Combined first line anti-TB drugs: New insights into stability

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“Excellence is never an accident. It is always the result of high intention, sincere effort, and intelligent execution; it represents the wise choice of many alternatives - choice, not chance, determines your destiny.”

— Aristotle

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Abstract

Tuberculosis (TB) is the most prevalent infectious cause of death globally, affecting approximately one-third of the world's population (Borgdoff *et al*., 2002). *Mycobacterium tuberculosis* (*M*. *tuberculosis*) is spread through small airborne droplets, generated through coughing, sneezing, or even by talking to a person with pulmonary or laryngeal TB (Knechel, 2009).

The treatment of tuberculosis with a multi-drug regimen requires therapy for a long period of time. This is associated with risks such as poor patient compliance, treatment failure and drug resistance. To limit these risks, the World Health Organisation and the International Union Against Tuberculosis and Lung Disease recommend the use of fixed-dose combination (FDC) tablets for the treatment of TB (WHO, 1999). The recommended multi-drug treatment approach of TB includes rifampicin, isoniazid, pyrazinamide and ethambutol, daily for 2 - 3 months. The use of FDCs may hence simplify treatment and encourage patient compliance, especially in patients who already take numerous medications, when co-infected with human immunodeficiency virus (Panchagnula *et, al*., 2004, WHO, 1999).

The four current anti-TB drugs, isoniazid, pyrazinamide, ethambutol hydrochloride and rifampicin, belong to two different classes of the Biopharmaceutical classification system (BCS). Isoniazid, pyrazinamide and ethambutol hydrochloride belong to class I (highly soluble and highly permeable) and rifampicin on the other hand, is the only hydrophobic ingredient of the FDC product (Ellard & Fourie, 1999). It has been postulated that polymorphism of rifampicin may be responsible for its variable bio-availability among its solid oral dosage forms (Agrawal *et al*., 2004). Rifampicin may react adversely with isoniazid to form isonicotinyl hydrazone (HYD) in formulation according to Singh *et al* (2000). Singh & Mohan (2003) further reported that pyrazinamide and ethambutol are catalytic towards the reaction between rifampicin and isoniazid, since FDCs that contain four-drug combinations have shown far more chemical instability than two-drug FDCs that only contain rifampicin and isoniazid (Singh & Mohan, 2003).

Various hypotheses have been put forward to explain inter-drug interactions that may occur in anti-TB FDC formulations and during oral administration. Therefore, for the purpose of

this study, the latest techniques were used to determine whether such reported chemical reactions indeed occur and under which conditions they would occur, if at all.

Hydrolysis experiments were done in distilled water to determine the extent of decomposition of RIF and INH using single, two, three and four anti-TB APIs. The aim of the investigation was to test the above hypotheses regarding the stability of especially RIF and INH in combination with EMB and PZA. Assays were done at 2, 3, 6, 12, 24 and 48 hours using solutions that were maintained at three different temperatures (5, 25, and 37°C – each ± 2°C). The results showed that EMB together with RIF and INH showed the greatest rate of degradation. Surprisingly the degradation of the four combination active pharmaceutical ingredients was less than that of the above mentioned three combination. Apart from a clear impact of INH and RIF on each other, the presence or absence of EMB and/or PZA also influences their rate of hydrolysis in water.

The microcalorimetry results showed at 40°C that no incompatibility exists with and without humidity. Previous studies have suggested that EMB together with humidity conditions is mainly responsible for the RIF degradation and the so called 'bleeding' of the tablets. However, it might be that the deliquescence of EMB masks any interaction or stability. It has been suggested that in the solid-state, HYD may also be formed because of a direct interaction between the imino group of RIF and the hydrazine group of INH. This interaction in the solid-state is exactly what we find with the microcalorimetry results at 50°C. The microcalorimetry results showed that an incompatibility exists between RIF and INH in the solid-state.

The moisture sorption results confirmed the hygroscopic nature of EMB, but the question remains is that moisture responsible for the degradation of RIF. The TAM and hydrolysis results were not conclusive about this. From the results it is not clear if the hygroscopic nature of EMB is solely responsible for the instability of four combination anti-TB drugs. The stability of the anti-TB FDC tablets remains a challenge to researchers and in future more analysis need to be proposed to solve this problem.

Keywords: Tuberculosis, rifampicin, isoniazid, ethambutol, pyrazinamide, fixed-dose combination solid-state stability, incompatibility

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List of abbreviations

AIDS	Acquired immunodeficiency syndrome
API	Active pharmaceutical ingredients
ASD	Amorphous solid dispersion
BCG	Bacilli Calmette-Guérrin
BCS	Biopharmaceutical classification system
BMI	Body mass index
CFA	Cefuroxime axetil
DOTS	Directly-observed therapy short course
DSC	Differential scanning calorimetry
EMB	Ethambutol
FDA	Food and drug administartion
FDC	Fixed dose combination
GIT	Gastrointestinal tract
HIV	Human immunodeficiency virus
HYD	Isonicotinyl hydrazone
HPLC	High performance liquid chromatography
ICH	International conference of harmonisation
INH	Isoniazid
IUATLD	International union against tuberculosis and lung disease
MDR-TB	Multi-drug resistant tuberculosis
M. Tuberculosis	*Mycobacterium tuberculosis*
PAS	Para-amino salicylic acid
PZA	Pyrazinamide

RH	Relative humidity
RIF	Rifampicin
SEM	Scanning electron microscopy
TAM	Thermal activity monitor
TB	Tuberculosis
TGA	Thermogravimetric analysis
Tg	Glass transition temperature
TM	Thermal microscopy
VTi	Vapour sorption analysis
WHO	World health organization
XDR-TB	Extensive drug resistant tuberculosis
XRPD	X-ray powder diffraction
3-FR	3-formylrifamycin

Research problem and aims of the study

Anti-TB FDC products have been reported to be unstable in formulation due to chemical interactions between the component drugs. It has been previously mentioned that ethambutol catalyzes the degradation of rifampicin and isoniazid in the formulation due to its hygroscopic nature. This was reported to result in the loss of rifampicin potency upon storage. Thus, for this study, the individual drugs of the FDC formulation will be individually characterised to investigate the reported drug-drug interactions, water uptake by ethambutol and possible degradation. Stability studies will also be performed to investigate the problem mentioned above.

Aim 1: Understand the relevant physico-chemical properties of anti-TB drugs. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) scans will be performed to monitor enthalpy changes and possible loss of solvent/moisture respectively. Thermal microscopy (TM) will be used to identify thermal events recorded in the DSC and TGA scans by directly observing the changes in the active pharmaceutical ingredients (APIs) relating to temperature. The presence or absence of any solid-state form changes that could possibly be induced by drug-drug interactions will be analysed by X-ray powder diffraction.

Aim 2: Investigate the degradation of rifampicin and isoniazid in the presence of ethambutol and pyrazinamide.

The aim of the investigation is to test the above hypotheses regarding the degradation of especially rifampicin and isoniazid in combination with ethambutol and pyrazinamide. Hydrolysis experiments will be done in distilled water to determine the extent of decomposition of rifampicin and isoniazid using single, two, three and four anti-TB APIs.

Aim 3: Perform compatibility and stability indicating tests on the four anti-TB drugs.

Gravimetric sorption analysis will be performed to determine the moisture sorption properties of the APIs. Compatibility studies on the API's will be performed using isothermal calorimetry to determine any incompatibilities with the individual drugs or with commonly use tablet excipients.

Chapter 1
Tuberculosis

1.1 Introduction

Tuberculosis is an ancient disease that has claimed the lives of humankind throughout history. It is the most prevalent infectious cause of deaths globally, killing around 1.8 million people in developing countries each year (Borgdorff *et al*., 2002). *Mycobacterium tuberculosis* (*M. tuberculosis*), a rod shaped, non-spore forming aerobic bacterium (Figure 1.1), is the causative agent of tuberculosis (TB), of which humans are the principal host. *M. tuberculosis* has a unique cell wall structure that is crucial to the survival of these acid-fast bacilli. The cell wall contains a considerable amount of fatty acid (mycolic acid) that is held together by the underlying peptidoglycan bound polysaccharide, arabinogalactan. The cell wall forms a barrier that is responsible for its medically challenging physiological characteristics (Knechel, 2009).

It is postulated that *M. tuberculosis* had originated in Africa, because the oldest known fossil records about TB in humans are from there (Daniel, 2004; Grange & Zumla, 2002). By utilising genetic data, Gutierrez *et al*. (2005) discovered an early progenitor of *M. tuberculosis* in East Africa, dating as far back as 3 million years ago. This suggests that early hominids already may have been infected with TB. It is believed that all modern members of the *M. tuberculosis* complex had originated from a common ancestral gene of 35 000 years ago. Modern strains of *M. tuberculosis*, however, may have originated from a common ancestor of 20 000 years ago. It is probable that *M. tuberculosis* had originated in Africa, and as humans left the continent to populate the world, they carried the disease with them (Gutierrez *et al*., 2005).

TB was certainly known in classical Greece, where it was referred to as phthisis. Hippocrates wrote about the disease and its clinical manifestations in his aphorisms, noting the prevalence of TB in young adults, especially. The Greek physician, Clarissimus Galen, also wrote about TB and recommended fresh air, milk and sea voyages for treatment (Coar, 1982).

TB had reached Europe and North America in the 18th and 19th centuries, respectively, after which it began to decline, probably because of the improved living conditions of people.

René Laennec was the first scientist to expound on the pathogenesis of TB. His work was made possible by his extensive experience gained from autopsies done on persons who had died of TB at the Necker Hospital in Paris. Despite their knowledge about the pathology of TB, health workers still struggled to comprehend its cause. In 1865, Jean-Antoine Villemin clearly demonstrated the infectious nature of TB, by inoculating a rabbit with purulent liquid from a tuberculous cavity. Although the rabbit had seemingly remained healthy, autopsies done 3 months later revealed that it had had extensive TB (Daniel, 2004; Daniel, 2006).

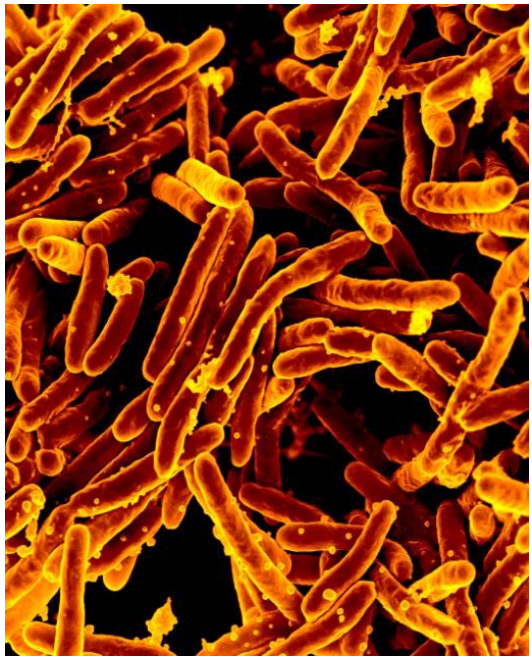

Figure 1.1: Electron microscopic image of the rod shaped, non-spore forming aerobic *Mycobacterium tuberculosis* bacteria (Flores, 2012).

In 1882, Robert Koch identified tubercle bacilli as the causative agents of TB. He also introduced his famous postulates, which, to this day, are still used as a standard for demonstrating infectious diseases. In 1907, Clemens Freiherr von Perquet developed the tuberculin test and noted that positive tuberculin reactions had reflected latent TB in asymptomatic children. In 1908, Charles Mantoux presented the use of a cannulated needle and syringe for injecting tuberculin intra-cutaneously (Shet, 2012). Albert Calmette and Camille Guérrin were the first to develop a vaccine, Bacille Calmette-Guérrin (BCG), against TB, which was first successfully used in humans in 1921. The history of TB had changed dramatically since the discovery of para-amino salicylic acid by Jorgen Lehman in 1944, and of thiosemicarbazone by Gerhard Domagk in 1945. These agents were unfortunately only

bacteriostatic in nature. In 1944, Schatz and his associates managed to isolate streptomycin, the first effective bactericidal agent against TB. Isoniazid was the first oral mycobacterial agent to be used, followed by the rifamycins in 1957 (Daniel, 2006).

1.2 Clinical manifestations

M. tuberculosis is spread through small airborne droplets, generated through coughing, sneezing, or even by talking to a person with pulmonary or laryngeal TB. When these droplets reach the lungs, they cause infection of the respiratory system. However, these organisms can also spread to other organs and cause extra-pulmonary TB (Knechel, 2009). The clinical manifestations of pulmonary TB are generally progressive (Table 1.1). The patient may present with a moderate or severe disease or may present with no symptoms at all. A productive cough of yellow or green sputum is the most common indication of a TB infection. Night sweats and dyspnoea may also occur, while haemoptysis (expectoration of blood) only occurs in cavitary pulmonary disease (Beers & Porter, 2006).

Table 1.1: Clinical manifestations of pulmonary tuberculosis (Wells *et al*., 2000)

Signs and symptoms	Patients typically present with weight loss, fatigue, a productive cough, fever and night sweats.
	Noticeable haemoptysis.
Physical examination	Dullness to chest percussions, rales and increased vocal fremitus are observed frequently on auscultation.
Laboratory tests	Moderate elevations in the white blood cell count with a lymphocyte predominance.
Chest radiograph	Patchy or nodular infiltrates in the apical area of the upper lobes, or in the superior segment of the lower lobes. Cavitation that may show air filled levels as the infection progresses.

1.3 Epidemiology

Approximately one-third of the world's population (2 billion) is infected with TB. It is estimated that more than 8.8 million patients develop active TB annually, with 1.6 million related deaths every year, of which 95% of all cases are reported in developing countries.

Twenty-two countries that have been labelled as “high burden” countries account for 80% of the global TB load. According to the World Health Organisation (WHO) (2007), among the fifteen countries that have the highest incidence rates of TB, twelve are in Africa. In 2005, 5.1 million TB cases were reported to the WHO, of which 2.4 million were new sputum smear-positive cases. Of these cases, 35% were diagnosed in the South-East Asian region, 25% in the West Pacific region, 23% in Africa, 4% in America, 5% in the East Mediterranean and 7% in Europe. Figure 1.2 geographically illustrates the global TB prevalence rates of 2005. This enormous imbalance of the TB burden has been attributed to insufficient funding of public health services in those worse affected countries, the human immunodeficiency virus (HIV) pandemic and the emergence of drug resistant TB (WHO, 2007; WHO, 2002). The 2013 WHO report estimated that 1.1 million of those people who had developed TB in 2012 had been HIV positive and that 75% of those cases were from Africa. The TB incidence rate is said to range between ±1 000 cases per 100 000 people living in South Africa and in Swaziland. According to estimates in the 2014 WHO report, there were 9 million TB cases in 2013 and 1.5 million related deaths, of which 1.1 million deaths were among HIV negative people, while the remaining 0.4 million were among HIV positive people (WHO, 2014; WHO, 2013). The 2015 WHO report estimated that of the 9.6 million people whom had fallen ill with TB in 2014, 5.4 million were men and 4.2 million were women. Of the 1.5 million people killed by TB in that year, 1.1 million were HIV negative and 0.4 million were HIV positive. On the positive side, the occurrence of TB has fallen by approximately 1.5% per year since 2000.

The World Health Assembly implemented its “End TB strategy” in 2014, aimed at ending the global TB epidemic, starting in 2016. Their goal is to reduce the number of TB deaths with 90% by 2030, to cut new cases by 80% and to reduce the financial costs incurred by families for the treatment of TB (WHO, 2016; WHO, 2015).

It is clear that the spread of TB is not only a matter of the transmission of TB infections but is it also a reflection on the global wealth distribution. In most parts of sub-Saharan Africa, where there is a serious lack of financial and medical resources, TB rates are the highest. In countries where TB therapies are more freely available, but not used appropriately, or where there are shortages in anti-TB drugs, multi-drug resistant TB has emerged. According to Holtz (2008), in regions where TB programs had not been funded and where there had been no political support, there were increases in TB infections and in multi-drug resistant TB.

High TB rates can be associated with a low socio-economic status, poor living conditions and limited access to public health systems. Urgent action is therefore needed to address such problems, before an absolute decline in TB infections will occur.

1.4 Socio-economic impact

TB is principally known as a disease of the poor. In Haiti it is known as "*maladi ti kay*", meaning "a sickness of the little house". Persons with TB are often ostracised and driven out of their communities. In the 19th century, however, TB was often romanticised. Lord Byron was quoted as saying, "I should like to die of a consumption, because the ladies would all say, 'Look at poor Byron. How interesting he looks in dying!'" (Daniel, 2004).

Studies that had been done to assess the TB burden on vulnerable populations, such as prisoners, the homeless and certain minority groups, concluded that there is a correlation between social deprivation and the risk of TB infection, which can be attributed to the following factors:

- People of a low socio-economic status are more frequently in close contact with persons suffering from active TB;
- Aspects of socio-economic status, such as mal-nutrition and low income make it difficult for patients to afford healthy foods;
- There is a higher likelihood of over-crowding and poorly ventilated living conditions; and
- There are lower levels of awareness regarding healthy lifestyles among these groups (Lönnroth *et al.*, 2009).

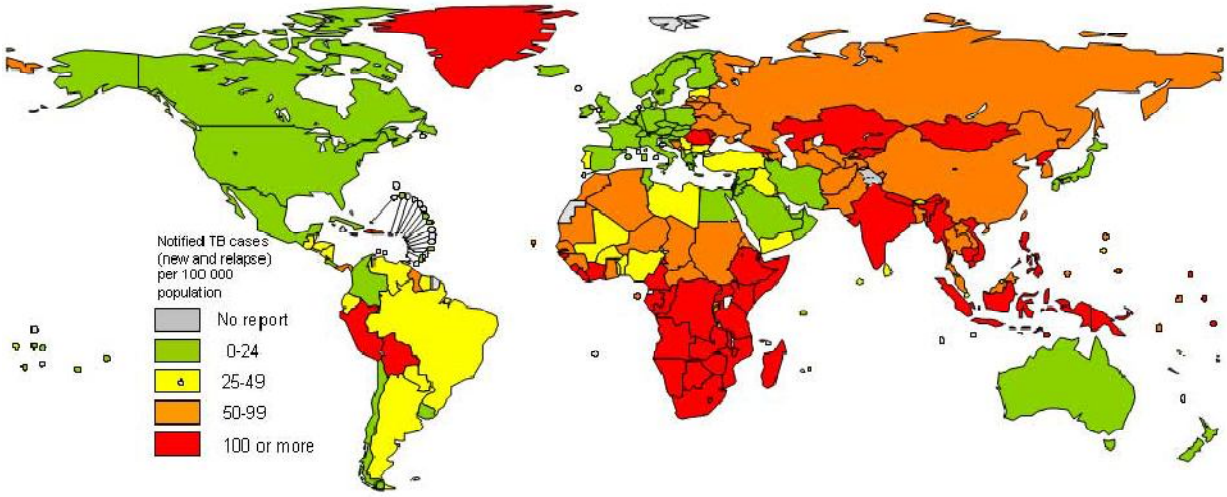

Figure 1.2: Geographic representation of reported tuberculosis incidences globally in 2005 (WHO, 2007).

1.5 The correlation between human immunodeficiency virus and tuberculosis

TB and human immunodeficiency virus (HIV) infections are inseparably linked. TB now ranks alongside HIV as the leading cause of human deaths globally. In certain communities, TB infection is seen as a sign of being infected with HIV. The stigmas that are associated with TB and HIV then lead to delayed health seeking by TB infected persons (Daftary, 2012). In 2009, 12% of the over 9 million new TB cases being reported to the WHO, were HIV positive (WHO, 2010). Countries, such as South Africa and Zimbabwe carry most of this HIV associated TB burden, with at least 50% of all new TB cases being related to HIV. The risk of developing active TB in persons being infected with HIV exceeds 10%. Contrary, the risk of developing active TB is less in persons infected only with TB (Myers & Sepkowitz, 2008; Corbett *et al.*, 2003).

TB is one of those opportunistic infections that mark the progression of HIV infections into acquired immunodeficiency syndrome (AIDS). It is also the leading cause of deaths amongst persons living with AIDS (Phillips, 2007). TB accelerates the course of an HIV infection by increasing the viral load in some patients, thus making TB the number one cause of deaths in HIV infected persons. Due to the HIV mortality rates, the life expectancy in sub-Saharan Africa has decreased to 45 years (Murray & Lopez, 1997; Holtz, 2008).

Co-infection of TB and HIV results in major public health challenges, such as the emergence of multi-drug resistant TB (MDR-TB). The prevalence of MDR-TB is much higher in HIV infected persons than in HIV negative persons. The presence of MDR-TB in persons with HIV makes therapy almost impossible, resulting in patients having a reduced survival rate. Although TB treatment is effective in HIV infected persons, adverse reactions of the drugs may complicate therapy. Medication that is used in TB therapy may have overlapping toxicities with anti-retroviral drugs. Rifampicin, the mainstay of TB therapy for instance, is a cytochrome P450 enzyme inducer, which metabolises all three the major classes of anti-retrovirals. Co-administration of rifampicin with anti-retrovirals can lead to reduced systemic levels of HIV medication, which in turn may lead to more resistant strains of *M. tuberculosis* (Myers & Sepkowitz, 2008).

1.6 Challenges with regards to drug resistance

Multi-drug resistant TB is defined as *M. tuberculosis* that is resistant to at least isoniazid and rifampicin. Since MDR-TB does not occur because of horizontal gene transfer, but rather through the natural selection of rare drug resistant strains in the human body, MDR-TB is a man-made phenomenon. In 2008, the WHO reported 440 000 cases of MDR-TB of which 360 000 cases were new, while 94 000 cases were persons who had previously been treated for TB. The global epidemic of MDR-TB is mainly caused by a combination of acquired and primary resistance (WHO, 2008). The latter is defined as the transmission of drug resistant strains of *M. tuberculosis*, giving rise to MDR-TB in individuals who had never been exposed to anti-TB drugs before.

MDR-TB can be caused by the following factors:

- Inconsistent, or interrupted treatment (public health systems and/or patient factors);
- The prescription of incorrect drugs for the relevant treatment phase;
- The prescription of drugs of poor quality;
- An interruption in the supply of drugs; or
- Direct exposure to and transmission from an MDR-TB infected individual.

While MDR-TB is generally treatable, it may take up to 2 years to complete the regimen and may the program use more toxic and expensive drugs than when treating a drug susceptible case (Gandhi *et al*., 2010a).

Co-infection of TB and HIV may also lead to an increased rate of spontaneous resistance-conferring mutations. Persons with HIV associated TB may have a lower compliance rate, due to the increased medication burden, the overlapping of adverse reactions, or as a result of the fragmentation of TB from HIV/AIDS in health care systems. These problems are direct results of inadequate infection control (Holtz, 2008).

In 2005, another phenomenon was described, referred to as extensively drug resistant TB (XDR-TB). XDR-TB is defined as TB resistance to any flouroquinoline and one of the three second-line injectable drugs, i.e. amikacin, kanamycin, or capreomycin. XDR-TB has been reported all over the world, with Asia and Eastern Europe carrying the largest burden. In 2006, the mortality rate of XDR-TB infected persons, co-infected with HIV, was as high as 98%. These alarming results were documented for a high prevalence setting in rural KwaZulu-Natal (KZN) in South Africa. Failing TB programs and the lack of support from government have created fertile conditions for a "perfect storm" of drug resistant TB (Gandhi *et al*., 2010b).

The need for long-term, multi-drug therapies has been postulated to stem from two different drug mechanisms. One is genetic resistance that is heritable and fixed, while the other is a phenotypic, reversible resistance to administered antibiotics. The higher the bacterial burden, the more likely it is to contain strains that are genetically resistant. Therapy failure is said to result from genetic resistance that is related to the frequency of pre-existing resistant mutants. In patients who relapse after appropriate therapy, the bacteria can remain susceptible to the initial antibiotics and can be eradicated by the same treatment. This can be attributed to non-replicating bacteria that survive until anti-TB therapy is stopped, which then cause relapse as soon as they resume their growth in the absence of therapy. Long-term therapy may cure the infection by eradicating these bacterial populations, as soon as they resume their replicating state (Connolly *et al*., 2007).

1.7 Tuberculosis control strategies

Currently, four anti-TB drugs are recommended by the WHO and must they be taken daily in the initial phase of treatment, i.e. rifampicin, isoniazid, ethambutol and pyrazinamide. In cases where ethambutol is contra-indicated, streptomycin may be used instead (Panchagnula *et al*., 2004; WHO, 1999). In addition to this standardised drug treatment regimen, further TB control strategies have been implemented globally to deal with the TB crisis.

Directly observed therapy short course (DOTS) was a global control strategy for TB treatment that was initiated by the WHO. It was the basis of the WHO's "Stop TB" campaign that was introduced in June 2005. The goal of this strategy was to decrease the prevalence of TB mortality by up to 50% in 2015 (WHO, 1997). Although the DOTS strategy might have been essential, it is, however, important to note that additional interventions, or TB based programs are necessary for establishing long-term TB control in countries that experience a higher occurrence of TB (Myers & Sepkowitz, 2008).

Alongside the DOTS strategy, as already mentioned, a more recent initiative was introduced in 2014, called the" End TB strategy", of which its three main goals are to:

- Reduce TB deaths with 90% by 2030;
- Cut new cases of TB by 80% between 2015 and 2035; and
- Ensure that no family is burdened with excessive expenses, due to TB treatments.

Two milestones have been set for 2020 and 2025, i.e. a 35% reduction in the number of TB deaths, and a 20% reduction in TB incidences, compared to the levels in 2015. It is estimated that if these two targets are met in 2025, TB could eventually be successfully eradicated (WHO, 2016; WHO, 2015).

1.8 Tuberculosis in South Africa

TB is a major public health problem in many countries, including in South Africa. TB has been documented as being the leading cause of morbidity and mortality in South Africa. In

2005, the incidence rate of TB was 600 new cases per 100 000 persons annually, which was the third highest rate per country in the world (Harling *et al*., 2008; WHO, 2007).

Studies done with regards to the relationship between TB and socio-economic determinants revealed that there is a positive relationship between TB infection and poverty, the lack of education and of government social support, and social deprivation (Munch *et al*., 2003).

Harling *et al*. (2008) conducted a study to analyse the social determinants of TB in South Africa. Their study revealed the following:

- Personal education served as a shield against TB infection;
- Employed individuals had a lower chance (40%) of contracting TB;
- Minors had a two-fold increase in the chance of having TB at some time during their lifetime;
- Missing meals, due to a lack of funds, increased the chance of having TB by two-fold; and
- Alcohol abuse, cigarette smoking and a low body mass index (BMI) were all risk factors for developing TB.

This study thus suggested that TB incidences could be associated with smoking, alcohol consumption, mal-nutrition, low levels of personal education, unemployment and a lower household income (Harling *et al*., 2008).

1.8.1 Multi-drug resistant and extensively drug resistant tuberculosis in South Africa

In 2008, the WHO reported 440 000 cases of MDR-TB, of which 13 000 had been diagnosed in South Africa. MDR-TB has reached epidemic proportions in South Africa and is it utilising resources that are necessary to combat drug susceptible TB. It has been estimated that MDR-TB treatments consume about 70% of the budget that is allocated to treat the entire TB epidemic in South Africa. The incidence rate of MDR-TB is a direct result of poor infection control and the poor treatment of new MDR-TB strains (Streicher *et al*., 2012; WHO, 2010).

The existence of MDR-TB has fuelled the emergence of XDR-TB, which requires even more expensive treatment regimens than MDR-TB. The occurrence of XDR-TB was first reported by the WHO on 1 September 2006. XDR-TB was first detected in the Tugela Ferry in KwaZulu-Natal (KZN) (South Africa) (Figure 1.3). XDR-TB was considered endemic to KZN, with thirty-nine hospitals harbouring patients with XDR-TB and thirty new cases being reported every month in KZN alone. Some of the factors that have been fuelling the outbreak of MDR and XDR-TB are as follows:

- High incidences of treatment interruptions of drug susceptible TB;
- Low cure rates and the HIV epidemic;
- Inadequate health care system response;
- Poverty and global inequity;
- The lack of infection control in institutions; and
- Government suspending welfare benefits to patients with MDR and XDR-TB for the duration of their hospitalisation (Singh *et al*., 2007; Yong *et al*., 2005; Verma *et al*., 2004).

Figure 1.3: Map of South Africa illustrating the geographical location of the Tugela Ferry in KwaZulu-Natal (KZN) (Singh *et al*., 2007).

With TB being one of the leading causes of deaths in South Africa and with MDR and XDR-TB spreading throughout the country, a new solution for combating resistant strains of TB is urgently needed. If the government and members of communities remain complacent, South Africa may reach an era where TB would no longer be curable, and could the country be left with an epidemic that is much greater than the long prevailing HIV epidemic.

1.8.2 Anti-tuberculosis medication adherence and culture in South Africa

Anti-TB medication is available at no cost in South Africa. However, obstacles exist that prevent patients from receiving efficient care and from adhering to prescribed medication. Some of these difficulties include poor nutrition, the lack of transport, the fear of stigma stemming from the correlation between TB and HIV, and the conflicts that arise with regards to Western medicines and traditional beliefs (McInerney *et al*., 2007).

Recent studies have shown that the delay in receiving Western medical treatment, due to TB patients seeking the help of traditional healers first, may have dire consequences to their health. Understandably, many patients in rural settings visit traditional healers prior to consulting with government health services, as they may have to travel long distances and incur transportation costs to reach the nearest primary health care clinic, which they cannot afford. It was found that patients who had visited traditional healers first, were in worse conditions by the time that they sought the help at government health services. It was hence concluded that the time spent with traditional healers could be ineffective and detrimental to the health of TB patients (Barker *et al*., 2006).

Colvin *et al*. (2003), however, proved that traditional healers could effectively contribute towards TB programmes in rural areas, in a study that was conducted in Hlabisa, a rural health district in KZN, South Africa. Twenty-five traditional healers had volunteered to serve as supervisors of TB treatments in a community-based TB control programme. At completion of this study, most of the patients were satisfied with their supervisors, with one of the reasons being that some of the traditional healers had often offered food to patients when going for treatment. The main advantage of this programme was the easy access to traditional healers, who were even as close as a few yards away from some patients, hence saving money on travel. The findings from the Hlabisa study suggested that traditional healers may play a positive role in TB control programs (Colvin *et al*., 2003; Wilkinson *et al*., 1999).

1.9 Conclusion

Tuberculosis treatment requires long-term, multiple drug treatment regimens, because of the organism's cell wall having the ability to form a barrier against anti-TB drugs and to therefore develop drug resistance. Drug resistance results in patients having to take multiple capsules and tablets throughout the day for long periods of time. Long-term treatment regimens, consisting of high dosage burdens result in many disadvantages that are non-conducive to patients' recovery from TB, as will be discussed in the following chapter. In Chapter 3, fixed-dose combination products, and their advantages and disadvantages in the treatment of TB are discussed.

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Chapter 2

Solid-State Forms and Physico-Chemical Properties of Pharmaceuticals

2.1 Introduction

A large number of organic and inorganic compounds that are pharmaceutically relevant exist in one or more solid-state forms. Such different forms of a single compound may have different physical and chemical properties. The existence of different solid-state forms of a drug offers pharmaceutical scientists the opportunity to select the best form in terms of solubility and stability, for use in formulations. It is therefore important to study the solid-state properties of active pharmaceutical ingredients (APIs) beforehand to identify potential development challenges, or opportunities and to avoid manufacturing problems.

2.2 Solid-state forms of pharmaceutical compounds

Different solid-state forms of APIs commonly include polymorphs, solvates, hydrates, desolvates, co-crystals and amorphous forms, depending on their compositions, inter-molecular bonds, molecular arrangements and/or conformations. The United States of America Food and Drug Administration (FDA) have issued certain guidelines to ensure that solid-state forms are controlled during manufacturing processes (FDA, 2007). The FDA states that appropriate analytical procedures should be used to detect polymorphic, solvated, or amorphous forms of a drug substance. They further emphasise the importance of controlling the solid-state form of the drug substance during manufacturing, storage and distribution processes.

2.2.1 Crystalline forms

Pharmaceutical solids can be classified as crystalline and amorphous forms. Crystalline solids are characterised by the presence of a three-dimensional, long range order. Crystalline systems can occur in a vast number of polymorphic forms that contain the same elemental compositions but are characterised by differences in unit cell structures that arise from packing, or conformational variances (Healy *et al.*, 2017).

2.2.1.1 Single component forms: Polymorphs

Polymorphism refers to the phenomenon whereby an API may exist as two or more distinct crystalline forms, in which the molecules have different arrangements and/or conformations in the unit cell (Lu & Rohani, 2009). Polymorphs are classified as either monotropes, or enantiotropes with regards to their stabilities over a temperature range. In an enantiotropic system, one of the polymorphs is stable across a certain temperature and pressure range, while the other polymorph is stable over another. Monotropic systems exist when only one polymorph is stable at all temperatures below the melting point, whereas all the other polymorphs are unstable (Purohit & Venugopalan, 2009; Byrn, 1999).

Burger and Ramburger (1979) proposed four rules for determining the enantiotropic, or monotropic nature of the relationship between polymorphs. These rules, as summarised in Table 2.1, are important for consideration during crystallisation processes, because when crystallisation occurs above the transition temperature, it will favour one form, while favouring the other below that transition temperature (Burger & Ramburger, 1979).

2.2.1.2 Ionic multi-component forms: Salts

Salt formation is said to be the most basic and cost-effective strategy for increasing the aqueous solubility and bio-availability of ionisable drugs. Salt formation consists of three components, an acid, a base and one or more solvents. A salt is formed through the transfer of a proton from an acid to a base. Salt formation may increase both the solubility and dissolution rate of acidic and alkali drugs. However, a compromise between solubility and stability must often be made, since the most soluble form may not be the most stable or optimal form, due to increased hydrolysis, a common degradation pathway (Elder *et al*., 2013). The conversion of an API into a particular salt form may modify and even optimise its physico-chemical properties. When changing the salt, however, one needs to consider the implications on the safety and toxicity of the drug. The change from a salt may also affect the biological properties of the drug. The most appropriate salt form of an API should be identified at an early stage of development to optimise the characteristics of the final formulation (Bastin *et al*., 2000).

The choice of a particular salt form can impact the physico-chemical properties of a drug, and hence the optimal formulation of the dosage form and large-scale manufacturing. The melting point of a particular salt plays an important role on the stability of a formulation. APIs with

low melting points are known to exhibit plastic deformation that can result in the caking and aggregation thereof. The latter can alter the flow properties, compression profile and subsequently impact negatively on the dose uniformity, friability, disintegration and dissolution rate of solid dosage forms (Verbeeck *et al*., 2006).

Table 2.1: Rules for determining the relationship between polymorphs of the same substance (Burger & Ramburger, 1979)

Rule	Explanation
Heat of transition rule	If an endothermic transition is observed at some temperature below the melting point, it may be assumed that there are two enantiotropically related forms. If an exothermic transition is observed below the melting point, it may be assumed that there are two monotropically related forms, or that the transition temperature is higher.
Heat of fusion rule	If the higher melting form has the lower heat of fusion, the two forms are usually enantiotropic.
Infrared rule	If the first absorption band in the infrared spectrum of a H_2-bonded molecular crystal is higher in the one modification than in the other, it may be assumed that the former has the larger entropy.
Density rule	If one modification of a molecular crystal has a lower density than the other, it may be assumed that the former is less stable at a certain temperature.

2.2.1.3 Non-ionic multi-component forms: Molecular adducts

Hydrates and solvates are multi-component, crystalline, solid, molecular adducts, as they contain both the host molecule (API/excipient) and the guest molecule within the crystal lattice structure. Water and other solvent molecules tend to form hydrogen bonds and co-ordinate covalent bonds in the crystal lattice with the APIs or excipients. APIs and excipients with small molecular weights usually readily form solvates and hydrates, due to their small molecular sizes. Water molecules consist of both hydrogen bond donor and acceptor atoms that can form inter-molecular hydrogen bonding with molecules. As a result, hydrates are known as the most common type of solvated organic compounds (Aaltonen *et al*., 2009).

a) Solvates and Hydrates

Solvates are formed when solvent molecules are included in an API's crystal lattice in either stoichiometric, or in non-stoichiometric amounts. Hydrates are solvates in which the included solvent is water. In stoichiometric solvates, the included solvent is an integral part of the crystal structure. The desolvation mechanism represents a comprehensive re-arrangement of the host molecules, resulting in a completely different crystal structure, or even an amorphous form. Solvates that contain solvent in their channels may often show non-stoichiometric behaviour. The solvent can fully or partly escape through these channels, with insignificant changes in the crystal structure. Non-stoichiometric hydrates may exhibit variable water contents, depending on the water activity, or the relative humidity of the environment. The structure expands or contracts an-isotropically to account for the variability of the lattice water (Braun & Griesser, 2016). Desolvated solvates exist when a solvate is desolvated and the crystal retains the structure of the solvate (Byrn *et al.*, 1995).

Although rare, anomalous stoichiometric variations may occur if the length of the guest molecule is disproportionate to the host channel that accommodates it (Stieger *et al.*, 2010).

Crystal hydrates (when water is the crystallisation solvent) are said to account for almost one-third of pharmaceutically active substances (Vippagunta *et al.*, 2001).

Crystalline solvates, based on their structures, may be classified into three distinct groups:

- **Class I: Isolated site solvates:** Solvent molecules are isolated from each other by intervening drug molecules.
- **Class II: Channel solvates:** Solvent molecules are included in the crystal structure by lying next to other molecules of neighbouring unit cells along the axis of the lattice, which results in the formation of channels which spread throughout the crystal lattice. Channel solvates are further sub-divided into expanded channel solvates, planar solvates and desolvated solvates. Expanded channel solvates take up additional vapour in their channels when exposed to high vapour pressure of that solvent. Vapour molecules may cause the crystal to expand or contract as the solvation or desolvation proceeds, causing changes in the dimensions of the unit cells. Planar solvates are channel solvates in which the guest solvent is localised in two-dimensional planes. Desolvated solvates are crystals that have desolvated, sometimes despite having been exposed to high vapour pressure. The term is usually reserved for

crystalline forms that do not immediately undergo structural transformation during desolvation. Desolvated solvates are therefore, at least initially, iso-structural to the solvates from which they originate.

