i.e., greater or less than 1). This criterion of dichroic ratio is applicable for assigning various vibrational modes.

### 2.3 Surface-enhanced infrared absorption (SEIRA)



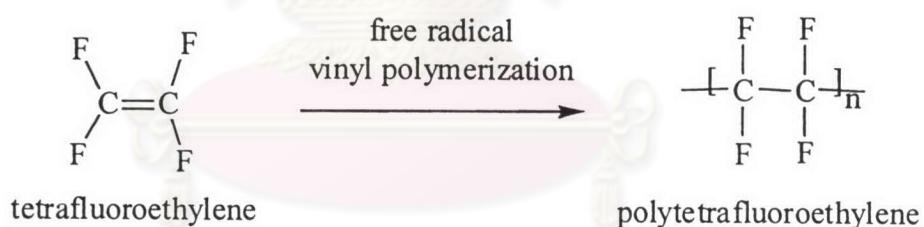
**Figure 2.6** Schematic mechanism of SEIRA phenomenon. Polarization of localizes electron resonance inside a metal particle, as a result of the incident electric field.  $E_i$  is represented by induced dipole  $p$ .  $\epsilon_m$  and  $\epsilon_h$  are the dielectric functions of a metal particle and an analyte molecule, respectively.

The spectral selectivity of IR spectroscopy allows for positive identifications of many chemical contaminants in industrial, biological and environmental samples. However, the relatively low molar absorptivities in the IR region limit its use in many applications<sup>7</sup>. In accordance with the methods for enhancing its sensitivity are great interested.

The phenomenon of surface-enhanced infrared absorption (SEIRA) was first observed by Hartstein *et al.* in 1980<sup>8</sup>. The SEIRA effect has been investigated with thin films adsorbed on noble metal surfaces (e.g., silver, gold, copper and tin)<sup>7,9</sup>. The metal films which show SEIRA must be discontinuous and consist of very small (i.e., less than wavelength of light) metal islands. Consequently, IR absorption of the adsorbed molecule was remarkably enhanced. The mechanism of SEIRA is not yet fully understood. However, it has been proposed that a polarized IR light cause an oscillating dipole inside metal islands coated with analyte molecules (as shown in Figure 2.6). Such a dipole establishes an additional electric field perpendicular to any point on metal surface. The electric field serves to channel the IR energy into the adsorbed analyte, causing a substantially enhanced absorption intensity of vibrations that are perpendicular to the metal surface.

## 2.4 Polytetrafluoroethylene (PTFE)

### 2.4.1 Processing and structure



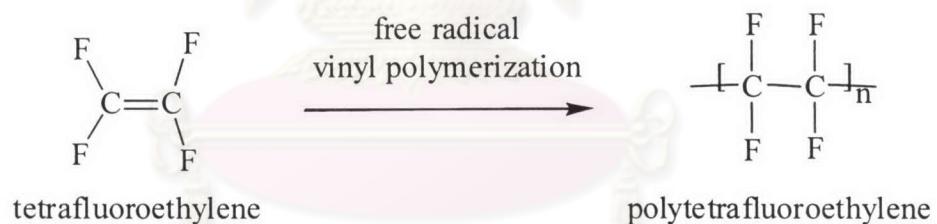
**Figure 2.7** Free radical polymerization of PTFE

The polymeric tetrafluoroethylene was discovered by R.J. Plunkett (Garrett 1962)<sup>10</sup>. It is usually polymerized with free-radical process, as shown in Figure 2.7. Redox initiation can be alternatively employed by using persulfates and hydrogen peroxide as initiators. Polytetrafluoroethylene (PTFE) composes of a carbon backbone chain with two fluorine atoms attached to each carbon. PTFE is a highly crystalline and orientable polymer. These facts indicate a regular structure, which implies the absence of any considerable amount of crosslinking. Branching is

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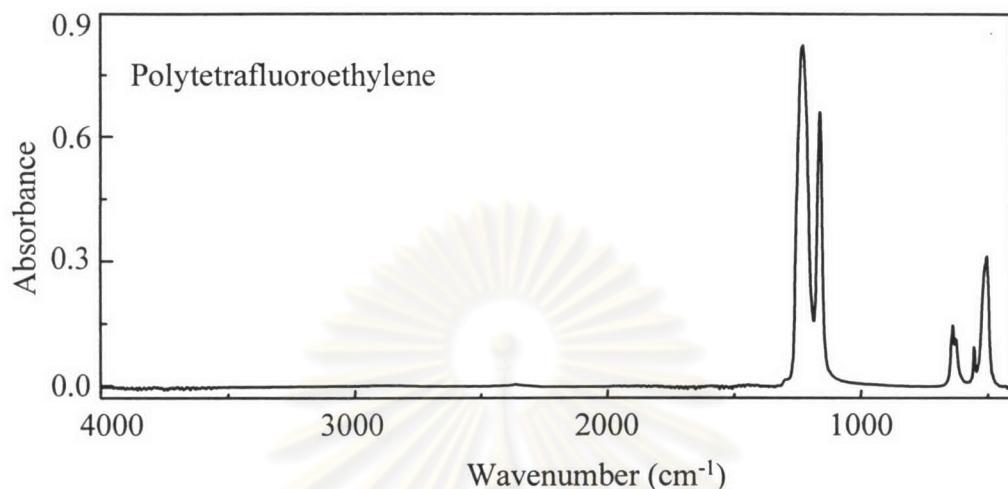
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**Figure 2.7** Free radical polymerization of PTFE

