CHAPTER 2

THEORETICAL BACKGROUND

2.1   Basic Concept of Fourier Transform Infrared Spectroscopy

Infrared spectroscopy is the measurement of the wavelength and the intensity of absorption of infrared light by a sample. When a sample is bombarded with infrared radiation, it absorbs some of the radiation and resulting in a shift of energy from a lower to a higher level within the sample. This is useful in regards to the identification of various compounds. Some of the many useful functions of the various data available range from quantitative to qualitative molecular information. Infrared radiation is divided into three regions according to the different molecular energy levels:

- Near infrared; 2.5-0.8 μm: low energy electrons in molecules and crystals,
- Mid infrared; 50-125 μm: the most stretching and bending fundamental vibrations of molecules occur, and
- Far infrared; 1000-50 μm: low frequency bending where rotational transitions in gaseous molecules occur.

Through the use of an infrared radiation source (such as a Nernst Globar), a broad range of infrared wavelengths is achieved. This continuum source of light is splitted into two paths using a half silvered mirror; this light is then sent from two mirrors back onto the beamsplitter where it is recombined. This technique is termed Fourier transform infrared (FT-IR) spectroscopy, which has significant advantages over traditional instrument. FT-IR spectroscopy has improved the quality of infrared data at the same time and made data collection faster and easier. Additionally, the computerized nature of FT-IR instrument allows manipulation of the digital data to obtain information that would be previously remained hidden.

The absorption of infrared radiation is associated with the vibration of chemical bonds. The absorption spectrum contains a wealth of information about the elements comprising a material and the structure in which those elements are bounded.[7-8]

2.2 Attenuated Total Reflectance Spectroscopy

Attenuated total reflectance (ATR) spectroscopy utilizes the phenomenon of total internal reflection (Figure 2.1). A beam of radiation entering a crystal will undergo total internal reflection when the angle of incidence at the interface between the sample and the crystal is greater than the critical angle. The critical angle is a function of the refractive indices of ATR crystal and the sample. The beam penetrates a fraction of a wavelength beyond the surface, which is in close contact with the ATR crystal, the beam loses energy at the wavelength where the material absorbs. The resultant attenuated radiation is measured and plotted as a function of a wavelength by the spectrometer and gives rise to the absorption spectral characteristics of the sample.
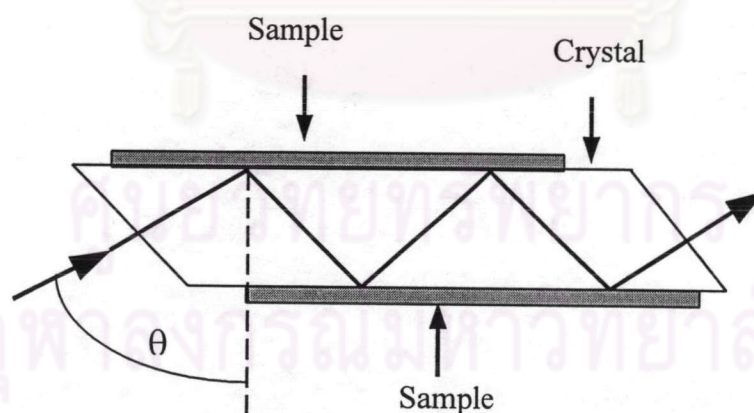

Figure 2.1   An attenuated total reflectance cell

The crystal used in ATR cell is made from material, which has low solubility in water and is of very high refractive index. Such material includes zinc selenide (ZnSe), germanium (Ge), and thallium/iodide complex (KRS-5). Different designs of

ATR cells allow both liquid and solid samples to be examined. The nature of the technique produces a less intense solvent contribution to the overall infrared spectrum. Moreover, solvent spectra can be easily subtracted from the sample spectrum of interest.[9]

2.3   Multicomponent Analysis by Infrared Spectroscopy

Measuring the concentration of an absorbing species in a complex mixture by infrared spectroscopy is accomplished through application of the Beer-Lambert law. The Beer-Lambert law is the linear relationship between the absorbance and concentration of an absorbing species or simply described that the spectrum of the mixture is a linear combination of the spectra of all pure components in the mixture.