- **Class III: Non-associated solvates:** These solvates are based upon the co-ordination of solvents and metal ions (Vippagunta *et al*., 2001; Morris, 1999).

b) Co-crystals

Co-crystal formation is an attractive alternative for non-ionisable drugs, or compounds with pKa values in a range where salt formation is limited. The FDA defines co-crystals as 'solids that are crystalline materials, composed of two or more molecules in the same crystal lattice'. Co-crystals consist of an API and a neutral guest compound conformer in the crystal lattice. Unlike salts, where the components in the crystal lattice are in an ionised state, the components of a co-crystal are in a neutral state and they interact through non-ionic interactions (Elder *et al*., 2013; FDA, 2011). Co-crystals are crystalline solids that are assembled through non-covalent interactions, such as hydrogen bonds, ππ or van der Waals interactions. Some co-crystals may exist as polymorphs, hydrates and solvates. Of the biggest advantages of co-crystals are the possibility to obtain better soluble and more stable forms of poorly soluble, neutral components, which are especially important to class II and class IV drugs. Another advantage is the possibility to extend the life cycle of old APIs such as sacubitril-valsartan, which was in 2015 approved by the FDA for the treatment of heart failure (Pindelska *et al*., 2017).

Co-crystals can be used to increase the solubility and dissolution rate of a drug, and subsequently its rate and extent of absorption. However, an important aspect regarding crystal formation, which often leads to its transformation back into the free base, or acid (pure API), has to be kept in mind. If the solubility of the co-crystal is higher than that of the API, and the conformer and the API dissociate completely in solution, dissolution will lead to an API concentration that exceeds the solubility of the API. This results in a super-saturated solution of the API, which means that the API is likely to precipitate (Elder *et al*., 2013).

2.2.2 Liquid crystalline forms

The liquid crystalline state combines both the qualities of the liquid and solid states. Liquid crystals thus represent an intermediate phase between the solid and liquid phases and are also

called mesopheres. Liquid crystals have several common characteristics, such as rod-like molecular structures, rigidness of the long axis and strong dipoles, or an easily polarisable substituent. The distinguishing characteristic of liquids is the tendency of the liquid crystal molecules to point along a common axis, known as the *director*, which leads to a state known as an-isotropy. This means that the properties of the material will be dependent upon the direction in which they are measured (Stevenson *et al*., 2005).

Three parameters describe the liquid crystalline structure name, i.e.:

- **Positional order**: The extent to which an average molecule shows transitional symmetry;
- **Orientational order**: A measure of the tendency of the molecules to align along the director on a long-range basis; and
- **Bond orientational order**: Describes a line joining the centres of the nearest neighbour molecules, without requiring a regular spacing along the line.

Liquid crystals can be further classified as *thermotropic* and *lyotropic* crystals. The thermotropic state can be achieved by increasing the temperature of the solid, while lowering the temperature of the liquid. If the temperature increase is too high, thermal motion will destroy the ordering of the liquid crystal phase, pushing the material into the isotropic liquid phase. If the temperature is sufficiently low, liquid crystal materials will form conventional crystals. Lyotropic liquid crystals form in solution, rather than in pure substances. The molecules do not align themselves, but rather come together to form an-isotropic aggregates, which align along the director. The advantages of liquid crystalline systems include their thermodynamic stability, high solubilisation levels, improved bio-availability, protection against oxidation, and the controlled release properties of such drugs (Maiti, 2012).

2.2.3 Amorphous forms

2.2.3.1 Single component amorphous forms

An amorphous form, or glass, is a solid that lacks the long-range molecular order, characteristic of a crystal, and is it basically formless, or without shape. The lack of a three-dimensional long-range order is commonly observed as a diffuse X-ray diffraction pattern and the absence of a melting endotherm. An amorphous API can therefore not be characterised as a crystal (Byrn *et al*., 1995). Generally, two types of amorphous solids exist,

i.e. molecularly pure amorphous solids, and amorphous solids that are combined with one or more other molecular entities in order to improve, or otherwise manipulate the physico-chemical properties of the resultant amorphous solid dispersion (Ilevbare *et al*., 2015; Konno *et al*., 2008).

Amorphous solids are generally known to have higher apparent solubility and higher dissolution rates than their crystalline counterparts. Amorphous systems do not require breakage of the crystal lattice and have higher energy, entropy and free energy than their crystalline counterparts, which are responsible for their higher apparent solubility and dissolution rates (Yu, 2001). Amorphous substances are, however, thermodynamically unstable and tend to crystallise with time, which is associated with an increase in density, a prominent characteristic of the crystalline form (Van den Mooter, 2012; Tobyn *et al*., 2009).

An amorphous solid can exist in mainly two states, i.e.:

- A super-cooled liquid (rubbery state), which is a viscous equilibrium form of a compound; and
- A glass, which is a solid, non-equilibrium form of the same compound (Cui, 2007).

The behaviour of amorphous materials can be explained by their changes in thermodynamic parameters (free volume, enthalpy, entropy), with a variation in temperature. Amorphous solids can be further characterised by their lack of distinctive melting points. When the molar volume of an amorphous material is plotted against temperature, these variables vary smoothly, until the compound approaches the region of the glass transition temperature (T_g), where they then change sharply. The temperature at which an amorphous material is converted from an equilibrium, super-cooled state into a non-equilibrium, glassy state during cooling or heating, is known as the glass transition temperature. This temperature is generally measured with differential or modulated differential scanning calorimetric techniques. Structural factors that affect the T_g include molecular size and shape, and the extent, strength, and direction of any hydrogen bonding. These factors affect the strength of inter-molecular interactions and packing (Healy *et al*., 2017; Cui, 2007).

2.2.3.2 Multi-component amorphous forms

Amorphous substances are chemically less stable than crystalline compounds. Being physically less stable, they tend to crystallise over time (Byrn *et al*., 1999). This has led to the

development of amorphous solid dispersions (ASDs) and their growing role within the pharmaceutical industry. ASDs refer to solid mixtures, at molecular level, of a pharmaceutical active and one or more polymer, with or without a surfactant(s). Polymers are used to stabilise amorphous drugs in the solid state. The combination of a polymer with an amorphous drug at a molecular level results in the improved stability, higher apparent solubility and faster dissolution rate of the drug (Singh & Van den Mooter, 2016).

2.3 Production of different solid-state forms

2.3.1 Polymorphs

Polymorphs can be manufactured through processing methods, such a re-crystallisation, by dissolving an API in an organic solvent, or through grinding, milling, heating and compression. Milling is the most common method used during production, as it can reduce the particle size, and can it be used to prepare a different crystal form, or the amorphous form. Sometimes a new crystalline form is produced alongside the amorphous phase. Higashi *et al*. (2017) employed liquid assisted milling and spray drying to generate two different polymorphic forms of sulfadimide:4-aminosalicylic co-crystals (1:1). Spray drying from ethanol led to the formation of the meta-stable form II of the co-crystal, while liquid assisted milling led to the formation of the thermodynamically stable form I of the co-crystal. Manufacturing conditions that include a solvent, such as wet granulation, may also facilitate conversion into a thermodynamic polymorph. Crystallisation, using a super-critical fluid (CO_2) in tolbutamide and barbital, has also been applied in the production of polymorphs. Three polymorphs of tolbutamide were successfully produced, while one barbital polymorph was consistently prepared (Higashi *et al*., 2017; Grossjohann *et al*., 2015; Shinozaki *et al*., 2006). Other methods that can be successfully employed to obtain polymorphs include solid-state transition through the re-crystallisation of meta-stable polymorphs and amorphous forms, or through the desolvation of solvates (Stieger & Liebenberg, 2012).

2.3.2 Solvates and Hydrates

The two most basic methods used for preparing solvates are evaporative and cooling re-crystallisation. Both methods result in super-saturation, nucleation and crystal growth. Evaporative re-crystallisation is achieved by dissolving the drug in an appropriate solvent.

The solution is then left to evaporate slowly, until crystals form. Cooling crystallisation is attained by heating a solvent to just below its boiling point and by dissolving adequate amounts of the drug powder in it to obtain a very concentrated solution, with no remaining non-dissolved particles. As the solution cools, it becomes super-saturated, resulting in crystals to precipitate. Different temperatures or rates of evaporation may result in different solvates, polymorphs, or even hydrates if moisture is absorbed from the atmosphere by hygroscopic solvents. Hydrates are often prepared by dissolving a hydrophobic API in an organic solvent and by adding water as anti-solvent, with a resultant decrease in the solubility and precipitation of the solid (Morissette *et al.*, 2004).

2.3.3 Amorphous solids

Amorphous solids may be prepared from the original crystalline form through common pharmaceutical processes, such as quench cooling of a melt, vapour condensation, desolvation, grinding, and rapid solvent evaporation, such as freeze drying or spray drying (Yu, 2001). Co-amorphous systems are also an alternative approach used for enhancing a drug's dissolution performance and the physical stability of poorly water-soluble drugs. The glass forming ability of the co-former plays a pivotal role in determining the formation of a co-amorphous system. Löbmann *et al*. (2011) developed co-amorphous forms of naproxen and indomethacin by employing a melt cool method, which resulted in the improved dissolution rates and physical stabilities of both drugs.

The formation of amorphous solids is illustrated in Figure 2.1.

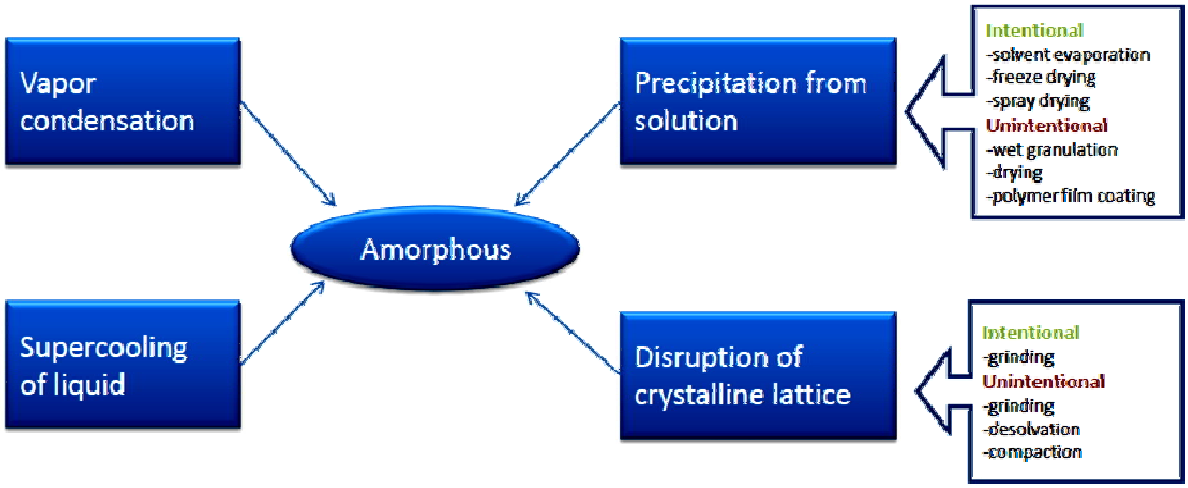

Figure 2.1: Schematic representation of the formation of amorphous solids (Hancock & Zografi, 1997).

2.4 Pharmaceutical impact of different solid-state forms

The differences in composition, molecular association and resultant energy states that are associated with different solid-state forms give rise to differences in their physico-chemical properties. Some properties that may be affected include melting point, hygroscopicity, dissolution rate, apparent solubility, equilibrium solubility, stability and thermal conductivity. Studies of possible phase transformations in the solid-state are important, because the sudden appearance or disappearance of a crystalline form may threaten process development and may lead to serious pharmaceutical consequences, if such transformation occurs in the final dosage form, or during manufacturing. An example of unpredicted and undesirable phase transformation was found in ritonavir, a novel protease inhibitor for treating human immunodeficiency virus (HIV). The drug had been launched in 1996 and was distributed for almost two years, before the company observed an unexpected change. The final product no longer had adequate dissolution properties and was the drug found to precipitate. After extensive investigations, it was concluded that a new thermodynamically stable and less soluble polymorph, form II, which had appeared spontaneously, was the cause. The two crystal forms differed significantly in their physico-chemical properties with regards to solubility and dissolution rate. Surprisingly, the company was unable to ever produce form I again, which forced the manufacturer to recall the original formulation from the market (Huang & Tong, 2004; Vippagunta *et al.*, 2001).

During the processes of milling or grinding, some pharmaceutical substances may undergo phase transitions into other polymorphic forms, or they may become amorphous. Such physical changes may be disadvantageous to the product and reduce the stability, or solubility of the product over time (Descamps *et al.*, 2007). It has been postulated that physical parameters that drive such transitions are the milling intensity and milling temperature. It has been shown in products, like cimetidine, that an increase in the milling intensity may drive the product towards its meta-stable state. Regarding the milling temperature, it has been shown that amorphization occurs when milling is performed below the T_g of the corresponding liquid, while polymorphic transitions occur when it is performed above T_g (De Gusseme *et al.*, 2008; Bauer - Brandl, 1996). It is thus important to control the whole milling process to regulate induced phase transitions in order to manufacture a stable and effective drug product.

Various solid forms have been produced by design in order to improve the performance of the final product. An example is the marketed amorphous form of cefuroxime axetil (CFA), which exists as two polymorphs and an amorphous form, of which the amorphous form is the most soluble. CFA is a poorly water-soluble drug and exhibits a low solubility and dissolution rate in the gastro-intestinal tract. These in turn limit the effective absorption and bio-availability of the drug. Amorphous CFA particles had been prepared by Zhang *et al.* (2006) through a controlled nano-precipitation method, which resulted in a higher dissolution rate, compared to the commercial, amorphous, spray dried product.

2.5 Known physico-chemical properties of first-line anti-tuberculosis drugs

The four current anti-TB drugs, isoniazid, pyrazinamide, ethambutol hydrochloride and rifampicin, belong to two different classes of the Biopharmaceutical classification system (BCS), according to differences in their solubility and intestinal permeability:

- **BCS class I**: High solubility, high permeability;
- **BCS class II**: Low solubility, high permeability;
- **BCS class III**: High solubility, low permeability; and
- **BCS class IV**: Low solubility, low permeability (Ellard & Fourie, 1999).

Isoniazid, pyrazinamide and ethambutol hydrochloride belong to class I (highly soluble and highly permeable). Rifampicin on the other hand, is the only hydrophobic ingredient of the

fixed-dose combination (FDC) product and is a borderline class II compound (Ellard & Fourie, 1999). There is some debate over whether rifampicin is in fact a class II, or class IV drug. It has been postulated that polymorphism of rifampicin may be responsible for its variable bio-availability among its solid oral dosage forms (Agrawal *et al*., 2004).

2.5.1 Rifampicin

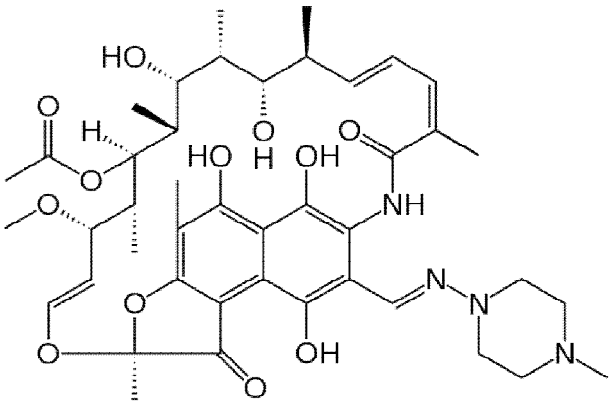

Figure 2.2: Chemical structure of rifampicin.

The rifamycins were discovered in the 1950's, which led to the synthesis of rifampicin in 1965. Rifampicin (Figure 2.2) is derived from the rifamycin family, which is produced by strains of *Norcadia mediterranei*. Rifampicin has a broad spectrum of anti-bacterial activity, and functions against intra- and extra-cellular micro-organisms, as well as bacteria in latent states (Alves *et al*., 2010; Gallo & Radaelli, 1976).

Synonyms: Rifampin; rifaldazine; rofact; 3-[[(4-methyl-1-piperazinyl)imino]methyl]; rifamycin SV
Molecular formula: $C_{43}H_{58}N_4O_{12}$
Molar mass: 822.95 g/mol
Melting point: 183 - 188°C
Boiling point: 937°C
Abbreviations: R, RMP, or RIF
Physical appearance: Crystalline powder that can vary from bright orange to reddish brown
Solubility: Soluble in dimethyl sulfoxide and slightly soluble in water.

Rifampicin has the following reported apparent solubility values in water (25°C) at different pH values:

- 10% m/v pH 2
- 0.4% m/v pH 5.3
- 0.3% m/v pH 2 (Lund, 1994).

It is believed that the stability of rifampicin is pH dependent and that it decomposes rapidly at low pH values. Rifampicin has two decomposition products, namely 3-formlyrifamycin SV and rifampicin quinone, formed though hydrolysis and oxidation, respectively. Rifampicin quinone is purple and inactive, while 3-formylrifamycin SV is insoluble and shows poor absorption. It shows anti-microbial activity *in vitro*, but is inactive *in vivo* (Bain *et al*., 1998; Lund, 1994).

Rifampicin has at least two reported crystalline forms, i.e. forms I and II, an amorphous form, as well as four solvates that convert into the amorphous form at room temperature, or after desolvation (Henwood, 2001; Pelizza *et al*., 1977). Form II is the preferred commercial form. Rifampicin shows polymophism due to the various possibilities of hydrogen bonding, conformational exchanges and ionisation states that enable different crystalline packings of the intricate structure (Pelizza *et al*., 1977). In a study by Henwood *et al.* (2001) to investigate the physico-chemical properties of rifampicin when crystallised from various solvents, it was found that the amorphous form of rifampicin was poorly soluble and had the slowest dissolution rate. In theory, amorphous forms are expected to have a faster dissolution rate and higher solubility than crystalline forms. However, this did not prove to be true for rifampicin. This behaviour was attributed to the electrostatic nature of the very fine particles in the amorphous powders. Electrostatic forces resulted in agglomeration, as observed during the dissolution tests (Henwood *et al.*, 2001).

2.5.2 Isoniazid

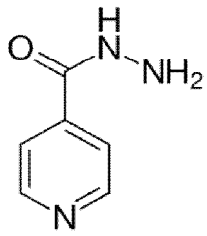

Figure 2.3: Chemical structure of isoniazid.

Synonyms: Isonicotinyl hydrazine; isonicotinic acid hydrazide; 4-pyridinecarbohydrazide
Molecular formula: $C_6H_7N_3O$
Molar mass: 137.14 g/mol
Melting point: 170 - 174°C
Flash point: 190°C
Abbreviations: H, or INH
Physical appearance: Colourless or white crystals, or a white crystalline powder
Solubility: Soluble in water and slightly soluble in ether and benzene.

Isoniazid (Figure 2.3) was first used clinically in 1952. It is a pro-drug, which converts into the active metabolite, isonicotinic acid. Isoniazid is slowly degraded by air and light over time.

Isoniazid reacts with reducing sugars, such as galactose, lactose and glucose in an acidic medium to form isonicotinyl hydrazones. Reducing sugars should hence preferably not be included as excipents in isoniazid formulations (Singh *et al.*, 2001).

To date, no reports on polymorphs, hydrates, or solvates of isoniazid exist in the available literature.

2.5.3 Pyrazinamide

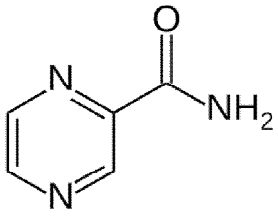

Figure 2.4: Chemical structure of pyrazinamide.

Synonyms: 2-carbamylpyrazine; 2-pyrazinecarboxamide; aldinamide; pyrazinoic acid amide
Molecular formula: $C_5H_5N_3O$
Molar mass: 123.113 g/mol
Melting point: 188 - 191°C
Boiling point: 357°C
Abbreviations: Z, or PZA
Physical appearance: White crystalline powder, or fine needles
Solubility: Soluble in water and slightly soluble in ether and benzene (Lund, 1994).

Pyrazinamide had first been discovered in 1940, but was not used until the 1980's as a first line anti-TB drug. Pyrazinamide (Figure 2.4) is a pro-drug that requires conversion into pyrazinoic acid for it to have bactericidal activity against *M. tuberculosis* (Singh *et al*., 2006; Lund, 1994).

Pyrazinamide, unlike rifampicin, is a conformationally rigid molecule with four polymorphs being reported to date, i.e. α, β, γ and δ. In 2009, Castro *et al.* (2009) reported form δ as the stable phase. However, a study conducted by Cherukuvada *et al*. (2010) found the α polymorph form as the stable form under ambient conditions, since forms β, γ and δ had transformed into the α phase under ambient conditions (Cherukuvada *et al*., 2010; Castro *et al*., 2009).

2.5.4 Ethambutol

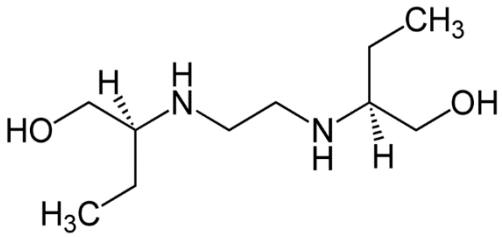

Figure 2.5: Chemical structure of ethambutol.

Synonyms: (+)-2,2'-(ethylenediimino)di-1-butanol; (+)-N,N'-bis(1-(hydroxymethyl)propyl) ethylenediamine; (2S,7S)-2,7-Diethyl-3,6-diazaoctane-1,8-diol; (+)-S,S-ethambutol
Molecular formula: $C_{10}H_{24}N_2O_2$
Molar mass: 204.31 g/mol
Melting point: 87.5 - 88.8°C
Boiling point: 345°C
Abbreviations: E, or EMB
Physical appearance: White crystalline powder
Solubility: Soluble in chloroform, methylene chloride, less soluble in benzene, sparingly soluble in water.

*2.5.5 *Ethambutol dihydrochloride*

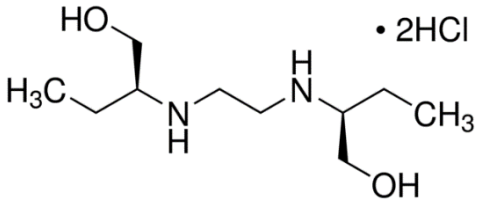

Figure 2.6: Chemical structure of ethambutol dihydrochloride.

Synonyms: ethambutol hydrochloride; (+)-ethambutol dihydrochloride; ethambutol dihydrochloride; (+)-(S,S)-ethambutol dihydrochloride; (+)-2,2'-(ethylenediimino)di-1-butanol dihydrochloride

Molecular formula: $C_{10}H_{24}N_2O_2 \cdot 2HCl$

Molar mass: 277.23 g/mol

Melting point: 198.5 - 200.3°C

Abbreviations: E, or EMB

Physical appearance: White crystalline powder

Solubility: Soluble in water and dimethyl sulfoxide (DMSO), sparingly soluble in ethanol, poorly soluble in acetone and chloroform (Merck Index).

> **The dihydrochloride salt was incorporated in all of the fixed dose combination drugs in this current study and is it the preferred form used in all other commercial products, but mistakenly only referred to as ethambutol. The physico-chemical properties and more specifically, the solubility of the two compounds differ significantly, as illustrated above.*

Ethambutol dihydrochloride has two dissociation constants, namely pKa1 = 6.35 and pKa2 = 9.35. Its melting point ranges from 199 - 204°C (Lund, 1994). It is intrinsically chiral and the S,S isomer is said to be five-hundred times more potent than the R,R isomer. Its therapeutically active stereo-isomer, (S,S)-ethambutol, is used in all commercial products (Becker *et al*., 2008). Ethambutol dihydrochloride exists in four polymorphic forms, of which only one form is used in commercial drug formulations (Rubin – Preminger *et al*., 2004).

2.6 Conclusion

As discussed in Chapter 1, the worldwide goal to eradicate tuberculosis, as well as the increase in resistance of *Mycobacterium tuberculosis* to current anti-TB drugs, dictate the need for for long-term, multi-drug regimens for the treatment of this disease. If given individually, the four anti-TB drugs, isoniazid, pyrazinamide, ethambutol hydrochloride and rifampicin, as described in this chapter, will lead to a high dosage burden and poor patient compliance, thereby exacerbating the problem of resistence. By combining these individual drugs into single, fixed-dose combination products would simplify treatment and improve patient compliance. However, the physico-chemical properties of each drug and in different combinations need to be well investigated beforehand, to ensure the stability and efficacy of any proposed FDC formulation.

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Chapter 3

Anti-Tuberculosis Fixed Dose Combination Products

3.1 Introduction

The treatment of tuberculosis with a multi-drug regimen requires therapy for a long period of time. This may lead to problems, such as poor patient compliance, treatment failure and drug resistance. To limit these risks, the World Health Organisation (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) recommend the use of fixed-dose combination (FDC) tablets for the treatment of TB. FDCs can be defined as formulations of two or more active ingredients in a single product, available in fixed doses (WHO, 1999).

The recommended multi-drug treatment approach of TB includes rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB), daily for 2 - 3 months. RIF and INH are used for a further 4 months, either daily, or three times a week. Alternatively, EMB and INH may be prescribed for administration daily for 6 months. In cases where patients have optic neuritis, ethambutol is contra-indicated and streptomycin may be used instead. However, streptomycin is generally not recommended, because of a higher reported incidence of resistance and because it requires parenteral administration (Panchagnula *et al.*, 2004). Where there is resistance towards INH or RIF during the continuation phase, EMB and PZA may be used daily for 6 months. In certain cases, where resistance exists towards INH in the absence of RIF resistance, levofloxacin or moxifloxacin may be used (Horsburgh *et al.*, 2015). Taken separately, typical treatment during the initial phase would require nine tablets per day, compared to two to five FDC tablets daily, depending on the weight of the patient. The use of FDCs may hence simplify treatment and encourage patient compliance, especially in patients who already take numerous medications, when co-infected with human immunodeficiency virus (HIV) (WHO, 1999).

3.2 Simplifying tuberculosis treatment and preventing drug resistance

One of the main complicating factors of TB chemotherapy is the necessity for multi-drug therapy. Contrary, the use of FDCs reduces the number of tablets to be taken for the whole duration of the treatment (Table 3.1). Having fewer tablets to ingest daily makes treatment with FDCs easier than monotherapies. For each of the anti-TB APIs in FDCs, a fixed recommended dose per kilogram body weight exists. The number of tablets required is calculated according to the patient's body weight, which makes the calculation of individual drug doses unnecessary. The dose: body-weight relationships in FDCs are correctly balanced to ensure adequate drug delivery of all the included anti-TB active materials.

The advantages and disadvantages of FDCs are listed below (Kaplan, 2004):

a. Advantages:

- Simpler dosage forms improve patient understanding and compliance;
- FDCs reduce inadvertent medication errors;
- FDCs prevent and/or slow the development of anti-microbial resistance by eliminating monotherapies;
- FDCs reduce drug shortages by simplifying drug storage and handling, and thus lowers the risk of being out of stock;
- Only one dosage form with a single expiry date simplifies dosing, as it eliminates multiple drugs with different expiry dates; and
- Stock management, procurement and distribution are simplified (important in state run facilities, especially primary health care facilities).

b. Disadvantages:

- FDCs are sometimes more expensive than single component drugs;
- The quality of individual drugs may be compromised if chemical interactions occur; and
- If a patient experiences an undesirable reaction towards one drug component, the FDC must be stopped and replaced by single drugs.

To date, not much information is available regarding the improvement of patient compliance when using FDCs. However, in a study conducted on the use of FDC products in Hong Kong, only 1% of three-hundred and twelve patients being treated with FDCs complained about the quantity, tablet size, or difficulty with swallowing, in contrast to the 5% of three-hundred and eight patients treated with single drug formulations. Problems that arise when using single drug formulations usually occur because of any combination of three main reasons, i.e. the absence of stock, delays in receiving stock and/or the lack of replacements when expiry dates are reached. These situations may lead to other drugs being given in isolation (monotherapy), while awaiting fresh stock. The use of FDCs reportedly reduces these risks (Blomberg *et al*., 2001; WHO, 1999).

Table 3.1: The number and composition of FDC tablets to be taken daily during the two phases of TB treatment, as recommended by the WHO (1999)

	Intensive phase 7 days a week for 2 months	**Continuation phase 7 days a week for 4 months**	
Pre-treatment body weight	RIF, INH, PZA, EMB (150, 75, 400, 275)	RIF, INH (150, 75)	RIF, INH (300, 150)
30 - 37 kg	2 tablets	2 tablets	
38 - 54 kg	3 tablets	3 tablets	
55 - 70 kg	4 tablets		2 tablets
> 70 kg	5 tablets		2 tablets

The use of FDC tablets of good quality ensures adequate drug delivery and may prevent resistance when given as directly observed therapy treatment. However, inadequate doses, especially of rifampicin in FDCs, may lead to treatment failure and drug resistance. If FDCs are given unsupervised, patients may interrupt treatment, which in turn may lead to drug resistance. To avoid the risk, FDCs ought to be given as directly observed therapies, especially in the initial phase (Blomberg *et al*., 2001).

3.3 Quality of anti-tuberculosis fixed-dose combination drugs

The bio-availability of rifampicin has reportedly been identified as the major quality challenge in using FDCs (Acocella *et al*., 1988). According to Acocella *et al*. (1988) of the University of Pavia, the bio-availability of RIF could be compromised when given as FDC tablets, especially as the three-drug combination product. The reasons that have been hypothesised for the compromised RIF bio-availability include raw material characteristics, changes in its crystalline structure, excipients, manufacturing variables, degradation in the gastro-intestinal (GI) tract and its inherent variability in absorption and metabolism. The versatile behaviour of RIF can best be described by physiological, physico-chemical, pharmaceutical and manufacturing factors (Figure 3.1). Furthermore, a dissolution test, which is a simple and robust mechanism of judging the quality of formulations, cannot guarantee acceptable RIF bio-availability. To ensure that FDC tablets of good quality are used, the WHO and the IUATLD have developed a simplified, effective protocol for the assessment of RIF bio-availability from FDC formulations. The RIF protocol uses six sampling points over the duration of only 8 hours, which is different to the number of points that are normally used for PZA and EMB, which can be anything between 0 and 48 hours. This protocol is known to be more convenient and more affordable. A worldwide mechanism for the pre-qualification of FDCs has been proposed by the WHO to ensure that only FDCs of good quality are used in TB therapies (Panchagnula & Agrawal, 2004; WHO, 1999).

3.4 Reported incompatibilities of the four anti-tuberculosis drugs

The four anti-TB drugs, i.e. rifampicin, isoniazid, ethambutol and pyrazinamide, belong to two different classes of the Biopharmaceutical classification system (BCS), according to differences in their physico-chemical properties, as introduced in Chapter 2. Isoniazid, pyrazinamide and ethambutol hydrochloride belong to class I (highly soluble and highly permeable). Rifampicin on the other hand, is the only hydrophobic ingredient of current FDCs and is a borderline class II drug. In addition, rifampicin exhibits pH dependent solubility, which is reported to affect its absorption in the gastro-intestinal tract. It has been reported that when rifampicin is taken on an empty stomach, peak serum concentrations are achieved within 2 hours, but if ingested with food, absorption may be delayed and

incomplete. Rifampicin is better absorbed under acidic conditions, than in alkaline conditions (Panchagnula & Agrawal, 2004; Ellard & Fourie, 1999).

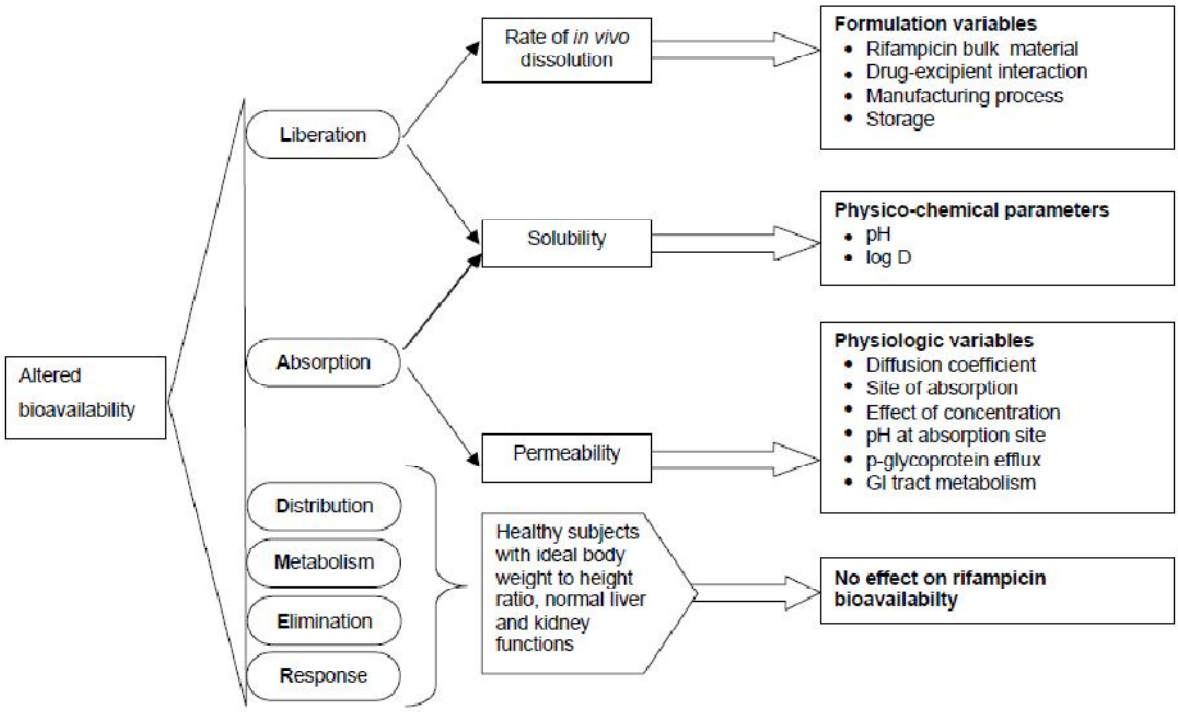

Figure 3.1: Probable reasons for the altered bio-availability of rifampicin in either separate dosage forms or in FDC formulations of anti-tuberculosis drugs (Panchagnula & Agrawal, 2004).

3.4.1 Reported incompatibilities in fixed-dose combination formulations

3.4.1.1 Reported incompatibilities between isoniazid and rifampicin

It has been postulated that below pH 2, rifampicin is converted into its inactive metabolite, 3-formylrifampicin. The reaction then proceeds to form isonicotinyl hydrazone (HYD) when rifampicin combines with isoniazid in formulation (Singh *et al*., 2000). It has been found that even under non-acidic formulation conditions, a direct reaction (although much slower) between rifampicin and isoniazid, yielding HYD as a product, is still possible (Bhutani *et al*., 2004). This direct interaction can best be explained by a trans-hydrazone formation (Figure 3.2) through a nucleophilic attack on the imine group of rifampicin, by the amino group in isoniazid, following a tetrahedral mechanism (Smith & March 2001). This explains the lack of appreciable decomposition observed in a mixture of rifampicin and isoniazid

alone (1 - 3%), as reported by Bhutani *et al.* (2005). It has furthermore been reported that pyrazinamide and ethambutol are catalytic towards the reaction between rifampicin and isoniazid, since FDCs that contain four-drug combinations have shown far more chemical instability than two-drug FDCs that only contain rifampicin and isoniazid (Singh & Mohan, 2003).

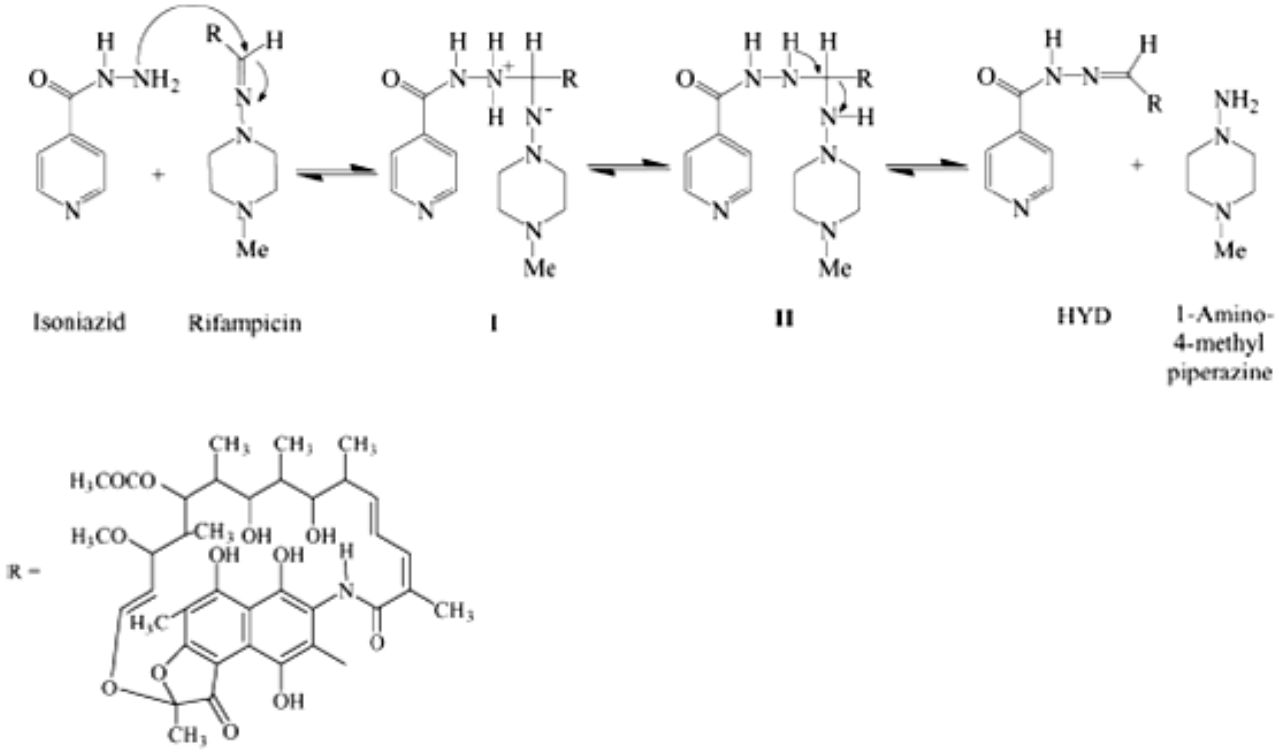

Figure 3.2: Mechanism of the formation of isonicotinyl hydrazone resulting from the direct interaction between rifampicin and isoniazid (Bhutani *et al.*, 2005).