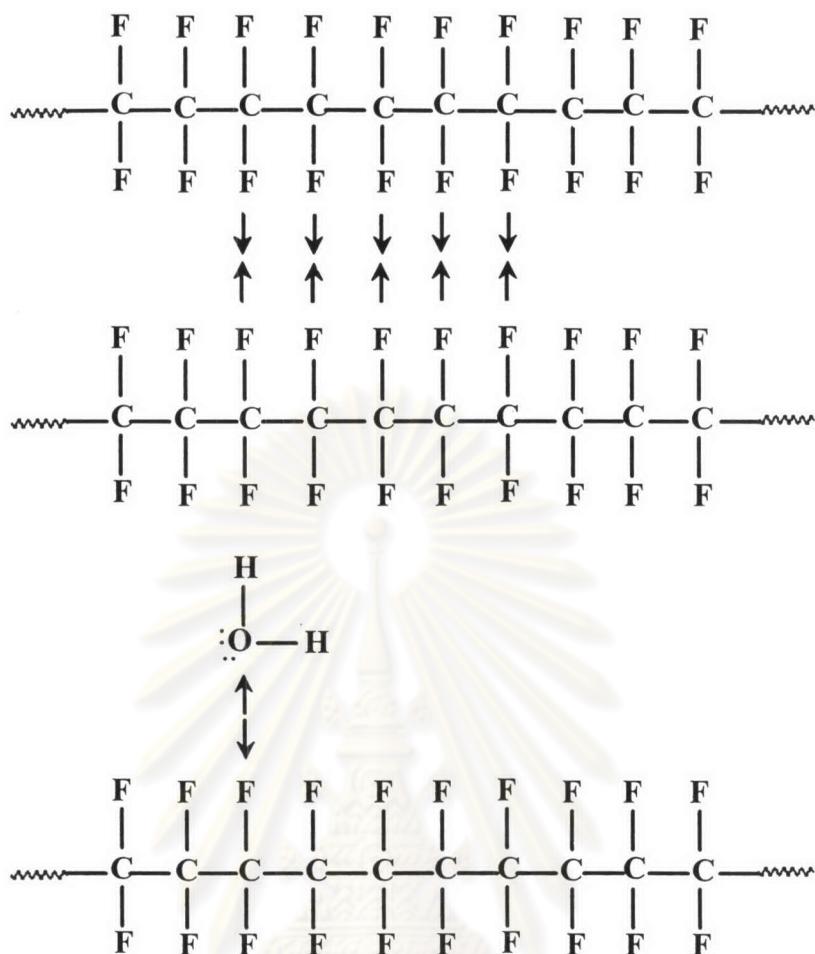
The polymeric tetrafluoroethylene was discovered by R.J. Plunkett.<sup>10</sup> It is usually polymerized with free-radical process, as shown in Figure 2.7. Redox initiation can be alternatively employed by using persulfates and hydrogen peroxide as initiators. Polytetrafluoroethylene (PTFE) composes of a carbon backbone chain with two fluorine atoms attached to each carbon. PTFE is a highly crystalline and orientable polymer. These facts indicate a regular structure, which implies the absence of any considerable amount of crosslinking. Branching is presumed to be absent, since

branching mechanisms would involve in breaking such a strong C-F bond. The IR spectrum of PTFE film is shown in Figure 2.8.



**Figure 2.8** IR spectrum of PTFE film

The fluorine atoms of PTFE prefer to be accommodated by the same fluorine atoms, while repelling any other kinds of molecule (e.g., water molecule), as shown in Figure 2.9. As a result, the surface of PTFE usually repel the outside molecules.



**Figure 2.9** Interaction of fluorine atoms on polytetrafluoroethylene chain with their own kind and other kinds of molecules.

The degree of crystallinity of PTFE is generally quite high (*i.e.*, 93-98%). The melting point of crystalline PTFE is 327°C, whereas its crystalline density is 2.30 g/cm<sup>3</sup>. By melting the polymer and subsequently cooling down, lower densities are consequently obtained. A subsequent thermal treatment or annealing generally increases the crystallinity of solid PTFE. The specific gravities for a various types of PTFE have been ranged between 2.15 and 2.28. This range of densities has been correlated with the molecular weight and thus the melt viscosity of samples.<sup>10,11</sup>

PTFE does not readily flow above its crystalline melting point. The viscosity of such a polymer is very high because of the restricted rotation due to the chain and its high molecular weight. Upon the continued application of stress to the

amorphous polymer above the crystalline melting point, strength of the polymer is exceeded causing a fracture before the stress for inducing a flow are reached.