The absorbance at a single wavelength due to the presence of a single component is given by:

$$A = KC \qquad\qquad [2.1]$$

where:

A is the absorbance at that wavelength,

K is the absorbance coefficient for that component and wavelength,

C is the concentration of the component.

for the multiple components and multiple wavelengths:

$$A_w = \sum_{c=1}^{n} K_{wc} C_c \qquad\qquad [2.2]$$

where:

A_w is the absorbance at the wth wavelength,

K_{wc} is the absorbance coefficient at the wth wavelength for the cth component,

C_c is the concentration of the cth component,

n   is the total number of components.

2.4   Factor Analysis

Factor analysis (FA) is a mathematical technique for interpreting multidimensional data set. Originally it was developed to solve the problems in psychology and sociology, but it is now successfully used in various areas of chemistry. Factor analysis is used to determine the number of component or factor creating the given physical or chemical system.

2.5   Factor Analysis in Infrared Spectroscopy

When factor analysis was applied in infrared spectroscopy, this technique assumes that the infrared spectra of the materials obey the Beer-Lambert law. The spectra of the mixture are linear combination of the individual spectrum of each component in the mixture proportional to the amounts of each component present. This assumption allows the use of factor analysis to condense the information of the infrared spectra into the manageable form of matrices.

2.5.1   Mathematical Considerations and Data Organization

The nature of factor analysis makes it extremely convenient to organize the data into matrices. In spectroscopy, when the absorbance spectra of a number of samples are measured and managed into one matrix, which called the mixture matrix. Factor analysis separates this matrix into the concentration values for each component of sample called concentration matrix and the data of pure component spectra called original data matrix.

2.5.1.1 Mixture Matrix

Using column-wise organization, the mixture matrix holds the spectral data. Each spectrum is placed into the absorbance matrix as a column vector:

$$\begin{bmatrix} A_{11} & A_{12} & A_{13} & ... & A_{1s} \\ A_{21} & A_{22} & A_{23} & ... & A_{2s} \\ ... & ... & ... & ... & ... \\ A_{wl} & A_{w2} & A_{w3} & ... & A_{ws} \end{bmatrix} \qquad [2.3]$$

where A_{ws} is the absorbance at the wth wavelength for sample s. If the spectra of 30 samples at 15 different wavelengths were measured, each spectrum would be held in a column vector containing 15 absorbance values. These 30 column vectors would be assembled into the mixture matrix, which would be 15X30 in size (15 rows, 30 columns).

2.5.1.2 Concentration Matrix

Similarly, a concentration matrix holds the concentration data. The concentrations of the components for each sample are placed into concentration matrix as a column vector:

$$\begin{bmatrix} C_{11} & C_{12} & C_{13} & ... & C_{1s} \\ C_{21} & C_{22} & C_{23} & ... & C_{2s} \\ ... & ... & ... & ... & ... \\ C_{cl} & C_{c2} & C_{c3} & ... & C_{cs} \end{bmatrix} \qquad [2.4]$$

where C_{cs} is the concentration of the cth component of sample s. If there are the concentration of 4 components in each of the 30 samples, the concentration for each sample would be held in a column vector containing 4 concentration values. The

total sum of 4 concentration values equal to 1. These 30 column vectors would be assembled into a concentration matrix, which would be 4X30 in size (4 rows, 30 columns).

2.5.1.3 Original Data Matrix

The original data matrix or pure component matrix holds the speatral data of each component that placed into the absorbance matrix as a column vector:

$$
\begin{bmatrix}
S_{11} & S_{12} & S_{13} & \ldots & S_{1s} \\
S_{21} & S_{22} & S_{23} & \ldots & S_{2s} \\
\ldots & \ldots & \ldots & \ldots & \ldots \\
S_{w1} & S_{w2} & S_{w3} & \ldots & S_{ws}
\end{bmatrix}
\qquad [2.5]
$$

where S_{ws} is the absorbance at the wth wavelength of component s. If 3 components are measured at 15 different wavelengths, the spectrum of each component would be held in a column vector containing 15 absorbance values. These 3 column vectors would be assembled into an absorbance matrix, which would be 15X3 in size (15 rows, 3 columns).