3.4.1.2 The roles of pyrazinamide and ethambutol

Bhutani *et al.* (2005) reported about the catalytic role of pyrazinamide and ethambutol hydrochloride in the reaction between rifampicin and isoniazid. Organic bases and amides with similar structures to pyrazinamide and ethambutol hydrochloride were mixed individually with rifampicin and isoniazid. These mixtures were exposed to accelerated stability test conditions of 40°C / 75% relative humidity (RH) for 15 days. In most cases, HYD was formed along with other degradation products of rifampicin. It was reported that mixtures containing pyrazinamide were accompanied by the formation of only HYD. In the presence of the ethambutol salt, HYD was the major product with minimal quantities of 3-formylrifamycin (Bhutani *et al*., 2005). Since pyrazinamide and ethambutol bases were able to quantitatively convert rifampicin and isoniazid into HYD, it was concluded that pyrazinamide and ethambutol had played a catalytic role in the reaction.

Bhutani *et al*. (2005) further postulated that the decomposition of rifampicin and isoniazid in an FDC product of rifampicin, isoniazid and pyrazinamide was not only the result of trans-hydrazone formation, but rather of pyrazinamide playing a catalytic role in the decomposition. They hence proposed that pyrazinamide had acted as a proton scavenger (Figure 3.3) through a five-membered ring transition state, involving intra-molecular hydrogen bond formation between one of $CONH_2$ and the adjacent nitrogen ring. Figure 3.4 illustrates the possible mechanism for the catalysis of the interaction between rifampicin and isoniazid in the presence of ethambutol. It was postulated that the reaction had followed the same path as the one described in Figure 3.3 for pyrazinamide (Bhutani *et al*., 2005).

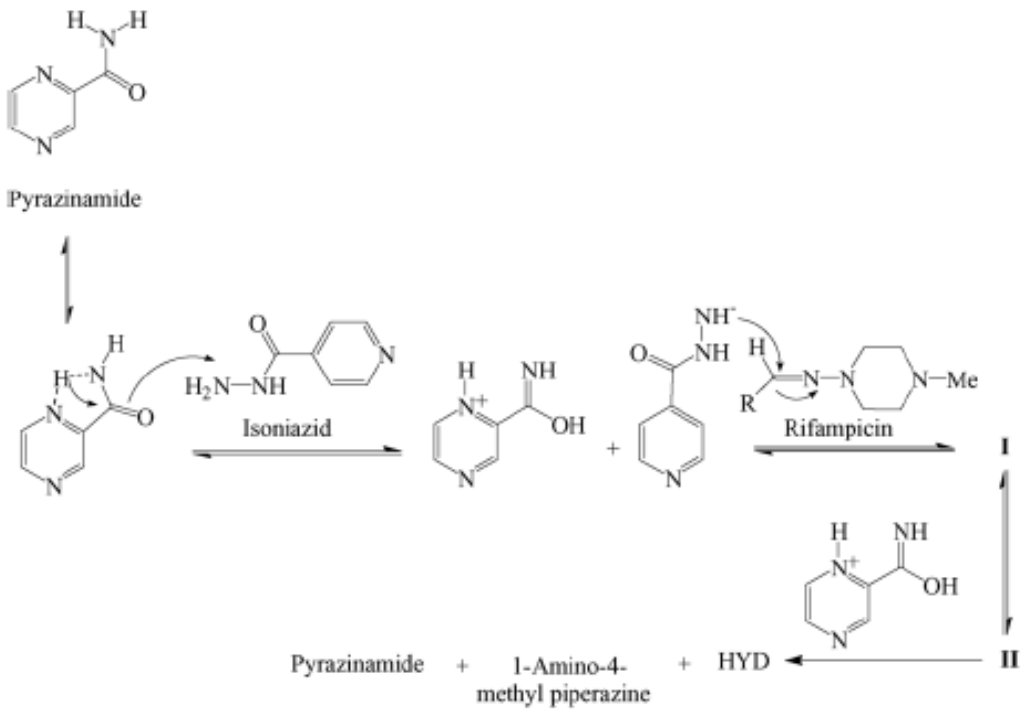

Figure 3.3: Mechanistic representation of the catalytic effect of pyrazinamide on the direct interaction between rifampicin and isoniazid (Bhutani *et al*., 2005).

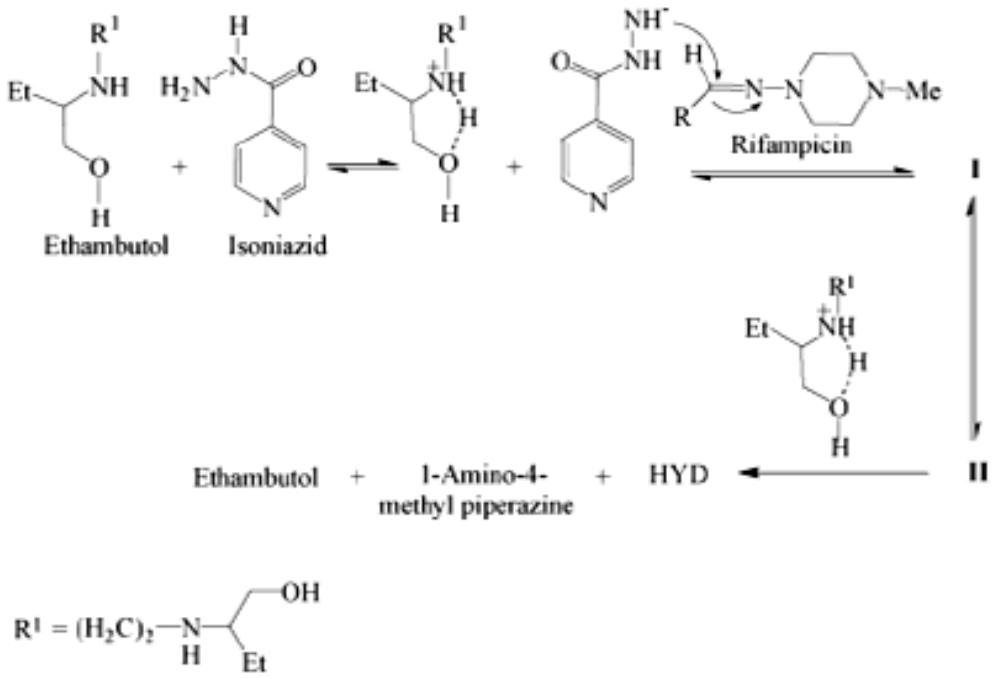

Figure 3.4: Mechanistic representation of the catalytic effect of ethambutol on the direct interaction between rifampicin and isoniazid (Bhutani *et al*., 2005).

3.4.2 Reported incompatibilities under acid stomach conditions

3.4.2.1 Degradation of rifampicin and isoniazid under acid conditions

It has been reported that rifampicin undergoes hydrolysis in an acidic medium to form 3-formyl rifamycin (3-FR) (Du Toit *et al.*, 2006; Shishoo *et al*., 2001). It is believed that isoniazid accelerates the degradation of rifampicin into 3-FR in an acidic medium through the reversible formation of the HYD of 3-FR with isoniazid. It has also been reported that when rifampicin is given in the presence of isoniazid, it may undergo higher decomposition in the stomach, compared to when rifampicin is administered alone (Du Toit *et al.*, 2006; Shishoo *et al*., 2001).

3.4.2.2 Degradation rate of anti-tuberculosis fixed-dose combination products under acid conditions

Singh *et* a*l*. (2000) conducted a study to determine the rate of degradation of rifampicin, isoniazid and pyrazinamide in commercial formulations, containing two, three and four APIs. This study was carried out in 0.1 M HCl at 37°C for 50 minutes. A control study was also

done in stimulated gastric fluid. Under both conditions, rifampicin was decomposed between 17.8 - 24.4%, isoniazid between 3.2 - 4.7%, while pyrazinamide was stable. The decomposition of rifampicin was reported to be influenced by the presence of isoniazid and not by pyrazinamide, nor by ethambutol.

Another stability study was done on the behaviour of rifampicin in the presence of isoniazid (Sankar *et al*., 2003). The study reported that rifampicin had decomposed to almost 34%, and isoniazid to approximately 10% in an acidic medium (stomach fasting condition). The decomposition of commercial FDC samples were also tested in fasted state pH conditions (0.01 M HCL solution), with the values ranging between 13 - 35% and 4 - 11% for rifampicin and isoniazid, respectively (Sankar *et al*., 2003). Many reports have suggested that rifampicin and isoniazid should be modified or segregated in such a way that they are not released simultaneously in the stomach.

3.4.3 The influence of temperature, humidity, light and packaging on the stability of fixed-dose combination products

Unsuitable storage and packing conditions may also cause stability problems in FDC products. Mohan (2001) conducted a comparative stability indicating assay on commercially available FDC products. These products were stored in an unpacked condition in light and humidity chambers, set at 40°C / 75% RH. Those products that had been stored in the light chambers showed more physical changes than those stored in dark chambers. Singh *et al*. (2002) later explored this phenomenon and concluded that if packaging is of a low quality, ethambutol, due to its reported hygroscopic nature, absorbs moisture from the atmosphere and gives rise to "bleeding" of the APIs, i.e. the APIs leak out of the formulation (Singh *et al*., 2002; Mohan, 2001). Ethambutol thus dissolves in the absorbed moisture and may even act as a solvent for the other APIs in formulation. This results in poor bio-availability of all the anti-TB drugs in formulation and ultimately therapeutic failure (Singh & Mohan, 2003).

3.5 Conclusion

Anti-tuberculosis fixed-dose combination products have been reported as being unstable in formulation, due to inter-drug interactions between the individual components. Various hypotheses have been put forward to explain these interactions that may occur in formulations and during oral administration. In Chapter 4 the importance of the dissolution, solubility and stability of APIs will be discussed to understand the reported interactions (if any) between the four anti-TB drugs.

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Chapter 4

Dissolution, Solubility and Stability

4.1 Introduction

The aqueous solubility of a drug plays an important role in the absorption of the drug after oral administration. Drug solubility depends on the physical and chemical properties of the solute and solvent, such as polarity, ionisation potential, crystal packing and the presence of solvates. The dissolution rate at which the drug passes into the solution is equally important. Only a small percentage of drugs reportedly have a high solubility and dissolution rate (Khadka *et al*., 2014; *York, 2002*).

4.2 Dissolution

There are a number of active pharmaceutical ingredient (API) properties that ought to be considered during the formulation of a pharmaceutical product. Dissolution and its dependence on size reduction is one of those critical properties. For a drug to be absorbed into the systemic circulation, it should be dissolved at the absorption site. For instance, a tablet can only be absorbed in the gastro-intestinal (GI) tract fluids, once the drug particles are dissolved or solubilised by the fluids. Dissolution describes this process by which the drug particles dissolve in fluids (York, 2002).

There are three theories that describe the manner in which materials dissolve. The first theory, termed the **diffusion-layer model**, postulates that there is a diffusion layer, or a static liquid film adjacent to the surface of the solid. The second theory, the **interfacial barrier model**, assumes that there is an interfacial barrier between the solid surface and the solution, and that the intrinsic dissolution rate is controlled by the surface reaction between the solute and solvent. The third theory, called the **Danckwert's model**, considers the groups, or packets of solvent molecules that reach the solid-liquid interface, which then absorb the solute through diffusion and carry it into the bulk of the solution (Byrn *et al*., 1999). Regardless of the particular model, dissolution is clearly affected by the interface between the solid drug and the liquid.

Other factors that might affect drug dissolution are listed below.

A. Dissolution factors relating to the physico-chemical properties of the drug

i. Solubility (the solubility of the drug will influence the dissolution rate by determining the magnitude of the drug's concentration gradient);

ii. Particle size and resultant surface area;

iii. Polymorphism and amorphism;

iv. Salt form of the drug; and

v. Hydrates / solvates.

B. Additional dissolution factors relating to the drug dosage form

i. Pharmaceutical excipients (diluents, reactive additives, colourants); and

ii. Manufacturing process (e.g. wet granulation or direct compression) (Lee *et al*., 2008).

4.3 Solubility

Solubility, expressed as a concentration, is the ability of a solute to dissolve in a given solvent at a defined temperature and pressure. Equilibrium solubility is defined as the concentration of the solute in a saturated solution, where excess solid in its most stable form is present, where dissolution and precipitation are at equilibrium, and where the solvent, temperature and pressure are specified. Under certain conditions, such as heating of a solution with excess solute, equilibrium solubility may be exceeded to yield a super-saturated solution, which is meta-stable when cooled *(*Bhattachar *et al*., 2006; Byrn *et al., 1999).*

The solubility of APIs plays a critical role in drug formulations. All drug substances should exhibit at least some degree of aqueous solubility for therapeutic efficacy. If the drug is insoluble, or sparingly soluble in an aqueous medium, it can exhibit erratic or incomplete absorption. Poor solubility is usually addressed by the incorporation of solubilising agents, micronisation, or complexation (Lee *et al*., 2008; Ostwald, 1900).

4.3.1 Ionisation

The logarithmic constant of the acid dissociation constant $pK_a = -\log_{10}K_a$ of a pharmaceutical active, is an expression of its acidic "strength". It is an important factor in predicting and determining drug solubility during pre-formulation. The lower the pK_a value of an active, the more acidic it is, and *vice versa*. The dissociation of a dissolved acid or base into an ionised state is at 50%, where its pK_a value equals the pH of the solution. The dissociation of acids is higher in basic environments, while bases' is higher in alkali media. The solubility of a drug in aqueous media is therefore higher in the ionised state, than in its neutral state. Many drugs are weak acids or weak bases and may be ionisable somewhere within the pH range of the GI tract. The solubility, dissolution and absorption of weak acids and bases can hence be manipulated through the co-administration of antacids, or by targeting intestinal drug delivery. Furthermore, if a compound is ionisable, then a salt may result, which may have a better dissolution profile, or show improvements in other properties, such as the stability of the chemical- or solid-state (Jia *et al.*, 2001).

4.3.2 Lipophilicity

Lipophilicity plays a crucial role in determining the solubility, permeability and sometimes protein binding and tissue distribution properties of a compound. It is described in terms of a partition coefficient, which is the concentration ratio of a compound, at equilibrium, in a non-polar organic- and polar aqueous phase. The higher the lipophilicity of a compound, the lower its aqueous solubility. However, some degree of lipophilicity is required for a dissolved drug to cross the intestinal mucosa and enter the systemic circulation. The partitioning of a drug depends on its ionisation state and is thus dependent on pH (Arnott & Planey, 2012; Comer & Tam, 2001). Methods for improving the solubility of lipophilic drugs include the use of cyclodextrin based formulations and solid dispersions (Thakur *et al.*, 2011).

4.3.3 Wettability and surface activity

Surface activity refers to the ability of a substance to change the nature of the surface between two substances. Certain compounds are surface active and are thus characterised by having two distinct regions, namely hydrophilic and hydrophobic. These compounds are capable of forming micelles. Surface activity can increase solubility and wettability in

aqueous media, due to micelle formation. However, surfactants can also cause a disruption in the membranes and lead to toxicity. Several methods can be used to evaluate the surface tension of solutions of surface active compounds, as well as their critical micelle formation (Olorunsula & Adedokun, 2014; Kodavanti, 1990).

4.4 Stability

Stability testing of pharmaceutical compounds is important, as it gives an overview of the stability of a compound in various environmental conditions, advises on the recommended storage conditions, as well assists with the calculation of the shelf-life of products. Factors that may affect drug stability include pH, temperature, moisture, light, particle size, additives and packaging. The International Conference on Harmonisation (ICH) requires that all pharmaceutical compounds be tested under different stress conditions that represent the environmental conditions that the compound will be exposed to. Stress testing of an API includes investigating the effect of temperature, relative humidity (RH 75% or higher), and where appropriate, oxidation and photolysis (Gudmundsson & Venkatesh, 2004; ICH, 2003; Kerns, 2001). For optimal drug performance, the best solid-state form of the specific drug needs to be chosen that would remain stable during processing, manufacturing, distribution and storage of the final product. Where a drug exhibits polymorphism, the most stable polymorph is usually chosen to avoid possible changes in form during processing. If the meta-stable form is chosen, it will dissolve and then crystallise into the more stable form that is less soluble. At times this may lead to precipitation of the API from the solution, physical instability and ultimately therapeutic failure (Zhang *et al*., 2004; Gu *et al*.,2001).

4.4.1 Physical stability

Solid-state stability of drugs can be studied as a function of temperature and humidity. Solid-state reactions that occur in pharmaceutical compounds can be driven by physical instabilities. A solid may convert from one form into another, crystallise, change its polymorphic form, or desolvate. Preventative measures have to be taken to avoid the unintentional transformation of pharmaceutical compounds, as they might lead to unexpected and undesirable drug properties (Gorman *et al.*, 2010). Different solid-state transformations, their causes and effects are discussed below.

4.4.1.1 Solid-state phase transformations

Solid-state transformation refers to any transition from one solid form of a drug into another, but with a different packing arrangement, or lack thereof. The transformations that occur in drugs can be classified under the following **six mechanisms**: *solid-solid* (no solvent interaction), *solid-melt-solid*, *solid-vapour-solid*, *solid-solution-solid* (solution mediated), *solid-solid* (solvent *mediated*), and *solid-solid* (solvent *catalysed*).

Solid-solid transitions occur without being dissolved, or without passing through the melt or vapour phases. **Melt induced** transitions occur when a compound is heated to its melting point and subsequently cooled. Quench cooling is most commonly employed, but slow cooling to room temperature or below may be more effective for certain APIs (Aucamp *et al.*, 2015; Zhang *et al*., 2004).

Solid-solid transformations can convert a drug from one form to the other without the aid of a solvent. However, the conversion occurs more rapidly in the presence of a solvent, due to less energy being required for the transformation. Spontaneous conversions need to be thermodynamically favoured in order to occur without mediation and can therefore only result in products that are more stable than the starting material (Aucamp *et al*., 2015). Zhang *et al*. (2004) and Mullin (2001) have reported that when a drug is partially dissolved in a solvent during processing, subsequent solvent removal may induce its transformation. Once the solvent is removed, the regenerated solid may be a single phase, or a mixture of amorphous and crystal forms. Aucamp *et al*. (2015), however, believe that it is much more likely that solvent mediated transformation had resulted in a meta-stable form that subsequently transformed, *via* regular, non-solvent interactive solid-solid transformation into a more stable form. Another suggestion is that the said meta-stable form could have undergone solvent-catalysed transformation into a more stable form. The term, **solvent-catalysed transformation**, is used for cases where a solvent or solvent vapour increases the rate of a non-mediated transformation (Aucamp *et al*., 2015).

Solution mediated transformation is driven by the difference in solubility of the meta-stable and stable phases. Solution mediated transformation occurs when the meta-stable phase is in contact with the saturated solution. The three consecutive steps that are involved in a solution mediated transformation include:

- Initial dissolution of the meta-stable phase in solution to reach and exceed the solubility of the stable phase;
- Nucleation of the stable phase; and
- Crystal growth of the stable phase, with continuous dissolution of the meta-stable phase (Zhang *et al.*, 2002; Rodríguez-Hornedo *et al.*, 1992).

These three general steps may lead to **four classes of phase transitions**, namely *solid-solid*, *solvent-interactive*, *vapour deposition* (solid-vapour-solid), *solution mediated* and *cooling of the melt* (solid-melt-solid) (Zhang *et al.*, 2004). Mediated processes and Ostwald's rule of stages offer researchers the opportunity to create and study meta-stable forms and new polymorphs.

4.4.1.2 Process induced phase transitions

Some of the common process induced phase transitions that occur during the manufacturing of solid dosage forms include the partial complete formation of meta-stable forms, depending on the compound and processing conditions. Meta-stable forms, including amorphs, generally have poorer physical stability, higher solubility and increased chemical reactivity. Re-crystallisation of an amorphous phase of a drug, or the excipients over an extended period of time may affect their solid-state properties. Excipients may induce conversion into the amorphous state, which can compromise the chemical stability of a drug. Hydration-dehydration cycles of a solid substance may lead to the formation of a meta-stable or stable form, an amorphous form, or mixtures of various crystalline forms. All of these possible solid phase changes may have undesirable effects on a product's quality and subsequent efficacy (Zhang *et al.*, 2004).

4.4.2 Chemical stability

Many drugs are susceptible to chemical degradation and such decomposition may lead to a loss in potency and a shorter shelf-life. The main chemical reactions that affect the stability of a drug are discussed in the four sections below.

4.4.2.1 Oxidative stability

Oxidation occurs when a drug molecule is exposed to oxygen. Oxidation refers to the loss of electrons, while reduction refers to gaining electrons. In the solid-state, oxidation occurs when oxygen diffuses through the crystal lattice to the labile sites. Oxidation stability of a molecular entity can be tested in solution in hydrogen peroxide, or in any other free radical initiator. Pharmaceutical compounds can be protected from oxidation by using anti-oxidants, or any chelating agent in formulations. Oxidative degradation can be reduced by storing susceptible drugs in amber vials (Horvorka & Shoneich, 2001; Maddux *et al*., 2001).

4.4.2.2 Hydrolysis

Hydrolysis is of importance in formulations that contain water and for drugs that are easily affected by moisture from the atmosphere. Many drugs contain hydrolysable moieties, such as ester or amide groups. Drugs can be protected from hydrolysis by using suitable packaging materials, such as blister packs and through storage in humidity and temperature controlled environments (Blessy *et al*., 2014). Hydrolysis of drugs, such as benzocaine, has previously been reduced by adding theophylline in the formulation. Other mechanisms of decreasing hydrolysis include pH adjustment of the aqueous vehicle, preparation of the insoluble salt of the drug, or chemical modification of the drug (Al-Maaieh & Flanagan, 2006). Hydrolysis can be prevented by chemically modifying the structure of the active compound in the early drug development stage. The sensitivity of a drug to hydrolysis can also be controlled by preparing a less hygroscopic salt of the drug, or by reducing the water content in the excipients that are used in the formulation (Snape *et al.*, 2010).

4.4.2.3 Photolysis

Exposure to light may affect the stability of a compound. Photolysis refers to the degradation of a drug substance, due to the absorption of radiation energy in the form of light. A drug that has photo-chemical reactivity *in vitro*, may cause adverse effects after administration. Sunlight may penetrate the skin to reach drug molecules circulating in surface capillaries, or even react with compounds accumulated in the eye. Light can induce unfavourable chemical reactions that can be toxic to human tissues. Testing of photolysis, according to the ICH (2003) guidelines requires the pre-weighed drug to be stored at high intensity light conditions at 25°C in a photo-stability chamber. When the experiment is completed, the solid is

dissolved in a suitable medium and analysed by HPLC (ICH, 2003; Tønnesen, 2001). *In vitro* photolysis can be reduced by storing products in the dark, using amber coloured bottles and through coating with polymer films, as in the case of the photolabile nifedipine microcrystals (De Villiers & Lvov, 2007).

4.4.2.4 Reaction with excipients

Reactivity of drugs with excipients often involves the reaction of either nucleophilic drugs with electrophilic excipients (e.g. esters), or electrophylic drugs with nucleophilic excipients. The reaction between primary and secondary amine drugs (e.g. isoniazid) with reducing carbohydrate excipients (lactose) is one of the most common reactions. This reaction is known as the Maillard reaction, which is often observed as a brown colour formation in dosage forms. Excipient impurities and degradants are able to react directly with drugs, or act as catalysts for other degradation pathways, such as hydrolysis or oxidation (Waterman & Adami, 2005; Kumar & Banker, 1998).

The photo-stability of a drug can also be affected by excipients and manufacturing processes. Titanium dioxide particles, for instance, can catalyse the decomposition of drugs. Photo-catalysis by titanium has also shown to be moisture sensitive. For tablets, film coatings can prevent exposure of the tablet core to light and thus reduce degradation (Kakinoki *et al*., 2004; Thoma & Kubler, 1997).

4.5 Techniques described for addressing reported anti-tuberculosis drug and fixed-dose combination shortcomings

It has previously been reported that the bio-availability of rifampicin (RIF) can be compromised when given as fixed-dose combination (FDC) tablets, especially the three-drug and four-drug combinations (Acocella *et al*., 1988). It has been postulated that RIF is converted into its inactive metabolite, 3-formylrifampicin, below pH 2. The reaction then proceeds to form isonicotinyl hydrazone (HYD) when RIF is combined with isoniazid (INH) in formulation. Furthermore, it has been reported that pyrazinamide (PZA) and ethambutol hydrochloride (EMB) are catalytic towards the reaction between RIF and INH, since FDCs that comprise of 4-drug combinations have shown far higher chemical instability than two-drug FDCs that only contain RIF and INH (Bhutani *et al*., 2005; Bhutani *et al*., 2004).

Certain drug delivery and formulation approaches have been suggested to resolve the problems associated with the decomposition of RIF in the presence of INH, as well as RIF's overall reported incompatibility with the other anti-TB drugs. These approaches are discussed below.

4.5.1 Dissolution enhancement

Rifampicin has a low solubility in water and varies with changes in pH and the type of solution. At human body temperature, 200 mg/ml is soluble in 0.1 M of HCl, while 9.9 mg/ml is soluble in phosphate buffer at pH 7.4. However, RIF is freely soluble in organic solvents. In 2009, Kumar *et al*. reported on the encapsulation of RIF. The process was achieved by the hydrogen-bonded, layer-by-layer (LbL) self-assembly of poly(vinyl pyrrolidone) and poly(methacrylic acid) capsules (PVA/PMA capsules) on sacrificial silica cores. RIF was encapsulated at pH 2, since PVP/PMA capsules are stable at this pH, whereas they disintegrate at pH 7.4, where the release of RIF is possible. The results showed a sigmoidal release pattern as a function of pH, with negligible release at low pH and a "burst" kind of release at a pH above 6.8, where the capsules disintegrated rapidly, thereby releasing the drug in a short period of time. A study conducted by Jindal *et al*. (1995) on the stability of rifampicin, however showed that RIF was unstable in highly acidic solutions. Kumar *et al*. later managed to stabilise RIF at a lower temperature of 4°C in order to delay the reaction in acidic media. With some modifications to this system, it can be used to formulate rifampicin loaded capsules that can be used for controlled release (Kumar *et al*., 2009; Jindal *et al*., 1995).

4.5.2 Solubility enhancement

Isoniazid is poorly absorbed from the stomach, but well absorbed from the other three segments of the GI tract. Considering this, Gohel & Sarvaiya (2008) formulated tablets containing RIF, together with enterically coated INH, prepared through the cold extrusion method, aimed at solving the reported issue of decreased bio-availability of RIF. Hydroxylpropylmethylcellulose phthalate was included in the core of these tablets to control the release of INH in alkaline media. The results of their study showed that INH had subsequently not released in acidic media, while the degradation of RIF, due to its reported interaction with isonicotinyl hydrazone, had been inhibited. Gohel & Sarvaiya (2008)

concluded that the degradation of rifampicin could be reduced in FDC products containing RIF and enterically coated INH.

4.5.3 Stabilisation

In vitro release studies have been conducted on anti-TB drugs entrapped in niosomes and liposomes, aimed at improving their stability and bio-availability. Niosomes are structurally related to liposomes, with both consisting of bilayers. The only difference is that the bilayer of niosomes consists of non-ionic surface agents. In liposomes it consists of phospholipids (*Makeshwar & Wasankar, 2013)*. Co-encapsulation of RIF and INH in liposomes has proven to be a satisfactory alternative for protecting sensitive APIs from adversely interacting. INH (water soluble) and RIF (lipid soluble) were successfully co-encapsulated in the same liposome formulation by loading RIF in the lipid layer and INH in the aqueous phase *(*Gürsoy *et al*., 2004). RIF was encapsulated in Triton niosomes and showed neither degradation, nor photo-instability after a 4-week test period. Both INH and PZA were successfully entrapped in the hydrophylic region. *In vitro* studies revealed that the extent of drug release from highest to lowest was as follows: INH > PZA > RIF. The slow release of RIF ensures that the drug is available for a longer period of time, which also decreases degradation. Niosomes can act as good reservoirs for continuous delivery of anti-TB drugs and thereby increase their oral bio-availability. This technique was reported by the authors as being a good alternative for combination therapies (Mehta *et al*., 2011).

A study was done to investigate the feasibility of formulating the segregated delivery of RIF and INH from FDC bilayer tablets. Since RIF is converted into its inactive metabolite 3-formylrifampicin below pH 2, while the reaction proceeds to form isonicotinyl hydrazone (HYD) when RIF is combined with INH in formulation, segregated delivery would be crucial in this instance. The tablet contained an immediate release layer composed of both RIF and PZA and a retarded release layer composed of INH only. RIF and PZA were released in the gastric fluid and isoniazid in the intestinal fluid. This tablet allowed the segregated delivery of RIF and INH in order to reduce the interaction between these two drugs and thereby increase the bio-availability of RIF. This proved to be a suitable strategy for preventing contact and thus interaction between RIF and INH under acidic conditions, and hence increased the bio-availability of both drugs (Silva *et al.,* 2014).

4.6 Conclusion

The augmentative techniques, as discussed above, are the results of studies aimed at addressing reported drug interactions, instability and/or poor bio-availability of APIs in formulation. The experimental work being done in this study, as discussed in Chapters 5 and 6, by using the latest techniques, aimed at determining which previously reported problems (observed or theoretical) were closest to the truth. In doing so, one would be able to judge which improvements and counter measures are indeed necessary during the formulation of anti-TB fixed-dose combinations.

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Chapter 5

Materials and Methods

5.1 Introduction

The solid-state properties of APIs are important in a pharmaceutical related study. When polymorphism or stability problems with a given API are reported, the investigator/researcher should be sure about the specific crystal form and all other physico-chemical properties of the specific API.

A solid-state study involves **thermal analyses** (i.e. thermogravimetric analysis, differential scanning calorimetry and thermal microscopy, **calorimetric analyses** (i.e. thermal activity monitor and vapour sorption analysis), **microscopy** (light microscopy and scanning electron microscopy), and X-ray powder diffraction.

5.2 Thermal analyses

According to Byrn *et al*. (1999), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and thermal microscopy are important methods in the study of solid-state chemistry. DSC involves measuring the difference in heat flow between a reference and a sample, whereas TGA measures the changes in mass with changes in temperature. TGA is often used to study the loss of solvent of crystallisation, or other solid to solid and gas reactions. Thermal microscopy is a tool used to observe physical changes in a solid with changes in temperature, through direct observation or recording.

For DSC and TGA determinations, a Shimadzu DSC-60A (Kyoto, Japan) and DTG-60 (Kyoto, Japan) were used, respectively. A Nikon Eclipse 50i (Nikon, Japan) thermal microscope, equipped with a digitally controlled and programmable Linkam (Tadworth, UK) heating stage, as well as a Nikon DS-Fi1 camera (Nikon, Japan) were used for the thermal microscopic data collection.

5.2.1 Differential scanning calorimetry (DSC)

DSC provides information about the energy change (enthalpy) of various solid-state processes. The result of a DSC analysis is a thermogram, i.e. a plot of $\Delta T = T_s - T_r$ (temperature difference). DSC can be used to discover a wealth of information about a compound. The physical properties of the compound, compound-excipients interactions and final drug stability can all be analysed, using DSC (Saunders & Gabbot, 2011; Byrn *et al*., 1999).

Experimental setup

Thermograms were generated, using a Shimadzu DSC-60A instrument. Samples weighing approximately 3 - 5 mg were placed in sealed aluminium crimp cells with a pin-hole in the lid, and heated at a rate of 10°C/min within the temperature range of 25 - 300°C, at a nitrogen gas flow of 35 ml/min. The resultant DSC traces were analysed, using the Shimadzu software.

5.2.2 Thermogravimetric analysis (TGA)

This method measures the change in mass with an increase in temperature and is often used to study the loss of solvent of crystallisation, or other solid to solid and gas reactions. A sample in an open crucible is heated at a specific rate and the weight gained or lost by the sample is monitored. This technique is often used in conjunction with DSC to compare the enthalpy transitions with the resulting weight gain or loss (Munson, 2009).

Experimental setup

TGA thermograms were generated by a Shimadzu DTG-60A (Kyoto, Japan) instrument. Samples weighing approximately 3 - 5 mg were placed in open aluminium crucibles. The samples were heated from 30 - 200°C at a heating rate of 10°C/min under a nitrogen gas flow of 35 ml/min.

5.2.3 Thermal microscopy

Thermal microscopy is a tool used to observe the changes in a solid related to changes in temperature through direct observation under a microscope, or by recording the changes.

According to Byrn *et al*. (1999), there are six observations that an analyst should take note of when using a thermal microscope, namely:

- A change in birefringence (the splitting of a light ray) without melting: Possibly indicative of changes in the crystal structure;
- A change in transparency, or outward appearance: Indicative of a solid-state transformation, or a chemical reaction;
- Any signs of gas evolution (when sample is covered with a drop of silicon oil): Indicative of a loss of solvent, or a decomposition reaction;
- Crystallisation of a melt or solution: Indicative of a phase transformation;
- The melting behaviour and melting equilibrium: An identification constant;
- Sublimation: Indicative of a phase change.

Experimental setup

A small amount of sample was placed on a microscope slide and covered with silicon oil (Fluka Chemika, Switzerland). A cover slide was then placed over this sample. Duplicate samples were prepared without silicon oil.

The hot-stage microscope used in this study was a Nikon Eclipse 50i microscope (Tokyo, Japan), fitted with a Nikon DS-Fil camera and a Linkam THMS600 heating stage (Surrey, UK), and equipped with a T95 LinkPad temperature controller (available in-house).

5.3 Scanning electron microscopy (SEM)

SEM enables analysts to examine pharmaceutical substances at a much higher resolution than is possible with a simple light microscope. SEM generally addresses questions, such as particle-particle interactions, surface characteristics and other details that encircle sub-optical to macro-molecular sizes (Nichols *et al*., 2011).

Experimental setup

A FEI Quanta 200 FEG SEM with an X-Max 20 EDS system (FEI, USA) (available on campus) was used to obtain micrographs of the various crystal and amorphous forms. In

preparation, each sample was adhered to a small piece of carbon tape, mounted onto a metal stub and coated with a gold-palladium film (Eiko Engineering ion Coater IB-2, Japan).

5.4 X-ray powder diffractometry (XRPD)

XRPD qualitatively determines the crystallinity of a solid compound. It offers a distinct XRPD pattern per material, derived from the unique three-dimensional spacing of planes in a given compound, or groups of iso-structural compounds. During this study, it was used to differentiate and identify various solid-state crystalline forms and to confirm the existence of amorphous forms (Bhattacharya *et al.*, 2002).

Experimental setup

X-ray powder diffraction patterns were obtained, using a PANalytical Empyrean diffractometer (PANalytical, Almelo, Netherlands). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2°/min (step size, 0.025°; step time, 1.0 sec).

5.5 Compatibility and stability indicating tests

5.5.1 Micro-calorimetry

Micro-calorimetry is a sensitive calorimetric technique that is used to determine the heat being released or absorbed by various processes. Iso-thermal calorimetry is a highly sensitive technique that is conducted at a constant temperature. It can easily measure small outputs of heat down to the micro-watt scale (10^{-6}). This extremely sensitive technique is often described as more sensitive than the DSC and is commonly used to detect small amounts of amorphous materials in the presence of crystalline material. One disadvantage of iso-thermal calorimetry is that it is non–discriminatory. As a result, when the purity of a material is analysed, its purity can only be calculated from the observed calorimetric data and knowledge of the reaction enthalpy for the pure material (Gaisford & O'Neill, 2007; Munson, 2009).

The thermal activity monitor (TAM), is one of the most commonly used iso-thermal micro-calorimeters. It has the ability of operating in both heat conduction and power compensation

modes. The TAM usually consists of a water bath, or in some cases an oil bath, which acts as a heat-sink that is maintained at a certain temperature. Iso-thermal calorimetry can be used for the determination of purity, polymorphism and the solubility of drug substances. It is noteworthy that iso-thermal micro-calorimetry experiments can be relatively time consuming, ranging from a few hours to several days, depending on the characteristic that is analysed (Hills, 2011).

5.5.2 Gravimetric sorption analysis

The gravimetric sorption analyser measures how quickly and how much of a solvent is absorbed by a sample. The TA Instruments VTI-SA, with modules for water was used for this purpose.

Experimental setup

The vapour sorption analyses were performed, utilising a VTI-SA2 vapour sorption analyser (New Castle, Delaware, USA). The micro-balance was calibrated prior to each vapour sorption run with a standard 100 mg weight. The micro-balance was set to zero prior to weighing each sample into the quartz sample container. The sample was carefully placed into and evenly distributed in the sample holder. The percentage relative humidity (% RH) per temperature programme was pre-set on the TA Instruments Isotherm software. The percentage RH ramp was set from 5 - 95% RH, followed by a decrease from 95 - 5% RH. The last absorption phase was set to also ramp from 5 - 95% RH. The temperature was set at a constant 25°C throughout the percentage RH ramp. The programme criteria were set to a 0.0001% weight change, or to a 2-minute stability of weight gained or lost, before the programme would continue to the next set parameter.

5.6 High performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) is a technique that is used to quantify and identify individual components present in a sample. In HPLC, an elevated pressure is generated by a pump that causes the mobile phase to move through a column. The column, packed with very small porous particles, represents the stationary phase.

