# CHAPTER 1

# INTRODUCTION

## 1.1 General introduction

Tuberculosis is an infectious and contagious disease caused by *Mycobacterium tuberculosis*. Although the primary infection always involves the lung, the other organ systems such as the central nerve system, bone and genitourinary system are susceptible. Despite the advancement of the therapeutic methods in both prevention and treatment of this infection, tuberculosis still causes concern because it is the world's second most common cause of death from infectious disease, after human immunodeficiency virus (HIV/AIDs).

The first discovery of the tuberculosis bacillus by Dr. Robert Koch was on 24$^{th}$ March 1882 but streptomycin was not introduced for tuberculosis until 1940 and isoniazid and rifampicin were discovered in 1952 and 1962, respectively. Although these drugs were discovered many years ago, every year about 8 million people develop tuberculosis and 3 million people die from complications associated with the disease. It is estimated that 30-60% of adults in developing countries are infected, with tuberculosis being the first cause of death among people over 5 years of age. In Taipei, patients were in the aged group 24-35 years and more than 65 years (Wang, 2002). In Europe and the south-east Asia, patients were in the aged group more than 65 years. In 2002, World Health Organization (WHO) notified 49,656 patients in Thailand (WHO Report, 2004) and 6,906 deaths (Health Information Group, 2003) so it is an important cause of death. The most populated countries of Asia have the largest number of cases: India, China, Indonesia, Bangladesh and Pakistan together account for more than half of the global burden. There were 22 high-burden countries, including Thailand that WHO particularly noticed. WHO recommended Direct Observation of Treatment strategies (DOTs). In Pakistan, the study showed that tuberculosis patients were cured by DOTs therapy. The DOTs has been introduced in Thailand since 1996, the patient rate reduced thereafter.

There are several reasons for the increasing incidences of tuberculosis. The current increase in cases of HIV/AIDs is an important reason, while the development of the multidrug-reistant tuberculosis mutants, patient non-compliance and drug quality are also the factors. Drug stability, which determines the quality of a drug, varies with time, temperature, humidity and light. This has become an area of research in order to determine the clinical effects, patient safety and drug quality. Although scientists have researched new drugs and improved formulations for tuberculosis treatment such fixed-doses combination, inhaler formulation and microsphere formulation (Bain *et al.*, 1998) but classical drugs-rifampicin, isoniazid, pyrazinamide and ethambutol now are important to cure. Although classical drugs have been used for a long time but stability problems are still reported. Stability of rifampicin, isoniazid and pyrazinamide capsule/tablets in several hospitals was reported that their colors were changed during storage (Rookkapan, 2001). The behavior of the moisture gain of rifampicin, isoniazid, pyrazinamide and ethambutol at 40° C and 75 % RH in the absence and presence of light was determined as weight gain. Only pure ethambutol was used both in dark and lighted conditions because ethambutol is hygroscopic drug (Singh *et al.*, 2002). Ethambutol tablets in strip pack showed moisture uptake behavior at 40°C and 75 % RH in the absence and presence of light and the aluminum foil had pinholes revealed by scanning electron microscopy (SEM). Fixed-dose combinations (FDC) containing ethambutol in marketed blister packs gained more moisture at accelerated conditions (Bhutani *et a*l., 2003). Rifampicin deteriorated in acid condition 0.1 M HCl at 37°C for 50 minutes and FDC rifampicin and isoniazid showed higher degradation than rifampicin alone (Singh *et al.*, 2000). Rifampicin and isoniazid single tablet and FDC formulations contained less than 85% of stated contents and were substandard for use against tuberculosis (Laserson *et al.*, 2001). Many publications revealed stability problems, which affect the quality and effectiveness of antituberculosis drugs so the stability of antituberculosis drugs is an important area of study. When we accumulate stability data of antituberculosis drugs, we will be able to manage the drug quality and tuberculosis treatment. We used non-isothermal stability studies of antituberculosis drugs for preliminary test in order to establish the stability methods because non-isothermal stability is not reliable to predict shelf-life of drug.