2.5.1.4 Matrix Multiplication

The intensity of a spectral signal of a mixture at every point is given by the sum of the increments of individual components. According to the Beer-Lambert law from Equation [2.1] for absorption spectroscopy and the analogous relation of matrix multiplication, the increment of mixture spectral intensity is given by the product of the molar coefficient (generally of the pure component spectrum) and the concentration of the components:

$$a_{ws} = \sum_{j=1}^{n} s_{wc}\, c_{cs} \qquad [2.6]$$

where a_{ws} is the wth element of the spectral data matrix of the sth mixture in the spectral matrix, s_{wc} is the wth point of the spectrum of the cth component, and c_{cs} is the concentration of the cth mixture. In a matrix form, Equation [2.6] can be rewritten as:

$$A = SC \qquad [2.7]$$

This equation is the key step in factor analysis. First, an experimental data is conversed into a mixture matrix. This matrix can be mathematically obtained from the original data matrix and concentration matrix, which also relates to significant physical parameter.[10-12]

2.6   Principal Component Analysis

Principal component analysis (PCA) is the first step of factor analysis. PCA is a feasible approach to determine the number of components in the mixture. To determine the number of components in a mixture, which contains the same component by varying composition, the spectra of various compositions of the components are collected. The spectra of individual mixtures are arranged in column in matrix A.

$$
A = \begin{array}{c} \\ 800\ \text{cm}^{-1} \\ 805\ \text{cm}^{-1} \\ 810\ \text{cm}^{-1} \\ 815\ \text{cm}^{-1} \end{array}
\overset{\begin{array}{cccc} & & \text{absorbance} & \\ \text{mix.1} & \text{mix.2} & \text{mix.3} & \text{mix.4} \end{array}}
{\begin{pmatrix}
0.1189 & 0.1955 & 0.3183 & 0.2569 \\
0.4252 & 0.8749 & 1.0508 & 0.9628 \\
0.3988 & 0.8371 & 0.9772 & 0.9071 \\
0.1833 & 0.3787 & 0.4521 & 0.4154
\end{pmatrix}} \qquad [2.8]
$$

The matrix of the mixture spectra data is not directly factor analyzable. Instead, a square symmetrical matrix, Z ,(also called the scatter matrix) is constructed:

$$Z = A^T A \qquad [2.9]$$

Eigen analysis of the scatter matrix, Z, determines an eigenvector matrix, L, which diagonalizes Z:

$$L^T ZL = [\lambda] \qquad [2.10]$$

where $[\lambda]$ is a diagonal matrix. The eigenvectors are required to be orthonormal:

$$L^T L = L L^T = I \qquad [2.11]$$

where I is the identity matrix. The number of non-zero element in diagonal elements of $[\lambda]$ can tell how many factors are involved in a mixture.[13]

2.7 Evolving Factor Analysis

Evolving factor analysis (EFA) is a calculation method in factor analysis. EFA is also used for determining the mixture composition and to isolate the component spectra from the set of mixture spectra.

EFA procedure starts from a mixture matrix, which consists of m row, n column, A (mXn) only of the first two mixture spectra. After the eigenvalues (λ) of this subset are found, a new one is formed by adding a spectrum of the third mixture. The eigenvalue is calculated for every spectral subset. The increment of the number of the mixtures increases the number of the obtained forward eigenvalues $(\lambda)_f$ of this forward EFA. The backward EFA uses the same procedure but starts with the last two mixture spectra and add the next spectrum up to the first spectrum in the mixture spectra is added, the backward eigenvalues $(\lambda)_b$, are then obtained. The eigenvalue sets for both forward $(\lambda)_f$ and backward $(\lambda)_b$ are plotted together as a function of the