Samples obtained from the degradation studies were assayed on HPLC. A Shimadzu (Kyoto, Japan) UFLC (LC-20AD) chromatographic system was used. The system comprised of a

SIL-20AC auto-sampler, fitted with a sample temperature controller, a UV/VIS Photodiode Array detector (SPD-M20A) and an LC-20AD solvent delivery module. A VanKel700 dissolution bath, thermostatically controlled at 25°C, 37°C and 45°C ± 5°C (Vankel VK 650A), was used for the degradation studies. A temperature controlled mechanical water bath shaker was used to analyse samples at 5°C.

Experimental setup

A VanKel700 (Palo Alto, USA) dissolution bath was used for the degradation studies. United States Pharmacopeia (USP) apparatus 2 (paddle) was set up at 25°C, 37°C and 45 ± 2°C at a rotational speed of 100 rpm and was 500 ml of deionised water added to each vessel. The dissolution apparatus was covered with foil to protect the light sensitive mixtures. Degradation studies that were done at 5°C were analysed by using a temperature controlled water bath auto-rotator (Labotec).

5.7 Hydrolysis studies

500 mg of powder of each isoniazid (INH), pyrazinamide (PZA) and ethambutol HCl (EMB) was added into separate beakers containing 500 ml of distilled water each and mechanically stirred to obtain clear solutions. Each mixture was then transferred into 900 cm^3 dissolution flasks.

50 mg of rifampicin (RIF) powder (*poorly soluble in water*) was also dissolved in a beaker containing 500 ml of distilled water and mechanically stirred to obtain a clear solution. The mixture was then transferred into a 900 cm^3 dissolution flask.

To investigate the possible influence of different APIs on each other, different combinations of these materials (500 mg of each) with RIF (50 mg) were also tested.

Rifampicin combinations prepared and tested

- Rifampicin and isoniazid;
- Rifampicin, isoniazid, and pyrazinamide;
- Rifampicin, isoniazid and ethambutol HCl; and
- Rifampicin, isoniazid, ethambutol HCl and pyrazinamide.

Tests were carried out by using the Vankel VK 700 (220 V) dissolution apparatus, equipped with the Vankel VK 650A Circulator to maintain the temperature at either 25°C or 37°C. The dissolution apparatus was covered with foil to protect the light sensitive mixtures. Flasks (900 ml), filled to 500 ml with distilled water were used and a stirring speed of 100 rpm was applied. A 48 h test run was conducted, and 2 ml samples were withdrawn at hourly intervals for 48 h and filtered into amber vials for analysis on HPLC.

Degradation studies that were conducted at 5°C were analysed, using a temperature controlled auto-rotator (Labotec). Each sample contained 500 mg each of INH/PZA/EMB in the said combinations and only 50 mg of RIF powder, due to its low solubility in water. Each sample was mixed in a beaker containing 500 ml of distilled water and mechanically stirred to obtain a clear solution. Each mixture was then transferred into 20 ml amber test tubes and placed in the temperature controlled auto-rotator. A 48 h test run was conducted, and 2 ml samples were withdrawn from each sample at hourly intervals for 48 h and filtered into amber vials for analysis on HPLC.

5.7.1 HPLC method (INH/PZA/RIF)

The HPLC separations were performed on a Phenomenex Luna 5 µm C_8 (250×4.6 mm) stainless steel column.

Preparation of standard solution

Solutions of RIF, INH, and PZA were prepared by dissolving accurately weighed powder in distilled water to get final concentrations of 0.1 mg/ml, 1 mg/ml and 1 mg/ml, respectively.

Chromatographic conditions

The experiment involving separation optimisation was conducted in a gradient elution. The gradient elution was carried out with a mobile phase of acetonitrile (A) and phosphate buffer (B: 8 Mm, pH 6.8). The gradient profile of the mobile phase composition was (A:B) 10:90 (v/v) at 0 min, after which a linear gradient to 60:40 (v/v) was reached over a period of 18 min, which was maintained at 60:40 (v/v) for 6 min. The solvent composition was returned to 10:90 (v/v) over a period of 2 min and equilibrated for another 2 min before the next injection. Chromatic conditions were: wavelength of 210 nm, flow rate of 1.0 ml per minute,

injection volume of 10 µl per sample, retention times for deriving INH, RIF and PZA were approximately 2.9 min, 18 min, 3.7 min, respectively.

5.7.2 HPLC method (EMB)

The HPLC separations were performed on a Phenomenex Luna 5 µm stainless steel column (15 cm x 4.6 mm), packed with silica gel particles, the surface of which was modified with chemically bonded octadecylsilyl groups.

Preparation of standard solution

Samples of EMB were prepared by dissolving accurately weighed amounts of powder in distilled water to get final concentrations of 1 mg/ml.

Chromatographic conditions

The mobile phase was prepared by dissolving 50 g of ammonium acetate and 0.2 g of copper (II) acetate in 1000 ml of distilled water and adjusted to pH 5.0 with glacial acetic acid R. 940 ml of this solution was mixed with 60 ml of methanol. Chromatic conditions were: detector used was an ultra-violet spectrophotometer set at a wavelength of about 270 nm, flow rate of 2.0 ml per minute, injection volume of 20 µl per sample.

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CHAPTER 6
Results

6.1 Introduction

Anti-tuberculosis fixed dose combination products have been reported to be unstable in formulation due to chemical interactions between the component drugs. It has been previously published that ethambutol HCL catalyses the degradation of rifampicin and isoniazid in the formulation due to its reported hygroscopic nature. This is said to result in the loss of rifampicin potency upon storage. The following characterisation and stability indicating methods were done to ascertain if these claims are true, i.e. solid-state characterisation of the four APIs, stability testing, hydrolysis, compatibility between the components through microcalorimetry, and hygroscopicity analysis.

All the measurements in this chapter were done according to the methods as described in Chapter 5.

6.2 Solid-state characterisation of the four anti -TB APIs

Product information of API samples use in this study

RIF: batch number RMP/XP-003/12
Supplier DB Fine Chemicals, South Africa
Manufactured by Linaria chemicals, Thailand

INH: batch number 12174/INH
Supplier DB Fine Chemicals, South Africa
Manufactured by GIDC Industrial estate, India

EMB: batch number ETH/P-015/12
Supplier DB Fine Chemicals, South Africa
Manufactured by Linaria chemicals, Thailand

PZA: batch number PYZ/P-062/12
Supplier DB Fine Chemicals, South Africa
Manufactured by Linaria chemicals, Thailand

6.2.1 Rifampicin (RIF)

According to the XRPD pattern (Figure 6.1), and melting points as determined with DSC and TM (Figure 6.3, 6.4), the commercial rifampicin used in this study most closely resembles that of Form II as described by Henwood *et al*. (2001). The relatively low peak intensity on the XRPD diffractogram (for our particular instrument) and noisy background could be indicative of an amorphous component. Melting was evident from the deformation (thermal microscopy) of the crystals at 180°C, the crystals later turned black indicating decomposition. The melting point of 193°C is the same as reported by Henwood *et al.* (2001). The TG trace did not show any weight loss over the temperature range of 25 to 190°C, indicating that this raw material is not a solvate or hydrate. Henwood *et al.* (2000) showed that rifampicin raw materials used by manufacturers of generic rifampicin products in South Africa were either Form II or a mixture of Form II and an amorphous form. None of the pharmacopoeias specify any particular polymorphic form for manufacturing of rifampicin (BP, USP, IP) products. The SEM micrographs (Figure 6.2) showing the morphology of rifampicin indicated amorphous and crystalline parts. The micrograph shows a mixture of prismatic crystals and amorphous particles that appear to have been milled or ground.

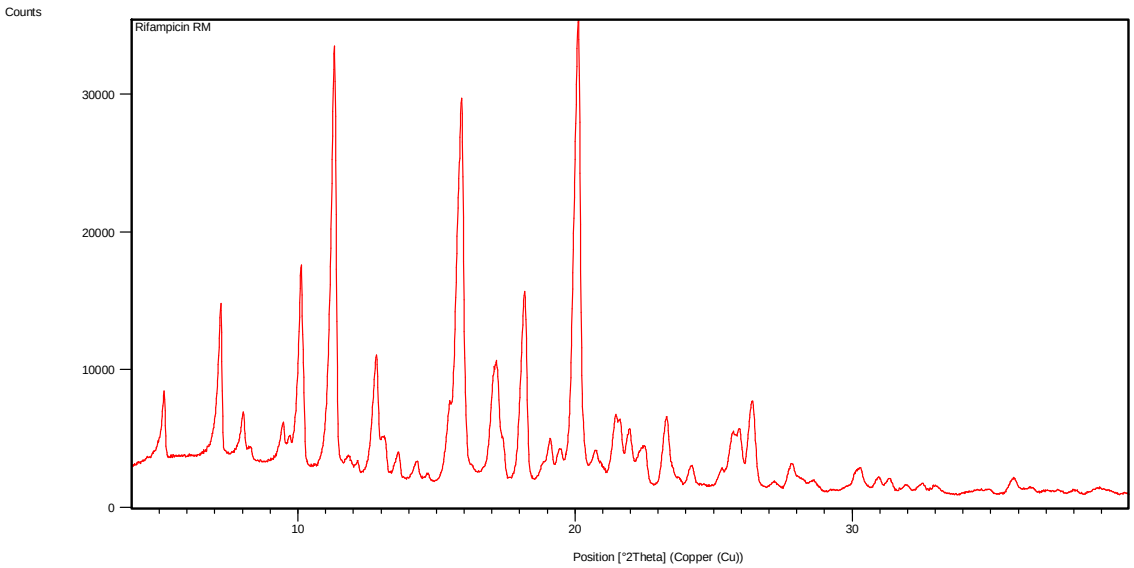

Figure 6.1: XRPD diffractogram of RIF.

Figure 6.2: SEM micrograph of RIF powder.

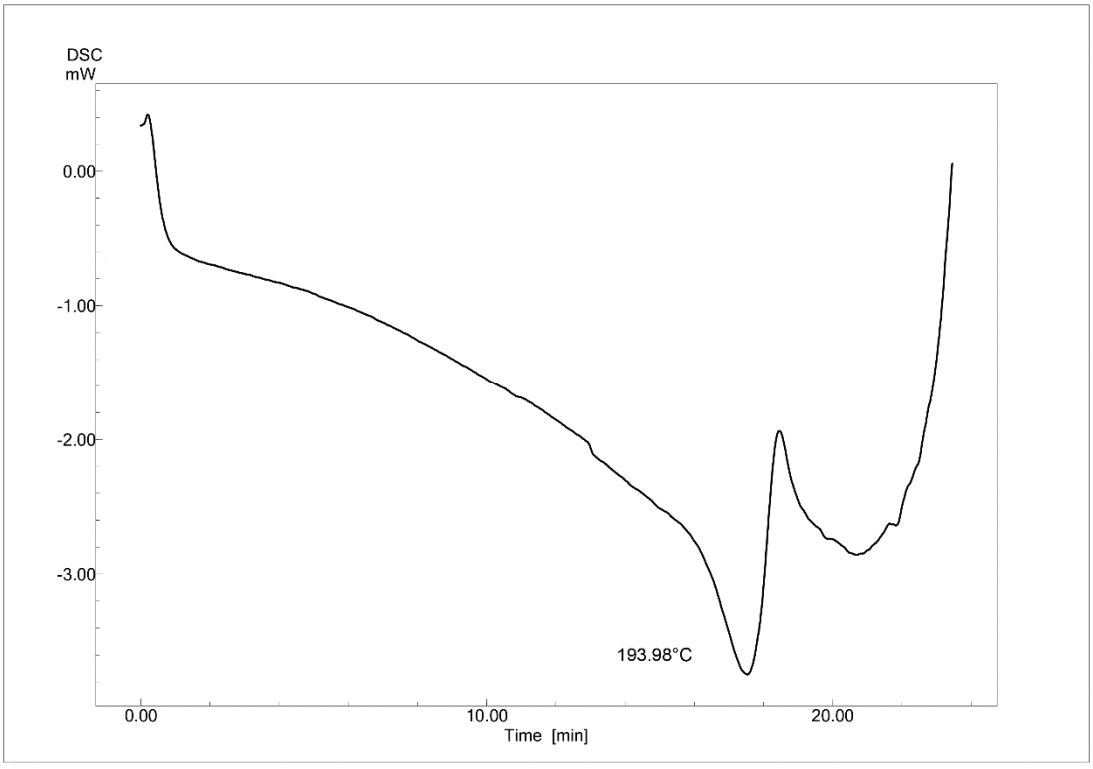

Figure 6.3: DSC thermogram of RIF.

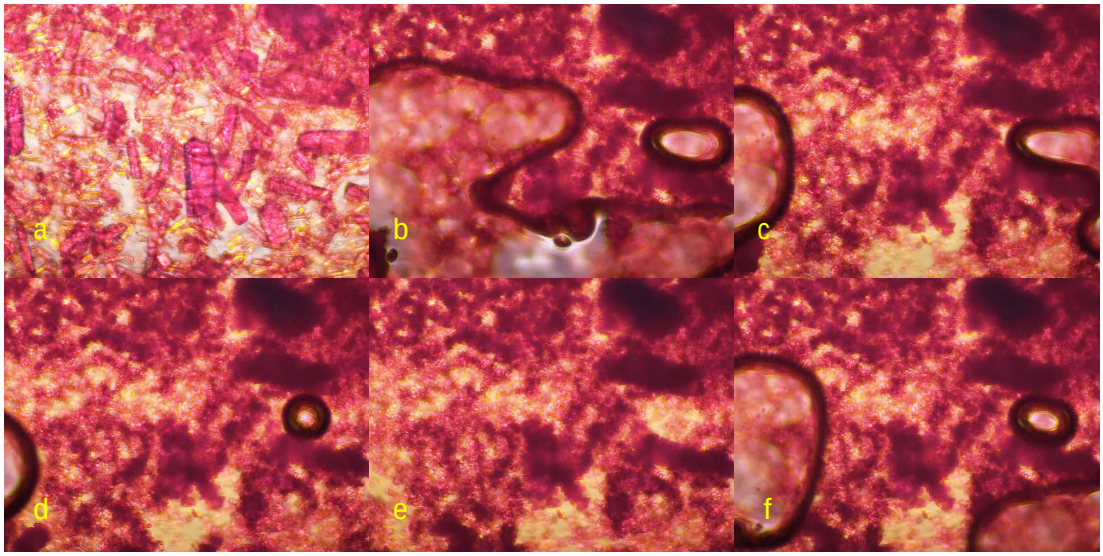

Figure 6.4: Thermal microscope events of RIF at different temperatures in silicone oil, a = 30°C; b = 180°C; c = 192°C; d = 197°C; e = 207°C; f = 210°C.

6.2.2 Ethambutol HCl (EMB)

As mentioned earlier in chapter 2, four polymorphic forms of ethambutol hydrochloride have been reported, and it is to believe that only form II is used in the manufacturing of EMB (Rubin-Preminger *et al.*, 2004). The XRPD diffractogram of EMB (Figure 6.5) resembles that of form II. On the SEM micrographs (Figure 6.6) the morphology of EMB indicated bladed crystals. The DSC thermogram (Figure 6.7) of EMB showed an endotherm at 77°C, which corresponds to the reversible phase transition from form II to form I that was previously reported by Rubin-Preminger *et al*. (2004). The phase transition can be clearly seen on the HSM photos (Figure 6.7b). Another phase transformation was observed at room temperature from Form IV to Form III upon cooling. This reaction is visible on the HSM photos (Figure 6.7e & f).

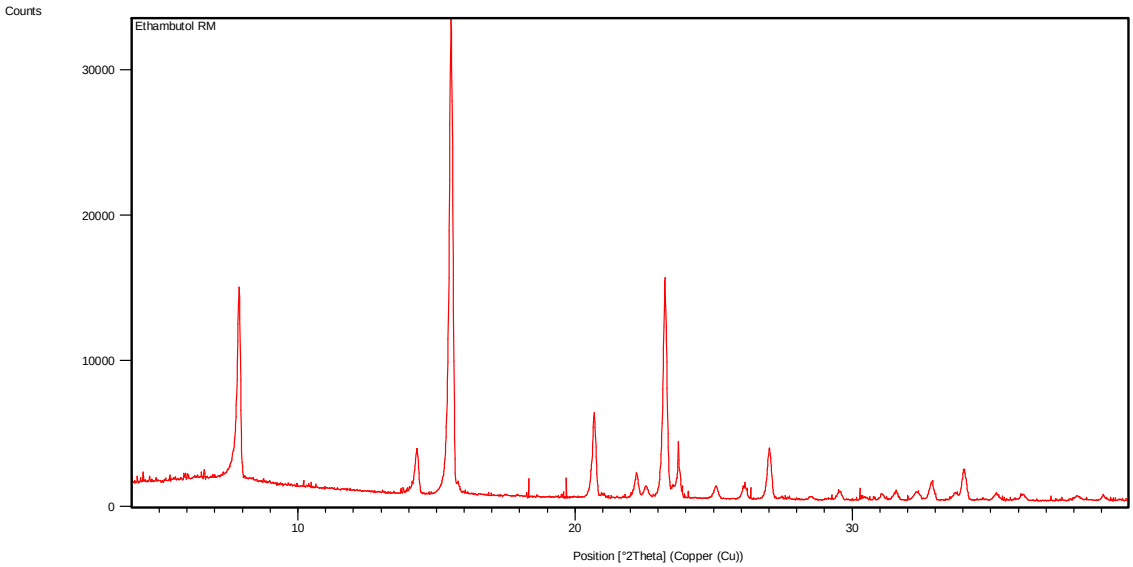

Figure 6.5: XRPD diffractogram of EMB.

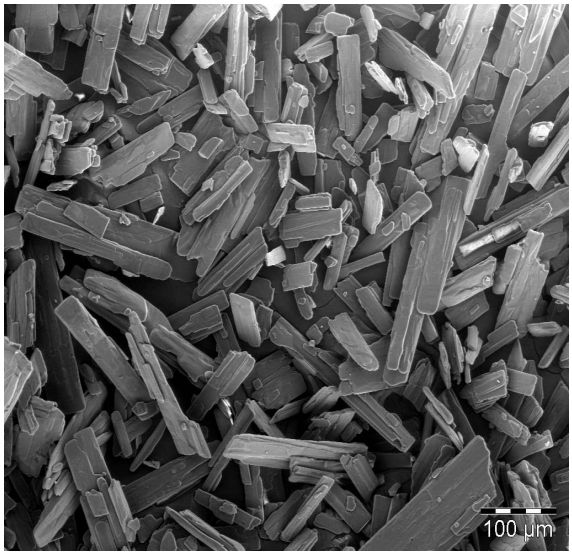

Figure 6.6: SEM micrographs of EMB powder.

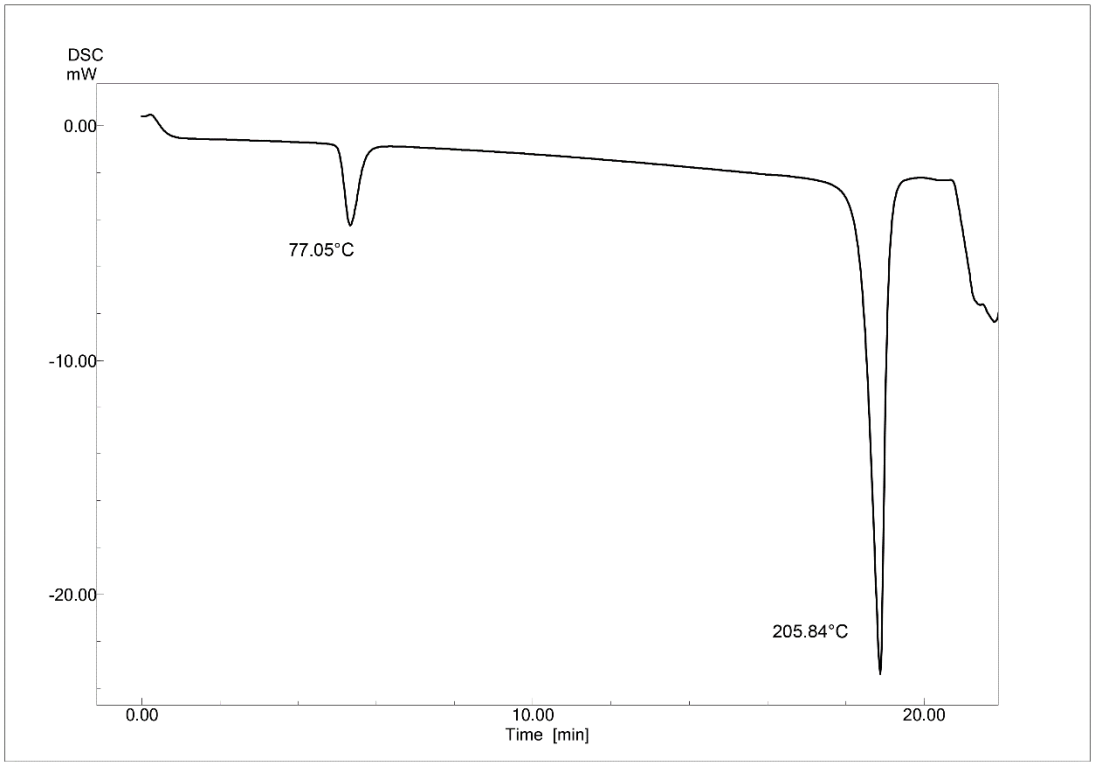

Figure 6.7: DSC thermogram of EMB.

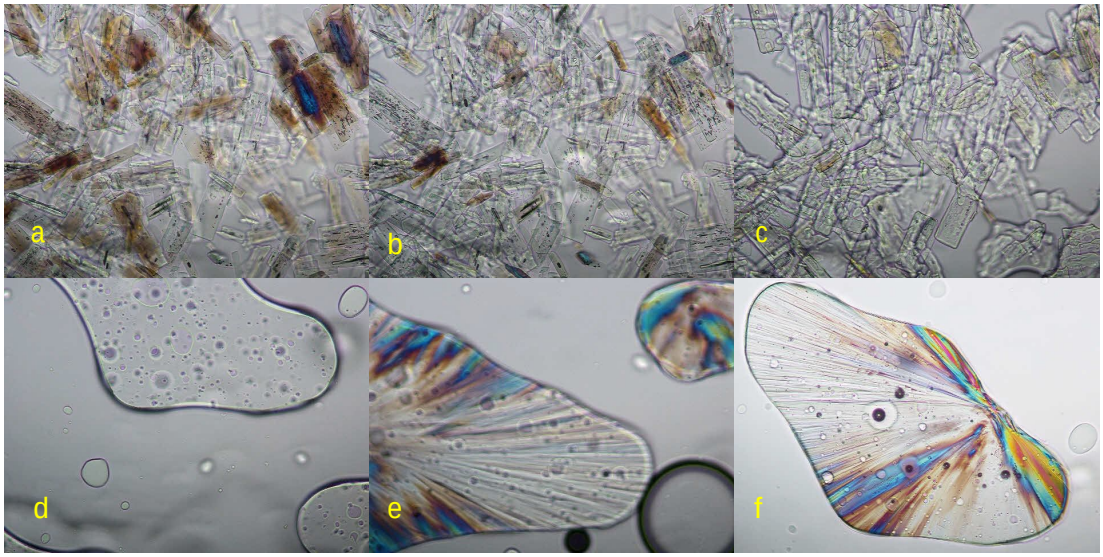

Figure 6.8: Thermal microscope events of EMB at different temperatures in silicone oil, a = 30°C; b = 77°C; c = 207°C; d = 209°C; e = room temperature; f = 60°C.

6.2.3 Isoniazid (INH)

As mentioned in chapter 2 there are no polymorphic forms of INH that have been reported yet. In-house studies prove to be unsuccessful to prepare any other form as that used in the commercial market. Becker *et al*. (2008) also reported that no polymorphic forms for INH were discovered, and INH also formed no hydrates. Only one crystalline form of orthorhombic habit was reported. The solid-state results of the INH used in this study are presented in Figures 6.9 – 6.12. A single endothermic event was observed between 172 – 180°C and the SEM micrographs showed that the INH morphology exhibited prismatic crystals.

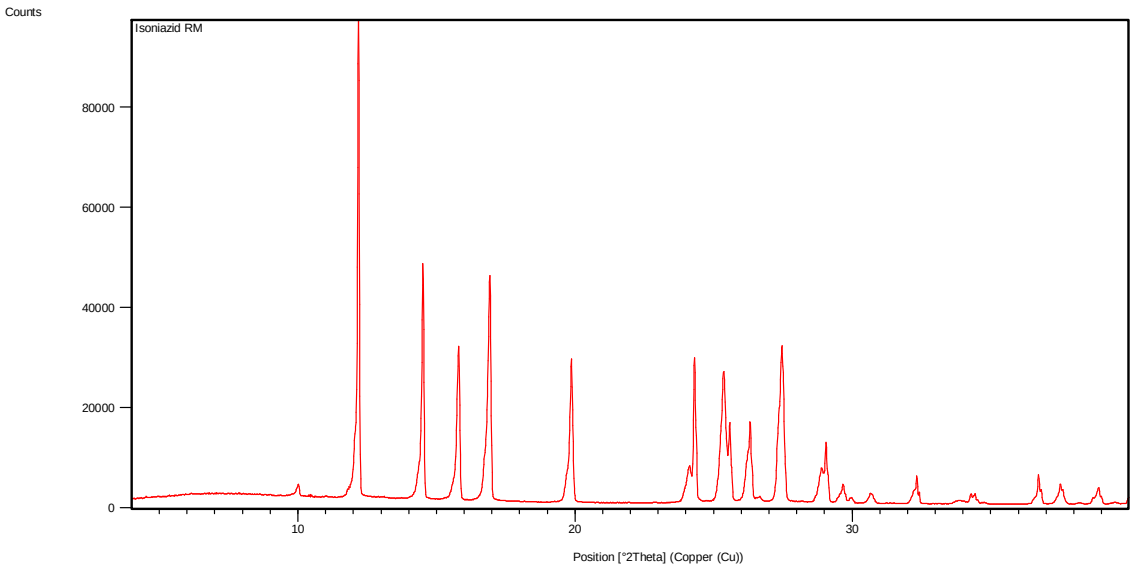

Figure 6.9: XRPD diffractogram of INH raw material.

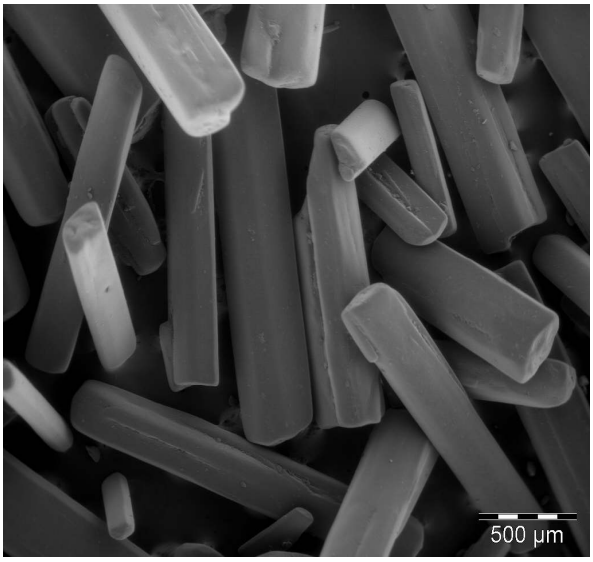

Figure 6.10: SEM micrograph of INH.

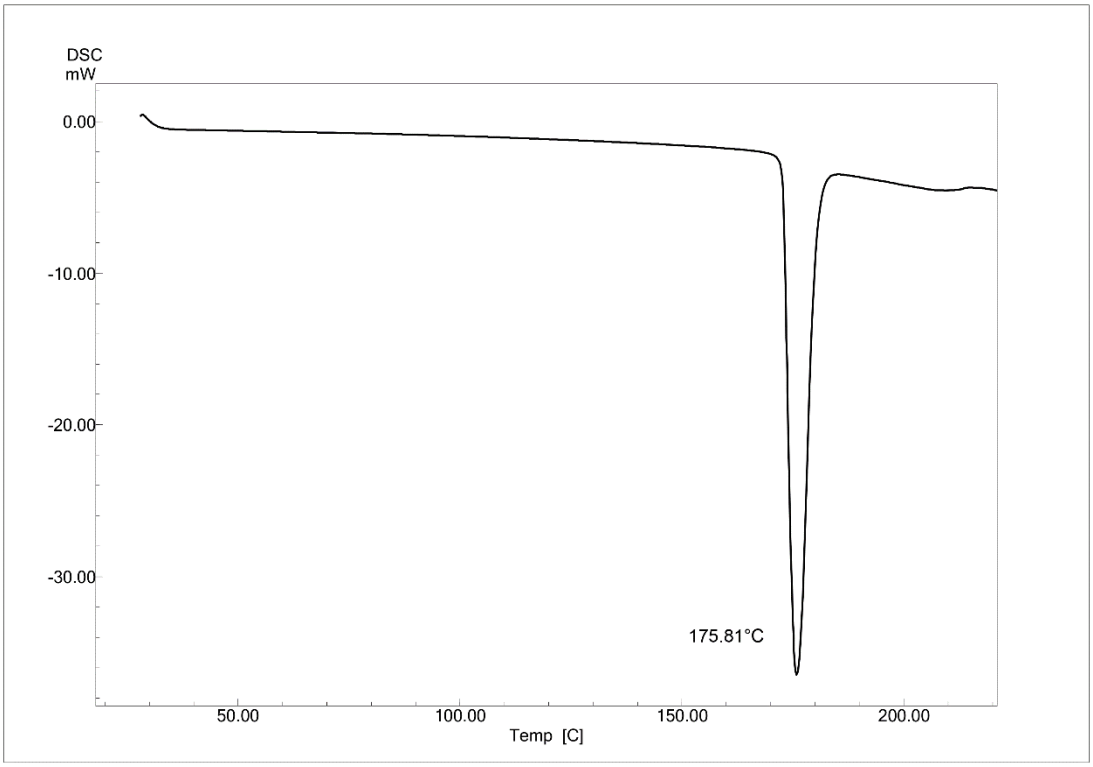

Figure 6.11: DSC thermogram of INH.

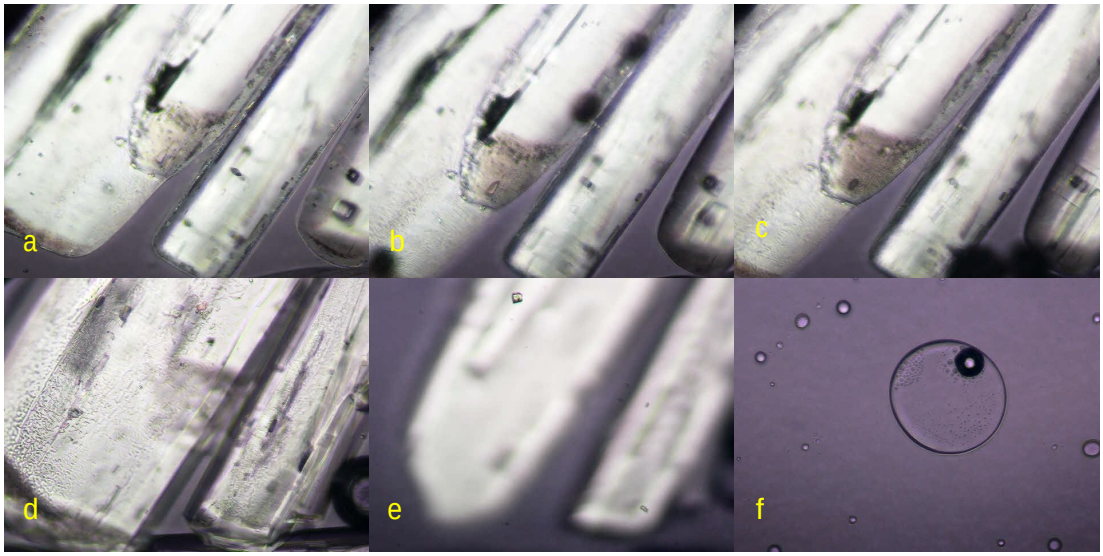

Figure 6.12: Thermal microscope events of INH at different temperatures in silicone oil, a = 30°C; b = 160°C; c = 172°C; d = 176°C; e = 179°C; f = 183°C.

6.2.4 Pyrazinamide (PZA)

Literature revealed that PZA presents itself in four packing polymorphic forms, each having different crystal arrangements, i.e. α-, β-, γ- and δ- pyrazinamide. The different forms were obtained by different recrystallisation solvents at different temperatures. According to the XRPD traces (Figure 6.13), the raw material being used in this study, represented the commercially available form, i.e. the α-form (Castro *et al.*, 2009). Castro *et al.* (2009) reported that PZA, the α-form underwent solid-solid phase transition upon heating. He showed that the α-form transforms into the δ-form upon heating. It is also reported that PZA undergoes sublimation at certain temperatures. The XRPD (Figure 6.13) showed the PZA raw material (α-form), which is also the commercial form. The SEM micrographs showed that the PZA morphology exhibited lamellar crystals (Figure 6.14). The DSC trace (Figure 6.15) showed a possible solid-solid transformation at about 157°C, and then melting at 193°C, however the solid-solid transformation was not observed on HSM (Figure 6.16a & b). The DSC thermogram was identical to that obtained by Castro *et al.* (2009). The thermal microscopy (TM) photos showed the melting events and also captured the sublimation product at 200°C (Figure 6.16f).

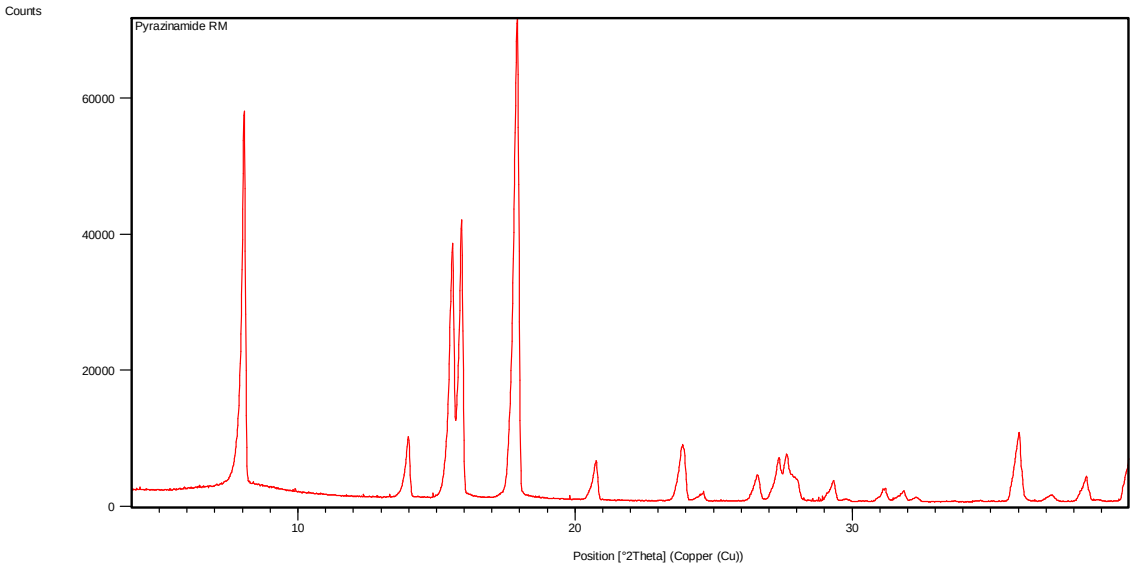

Figure 6.13: XRPD diffractogram of PZA raw material (α-form).

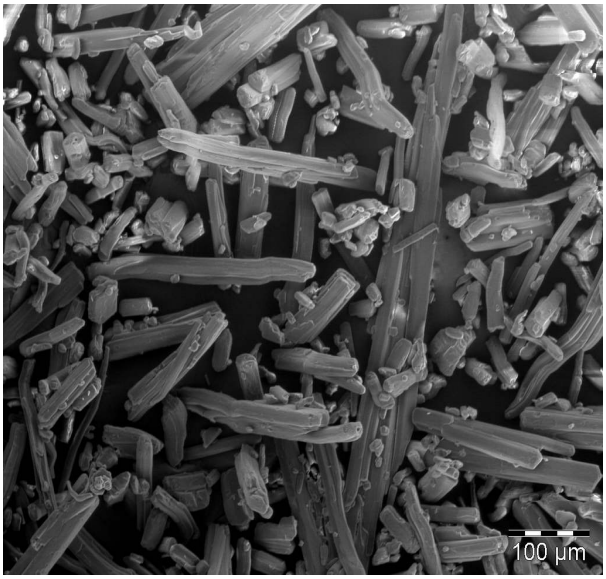

Figure 6.14: SEM micrographs of PZA crystals.

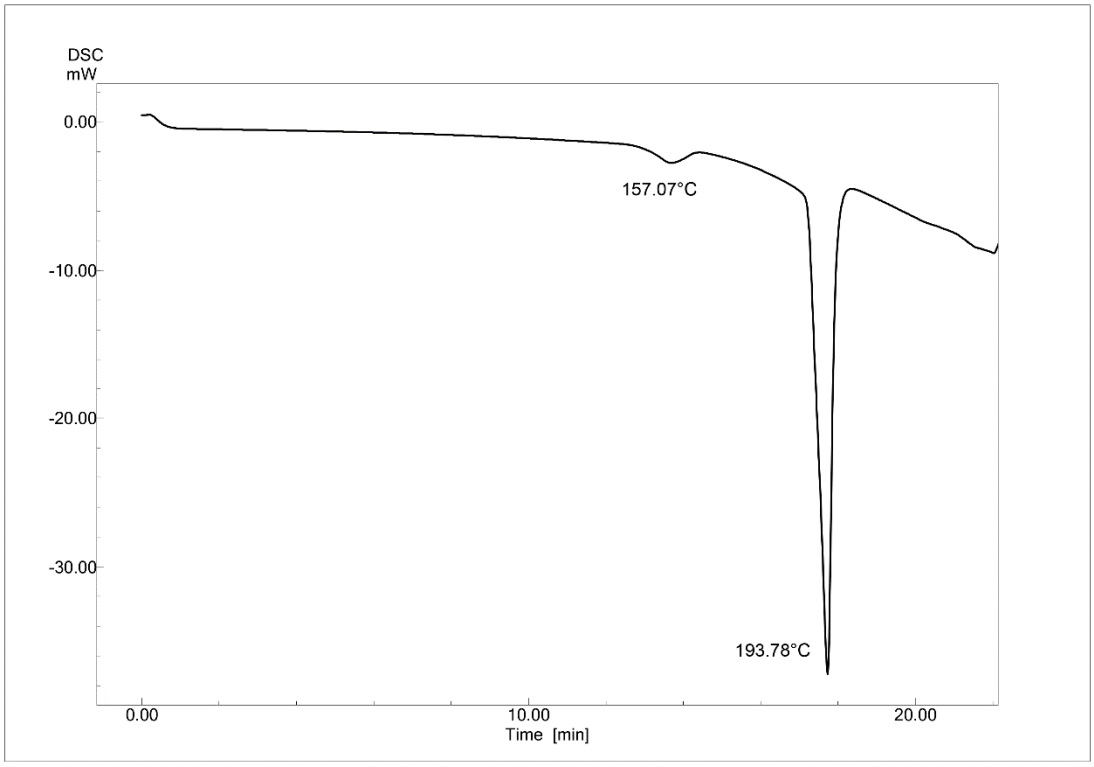

Figure 6.15: DSC thermogram of PZA.