In addition, PTFE can be decomposed at an elevated temperature. Under a vacuum condition, monomer is the main product. The vapor pressure of a monomer in equilibrium at 500°C is 1 mmHg. At low temperatures (250-350°C), the degradation starts from the chain ends, on the other hand, a random cleavage becomes more important at a higher temperature. A present of air results in a more complicated degradation process.

#### **2.4.2 Material properties**

PTFE is extremely resistant to any corrosive reagents or solvents. The thermal stability of PTFE is that its electrical and mechanical properties do not change for a long interval at temperature as high as 250°C. However the properties of PTFE are widely varied as a function of the molecular structure and the fabrication process.

Molded PTFE has a high impact strength, but it is easily strained. The surface is not hard, but slippery and waxy to the touch. In addition, it possesses a very low coefficient of friction on most substances as well as its high density and low refractive index.

#### **2.4.3 Practical applications**

PTFE is widely employed in many fields due to its unique properties: toughness, electrical properties, heat resistance, low frictional coefficient, or a combination of these as well. The electrical applications compose of wire and cable insulation, insulation for a motor, transformer, coil, capacitor and high-frequency electric uses. Chemical equipment such as gaskets, pump and valve packings, as well as pump and valve parts are also made of PTFE. Low-friction and antistick applications include nonlubricated bearing, lining for a tray in bakery and other food-processing equipments, mold-release devices, and covers for heat-sealer plates of a packaging machine. Low-molecular-weight polymers of PTFE dispersing as aerosols are effective dry lubricants. Uses as a fiber consist of gasketing, belting, pump and valve packing, filler cloths, and other industrial functions where essentially complete

chemical and solvent resistance, as well as heat resistance to above 250°C are dominantly.

## 2.5 Literature Survey

In a decade, a number of researches concern on uses of the disposable IR card for sample analysis both in industry and academia.

Wigman<sup>12</sup> *et al.* investigated an efficiency of the disposable polyethylene IR card and KBr pellet for sample analysis. Fluoroquinolone antibiotic was the sample of interest. They found that a disposable polyethylene IR card can reproduce spectra almost to those observed by KBr pellets.

Hoyt and Mitschele<sup>13</sup> found that a single sodium chloride (NaCl) plate can be used instead of a polyethylene IR card. The resulting spectra of *trans*-1, 2-dibenzoylethylene are the same for both methods, but cost of NaCl plate is minimized, because the NaCl plate is generally reusable. However, the disadvantage is that the NaCl plate should be cleaned off immediately, as compared to KBr pellets and the disposable cards.

Moeur and Pinnell<sup>14</sup> utilized the disposable PTFE card to compare to a polyethylene card for analyzing products from the synthesis of *N, N*-diethyl-*m*-toluamide, a high-boiling point liquid. They also used the card to aid in structural determination of unknown compounds. In conclusion, the cards were clearly useful for solids and liquids with a boiling point higher than 130 °C.

Christensen<sup>15</sup> studied mineral salts collected from monuments by combining FT-IR spectroscopy with microchemical tests and optical microscopy. Other different sampling techniques operated were Nujol mull and KBr pellet methods as well as disposable IR cards. Extracted salts from the sample were screened for nitrate, sulfate, chloride by dropping the sample solution onto a disposable polyethylene IR card and then evaporating with nitrogen. If the particle size of sample is left on the card large, the spectrum thereby becomes distorted.

Oberg and Daniel<sup>16</sup> invented an IR window from Teflon tape in order to analyze solid samples. They made the home-made IR card, which is similar to the

commercial PTFE card, by using Teflon tape available in any hardware stores. Experimentally, Teflon tape was pressed down with different stretching ratio values. They observed that the scattering of IR radiation was considerable in case of the unstretched tape. However, by stretched Teflon tape, light scattering is substantially reduced. In addition, the home-made IR card can be used effectively as a sample holder for IR measurements. This technique is simple, rapid and good quality spectra are obtained.

Lephardt<sup>17</sup> applied Teflon tape as an IR window to study curing process of epoxy resin. Two procedures were examined with comparable results. In the first procedure, resin and hardener were mixed externally and applied to the Teflon tape. In contrast, the resin was first applied to one end of the tape, whereas the hardener was applied to the other end for another approach. In the latter case, the sample was later mixed when both ends were pressed together. He found that Teflon tape cells can be used to perform many kinds of IR investigations. Teflon tape method is a convenient, cheap and easily alternative for some applications.

Mossoba and Fry<sup>18</sup> characterized bacterial strains by using a microporous polyethylene disposable optical film (DOF) as the transmission IR substrate. In addition, they used both the principal component analysis (PCA) and hierachocal cluster analysis (HCA) for classification of specific groups of bacteria. It was summarized that a disposal film is advantageous to characterizations of bacteria by mid-IR spectroscopy, in order to get rid of any contaminations possibly occurring in experiments of pathogenic microorganisms. No need of clean expensive IR crystal was required. The preparation of a bacterial test sample was simple as a routine procedure because bacteria consistently adhered to the DOF every time they were applied. Besides, bacterial test samples can be stored for reference and future use. As a consequence, the DOF is an alternative method against those expensive IR materials (e.g., ZnSe).