evolutionary variable. The concentration profiles of the kth component found as the boundary of the region under the curves of the forward and backward eigenvalues and they are shown in Figure 2.2.
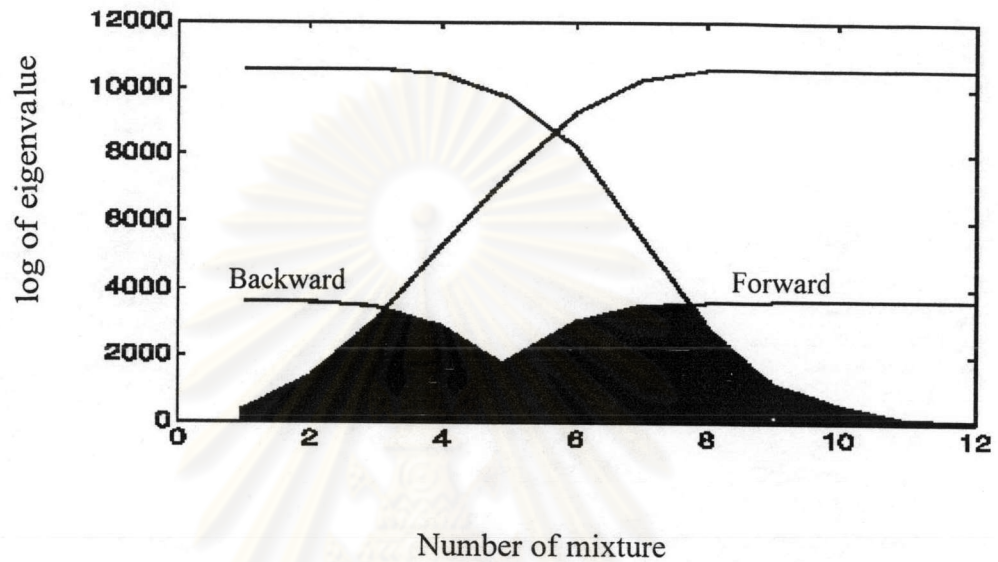

Figure 2.2   Forward and backward eigenvalue plot

2.8   Simple Linear Regression

The concentration profiles obtained by EFA are usually relatively rough. The iterative procedure called simple linear regression (SLR) will be applied for their improvement. SLR is started from the concentration profile. The individual components are normalized and arranged into rows of the matrix C* and the spectra of the component S* are obtained by solving the following equation:

$$S^* = A \, C^{*T} (C^* \, C^{*T})^{-1} \qquad [2.12]$$

Physically incorrect negative values in the matrix S* are set equal to zero, and matrix S* thus corrected is used to calculate the new concentration matrix:

$$C^* = (S^{*T} S)^{-1} S^{*T} A \qquad [2.13]$$

Any negative values in the elements of the matrix C* are set equal to zero, the rows of matrix C* are normalized to the matrix C and iteration is repeated from Equations [2.12] and [2.13] until convergence is achieved.[14]
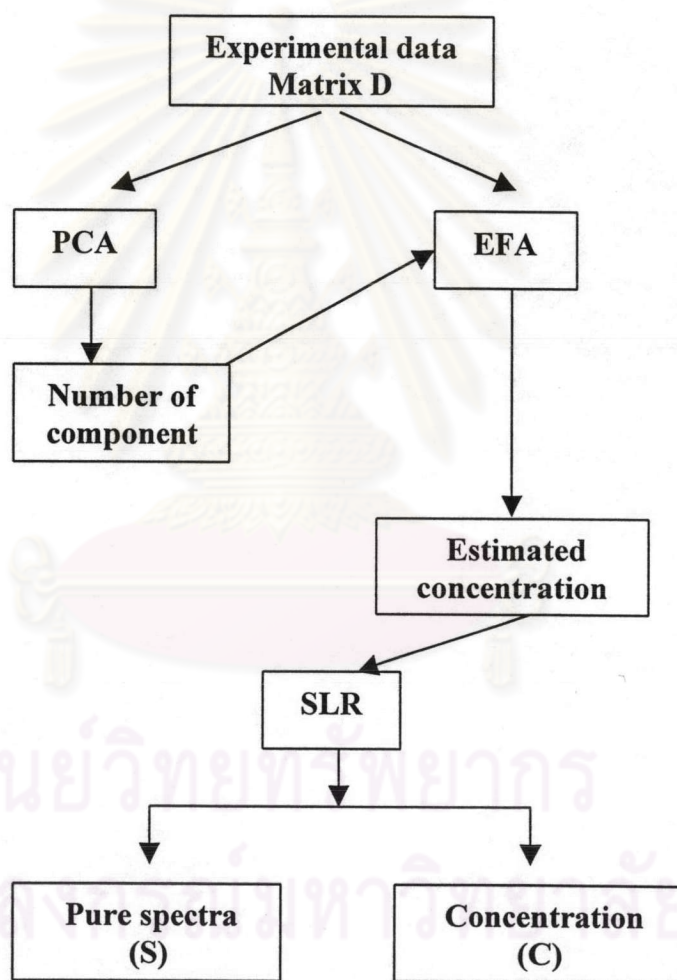