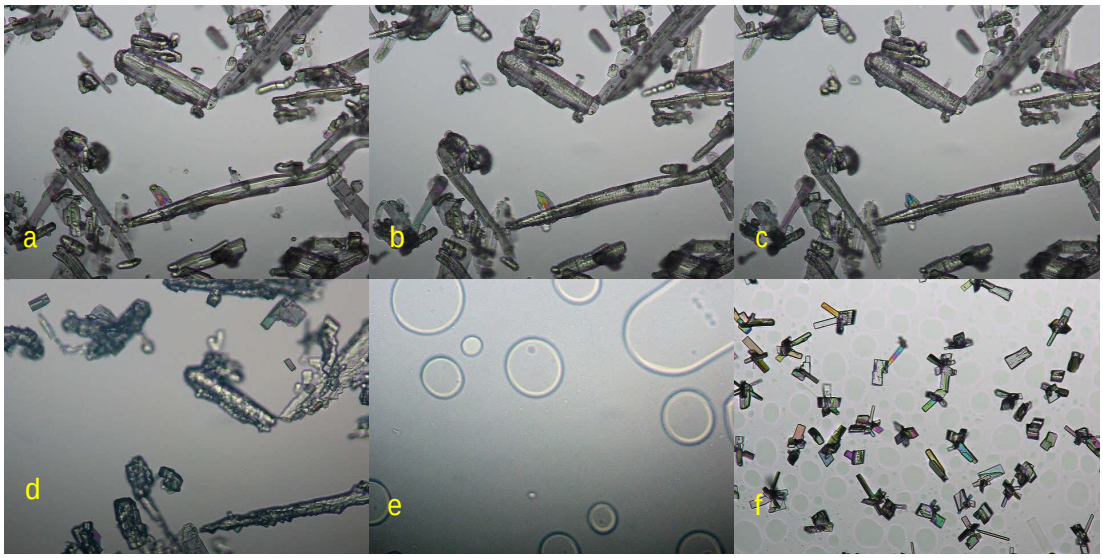

Figure 6.16: Thermal microscope events of PZA at different temperatures in silicone oil, a =30°C; b = 157°C; c = 170°C; d = 193°C; e = 215°C; f = sublimate.

6.3 Visual inspection of the influence of temperature and humidity on anti-TB APIs in the solid state

Mohan (2001) conducted a comparative stability indicating assay on marketed FDC products. The FDC products were stored unpacked in light and humidity chambers set at 40°C / 75% relative humidity (RH). The products that were stored in the light chambers showed more physical changes than those stored in dark chambers (Mohan, 2001). Singh *et al.* (2001) later explored this phenomenon and postulated that if packaging is of a low quality, EMB, due to its hygroscopic nature absorbs moisture from the atmosphere and gives rise to "bleeding" of APIs, i.e. the APIs leak out of the formulation (Singh *et al*., 2001). EMB is believed to dissolve in the absorbed moisture and may even then act as a solvent for the other APIs in the formulation. It was reported that this sequence of events results in poor bioavailability of all the anti-TB drugs in formulation and eventually therapeutic failure (Bhutani *et al*., 2004a; Singh *et al*., 2001). The proposed incompatibility of the four anti-TB drugs has been reported to lead to two major problems, namely; (1) a decrease in bioavailability of RIF upon oral administration and (2) instability of the drugs within the formulation environment during storage (Bhutani *et al*., 2004a).

For the purpose of this study, powder mixtures were stored in a climatic chamber at 40°C / 75 % RH for 15 days. The powders were mixed according to the commercial drug Rifafour® (RIF-150mg/INH-75mg/PZA-400mg/EMB-275mg). The control samples (Figure 6.17) were stored in desiccators and remained unchanged throughout the experiment. Samples containing mixtures of RIF/INH and RIF/INH/PZA did not show any visual or physical changes. However, all mixtures containing EMB (Figure 6.18) showed physical changes. The EMB deliquesced and there were colour changes observed with all the samples containing EMB when compared to the control samples. These changes likely confirm the reported hygroscopic nature of EMB.

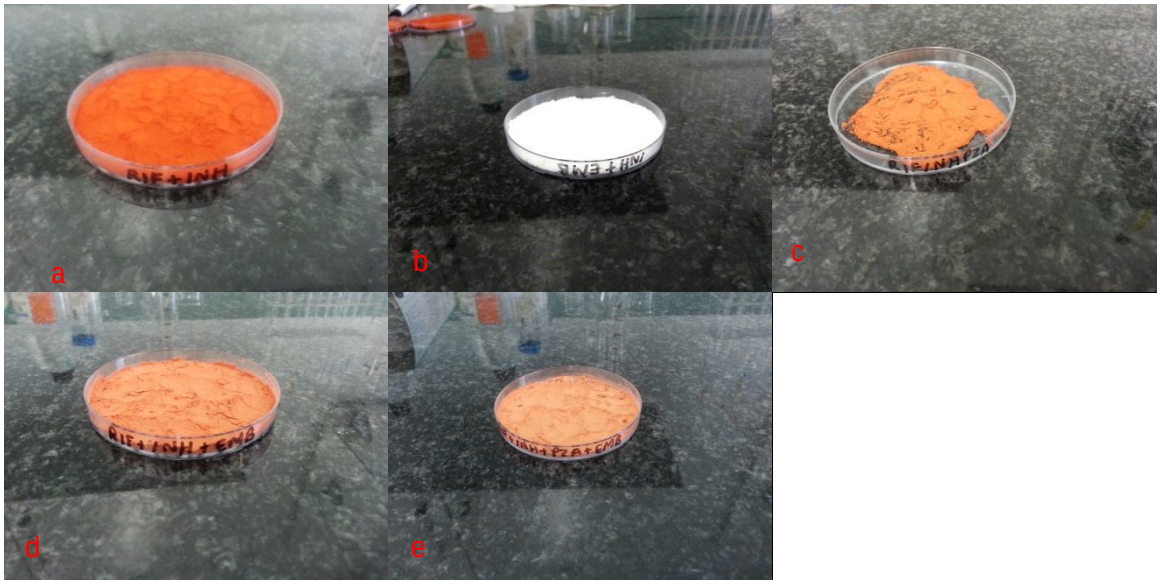

Figure 6.17: Control samples at ambient conditions a = RIF/INH, b = INH/EMB, c = RIF/INH/PZA, d = RIF/INH/EMB, e = RIF/INH/PZA/EMB.

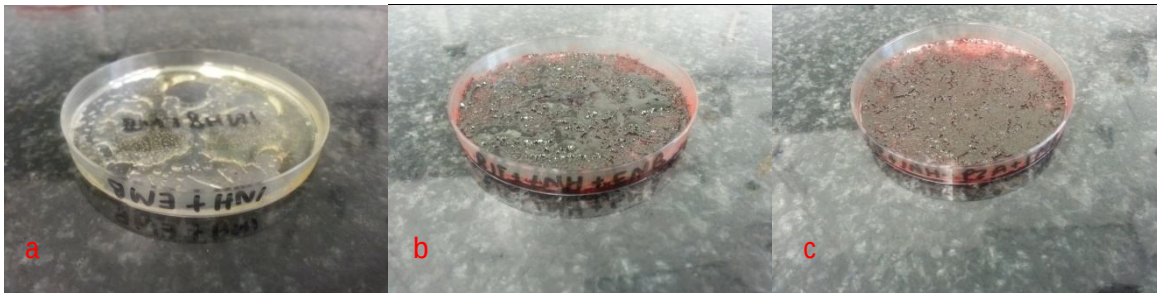

Figure 6.18: Samples containing EMB at 40°C / 75 % RH, a = INH/EMB, b = RIF/INH/EMB, c = RIF/INH/PZA/EMB.

6.4 Hydrolysis

It has been postulated that below pH 2, RIF is converted to its inactive metabolite 3- formylrifampicin SV. The reaction then proceeds to form isonicotinyl hydrazine (HYD) when RIF is combined with INH in formulation (Bhutani *et al.*, 2005). It has further been speculated that both PZA and EMB exhibit catalytic roles through the involvement of intra-molecular proton transfer during the reaction between RIF and INH, which is believed to occur through a base-catalysed transhydrazone formation process entailing a tetrahedral mechanism (Bhutani *et al.*, 2005; Smith & March, 2001). Sankar *et al.*

(2003) have also suggested that, even under non-acidic formulation conditions, a direct reaction (although much slower), between RIF and INH, yielding HYD as a product is still possible.

For the purpose of this study hydrolysis experiments were done in distilled water to determine the extent of decomposition of RIF and INH using single, two, three and four anti-TB APIs. The aim of the investigation was to test the above hypotheses regarding the stability of especially RIF and INH in combination with the other two APIs. Assays were done at 2, 3, 6, 12, 24 and 48 hours using solutions that were maintained at four different temperatures (5, 25, and 37°C – each ± 2°C). Mixtures containing rifampicin were protected from light by covering the vessels containing the solutions with aluminium foil as RIF has been reported to be photolabile.

Since pH may influence the solubility and degradation of many APIs, the pH of aqueous solutions of individual components as well as mixtures of the anti-TB FDC drugs (1 mg per 10 ml) were determined in distilled water (Table 6.1). The pH at T_0 was measured as soon as the powders appeared to have dissolved completely. It was not possible to determine the pH of the water because is deionised and therefore will not give an accurate pH value.

For each mixture and temperature, pH measurements were taken in distilled water.

Table 6.1: Individual components and mixtures of anti-TB FDC drugs (1 mg per 10 ml) in distilled water.

APIs	pH					
	5°C T_0	**5°C T_{2h}**	**25°C T_0**	**25°C T_{2h}**	**37°C T_0**	**37°C T_{2h}**
RIF	6.23	6.83 (Δ = 0.60)	6.04	6.38 (Δ = 0.34)	6.79	6.92 (Δ = 0.13)
INH	6.46	6.35 (Δ = -0.11)	8.05	6.36 (Δ = -1.69)	7.28	6.34 (Δ = -0.94)
PZA	6.14	6.95 (Δ = 0.81)	5.92	6.03 (Δ = 0.11)	5.84	5.96 (Δ = 0.12)
EMB	5.10	5.16 (Δ = 0.06)	4.59	4.67 (Δ = 0.08)	4.54	4.39 (Δ = -0.15)
RIF + INH	7.12	7.65 (Δ = 0.53)	6.29	6.31 (Δ = 0.02)	6.05	6.09 (Δ = 0.04)
RIF + INH + EMB	5.66	5.68 (Δ = 0.02)	4.99	5.15 (Δ = 0.16)	4.88	4.85 (Δ = -0.03)
RIF + INH + PZA	6.3	6.43 (Δ = 0.13)	6.02	6.12 (Δ = 0.10)	6.04	6.19 (Δ = 0.15)
RIF + INH + PZA + EMB	5.55	5.58 (Δ = 0.03)	4.93	5.07 (Δ = 0.14)	4.89	4.85 (Δ = -0.04)

RIF: At all three temperatures, the pH increased slightly over 2 hours. The larger ΔpH for 5°C might indicate that small crystallites, not visible to the naked eye in the bright orange liquid, might not yet have fully dissolved at this low temperature. The solubility of very poorly soluble APIs like RIF is often very dependent on temperature. At 37°C, it seems RIF might have dissolved more rapidly, thereby providing the API with its own near neutral pH environment. The pH at 25°C was lowest, indicating that some change might have occurred.

INH: The initial pH values for INH were extremely temperature dependent even though all three stabilised to near exact values after 2 hours.

PZA: The measured pH values were inversely proportional to the temperature of each solution. The ΔpH at 5°C was much higher and, as with RIF, most likely due to PZA's poor aqueous solubility which resulted in difficulty attaining equilibrium.

EMB: The ΔpH values for solutions of this API at different temperatures were small thanks to EMB being very soluble and having rapid dissolution. The measured pH values were inversely proportional to the temperature of each.

RIF + INH: The measured pH values for this mixture was inversely proportional to the temperature of each. As with the pure RIF solution the 5°C ΔpH value is larger than with the higher temperatures. Again, we attribute this to RIF's poor solubility.

RIF + INH + EMB: This mixture had measured pH values lower than RIF, INH and RIF + INH, but higher than EMB alone. Measured pH values for this mixture was inversely proportional to the temperature of each. The ΔpH values were low throughout. A 5°C ΔpH value lower than that of RIF + INH could indicate that EMB facilitates faster dissolution of RIF.

RIF + INH + PZA: The pH values measured appeared to be relatively independent of temperature in this limited range. The solutions were not as acidic as RIF + INH + EMB. Although improvement is evident, the addition of PZA to RIF + INH did not seem to enhance the dissolution rate of RIF to the same degree as EMB if one is to judge this by the 5°C ΔpH value.

RIF + INH + PZA + EMB: The pH profile of the complete four-drug mixture resembles that of RIF + INH + EMB very closely with almost identical values. If anything, the values are ever so slightly lower which is difficult to explain considering that PZA and RIF + INH + PZA solutions had higher pH values.

Table 6.2: Degradation of RIF at different temperatures.

API Combinations	Degradation of RIF (%)					
	$5°C_{T2H}$	$5°C_{T48H}$	$25°C_{T2H}$	$25°C_{T48H}$	$37°C_{T2H}$	$37°C_{T48H}$
RIF	1.2	5.6	3.3	19.4	0.9	15.1
RIF [+ INH]	0.04	60.7	1.4	28.5	0.4	54.0
RIF [+ INH + EMB]	5.3	67.9	1.0	73.7	6.8	71.4
RIF [+ INH + PZA]	4.5	7.7	3.1	19.0	4.7	60.8
RIF [+ INH + EMB + PZA]	0.6	74.3	12.6	63.2	6.2	68.2

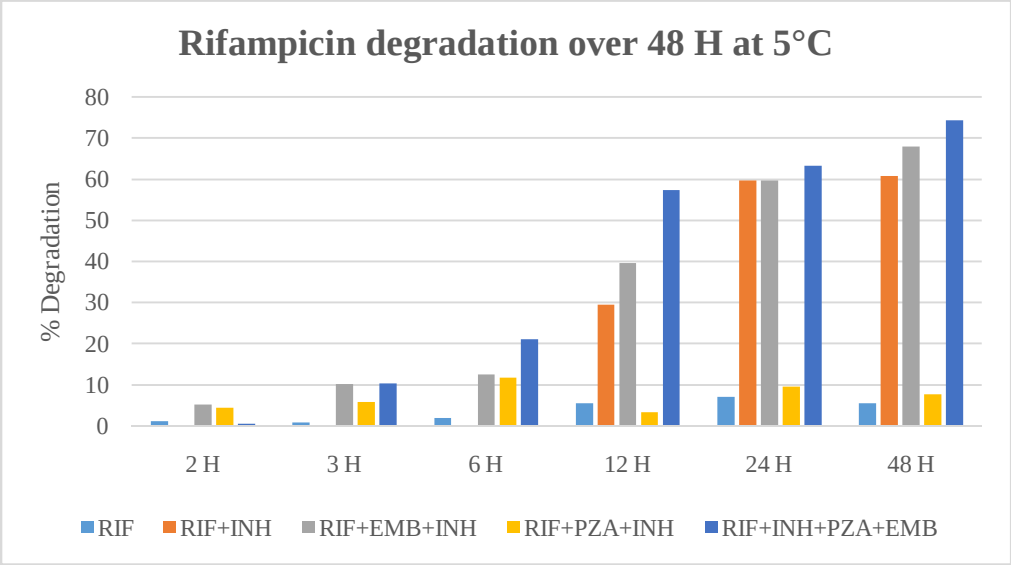

Figure 6.19: Rifampicin degradation as single component and in different combinations over 48 h at 5°C.

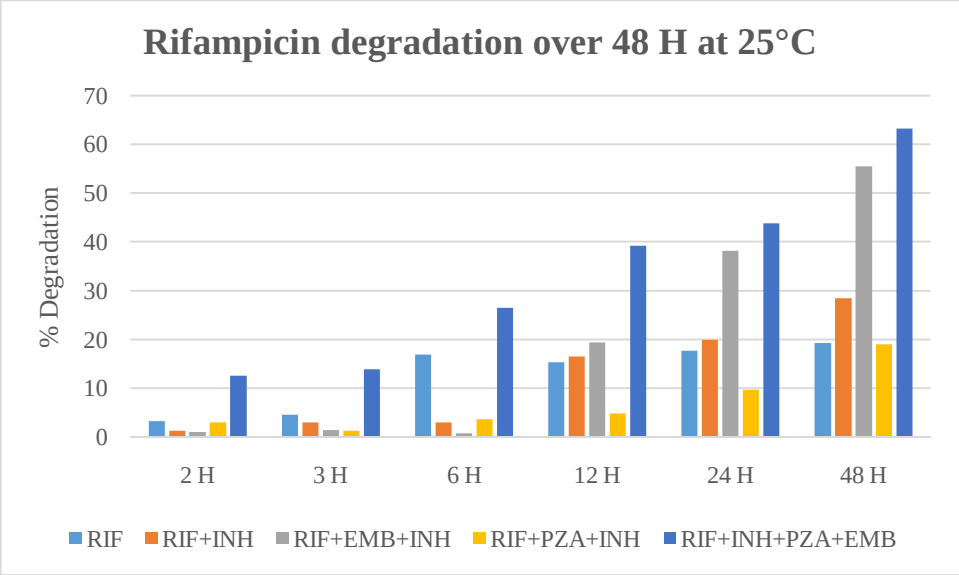

Figure 6.20: Rifampicin degradation as single component and in different combinations over 48 h at 25°C.

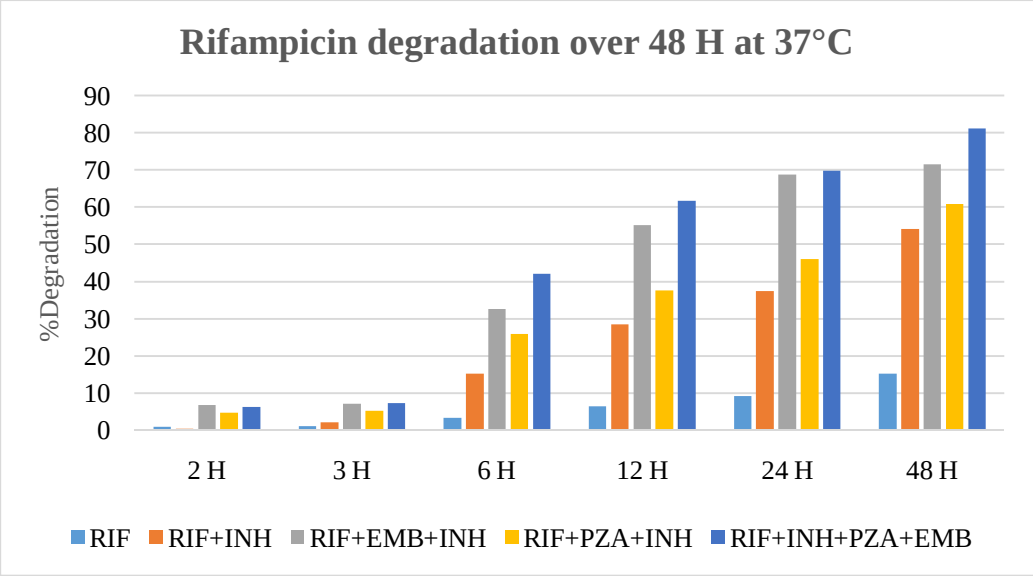

Figure 6.21: Rifampicin degradation as single component and in different combinations over 48 h at 37°C.

RIF: Rifampicin decomposes rapidly in acidic or basic conditions at 25°C, but slowly in neutral conditions (Maggi *et al*, 1966; Gallo & Radaelli, 1976). This is supported by our observations. During the first two hours, degradation was lowest at 37°C which represented the solution with the most neutral pH. However, by the time that 48 hours

had passed degradation was lowest at 5°C, presumably because the low temperature slowed any degradation processes. The highest percentage was observed for 25°C at every interval tested (Figure 6.19-21).

RIF + INH: During the first two hours, no significant degradation occurred (Table 6.2).

RIF + EMB + INH and RIF + PZA + INH: Stable over the first two hours, then the RIF degradation increased significantly with every time interval and different temperature. RIF + INH + PZA + EMB: Surprisingly stable until 3 hours, (7% degradation), but after 6 hours the degradation was more than 40% and increases from there with every time interval.

Table 6.3: Degradation of INH at different temperatures

API Combinations	**Degradation of Isoniazid (%)**					
	5°C$_{T2H}$	**5°C$_{T48H}$**	**25°C$_{T2H}$**	**25°C$_{T48H}$**	**37°C$_{T2H}$**	**37°C$_{T48H}$**
INH	0.6	0.6	0.02	0.23	0.04	2.5
INH [+ RIF]	0.2	5.9	0.2	2.9	5.9	10.7
INH [+ RIF + EMB]	0.2	1.6	0.2	3.0	0.2	6.4
INH [+ RIF + PZA]	1.0	2.7	0.03	3.3	3.7	11.8
INH [+ RIF + EMB + PZA]	0.02	1.2	15.2	17.7	11.4	13.6

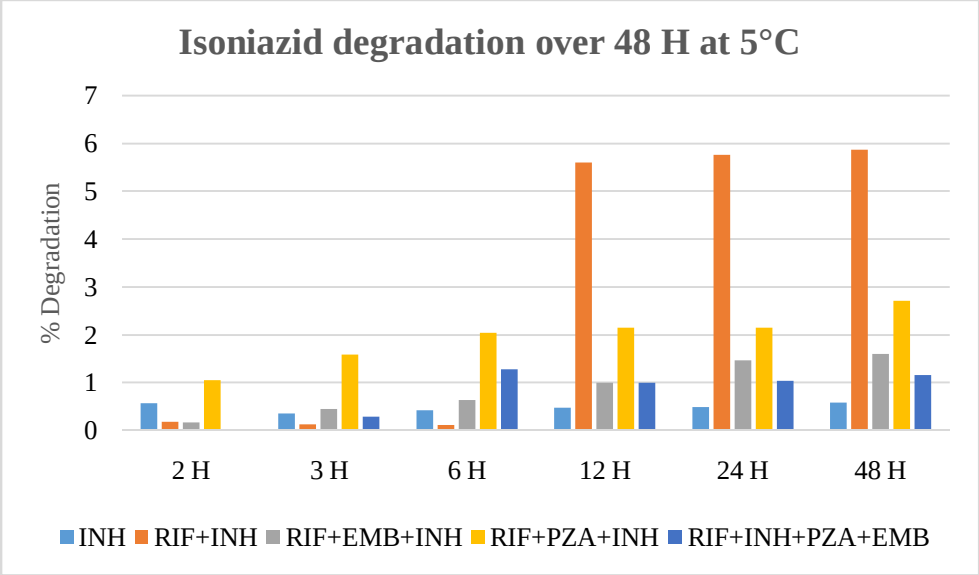

Figure 6.22: Isoniazid degradation as single component and in different combinations over 48 h at 5°C.

INH: In isolation INH aqueous solution was unexpectedly stable over a 48 hour period with just the 37°C solution showing degradation in excess of 2%. In terms of stability criteria, a deviation of 2% in drug purity is still acceptable (Table 6.3).

INH + RIF: Only the sample at 37°C and 24 hour showed degradation in excess of 10%.

INH + RIF + EMB: INH remains stable and degradation at 37°C and 24-hour period less than 7% (Figure 6.24).

INH + RIF + PZA: Again, the INH remains stable and the highest degradation occurred at 37°C and 24-hour period, less than 12%.

INH + RIF + PZA + EMB: 5°C showed minimal degradation (Figure 6.22-23), but strangely the 25°C at 2 and 24 hours exhibit the highest degradation (15.2 and 17.7%).

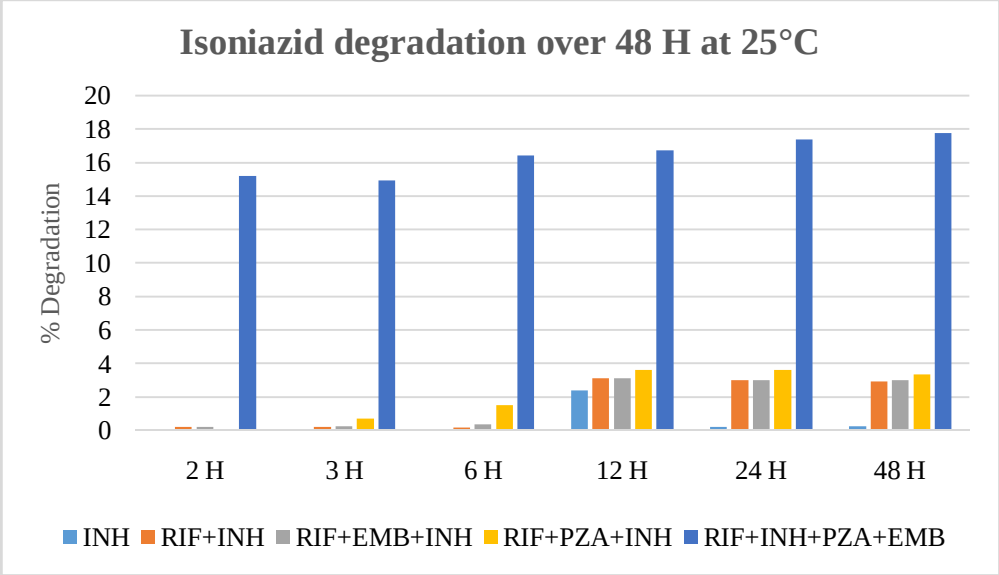

Figure 6.23: Isoniazid degradation as single component and in different combinations over 48 h at 25°C.

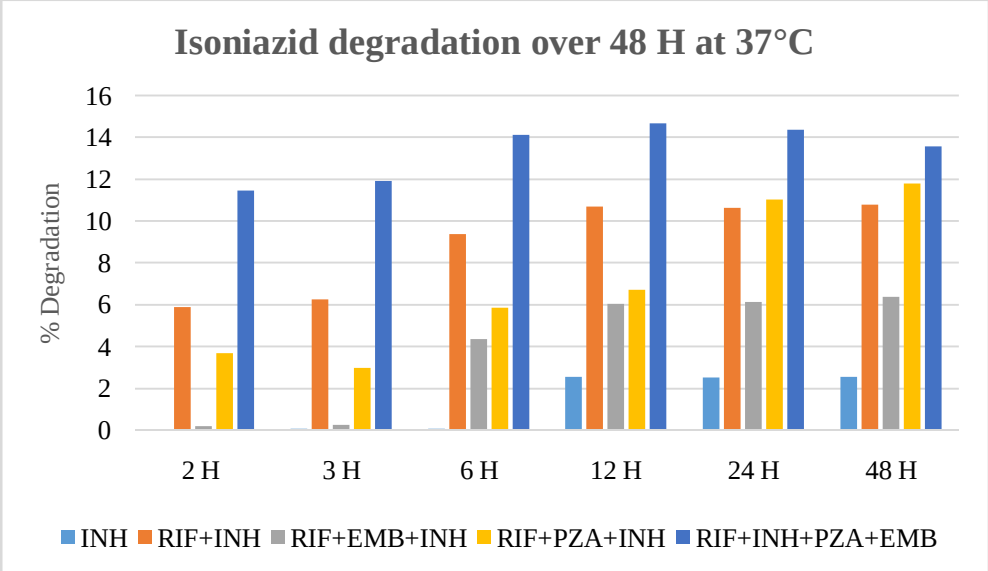

Figure 6.24: Isoniazid degradation as single component and in different combinations over 48 h at 37°C.

6.4.1 Discussion of hydrolysis results

It is conceivable that some of the degradation values at 5°C might be somewhat higher than expected due to the APIs taking longer to dissolve at low temperature and T_0 thus having been delayed.

Table 6.4: Relative Degradation of RIF at 48 Hours

RIF	5°C < 37°C < 25°C
RIF + INH	25°C < 37°C < 5°C
RIF + INH + EMB	25°C < 5°C < 37 °C
RIF + INH + PZA	5°C < 25°C < 37 °C
RIF + INH + EMB + PZA	25°C < 5°C < 37 °C

Table 6.5: Relative Degradation of INH at 48 Hours

INH	25°C < 5°C < 37 °C
INH + RIF	25°C < 5°C < 37 °C
INH + RIF + EMB	5°C < 25°C < 37 °C
INH + RIF + PZA	5°C < 25°C < 37 °C
INH + RIF + EMB + PZA	5°C < 37°C < 25 °C

The greatest rate of degradation for RIF in water at 37± 0.5°C was observed for the combination RIF+INH+EMB (68.6%). The degradation of the RIF+INH+PZA was at 37°C and 24 hours 46%. The degradation of the four drug combination at identical conditions was 63.2% (Table 6.4). It has been hypothesised that PZA and EMB are catalytic towards the reaction between RIF and INH as FDC's containing four-drug combinations have been reported to show far more chemical instability than 2-drug FDC's containing only RIF and INH (Bhutani *et al.*, 2004b).

For INH, the degradation rate was much lower, but greatest with the four-drug combination. Apart from a clear impact of INH and RIF on each other, the presence or absence of EMB and/or PZA also influences their rate of hydrolysis in water (Table 6.5).

The catalytic role of EMB has been attributed to its reported hygroscopic and acidic nature. Stability studies done by Sankar *et al*. (2003) on the behaviour of RIF in the presence of INH showed that 34 % of RIF was decomposed, and approximately 10% of INH was decomposed in acidic medium (stomach fasting condition). The decomposition of marketed FDC samples

were also tested in fasted-state pH conditions (0.01 M HCL solution) and the degradation values ranged from 13 – 35 % and 4 – 11 % for RIF and INH respectively. Unsuitable storage and packing conditions may also cause stability problems in FDC products.

6.5 Solutions for problems associated with decompositions

Certain drug delivery and formulation approaches have been suggested to solve the reported problems associated with the decomposition of RIF in the presence of INH as well as RIF's reported overall incompatibility with the other anti-TB drugs. It has been hypothesized that the following approaches may serve as solutions: (1) enteric coating of solid formulations or drug granules/powders; (2) using an alkaliniser at the time of administration of FDC formulations, the use of sodium bicarbonate during the administration of RIF containing FDC formulations may be advantageous as there will be less decomposition of RIF when it is in insoluble form; and (3) exploitation of formulation factors, by including additives (pH polymers) in the formulation and also (4) segregating the method of delivery of RIF and INH (Silva *et al.*, 2014).

Since one of the proposed problems associated with anti-TB FDC products is the hygroscopic nature of ethambutol hydrochloride, Bhutani and co-workers suggested that film coating of the tablets with water-resistant polymers may be essential in preventing moisture entry in the dosage form (Bhutani *et al.*, 2004b). Whilst film coating will prevent moisture entry, it cannot preclude the non-acid catalysed reaction that is reported take place due to direct contact between the individual drugs.

It was further shown that ethambutol hydrochloride influenced the reaction between rifampicin and isoniazid to a greater extent than pyrazinamide. This was attributed to the reported hygroscopic and acidic nature of ethambutol hydrochloride (Bhutani *et al.*, 2004b; Singh & Mohan, 2003).

Therefore, the main stability concern with anti-TB FDC products is the interaction between isoniazid and rifampicin. It was observed in a recent study that the three/four-combinations containing rifampicin and isoniazid along with pyrazinamide and or ethambutol hydrochloride showed more instability than the two-drug FDCs containing only rifampicin and isoniazid (Bhutani *et al.*, 2004a).

6.6 Compatibility and stability indicating tests

Introduction

In this section, the results obtained during vapour sorption analysis and isothermal microcalorimetry will be discussed. Vapour sorption analysis indicates a material's response to changes in relative humidity. In a typical experiment the relative humidity (%RH) is started at a low level and increase to a high level and then back to a low level, and finally increasing again to a high level of relative humidity (%RH). The shape of the adsorption/desorption profiles, provides information about the API (Hassel & Hesse, 2007). Isothermal microcalorimetry measures the heat change in a test sample as it is kept at a constant temperature in an oil bath. A typical experimental set-up will involves the placement of 100 mg of an API into an ampoule. The power output is recorded over time. Any energetic change in the sample such as degradation, amorphous to crystalline transformation will be recorded as a certain power output (Gaisford, 2016).

6.6.1 Vapour sorption analysis

The vapor sorption analyses were performed utilizing a VTI-SA vapor sorption analyzer (TA Instruments, New Castle, Delaware, USA). The microbalance was calibrated prior to each vapor sorption run with a 100 mg standard weight. The microbalance was set to zero prior to weighing of the sample into the quartz sample container. The sample was carefully placed into the sample holder and care was taken to evenly distribute the sample. The percentage relative humidity (% RH)/temperature program was set using TA Instruments Isotherm software. A drying phase at 40°C was utilized until a weight change of less than 0.0001% occurred, was incorporated into the program. Where possible, the % RH ramp was set from 5 to 95% RH, followed by a decrease in % RH from 95 to 5%. The last absorption phase was set to also ramp from 5 to 95% RH. This humidity ramping setup was not possible in all instances due to sample deliquescence. The temperature was set at a constant 25°C throughout the % RH ramp. The program criteria were set to 0.0001% weight change or 2-minute stability of weight gained or lost before the program would continue to the next parameter.

6.6.1.1 Discussion of results obtained

Rifampicin (RIF)

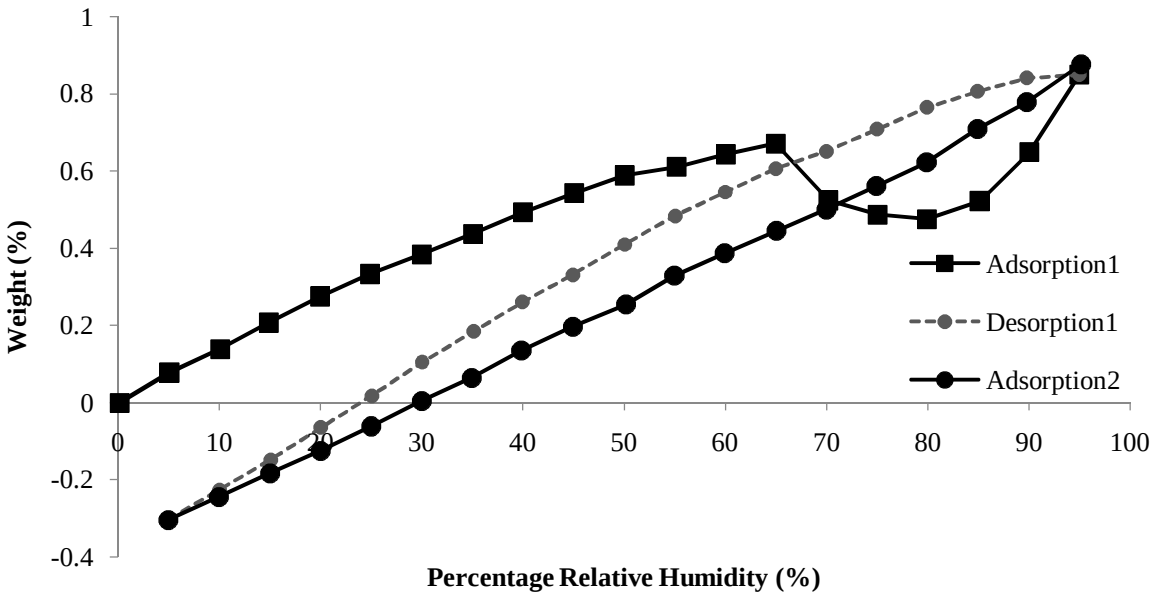

Figure 6.25: Vapour sorption isotherms obtained with RIF raw material.

Figure 6.25 depicts the adsorption, desorption and subsequent adsorption isotherms obtained with rifampicin collected at ambient temperature. During the initial adsorption phase a gradual increase in the sample weight was observed from 0.00% up to 0.67%.

At a relative humidity percentage of 65% upwards a decrease in the sample weight was observed with a value of 0.52% measured at 80% RH. This could signify a phase transformation that possibly occurred and therefore for this particular solid-state form of rifampicin relative humidity of 65 – 80% RH proved to be critical. At 85% RH the sample weight gradually increased as the %RH increased showing that the phase transformation is complete (if a phase transformation occurred).

The subsequent desorption phase showed a gradual weight loss over the range of 95 – 5% RH with no critical RH points observed. It was observed that the sample weight at 5% RH was 0.2% less than the initial starting weight of the sample. The subsequent adsorption phase showed the same isotherm shape as the desorption phase and therefore little hysteresis was identified between the desorption and second adsorption phase, showing the sample to be

stable in terms of desorption and adsorption characteristics. During the second adsorption phase no critical %RH was identified proving that the phase transformation (if any) occurred only during the initial adsorption phase.