## 1.2 Tuberculosis

Tuberculosis, the most common infectious disease worldwide, has probably killed 100 million people over the past 100 years, 5-10% of people become sick or infectious at some time during their life (Singh, 2004). *Mycobacterium tuberculosis* is spread by airborne droplet nuclei in size range of 1-5 μm diameter. The droplets can remain airborne for minutes to hours after expectoration by patient during coughing, sneezing or talking because their particle sizes are very small. The droplets can be inhaled into distal airway, and alveoli. *M. tuberculosis* is taken up by alveolar macrophages, replicates slowly but continuously and spreads via the lymphatic system. Cell-mediated immunity develops within 2-8 weeks to stop the progression. T-lymphocytes and macrophages are activated from granulomas that limit further replication and spread of M. tuberculosis. The results will be either successful containment of the infection or progression to active disease. The development of cell-mediated immunity against M. tuberculosis is associated with development of positive result in the tuberculin skin test.

*M. tuberculosis* is an aerobic, non-motile and non-spore-forming bacillus. Its cell wall is complex and contains a large amount of high-molecular-weight lipid (Yepes *et al.*, 2004). The cell wall is composed of two segments, upper and lower. Beyond the membrane is peptidoglycan in covalently attached to arabinogalactan, which in turn is attached to the mycolic acids with their long meromycolate and short α-chains. The uppersegment is composed of free lipids, some with longer fatty acid complementing the shorter α-chains, and some with shorter fatter acid complementing the longer chains. Interspersed somehow are the cell-wall proteins, the phosphatidylinositol manosides, the phathiocerol-containing lipid, lipomannan, and lipoarabinomannan. Mycolic acid-arabinogalactan-peptidoglycan complex the insoluble residues are essential for the viability of mycobacteria (Brennan, 2003). Mycobacteria can be detected in sputum smears by staining with either carbofuchsine (Ziehl-Neelsen) or flunochrome dyes. The latter is a more sensitive method. An estimated 10,000 organisms per milliliter of sputum are required for smear positive.

The most common clinic manifestation of tuberculosis is pulmonary disease. Symptoms of tuberculosis were mild and not specific and include night sweats, fever,

weight loss, anorexia and weakness but organ-specific symptoms of pulmonary tuberculosis include cough, pleuritic pain and hemoptysis. Sometimes the lack of specificity can result in a delayed diagnosis.

The goals of therapy are to ensure cure without relapse or die, to stop transmission and to prevent drug resistance. There are two treatment regimens, the first is the initial phase designed to kill actively growing and semidormant bacilli. Another is the continuation phase, which eliminate most residual bacilli and reduces numbers of failures and relapse. WHO recommended a guideline for tuberculosis treatment as shown in table 1 (Frieden, 2003).

Table 1 Guideline for tuberculosis treatment (Frieden, 2003)

| Treatment category | Patients | Tuberculosis treatment | |
|---|---|---|---|
| | | Initial phase | Continuation phase |
| I | New cases of smear-positive pulmonary tuberculosis or severe extrapulmonary tuberculosis or severe smear-negative pulmonary tuberculosis or severe concomitant HIV disease. | 2 months HRZE or 2 months HRZS | 4 months HR |
| II | Previously treated smear-positive pulmonary tuberculosis; relapse; treatment failure; treatment after default | 2 months HRZES/1 month HRZE | 5 months HRE |
| III | New case of smear-positive pulmonary tuberculosis or with less severe forms of extrapulmonary tuberculosis | 2 months HRZE | 4 months HR<br>6 months HE |

H=isoniazid, R= rifampicin, Z= pyrazinamide, E= ethambutol, S= streptomycin

### 1.3 Stability study

Stability is the capacity of a drug product to remain within specifications established to ensure its identity, strength, quality and purity. The purpose of pharmaceutical stability testing is to provide evidence on how the drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and to establish a re-test period for the drug substance or a shelf-life for the drug product and recommended storage conditions (ICH guidance, 2000).