Figure 2.3   Key steps in Factor Analysis

2.9 Applying Factor Analysis for Quantitative Analysis of Multicomponent Mixture by Infrared Spectroscopy

When factor analysis is applied for quantitative analysis of multicomponent mixture by infrared spectroscopy, there are many problems to be considered for making the analysis successful.

2.9.1 Number of Calibration Samples in the Mixture Matrix

The analytical performance of the accuracy of the result from factor analysis is based on the number of data in mixture data matrix. The mixture data matrix is separated into two sets, the calibration set and sample set. The calibration set contains the data of the mixture at various proportions of all components and the data of pure component consisting in the mixture. The improvement in the accuracy of a concentration values can result in major improvement in the analytical performance of the calibration developed from that calibration set.

There are three rules of number of experimental data in the calibration set that based on the number of components in the system being considered. The three rules to be considered are: [15]

1. The rule of Three is the minimum number of sample should be normally attempted to prepare. It says "Use three times the number of component in the mixture". If the mixtures of three-component system are considered, at least nine samples are expected for the calibration set. This rule is used when doing preliminary or exploratory work.

2. The rule of Five is a better guide for the minimum number of sample to use. It says "Use five times the number of component in the mixture". This rule allows enough samples to reasonably represent all possible combinations of concentration values for a three-component system increase, the number of sample should be use for systems with large numbers of components.

3. The rule of Ten is better still "Use ten times the number of component in sample". This rule will usually be able to create a solid calibration for typical applications.

2.10   Literature Review

There are several techniques of chemometrics, which have been used for quantitative analysis of multicomponent using many analytical techniques.

Haaland and Easterling[16] observed the interaction spectrum when the spectrum of pure xylene was subtracted from the spectrum of a mixture. They also applied the weighted least-squares prediction by using baseline adjusted to peak absorbance value to estimate prediction error in a 1:1:1 mixture of xylene.

Lesia et al.[17] used two data-handling techniques, least-squares fitting and cross-correlation for three-component analysis by infrared absorption spectroscopy. The research was studied under the effect of peak width variations in degree of peak overlap and signal-to-noise ratio. The result showed that both least-squares and cross-correlation can be used with samples under the deviation. The comparison of the result showed that the result from least-squares was better than that from cross-correlation.

Chan and Compton[18] studied the quantitative infrared analysis by using the principle component regression (PCR) and partial least-squares (PLS) method. The systems of two- and three-component xylene were used to study the performance of both methods.

Factor analysis method was studied to solve the problem in quantitative analysis of the raman spectra of weakly interacting component by Sasic et al.[19] Target factor analysis (TFA) and evolving factor analysis (EFA) were applied to the raman spectra of dichloromethane, 1,2-dichloroethane, and carbon tetrachloride. The

results demonstrated the concentrations of substances with low intense bands are successfully determined. However, the result obtained from EFA is better than that from TFA.