Isoniazid (INH)

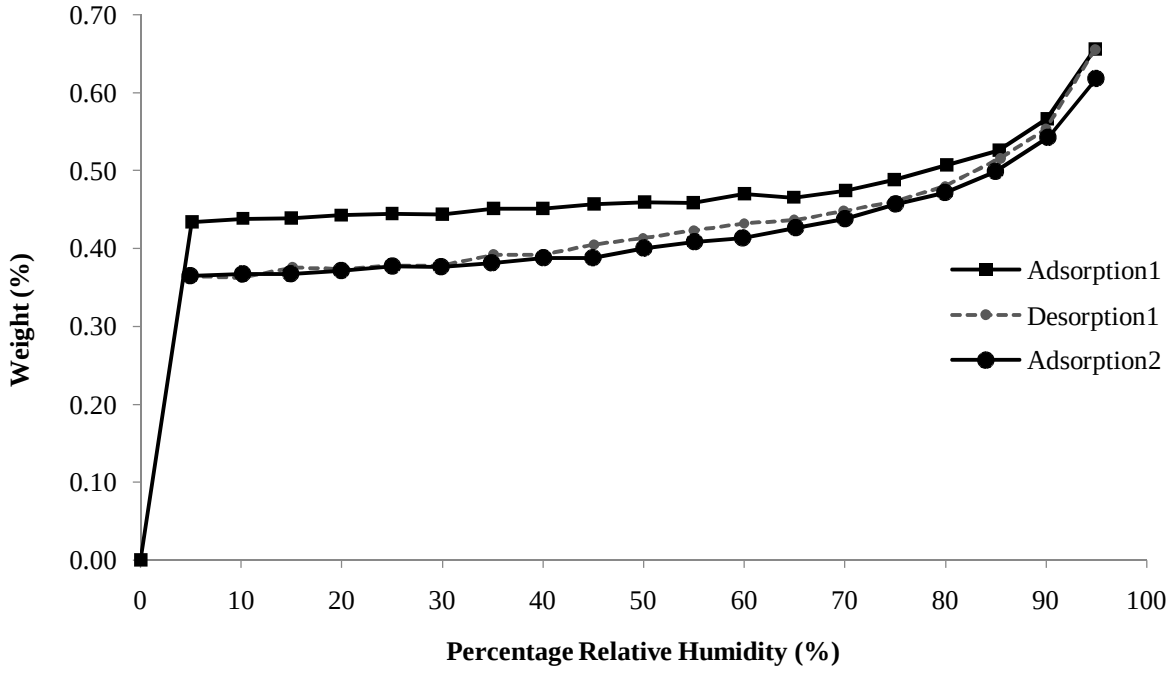

Figure 6.26: Vapour sorption isotherms obtained for INH raw material at ambient temperature.

The vapour sorption isotherms obtained with INH raw material showed the drug to remain stable during the up-ramping and de-ramping of the %RH. An initial moisture adsorption of 0.4% was obtained followed by the sample weight remaining stable up to a %RH of 75%. Thereafter a slight increase was observed up to a total sample weight of 0.66%. The subsequent desorption and adsorption phases did not show any significant change in the isotherms and therefore it is concluded that the sample remained stable during the complete up-, down- and up-ramping of the %RH (Figure 6.26).

Pyrazinamide (PZA)

The moisture sorption isotherms of commercially obtained PZA showed the sample weight to remain stable up to a %RH of 70%. A further increase in the %RH resulted in a weight gain of 0.4%. Considering this, a %RH point of 70% is deemed critical for PZA, although, from the subsequent desorption and adsorption steps no deviation was observed in terms of the shape of the isotherms. This is considered indicative that the sample remained physically stable during the moisture sorption analysis (Figure 6.27).

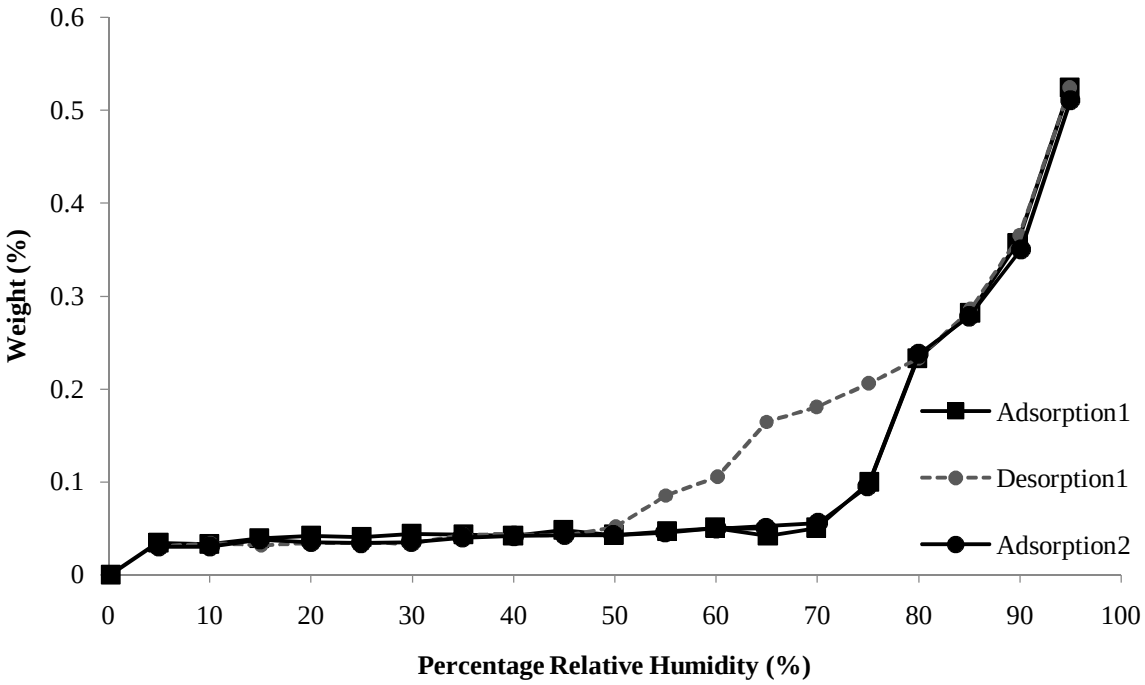

Figure 6.27: Vapour sorption isotherms obtained for PZA raw material at ambient temperature.

Ethambutol hydrochloride (EMB)

In terms of EMB, interesting results were obtained. Initially the commercially obtained EMB sample was analysed with a drying phase of 40°C. Figure 6.28 depicts the adsorption isotherm obtained with EMB. The sample weight remained stable up to a %RH of 75% followed by a sharp increase in weight of 90%. Upon inspection of the sample, it was found that the sample was completely deliquesced. Subsequently, no desorption and second absorption phase could follow.

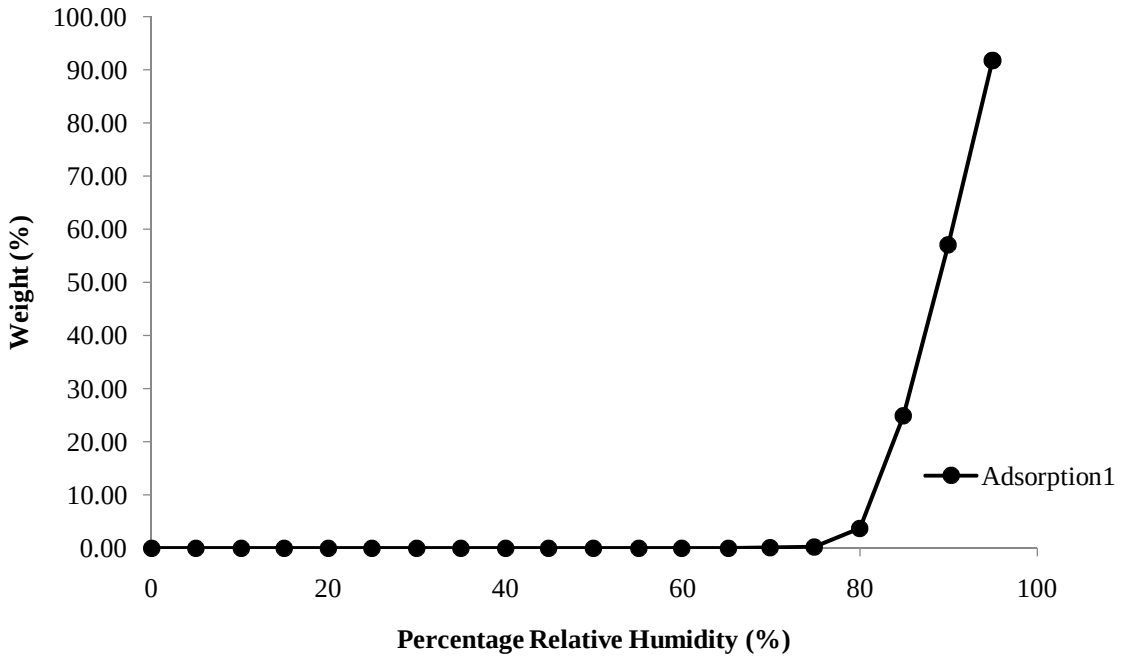

Figure 6.28: Vapour sorption isotherms obtained for EMB raw material at ambient temperature with a drying phase of 40°C.

Subsequently the drying phase temperature was changed to 80°C and thereafter 60°C. In both instances the same absorption isotherm was obtained (Figure 6.29). Furthermore, it was decided that the %RH will only be set to a maximum of 80%RH because the rationale was to investigate if deliquescence is a function of the amount of possible surface moisture or a characteristic of ethambutol. The same sharp increase in the sample weight was observed at 75%RH. Upon inspection of the sample after the initial adsorption phase the sample showed some degree of deliquescence and due to the fact that the chamber temperature of the instrument was set at 25°C it was not possible to attain desorption of the sample. Since the %RH program was only ramped to 80%RH and not 95% , the extent of deliquescence was not as extreme, however the fact remains that irrespective of the initial drying temperature deliquescence remains a characteristic of EMB.

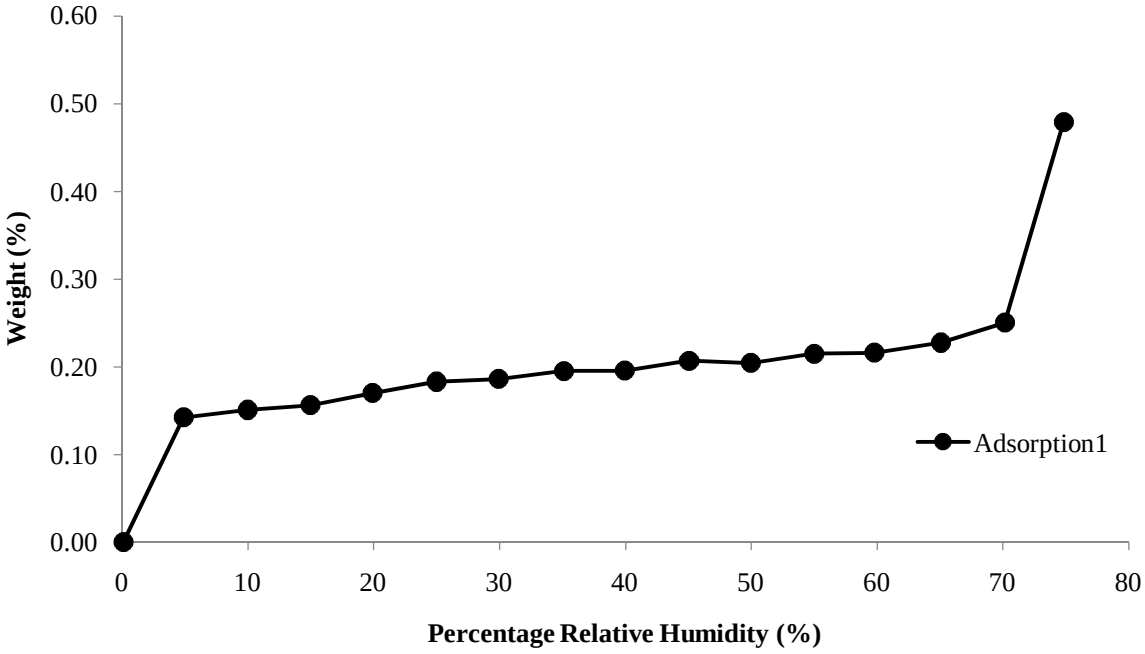

Figure 6.29: Vapour sorption isotherms obtained for EMB raw material at ambient temperature with a drying phase of 80°C.

6.6.2 Microcalorimetry

Compatibility testing between rifampicin, isoniazid, pyrazinamide and ethambutol hydrochloride at 40°C

A 2277 Thermal Activity Monitor (TAMIII) (TA Instruments, USA) equipped with an oil bath with a stability of ±100 μK over 24 hours was used during this study. The temperature of the calorimeters was maintained at 40°C. For compatibility studies the heat flow is measured for the single components as well as the mixtures. The observed calorimetric outputs observed for the individual samples are summed to give a theoretical response. This calculated hypothetical response represents a calorimetric output that would be expected if the two materials do not interact with each other. If the materials interact the measured calorimetric response will differ from the calculated theoretical response.

Combinations consisting of RIF, INH, PZA and EMB were tested in terms of compound compatibility. The combinations were made according to concentrations included in a commercially available fixed dose combination (FDC) tablet.

The mixtures were as follows:

No moisture (40°C):

1. Single-drug compounds (each in triplicate)

    - RIF
    - INH
    - PZA
    - ETH

2. Two-drug combinations (each in triplicate)

    - RIF/INH
    - RIF/PZA
    - RIF/ETH
    - INH/PZA
    - INH/ETH
    - PZA/ETH

3. Three-drug combinations (each in triplicate)

    - RIF/INH/PZA
    - RIF/INH/ETH
    - RIF/PZA/ETH
    - INH/PZA/ETH

4. Four drug combination (in triplicate)

    - RIF/INH/PZA/ETH

Moisture (40°C & 75% RH):

5. Single-drug compounds (each in triplicate)

    - RIF
    - INH
    - PZA
    - ETH

6. Two-drug combinations (each in triplicate)

    - RIF/INH
    - RIF/PZA
    - RIF/ETH
    - INH/PZA
    - INH/ETH
    - PZA/ETH

7. Three-drug combinations (each in triplicate)

    - RIF/INH/PZA
    - RIF/INH/ETH
    - RIF/PZA/ETH
    - INH/PZA/ETH

8. Four drug combination (in triplicate)

    - RIF/INH/PZA/ETH

6.6.2.1 Compatibility results of isoniazid, rifampicin, pyrazinamide and ethambutol

Isoniazid (INH) and rifampicin (RIF) without percentage relative humidity (%RH)

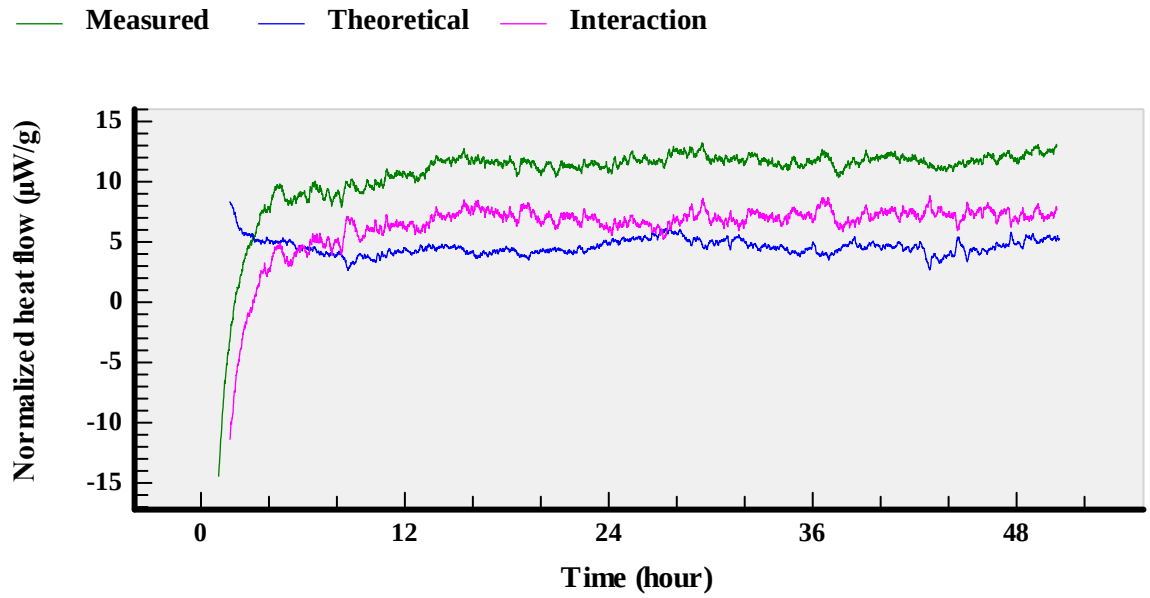

Figure 6.30: INH and RIF without percentage relative humidity (%RH).

No incompatibility was identified. An interaction average heat flow of 6.367 µW/g and interaction error of 6.740 µW/g was measured.

Isoniazid (INH) and rifampicin (RIF) with relative humidity (RH)

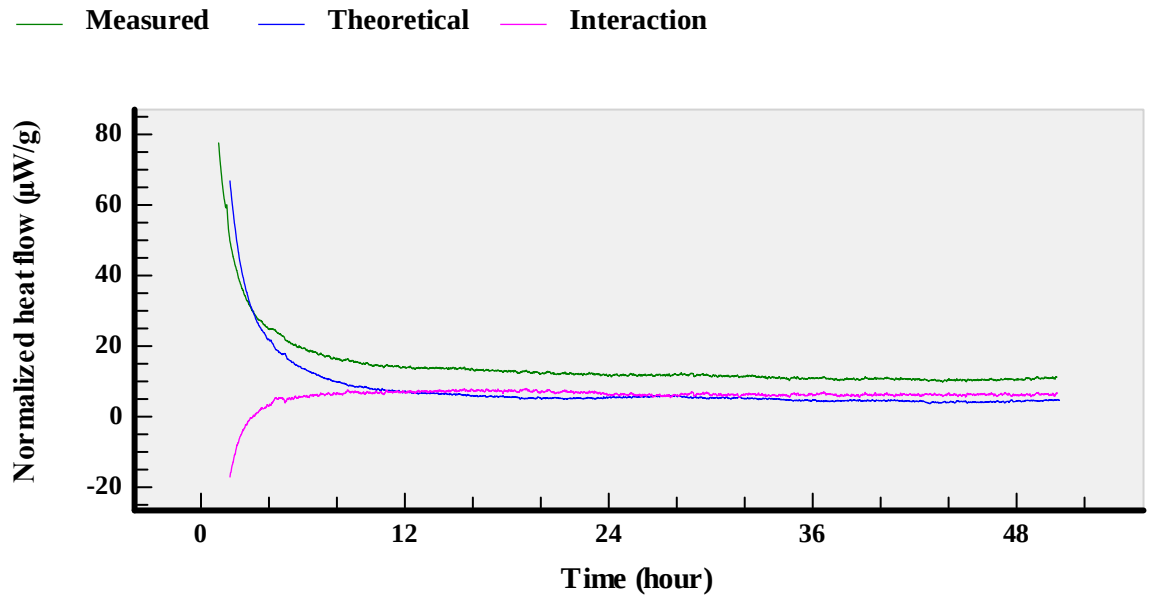

Figure 6.31: INH and RIF with RH.

No incompatibility was measured. Interaction average heat flow: 6.024 µW/g and interaction error: 6.472 µW/g.

Isoniazid (INH) and ethambutol (EMB) without RH

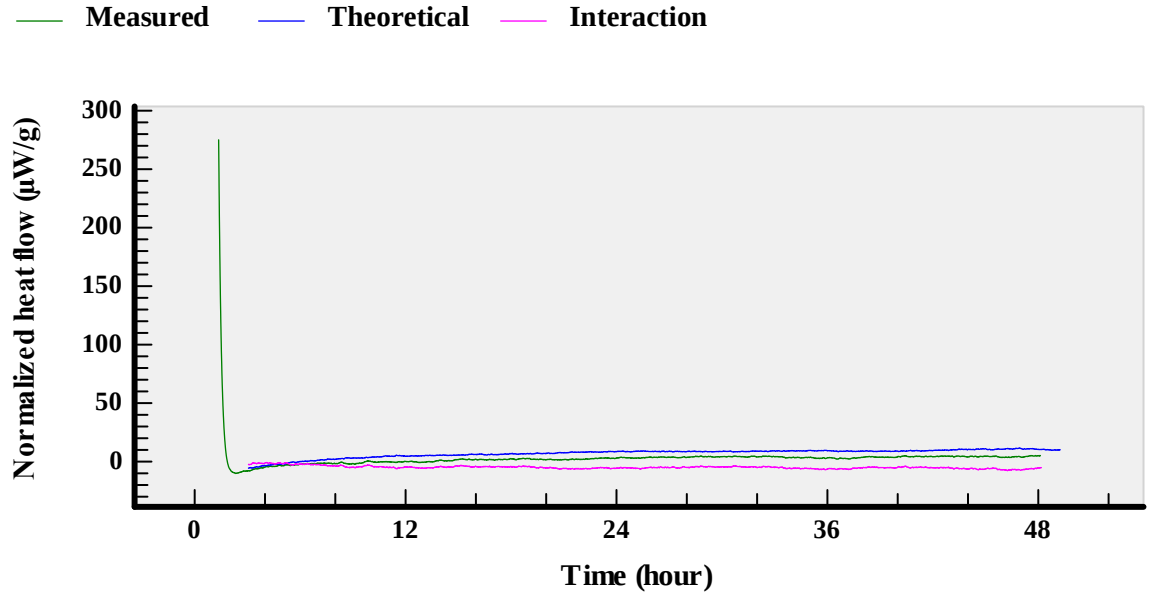

Figure 6.32: INH and EMB without RH.

No incompatibility was identified. Interaction average heat flow: -4.837 µW/g and interaction error: 4.998 µW/g.

Isoniazid (INH) and ethambutol (EMB) with RH

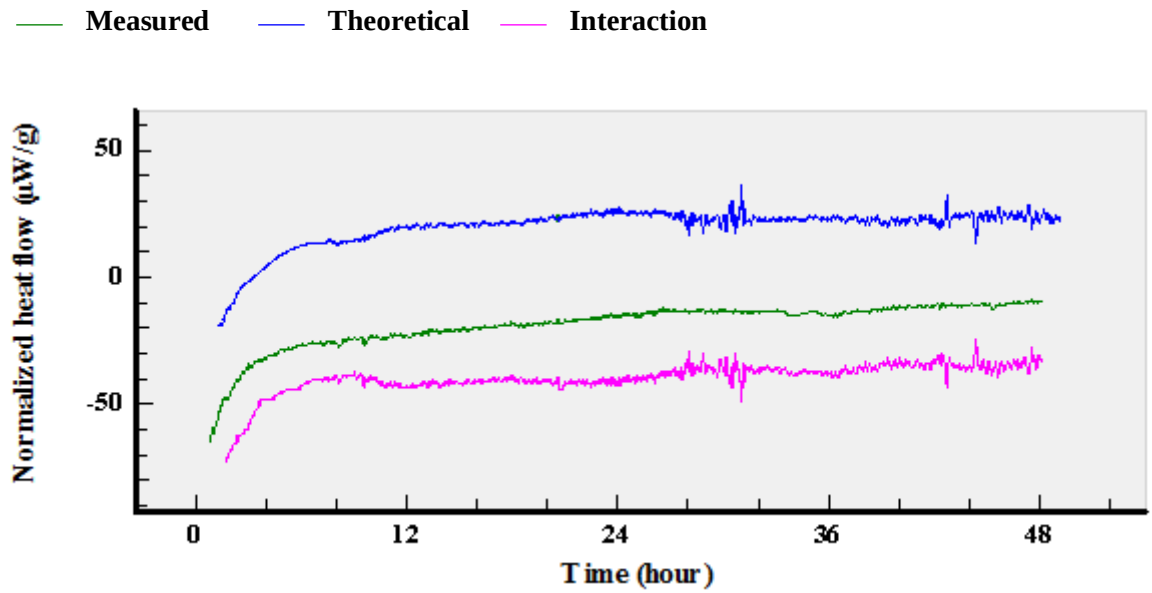

Figure 6.33: INH and EMB with RH.

Despite some baseline interference / noise no incompatibility was measured. The interaction average heat flow was calculated to -38.135 µW/g being a 38.292 µW/g difference between the measured and theoretically calculated heat flow.

Isoniazid (INH) and pyrazinamide (PZA) without RH

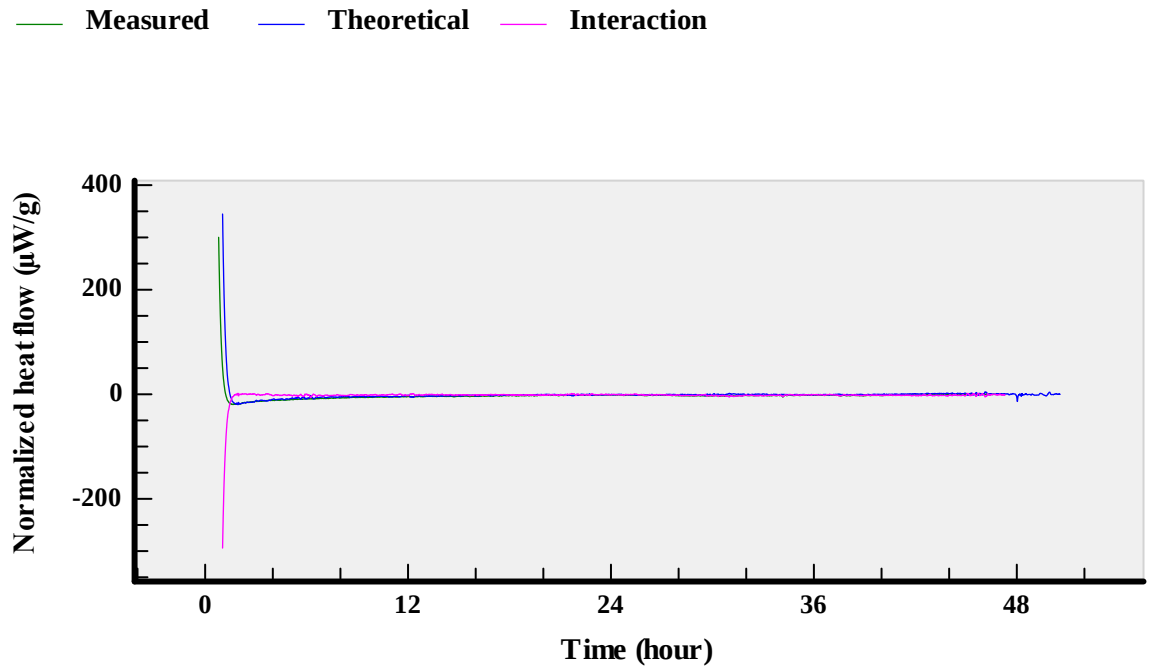

Figure 6.34: INH and PZA without RH.

No incompatibility was measured. Interaction average heat flow: -2.419 µW/g and interaction error: 2.50 µW/g.

Isoniazid and pyrazinamide with RH

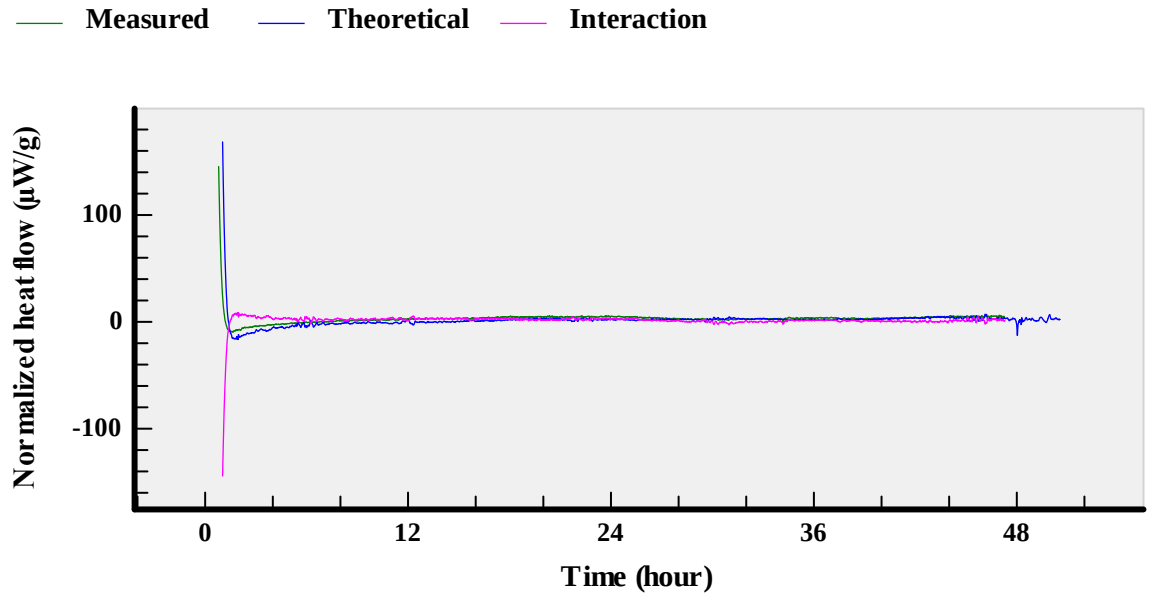

Figure 6.35: INH and PZA with RH.

No incompatibility was identified. An interaction average heat flow: 1.45 µW/g was measured, and the interaction error was calculated to: 6.50 µW/g.

Isoniazid, pyrazinamide and ethambutol without RH

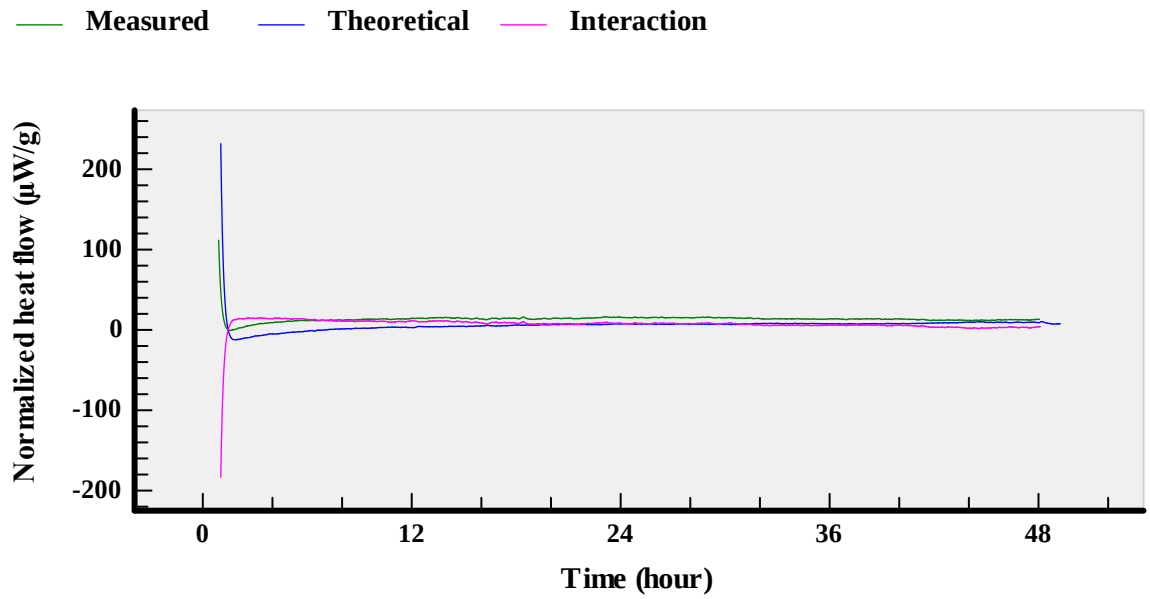

Figure 6.36: INH, PZA and EMB without RH.

No incompatibility was identified. Interaction average heat flow: 7.45 µW/g interaction error: 11.17 µW/g.

Isoniazid, pyrazinamide and ethambutol with RH

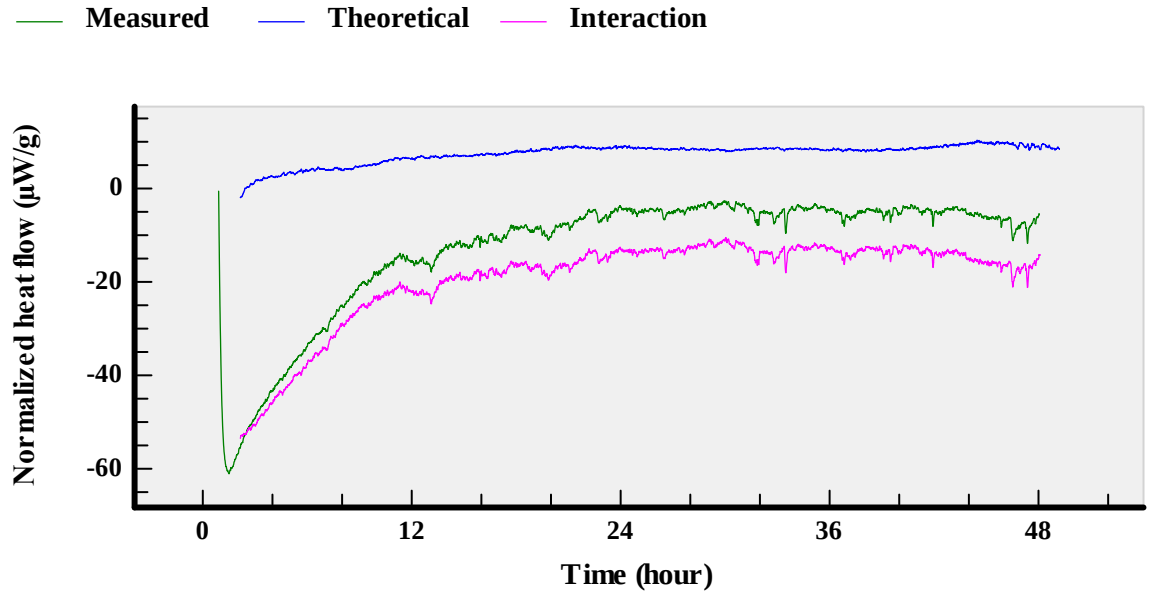

Figure 6.37: INH, PZA and EMB with RH.

Despite baseline noise no incompatibility was measured. Baseline noise can possibly be ascribed to the deliquescence of the ethambutol? Interaction average heat flow: -19.22 µW/g interaction error: 21.47 µW/g.

Isoniazid, pyrazinamide, rifampicin and ethambutol without RH

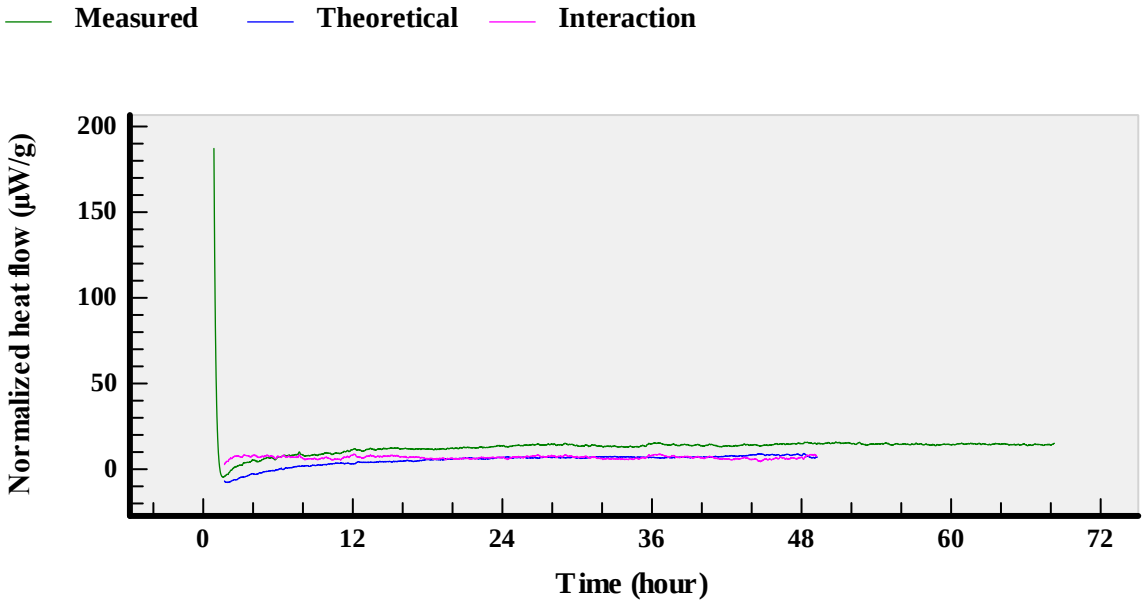

Figure 6.38: INH, PZA, RIF and EMB without RH.

No incompatibility was measured. Interaction average heat flow: 6.803 µW/g interaction error: 6.851 µW/g.

Isoniazid, pyrazinamide, rifampicin and ethambutol with RH

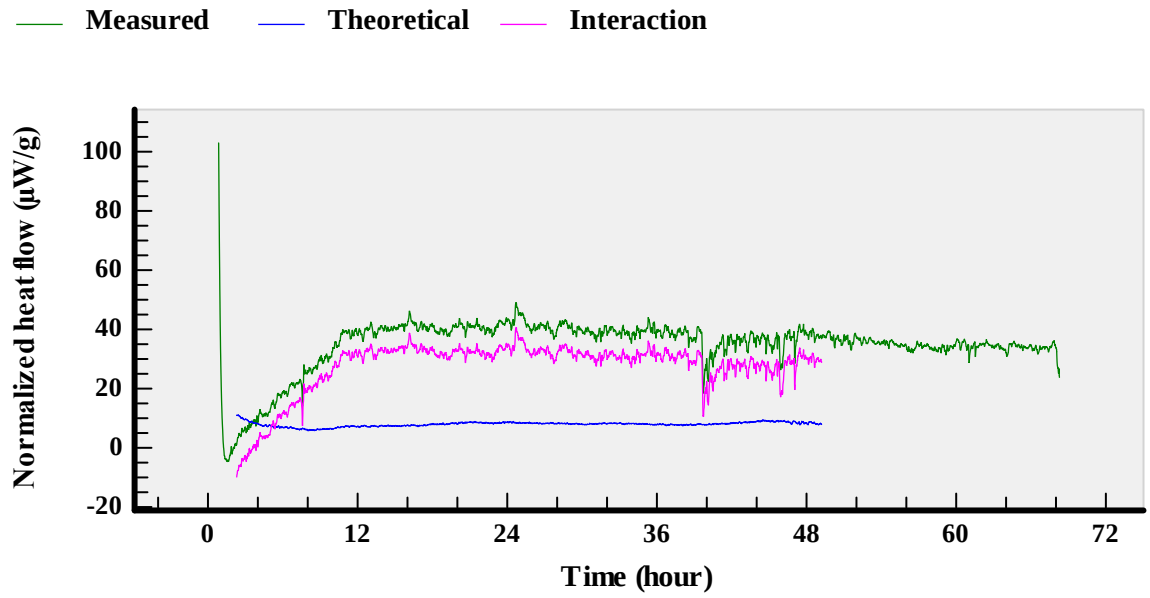

Figure 6.39: INH, PZA, RIF and EMB with RH.