#### 1.3.1 Theoretical part

Chemical stability was generally expressed in term of the rate constan ($k$) representing either product formation or drug degradation. For any mechanism, the rate of reaction ($k$) can be described by the general rate equation (Cartensen, 1990).

$$\frac{d\alpha}{dt} = kf(\alpha)$$

Where $\alpha$ is the conversion of the reaction and f ($\alpha$) is the conversion function. For the zero reaction, the reaction rate is independent of the drug concentration, while for the first order reactions, the rate depends linearly on drug concentration (Cartensen, 1990).

Zero order $\frac{d\alpha}{dt} = k$

First order $\frac{d\alpha}{dt} = k\alpha$

The temperature dependence on the rate constant, k is usually expressed by the Arrhenius equation that is linear dependence of the natural logarithm of the reaction rate, k, versus the absolute temperature (T).

$$k = A_k e^{-\frac{Ea}{RT}}$$

Where A is the pre-exponential factor, $E_a$ is the activation energy (cal/mole), T is absolute temperature (K) and R stands for the gas constant (g/mole).

### 1.3.2 Non-isothermal stability

Traditionally, accelerated pharmaceutical stability study was carried out at the fixed temperature. Isothermal stability study needs longer periods to carry out and considerably expensive equipment, such as temperature controlled cabinet. Non-isothermal method, where samples are placed in temperature ramping and sampling at various time points during a heat cycle had been suggested (Zhan *et al.*, 1997, Yoshioka *et al.*, 1987, Tucker, 1985, Crespo and Alvarez, 1985, Hempenstall *et al.*, 1983 and Tucker and Qwen, 1982). Different temperature program such as flexible, cycle, linear, logarithmic, hyperbolic, stepped had been used with non-isothermal method. Non-isothermal stability had been reported as an alternative to isothermal stability study. Therefore this method is a useful method for the quick and cheap assessment of stability characteristics of pharmaceutical formulation but it is not suitable for all kind of dosage forms. It is suitable for solution and suspension dosage forms though it had been published for solid dosage forms (Degim *et al.*, 2002). Non-isothermal kinetic method is based on the Arrhenius relationship. It was hard to determine the rate order of reaction in non-isothermal stability because non-isothermal stability literature used the specific computer program for each experiment (Zhan, 1997, Yoshioka, 1987 and Kipp, 1985). The Arrhenius parameters obtained from non-isothermal stability data were often reported to disagree with the values derived from isothermal stability study (Galway, 2003 and Vyazovkin *et al.*, 1999) there were two major reasons for this disagreement (Vyazovkin *et al.*, 1999). First, the Arrhenius parameters were determined by the term of conversion function ($f(\alpha)$) assumed but did not know the true reaction model decomposition. For this reason, the model-fitting methods tend to produce highly uncertain values of the Arrhenius parameters. Secondly, if decomposition involves several steps with different activation energies, the contributions of these steps to overall decomposition rate measured in the stability study would vary with both temperature and the conversion of reaction. The parameter values were in the fact an average that did reflect changes in the mechanism and kinetics with a temperature and a conversion of reaction (Vyazokin *et al.*, 1999).

### 1.3.3 Humidity

Humidity can have an effect on solid drug substances or drug products even for reactions which themselves do not involve water. The role of moisture in causing physical changes is related to water in the system rather than the moisture content of either the dosage form or the surrounding air (Waterman, 2005). Pharmaceutical solid form may contact with moisture during manufacturing processes and storage at high relative humidity (RH). The amount of moisture that is sorbed is dependent on the chemical properties, temperature, relative humidity and porosity of the packing material. The characteristics of excipients in solid dosage form affect the stability of formulation. The more amorphous excipient used in the formulation the more water is absorbed into the structure of excipients (Airaksinen *et al.*, 2005).

### 1.3.4 Packaging

The primary role of packaging is to protect the dosage form from the moisture and oxygen present in the atmosphere, light and other types of exposure especially if these factors affect the overall quality of product on long-term storage. There are many types of packaging materials such as glass, plastic, rubber, metal and paper. Plastic have become the most popular material for packaging pharmaceuticals because it is strong, lightweight, reasonably inert and chemical resistant. Most commonly plastic includes polyethylene (Low Density-LDPE; High Density-HDPE), polystyrene, polypropylene and polyvinyl chloride used in pharmaceuticals. Solid dosage form is popularly packaged in blister pack and aluminum foil. Two major factors, which affect the packaging, are leachable impurities and permeability of moisture and oxygen. Two blister packs, polyvinyl chloride and polyvinyl chloride with laminate of polymonochlorotrifluoroethylene both of which backed with impermeable foil. It was found that polyvinyl chloride laminated with polymonochlorotrifluoroethylene blister pack could protect solid dosage form better than polyvinyl chloride one (Amidon *et al.*, 1988). The stability of moisture sensitive compound (PEG-7763928) in polyvinyl chloride blisters, cyclic olefin blisters, aclar blister and cold-form aluminum blister was studied at 40°C and 75%RH for six months. The active ingredient was determined 84, 91, 97 and 100%, respectively

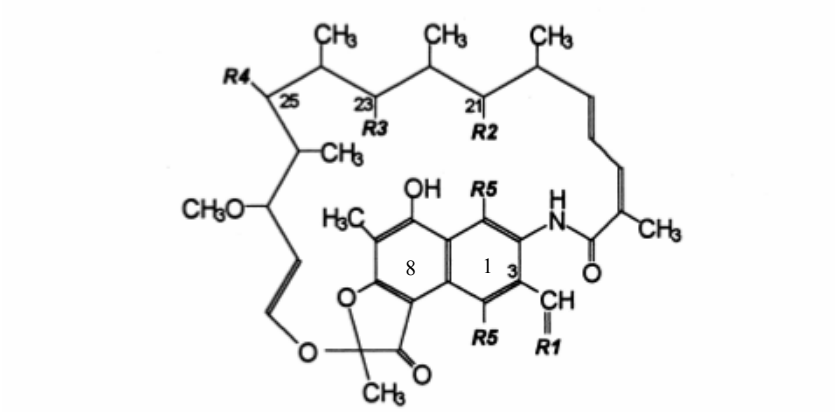
(Allison *et al*., 2001). For prednisone tablets in blister pack and aluminum foil stored in 40°C and 75%RH for eight weeks, their dissolution changed to 59 and 74%, respectively (Jenkin *et al*., 1993).

**1.4 Rifampicin**

Rifampicin is the key component of tuberculosis chemotherapy along with other first line anti-tuberculosis drugs. It is used in both intensive and continuation phase for the treatment of all patient categories (Agrawal *et al*, 2004). Rifampicin is designated by IUPAC as 2,7-(Epoxypentadeca[1,11,13]trienimino) naphtho[2,1-b]furan-1,11(2H)-dione,5,6,9,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl 1-8-[N-(4-methyl-1-piperazinyl) formimidoyl] -21-acetate. It is a semi-synthetic rifamycin obtained by condensation of 1-amino-4-methylpiperazine with 3-formyl-rifamycin SV in peroxide-free tetrahydrofuran at 10°-15°C (Gallo and Radael, 1976). Rifampicin melts with decomposition at 183-188°C. Rifampicin exists in water solution as the zwitterion with iso-electric point equal to 4.8. Rifampicin is a borderline class II drug of BCS from Biopharmaceutic Classification System (BCS)-i.e. solubility and permeability are considered as fundamental properties in predicting the in vivo performance of oral drug product.