Some baseline noise was detected but no incompatibility was measured. Interaction average heat flow: 27.51 µW/g interaction error: 28.922 µW/g.

Pyrazinamide and ethambutol without RH

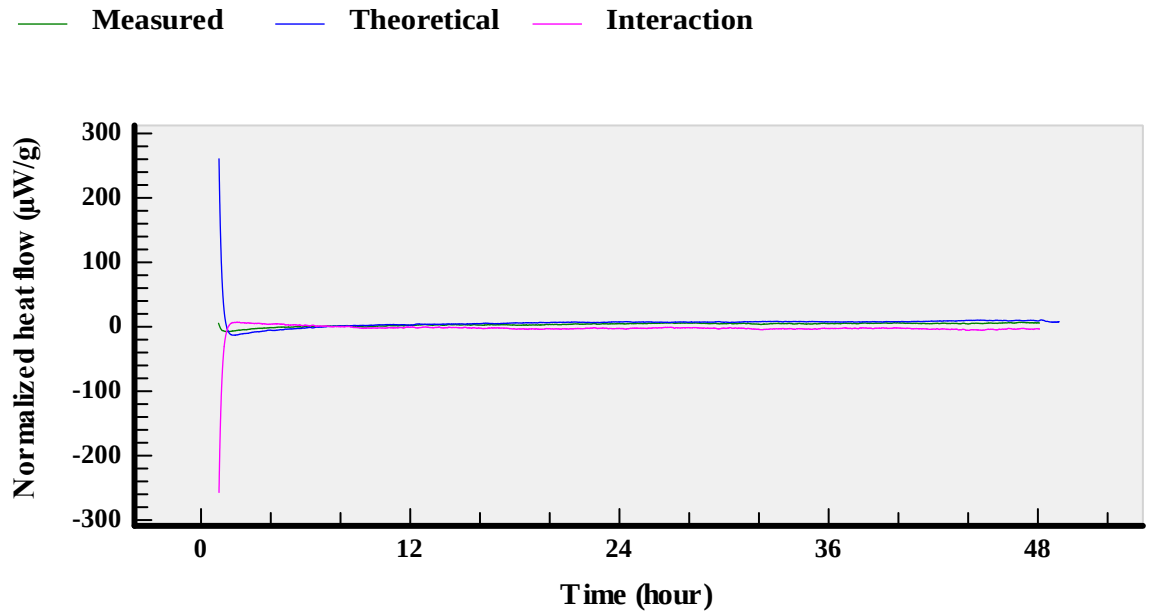

Figure 6.40: PZA and EMB without RH.

No incompatibility was measured. Interaction average heat flow: -2.479 µW/g interaction error: 1.632 µW/g.

Pyrazinamide and ethambutol with RH

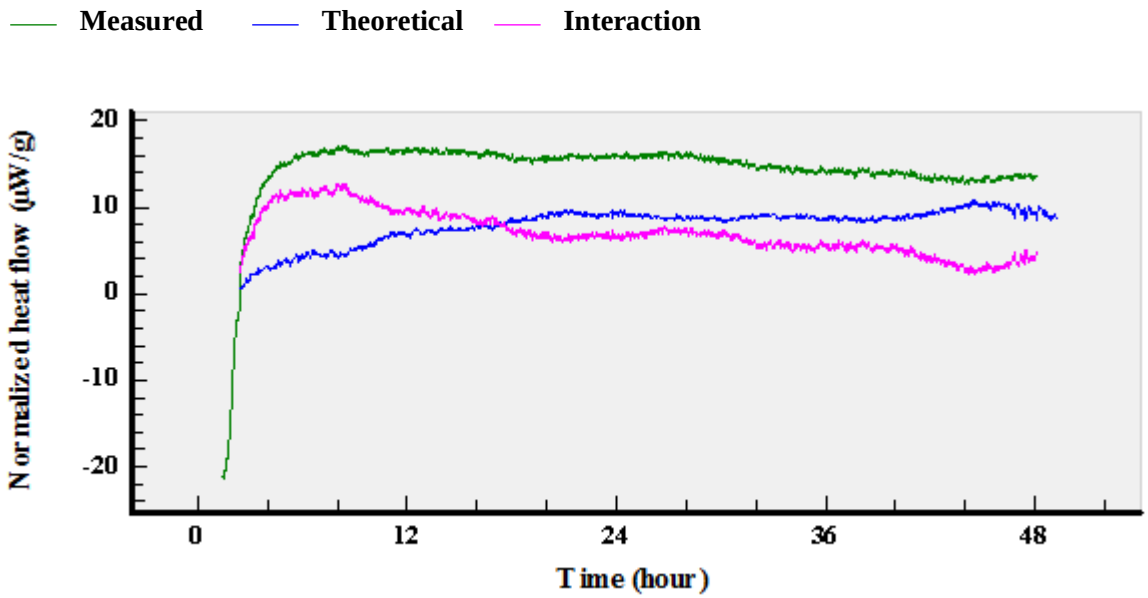

Figure 6.41: PZA and EMB with RH.

No incompatibility was measured. Interaction average heat flow: 7.040 µW/g interaction error: 7.467 µW/g.

Pyrazinamide and rifampicin without RH

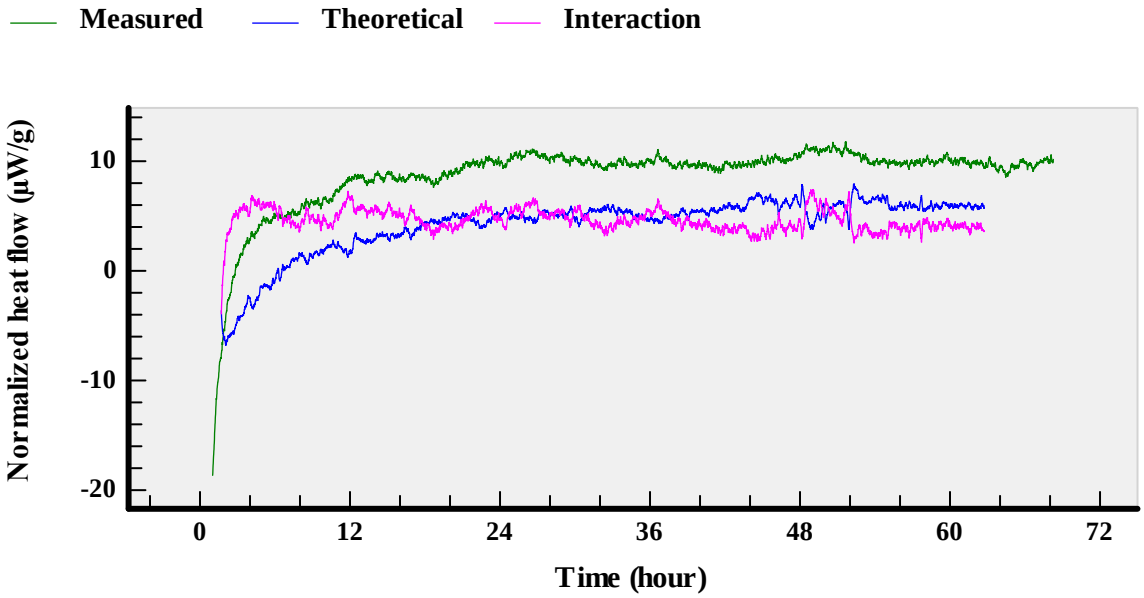

Figure 6.42: PZA and RIF without RH.

No incompatibility occurred. Interaction average heat flow: 4.64 µW/g interaction error: 4.74 µW/g.

Pyrazinamide and rifampicin with RH

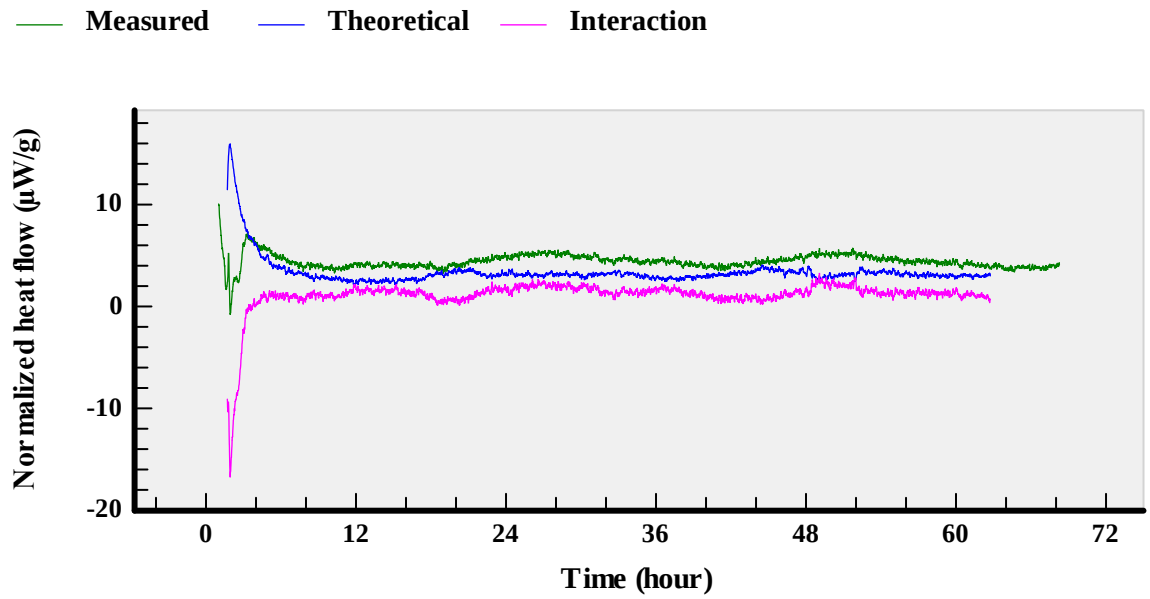

Figure 6.43: PZA and RIF with RH.

No incompatibility was measured. Interaction average heat flow: 1.09 µW/g interaction error: 2.04 µW/g.

Rifampicin and ethambutol without RH

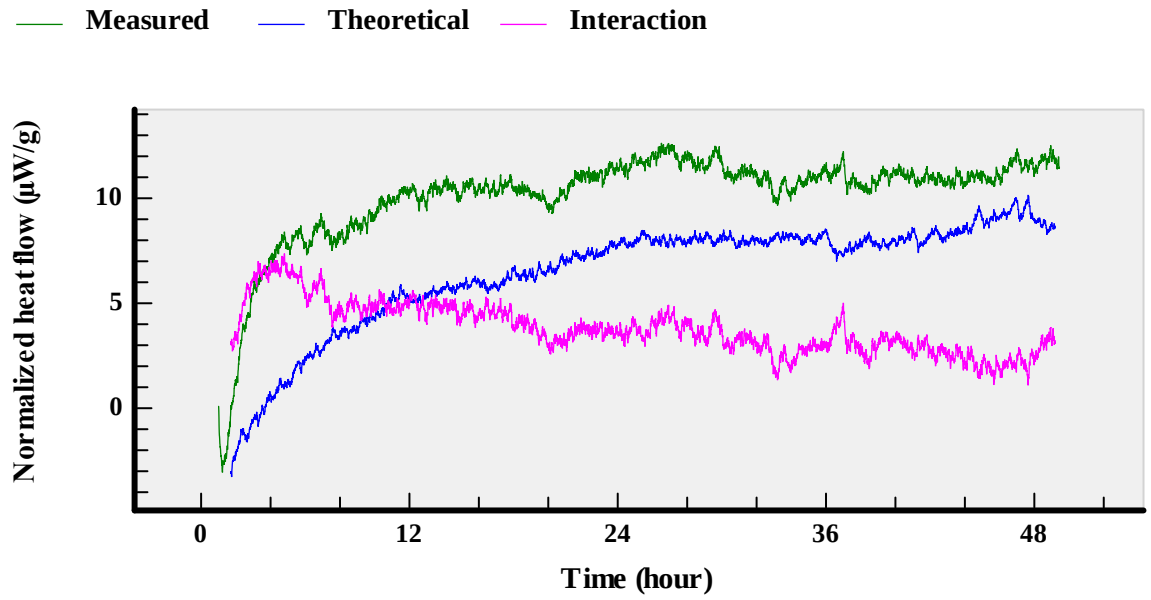

Figure 6.44: RIF and EMB without RH.

No incompatibility was measured. Interaction average heat flow: 3.813 µW/g interaction error: 3.999 µW/g.

Rifampicin and ethambutol with RH

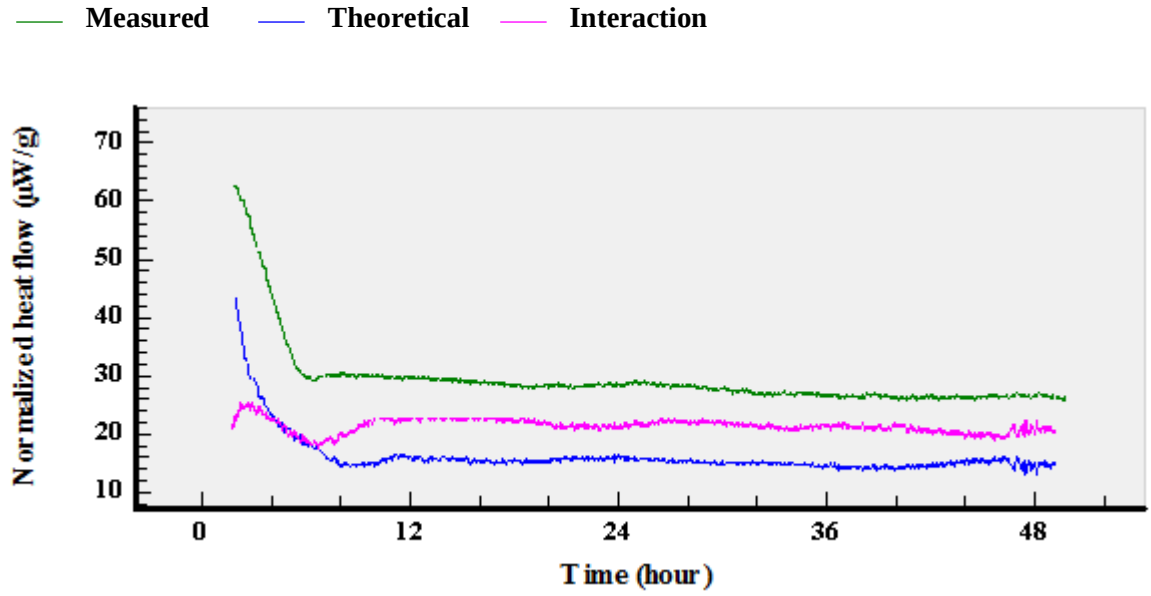

Figure 6.45: RIF and EMB with RH.

No incompatibility was identified. Interaction average heat flow: 21.49 µW/g interaction error: 21.52 µW/g.

Rifampicin, isoniazid and ethambutol without RH

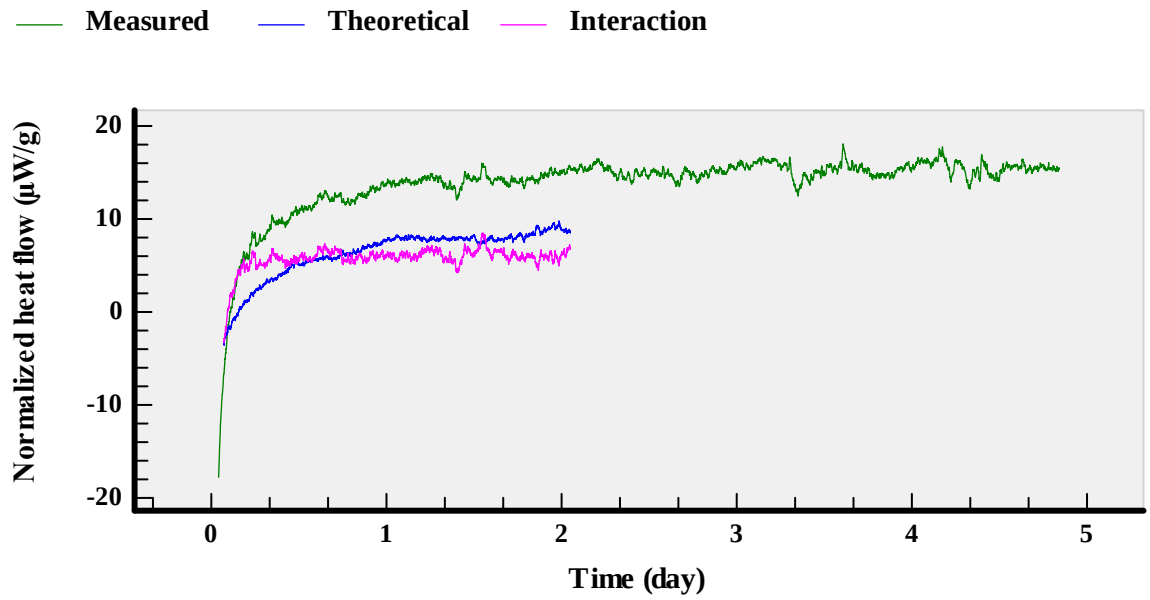

Figure 6.46: RIF, INH and EMB without RH.

No incompatibility was identified. Interaction average heat flow: 5.78 µW/g interaction error: 5.91 µW/g.

Rifampicin, isoniazid and ethambutol with RH

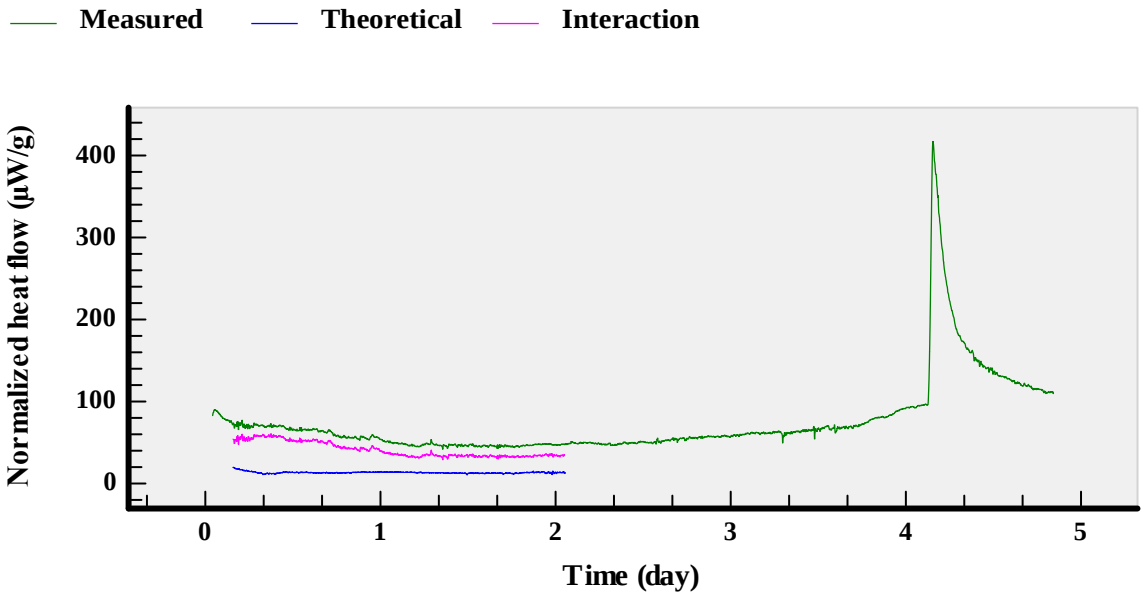

Figure 6.47: RIF, INH and EMB with RH.

No incompatibility was identified in the data that correlated up to the 2 day time period. The signals that were obtained by running the experiment longer showed an increase in heat flow in the time period of 4 days. Interaction average heat flow: 41.32 µW/g interaction error: 42.33 µW/g.

Rifampicin, isoniazid and pyrazinamide without RH

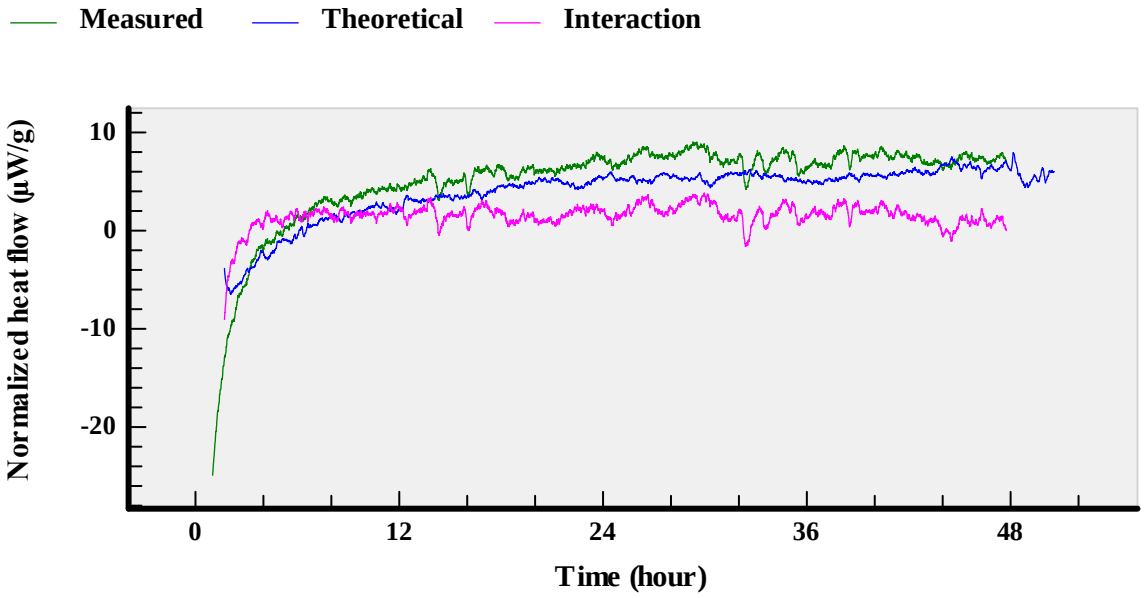

Figure 6.48: RIF, INH and PZA without RH.

No incompatibility was identified. Interaction average heat flow: 1.52 µW/g interaction error: 1.91 µW/g.

Rifampicin, isoniazid and pyrazinamide with RH

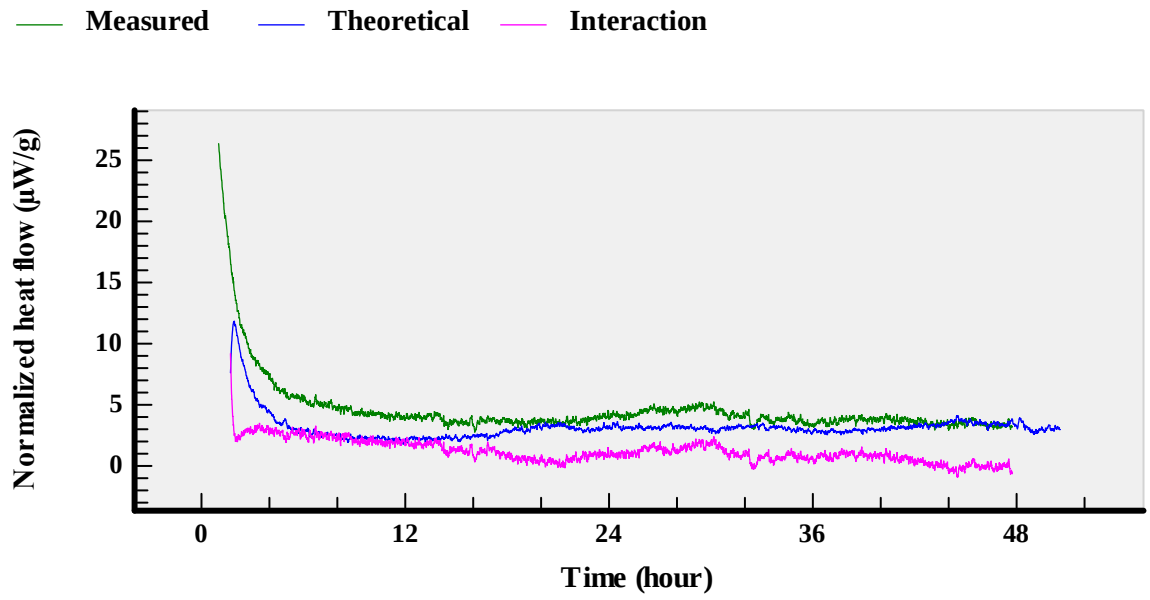

Figure 6.49: RIF, INH and PZA with RH.

No incompatibility was measured. Interaction average heat flow: 1.202 µW/g interaction error: 1.504 µW/g.

Rifampicin, pyrazinamide and ethambutol without RH

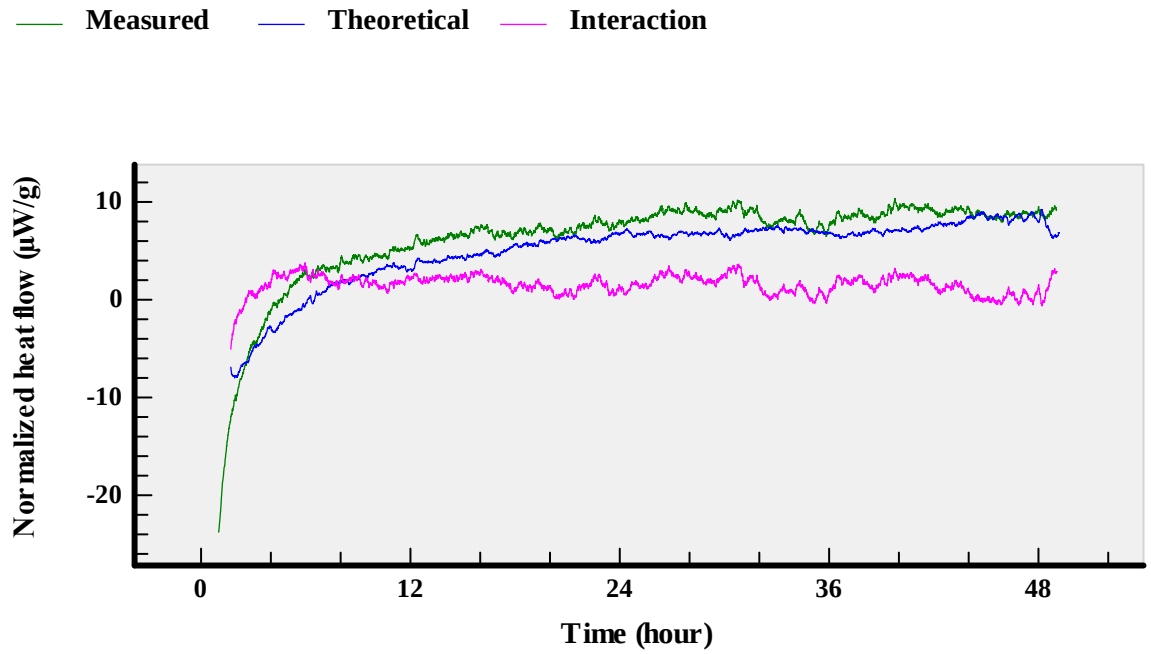

Figure 6.50: RIF, INH and PZA without RH.

No incompatibility was measured. Interaction average heat flow: 1.535 µW/g interaction error: 1.822 µW/g.

Rifampicin, pyrazinamide and ethambutol with RH

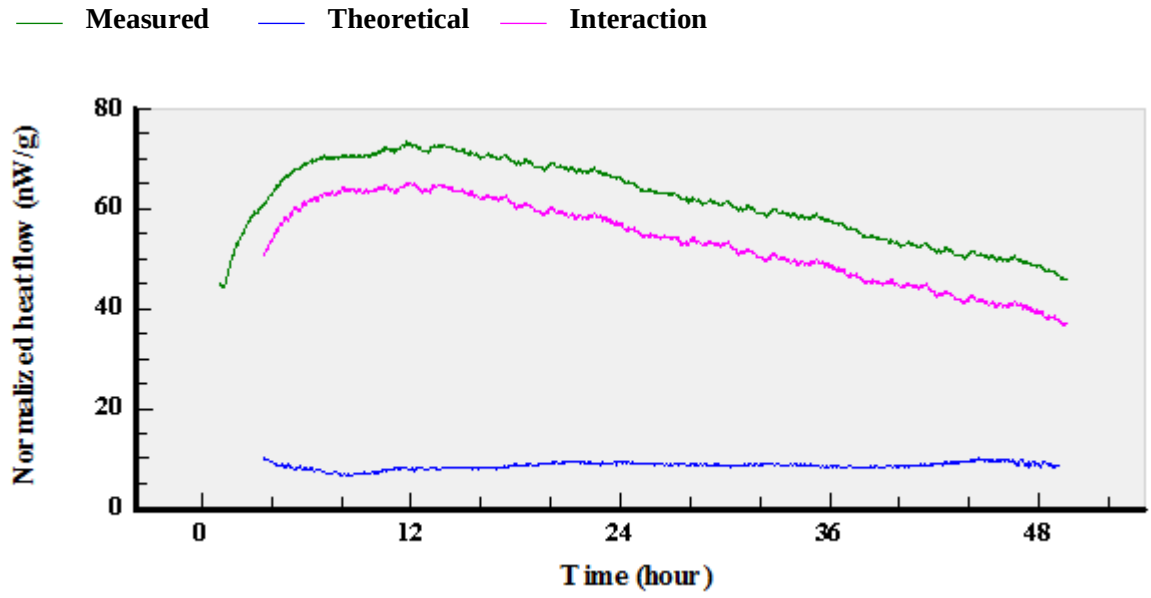

Figure 6.51: RIF, INH and PZA with RH.

No incompatibility was measured. Despite the slope that was measured the heat flow is in the nW/g range and therefore it cannot be identified as an incompatibility. Interaction average heat flow: 55.33 nW/g interaction error: 55.73 nW/g.

6.6.2.2 Compatibility testing rifampicin, isoniazid, pyrazinamide, ethambutol hydrochloride and tablet excipients at 50°C

The previous isothermal microcalorimetric studies at 40°C, with and without humidity (RH 75%), showed no incompatibility between rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol HCl (EMB). The purpose of this part of the compatibility study was to explore any interactions at an increased temperature of 50°C and to investigate the possibility of incompatibilities between the drugs and the tablet excipients used in the most commonly prescribed commercial four-drug TB FDC.

Combinations consisting of RIF, INH, PZA, EMB and several tablet excipients were tested in terms of compound compatibility. The combinations were made according to concentrations included in a commercially available fixed dose combination (FDC) tablet.

Analysis of the four APIs

The first tested set consisted of RIF, INH, EMB and PZA combined in the weight ratio of the commercial tablet formulation. The heat flow data of each individual active ingredient was measured to obtain reference heat flow curves for each of them. The obtained heat flow results obtained for the tablet mixture containing only the four active ingredients are provided below (Figure 6.52).

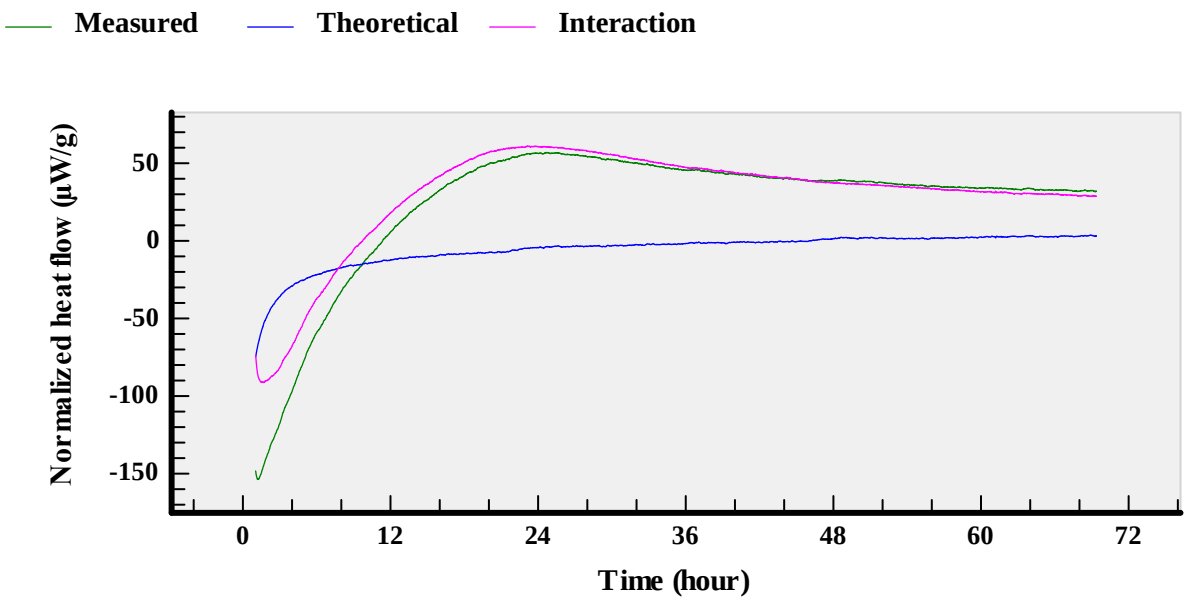

Figure 6.52: Heat flow graph obtained with the combination of RIF, INH, PZA and EMB.

The average interaction heat flow obtained during this run was calculated to be: 29.8 µW/g with an interaction error of 44.8 µW/g. This is indicative of an interaction occurring between the different active ingredients that forms the active ingredient component of the FDC tablets.

Analysis of the tablet mixture

Next the tablet mixture was tested in relation to the different excipients that are included in the tablet formulation. The following excipients were tested: Ac-Di-Sol = 10.5mg, PVP = 7mg, ascorbic acid = 3.5mg, starch = 113.31mg, lactose = 210.44mg, sodium lauryl sulphate = 3.5mg and magnesium stearate = 5.25mg. Figure 6.53 depicts the heat flow graph obtained during this experiment. No incompatibilities between the excipients included in the tablet formulation were observed. The average heat flow was calculated to be: 42.07 µW/g and the interaction error: 43.17 µW/g. The closeness of the average heat flow and that of the interaction error indicated that no incompatibility exists between the different excipients.

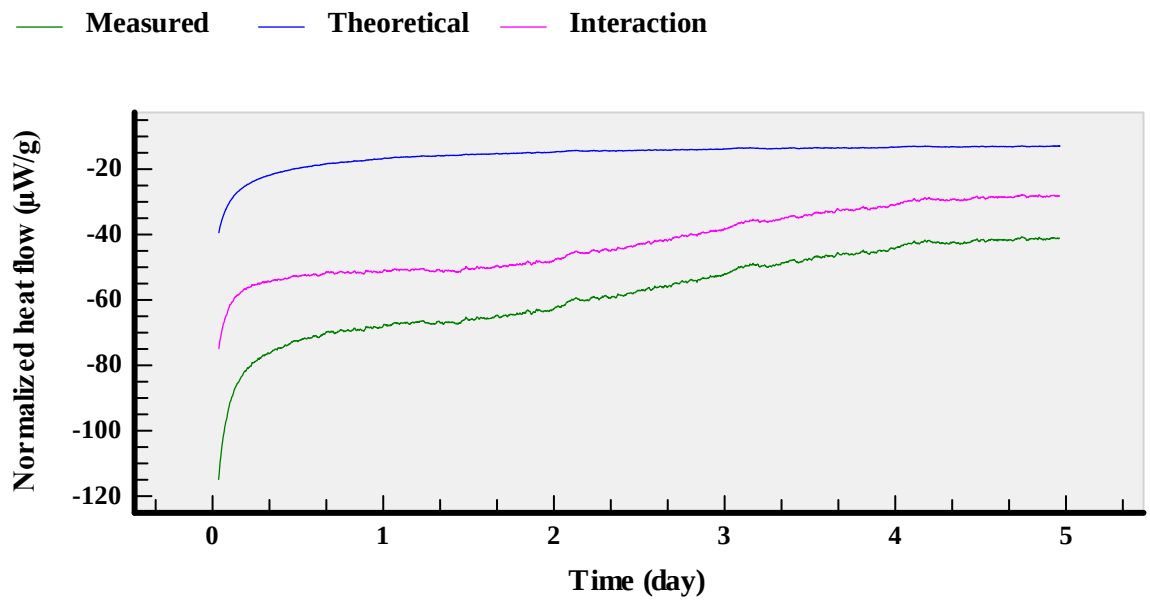

Figure 6.53: Heat flow graph obtained during compatibility testing of ascorbic acid, starch, lactose, sodium lauryl sulphate and magnesium stearate.

Since an incompatibility was identified between the four drugs but not between the different excipients, it was important to clarify if any of the drugs alone but in combination with the excipients would result in an incompatibility.

Analysis of the excipient mixture and single APIs

Rifampicin and excipient mixture

The next step was therefore to combine the excipient mixture with only one drug each time. Figure 6.54 depicts the heat flow data obtained with rifampicin combined with the excipient mixture. The heat flow was measured to be 3.43 µW/g and interaction error 5.91 µW/g. The closeness of the theoretical calculated and actual measured heat flow values is indicative that no incompatibility exists between rifampicin and the excipient mixture.