Stability study of rifampicin showed many degraded products and their chemical structures are shown in Figure 1. Two major decomposition products exist, namely, 3-formylrifamycin SV and rifampicin quinone formed by hydrolysis and oxidation, respectively. Rifampicin quinone is purple and inactive, due to the fact that the free hydroxyl groups on C-1 and C-8 are essential for binding the drug to bacteria (Foye *et al*., 1995). 3-Formylrifamycin SV is insoluble and has poor absorption. Although it shows high antimicrobial activity in vitro but it is inactive in vivo. 25-deacetyl-21-acetylrifampin and 25-deacetyl-23-acetylrifampin have negligible antimicrobial activity (Bain *et al*., 1998). The four oxygen atoms at the C-1, C-8, C-21 and C-23 positions of rifampicin serve as the essential structural activity requirement to bind with the RNA-polymerases of *M. tuberculosis* to prevent further cell multiplication (Woff, 1994). The interaction between rifampicin and excipients was investigated. The results showed the possiblity of interaction between rifampicin and the following excipients;

methylparaben, manitol, citric acid monohydrate solution, magnesium sulfate, potassium hydrogen phosphate and ∝-lactose monohydrate. Moreover, photochemical testing of rifampicin powder was performed by exposing rifampicin to different light intensities. The results showed the first order process of rifampicin photodegration (El-Bary *et al.*, 2004). A pilot stability study of fixed dose combination (FDC) at 40 °C and 75% RH showed both physical and chemical changes in the blister packed products and unpacked products, and severe decomposition of rifampicin and intensive physical changes. The main decomposition product in solid state was isonicotinyl hydrazone of 3-formylrifamycin and isoniazid that were stronger under lighted condition (Singh and Mohan, 2003, Bhutani *et al.*, 2004). A significant finding is that pyrazinamide and perhaps ethambutol may play a catalytic role in the interaction between isoniazid and rifampicin. The mechanism of rifampicin degradation was found that pyrazinamide and ethambutol hydrochloride exhibit catalytical role through involvement of intra-molecular proton transfer during reaction between rifampicin and isoniazid to form isonicotinyl hydrazone (Bhutani *et al.*, 2005). The results from the stability study ofrifampicin in the presence of isoniazid in the pH range of 1-3 at 37°C in 50 minutes showed that rifampicin degraded to higher extent at pH 2, maximum pH in fasting conditions (Sankar *et al.*, 2003). For rifampicin eye lotion which composed of borate buffer and anhydrous ethanol, the activity was decreased to 21.84% and 76.43% at room temperature for 1 month and in refrigerator for 6 months (Guangrong, 1982). The reaction rate of rifampicin in aqueous solutions depended on the initial rifampicin concentration so the kinetic was the first order rate reaction. The half-life of solution was range from 1.08 to 5.9 hours (Connors *et al.*, 1986). The rifampicin capsule had a shelf-life for three years and five years for rifampicin powder (Pharmabiz.com, 2006 and IPCS Homepage).



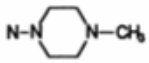
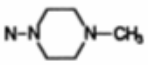
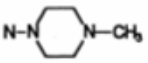
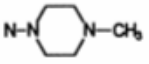
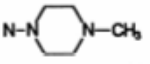
| Analyte | R1 | R2 | R3 | R4 | R5 |
|---|---|---|---|---|---|
| RIF |  | OH | OH | $CH_3COO$ | OH |
| RSV | O | OH | OH | $CH_3COO$ | OH |
| RQU |  | OH | OH | $CH_3COO$ | =O |
| DAR |  | OH | OH | OH | OH |
| 25-21 |  | $CH_3COO$ | OH | OH | OH |
| 25-23 |  | OH | $CH_3COO$ | OH | OH |

Figure 1. Chemical structures of rifampicin and its decomposition products.

### 1.5 Isoniazid

The chemical names of isoniazid are 4-pyridinecarboxylic acid hydrazide, pyridine-4-carboxyhydrazide and pyridine-ν-carboxylic acid hydrazide. It is a prodrug which gives an active metabolite, isonicotinic acid. Several hypotheses have had been proposed mode of isoniazid. It believed that isoniazid activation leads to inhibition of the synthesis of mycolic acid, a long chain fatty acid-containing component of the cell wall.

The two enzymes that believed to be targets of isoniazid are enoyl-acyl carrier protein reductase involved in the elongation cycle of fatty acid biosynthesis, and β-ketoacyl-acyl carrier protein synthase (Lei *et al.*, 2000). Another mechanism was depletion of intracellular nicotinamide adenine dinucleotide and reaction with tyrosine residues in mycobacterium protein (Klopmam *et al.*, 1996).

The literature reported that isoniazid syrup formulation major degradation product, hydrazine, was occurred by hydrolysis reaction at pH 3 in several different storage conditions over a period of 4 months (Carlin *et al.*, 1998). A stability study of isoniazid aqueous solution under anaerobic condition found the mixture of isonicotinic acid, isonicotinamide and 1, 2 diisonicotinyl hydrazine plus small amount of unidentified products (Brewer, 1966). The oxidation study of isoniazid in the presence of copper (II) ions brought about degradation product namely, isonicotinic acid, isonicotinamide, isonicotine-carboxaldehyde and 1, 2-isonicotinoyl hydrazone. The copper chelates of isoniazid were degraded by first order reaction (Brewer, 1977).

**1.6 Ethambutol**

Ethambutol dihydrochloride is an oral chemotherapeutic agent that is effective against *M. tuberculosis*. The structural formula is (+)-2,2′ (ethylenediimino)-di-1-butanol dihydrochloride. The (S,S)-configuration of ethambutol is found to be esssential for antibacterial activity compared with (R,R) and meso-isomer which configuration exhibit only 0.2 and 8.3% antibacterial activities. Mechanisms of action are not clear. Several hypotheses related to mode of action have been proposed. The hypotheses showed that ethambutol primarily affects the biosynthesis of arabinan in the arabinogalactan and sequentially lipoarabinomannan cell wall of *M. tuberculosis*. The targets might possibly be arabinosyltransferase enzyme (Ramalho et al., 2004). The important problem of ethambutol is hygroscopic compared with other antituberculosis agents. The stability study of ethambutol tablets packaged in strip packs at 40±1°C and 75±3% RH showed a minimal moisture gain existed in darkness and light. This indicated aluminum was impervious to moisture as well as light (Bhutani et al., 2003). The combined of ethambutol and isoniazid tablet was found white powder inside strip pockets that caused the

formation of saturated layer of drugs upon moisture gain through the defective packaging material and drying of this layer with time (Bhutani *et al*., 2004).

### 1.7 Pyrazinamide

Pyrazinamide together with the isoniazid and rifampicin is a key in chemotherpy tuberculosis. Its chemical name is pyrazine-2-carboxamide. It kills semi-dormant tubercle bacilli in acid environment that could not be killed by the other antituberculosis drugs. This condition occurs during active inflammation in which pyrazinamide kills mycobacterium slowly and incompletely. Pyrazinamide is a prodrug that is converted into active form pyrazinoic acid by bacterial nicotinamidase/pyrazimidase enzyme. Pyrazinoic acid inhibits both RNA and protein synthesis. The mechanism of action pyrazinamide is the reduction of transport of uracil and methionine which needed for RNA and protein synthesis (Zhang et al., 2003). For stability, pyrazinamide exhibits good stability in solid state. There was no apparent degradation of bulk sample, either in wet and dry atmospheres. Pyrazinamide is also stable when exposed to natural daylight. (Felder *et al*., 1983).

### 1.8 Objectives of this thesis

1. To study accelerated non-isothermal stability for accelerated isothermal stability study design.

2. To study accelerated isothermal stability of isoniazid, pyrazinamide, ethambutol tablets and rifampicin capsules associated with temperatures and relative humidity.

3. To predict drugs' shelf-life at the specified temperature and relative humidity.