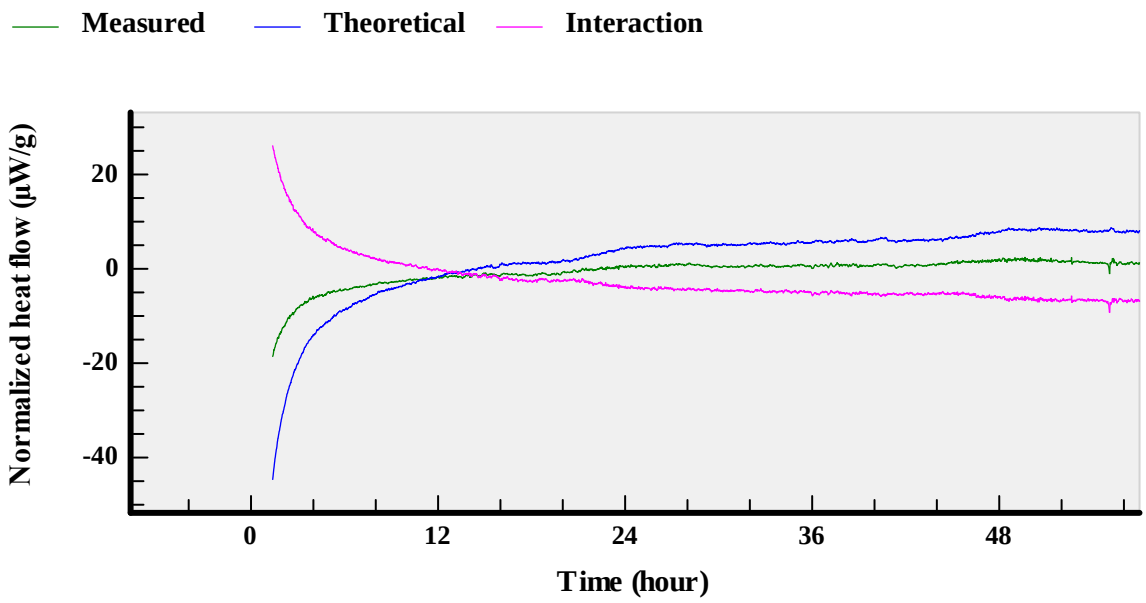

Figure 6.54: Heat flow graph of the tablet excipient mixture combined with RIF.

Isoniazid and excipient mixture

The next combination that was tested was that of INH with the excipient mixture. The average heat flow was measured to be 4.20 µW/g and interaction error 7.79 µW/g (Figure 6.55). Although a difference between the measured and theoretically calculated heat flow was observed, it was still considered a too small of a difference to signify an incompatibility between INH and the excipient mixture.

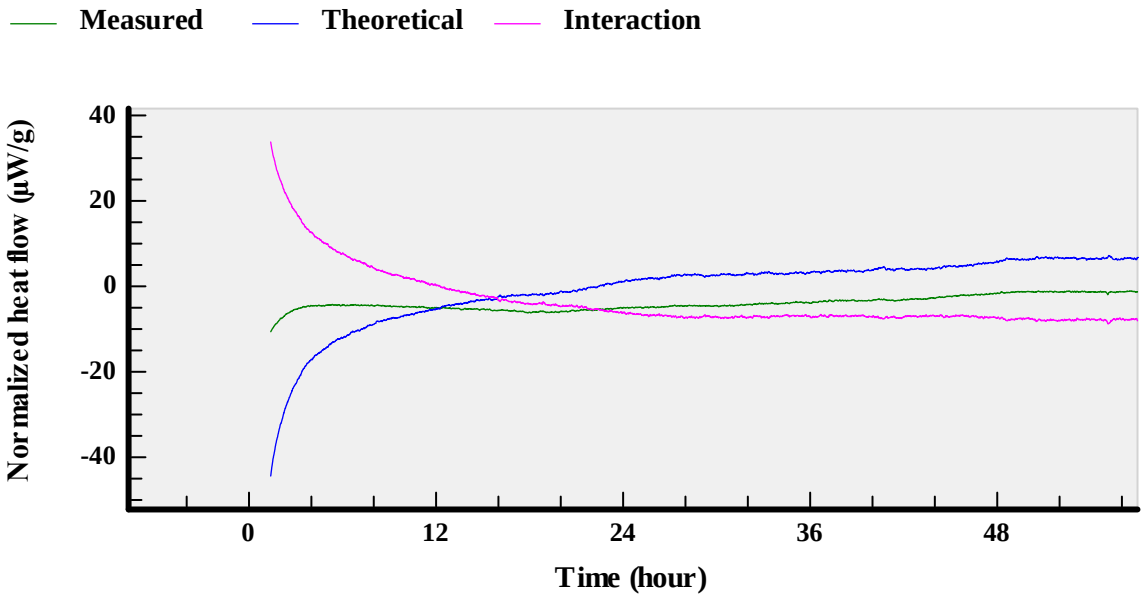

Figure 6.55: Heat flow graph of excipient mixture combined with INH.

Pyrazinamide and excipient mixture

Subsequently, the excipient mixture was tested for compatibility with pyrazinamide. The average heat flow was calculated to 5.24 µW/g and the interaction error 6.38 µW/g (Figure 6.56). Once again, the small difference is indicative of compatibility between PZA and the excipient mixture.

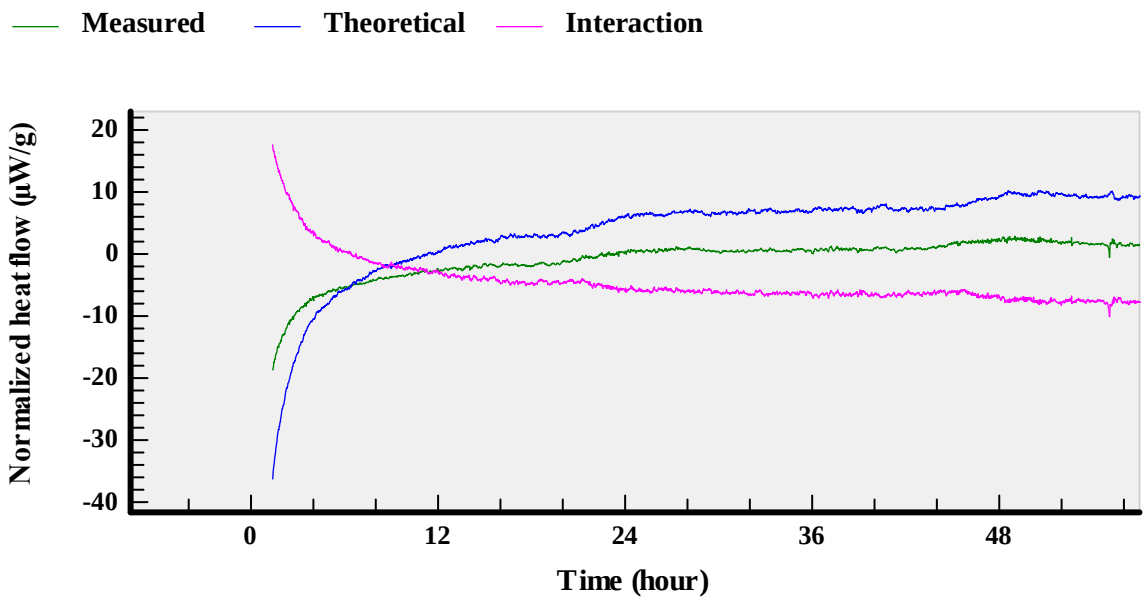

Figure 6.56: Heat flow graph obtained with the mixture of the tablet excipients with PZA.

Ethambutol hydrochloride and excipient mixture

The heat flow was also determined for EMB combined with the tablet excipient mixture. The interaction heat flow was calculated to be 5.20 µW/g and the interaction error 6.61 µW/g (Figure 6.57). From the data it became apparent that EMB is also compatible with the tablet excipient mixture.

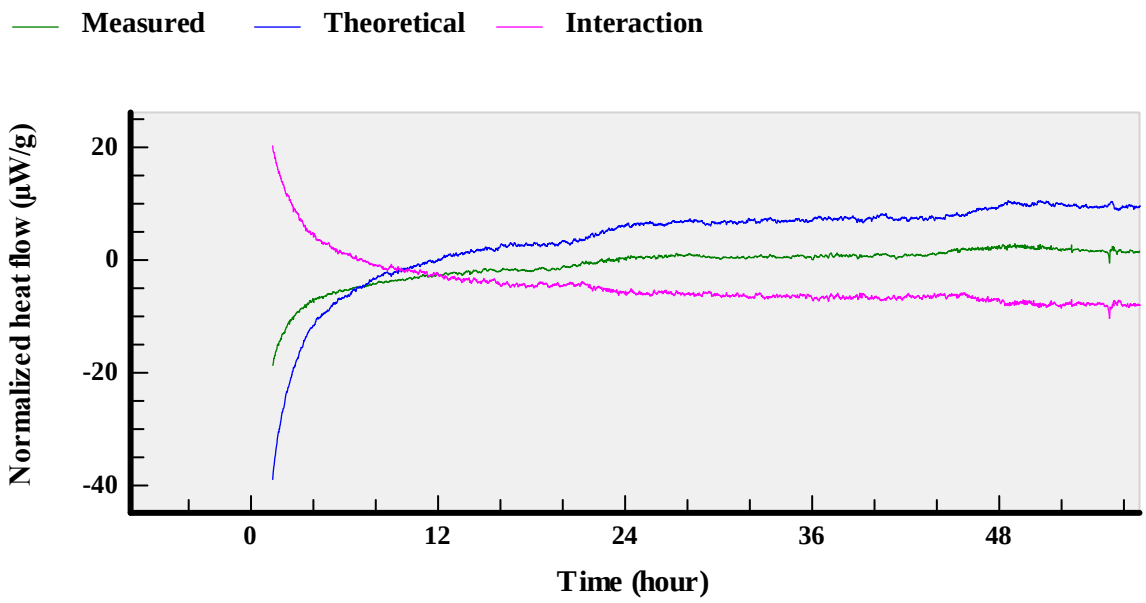

Figure 6.57: Heat flow graph obtained with a combination of the tablet excipients with EMB.

Conclusion:

It can therefore be concluded from the microcalorimetry experiments that an incompatibility exists between the four drugs but that no incompatibility exists between the different excipients and also not between the different excipients and each drug individually.

Incompatibility testing between INH, PZA and EMB

In order to clarify which drug(s) is/are responsible for the incompatibility, mixtures were tested by removing only one drug at a time. The first mixture consisted of INH, PZA and EMB. The heat flow graph obtained is depicted in Figure 6.58. An average interaction heat flow of 14.26 µW/g with an interaction error of 16.20 µW/g was obtained. From the obtained data it became apparent that no incompatibility exists between INH, PZA and EMB.

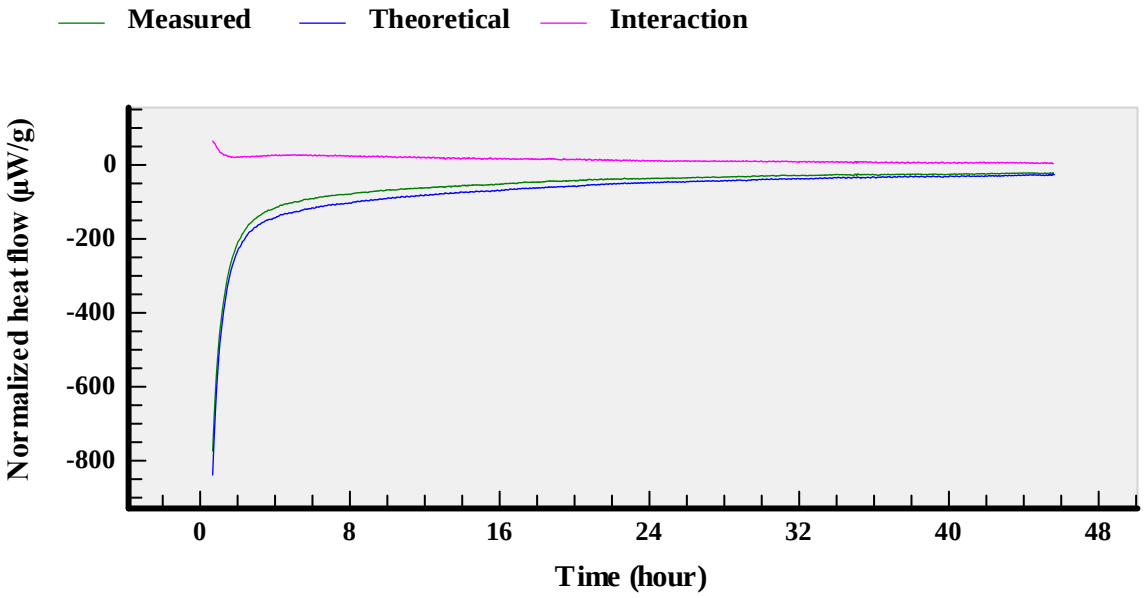

Figure 6.58: Heat flow graph obtained with a combination of INH, PZA and EMB.

Incompatibility testing between INH and EMB

During the same experiment the sample in which PZA was excluded from the mixture was also tested and the results showed that no incompatibility exist between INH and EMB. The average heat flow was determined to be 9.78 µW/g and the interaction error was found to be 10.36 µW/g. This is depicted in Figure 6.59 which doesn't show any slope deviation in the measured heat flow.

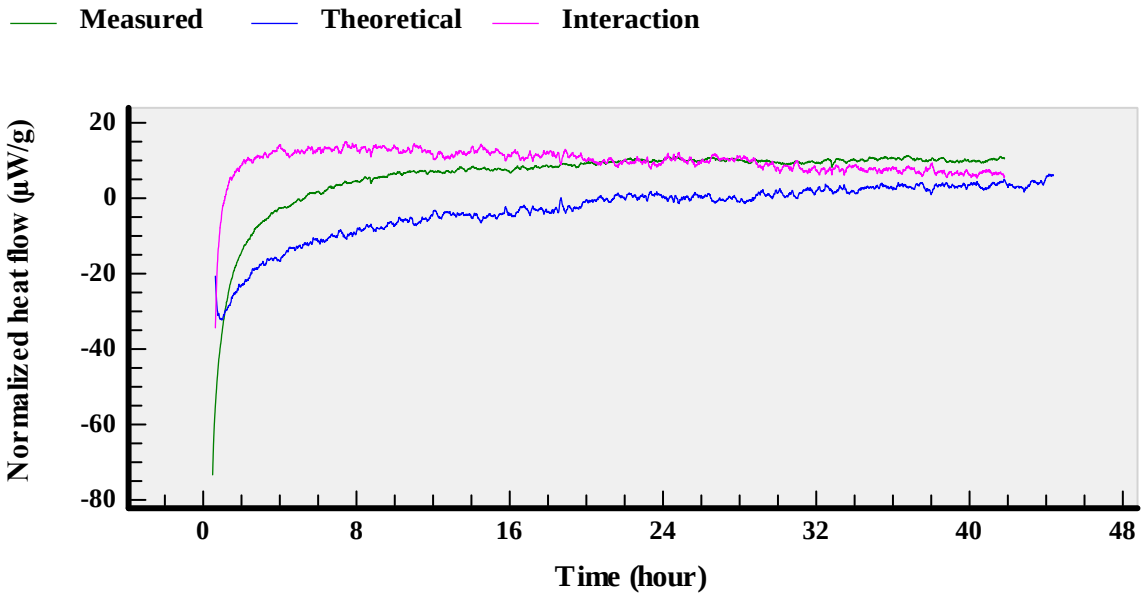

Figure 6.59: Heat flow graph obtained with a combination of INH and EMB.

Incompatibility testing between PZA and EMB

The heat flow data obtained with the combination of PZA and EMB showed an interaction average heat flow of 6.75 µW/g and an interaction error of 7.94 µW/g. The closeness of the actual measured heat flow signal to that calculated theoretically show that no incompatibility exists between PZA and EMB (Figure 6.60).

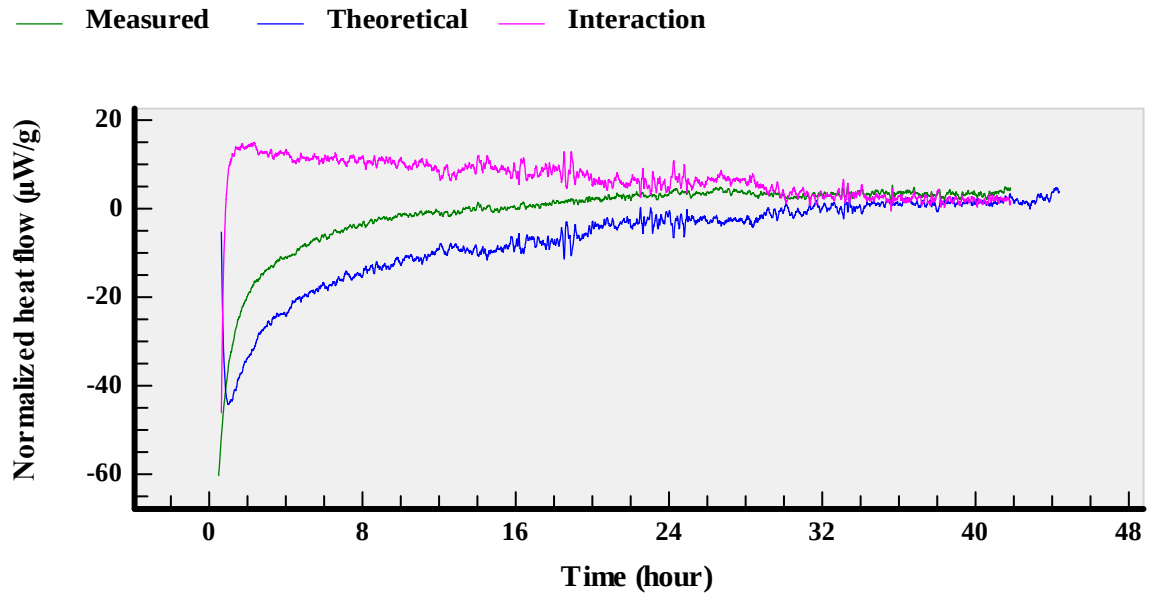

Figure 6.60: Heat flow graph obtained with a combination of PZA and EMB.

Incompatibility testing between INH and PZA

The next combination that was tested was that of INH and PZA. This combination showed no incompatibility between the two drugs. An average heat flow of 2.94 µW/g was measured with an interaction error of 2.22 µW/g calculated between the measured and theoretical heat flow data. The resulting heat flow curves are depicted in Figure 6.61.

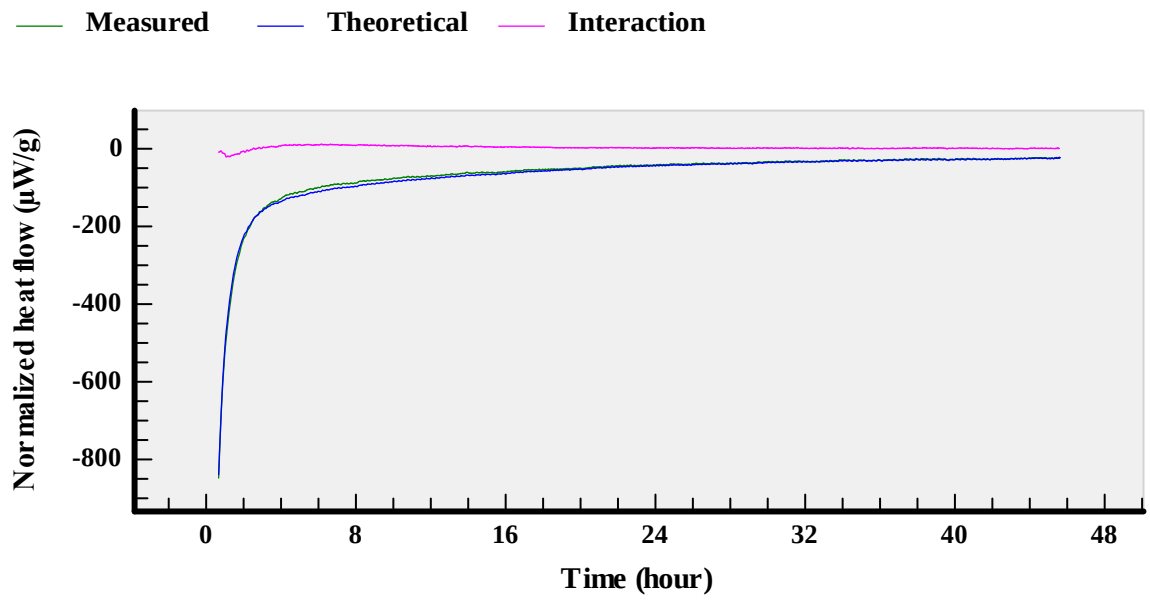

Figure 6.61: Heat flow graph obtained with a combination of INH and PZA.

Incompatibility testing between RIF and INH

The next step was to test the effect that the inclusion of RIF has on the four drug combination since no incompatibility was identified between INH, PZA or EMB. Figure 6.62 depicts a heat flow graph obtained with the combination of RIF and INH. It became apparent that an incompatibility exists between RIF and INH. An average heat flow of -64.05 µW/g and an interaction error of 73.41 µW/g were calculated for the mixture of RIF and INH. The presence of a distinctive slope and a clear difference in the physically measured and theoretically calculated heat flow curves are indicative of an incompatibility.

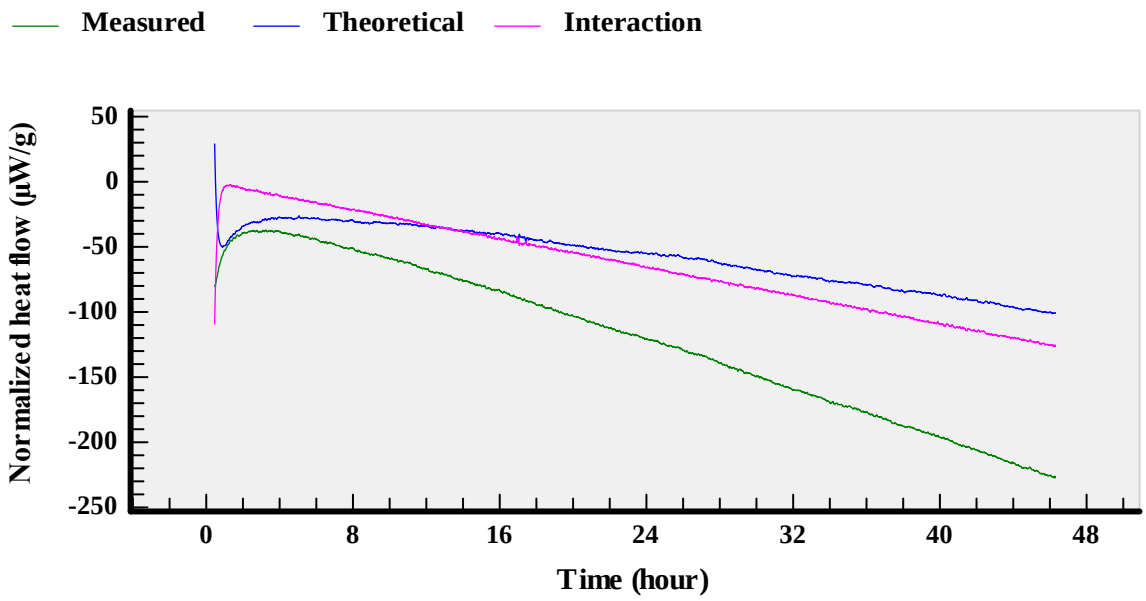

Figure 6.62: Heat flow graph obtained with a combination of RIF and INH.

Incompatibility testing between RIF and PZA

Subsequently, the combination of RIF and PZA was tested. Once again, no incompatibility was identified. Figure 6.63 depicts the heat flow graph obtained with this combination. An interaction heat flow of 18.44 µW/g was measured, and an interaction error of 19.59 µW/g was calculated.

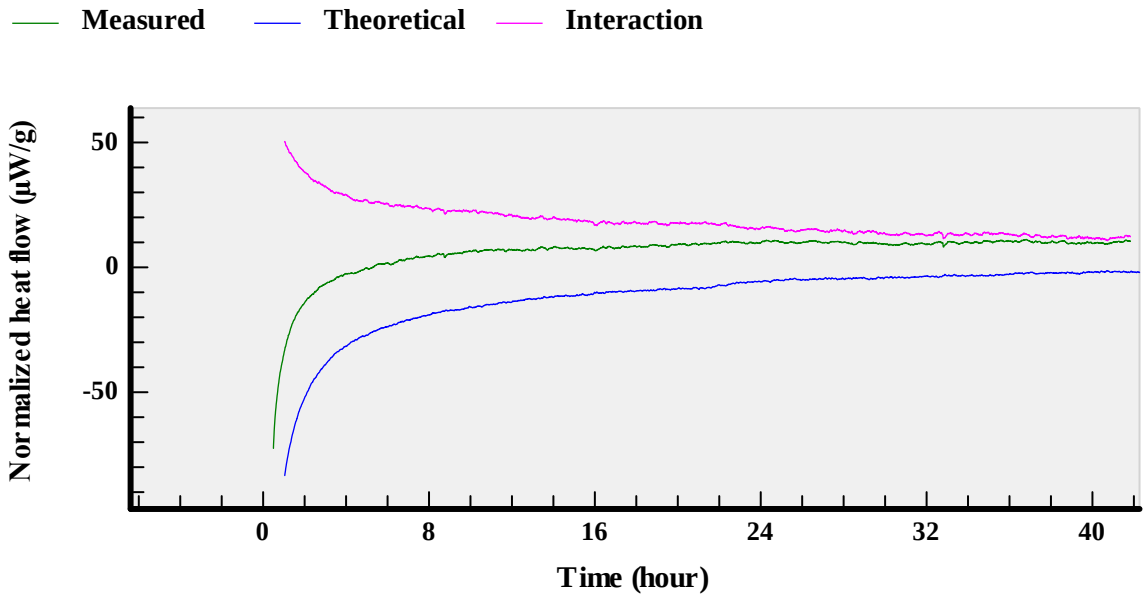

Figure 6.63: Heat flow graph obtained with a combination of RIF and PZA.

Incompatibility testing between RIF and EMB

Lastly, the combination of rifampicin and ethambutol was tested in terms of compatibility. No incompatibility was identified. Figure 6.64 shows the heat flow curves obtained with an interaction average heat flow of 6.49 µW/g and an interaction error of 8.38 µW/g.

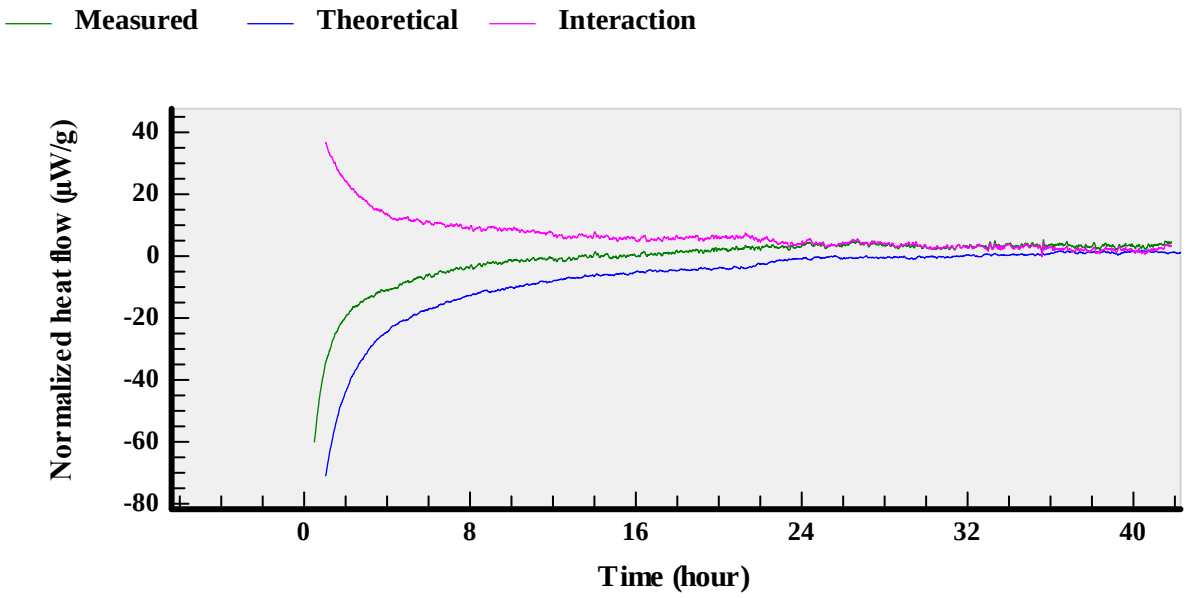

Figure 6.64: Heat flow graph obtained with a combination of RIF and EMB.

To summarise: The only incompatibility observed with this series of experiments was with the combination of RIF and INH.

Concluding remarks

We hoped that the hydrolysis results could shed more light on the subject of the incompatibilities and the reported problems associated with the FDC, but the results were inconclusive as to point out which of the APIs are mainly responsible for the RIF degradation.

Our findings partly support the results obtained by Bhutani *et al.* (2004b) where he stated that PZA and EMB act catalytic towards the reaction between RIF and INH. Our results showed that EMB together with RIF and INH showed the greatest rate of degradation. Surprisingly the degradation of the four combination API was less than that of the above mentioned three combination.

The hydrolysis experiments highlighted again the complexity of the chemistry involved with these four drugs.

The microcalorimetry results showed at 40°C that no incompatibility exists with and without humidity. The old school belief is that EMB together with humidity conditions is mainly responsible for the RIF degradation and the so called 'bleeding' of the tablets. Although the microcalorimetry results at 40°C and 75% RH showed no interaction, more analysis should be done to verify this. It might be that the deliquescence of EMB masks any interaction or stability. Isonicotinyl hydrazone (HYD) formed when RIF is hydrolysed to 3-formylrifamycin, the latter reacts then with INH to form HYD. This reaction occurs normally in acidic conditions (Singh *et al*., 2006). Dekker and Lotter (2003) suggested that in the solid-state, HYD may also be formed because of a direct interaction between the imino group of RIF and the hydrazine group of INH. This interaction in the solid-state is exactly what we find with the microcalorimetry results at 50°C. The microcalorimetry results showed that an incompatibility exists between RIF and INH in the solid-state.

The moisture sorption results confirmed the hygroscopic nature of EMB, but the question remains is that moisture responsible for the degradation of RIF. The TAM and hydrolysis results were not conclusive about this.

The stability of the FDC tablet is still a challenge for researchers and more sophisticated analysis should be proposed to solve this complex problem.

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Chapter 7
Summary

In chapter 1 a brief history was given of tuberculosis (TB) and the challenges around its treatment. Tuberculosis requires long-term treatment, multiple drug treatment regimens, because of the organism's cell wall structure that forms a barrier against anti-TB drugs. This cell wall barrier results in patients having to take multiple capsules and tablets throughout the day for long periods of time. Long-term treatment regimens, consisting of high dosage burdens result in many disadvantages that are non-conducive to patients' recovery from TB.

In chapter 2 the solid-state forms and physico-chemical properties of pharmaceuticals were discussed. Different solid-state forms of active pharmaceutical ingredients (APIs) commonly include polymorphs, solvates, hydrates, desolvates, co-crystals and amorphous forms, depending on their compositions, inter-molecular bonds, molecular arrangements and/or conformations. As discussed above, TB dictates the need for long-term, multi-drug regimens for the treatment of this disease. By combining the individual four anti-TB drugs (rifampicin, isoniazid, ethambutol, pyrazinamide) into single, fixed-dose combination products (FDC) would simplify treatment and improve patient compliance. However, the physico-chemical properties of each drug and in different combinations need to be well investigated beforehand, to ensure the stability and efficacy of any proposed FDC formulation.

In chapter 3 the anti-TB fixed-dose combination products are discussed. The World Health Organisation and the International Union Against Tuberculosis and Lung Disease recommend the use of fixed-dose combination (FDC) tablets for the treatment of TB to limit treatment failure, drug resistance and improve patient compliance. Fixed-dose combinations can be defined as formulations of two or more active ingredients in a single product, available in fixed doses. Anti-tuberculosis FDC products have been reported as being unstable in formulation, due to inter-drug interactions between the individual components. Various hypotheses have been put forward to explain these interactions that may occur in formulations and during oral administration.

In chapter 4 the dissolution, solubility and stability of APIs is discussed. The aqueous solubility of a drug plays an important role in the absorption of the drug after oral administration. Drug solubility depends on the physical and chemical properties of the solute and solvent, such as polarity, ionisation potential, crystal packing and the presence of solvates. The dissolution rate at which the drug passes into the solution is equally important. Only a small percentage of drugs reportedly have a high solubility and dissolution rate. It has previously been reported that the bio-availability of rifampicin (RIF) can be compromised when given as fixed-dose combination (FDC) tablets, especially the three-drug and four-drug combinations. Thus, certain drug delivery and formulation approaches have been suggested in previous studies to resolve the problems associated with the decomposition of RIF in the presence of INH, as well as RIF's overall reported incompatibility with the other anti-TB drugs.

In chapter 5, the latest techniques were used to determine whether the reported chemical reactions indeed occur and under which conditions they would occur, if at all. The following characterisation and stability indicating methods were done to ascertain if the above claims are true, i.e. solid-state characterisation of the four APIs, stability testing, hydrolysis, compatibility between the components through microcalorimetry, and hygroscopicity analysis. The solid-state properties of APIs are important in a pharmaceutical related study. When polymorphism or stability problems with a given API are reported, the investigator/researcher should be sure about the specific crystal form and all other physico-chemical properties of the specific API.

In chapter 6 the findings from the above mentioned methods were discussed. For the degradation studies, the greatest rate of degradation for RIF in water at 37°C was observed for the combination RIF+INH+EMB (68.6%). The degradation of the RIF+INH+PZA was at 37°C and 24 hours 46%. The degradation of the four drug combination at identical conditions was 63.2%. Apart from a clear impact of INH and RIF on each other, the presence or absence of EMB and/or PZA also influences their rate of hydrolysis in water. The microcalorimetry results showed at 40°C that no incompatibility exists with and without humidity. Potential chemical interaction between RIF and INH in the absence of water has been theorized, but if it cannot be measured with microcalorimetry at 40°C it is unlikely to be a major contributor to RIF and INH degradation in anti-TB FDCs. The microcalorimetry results showed that an incompatibility exists between RIF and INH in the solid-state at 50°C.

The moisture sorption results confirmed the hygroscopic nature of EMB, but the question remains: is that moisture responsible for the degradation of RIF? The TAM and hydrolysis results were not conclusive about this; therefore we cannot conclude that the hygroscopic nature of ethambutol is responsible for the degradation of rifampicin.

Judging from the results above, the stability of anti-TB FDC products remains a challenge to researchers. More sophisticated analyses ought to be done to answer the stability question of anti-TB FDC products.

Annexure A

Title of publication: Determining compatibility between four anti-TB drugs and tablet excipients using microcalorimetry

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Determining compatibility between four anti-TB drugs and tablet excipients using microcalorimetry

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Summary

Previous isothermal microcalorimetry studies at our laboratories at 40°C, with and without humidity (RH 75%), showed no incompatibility between rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol HCl (EMB). The purpose of this study was to explore any interactions at an increased temperature of 50°C and also to investigate the possibility of incompatibilities between the drugs and the tablet excipients used in the most commonly prescribed commercial four-drug TB FDC. No incompatibilities were observed between the excipients, or when the excipients were tested with the four drugs individually. Incompatibility was observed with the four drugs combined.

IMC (isothermal microcalorimetry) is a sensitive and useful tool to study thermal activities like chemical degradation, crystallization, compound interactions, to name but just a few. Microcalorimetry prove to be highly sensitive towards heat flow (± 0.1 uW) and temperature changes (Ball and Maechling 2009; Gaisford, 2005; Skaria et al. 2005). According to Skaria et al. (2005), the use of isothermal microcalorimetry is not widespread in the pharmaceutical field, because the data obtained are more complex of nature and often more than one process contribute to the data obtained. We used isothermal microcalorimetry exclusively to investigate if there is a possible interaction between the different anti-tuberculosis (anti-TB) drugs as combined in a well-known four drug containing fixed dose combination (FDC) product.

Anti-TB FDC products have been reported to be unstable in formulation due to chemical interactions between the drugs. It has been previously published that ethambutol HCL catalyses the degradation of rifampicin and isoniazid in the formulation due to its reported

hygroscopic nature. This is said to result in the loss of rifampicin (RIF) potency upon storage. The proposed incompatibility of the four anti-TB dugs has been reported to have led to two major problems, namely; (1) the decrease in bioavailability of RIF upon oral administration and (2) instability of drugs within the formulation environment (Bhutani et al., 2005; Sankar et al. 2003; March, 2001).

The first tested set consisted of RIF, isoniazid (INH), ethambutol hydrochloride (EMB) and pyrazinamide (PZA) combined in the weight ratio as present in the commercial tablet formulation. The heat flow data of each individual active ingredient was measured to obtain reference heat flow curves for each of them. The obtained heat flow results obtained for the tablet mixture containing only the four active ingredients are provided below (Figure 1).

During a step-wise elimination experiment it was deduced that no interaction exist between INH, PZA or EMB. Meaning that RIF is the drug interacting with any of the other three anti-TB drugs. This was further tested through microcalorimetry studies and it was identified that the incompatibility exist between INH and RIF (Figure 2) at 50°C (isothermal). An average heat flow of -64.05 µW/g and an interaction error of 73.41 µW/g were calculated. The presence of a distinctive slope and a clear difference in the physically measured and the theoretically calculated heat flow curves are indicative of an incompatibility.

No incompatibilities were observed between the excipients, or when the excipients were tested with the four drugs individually. Incompatibility was observed with the four drugs combined. Interaction of single drugs, combined with the excipients was also excluded. Combinations of INH+PZA+EMB, PZA+EMB, INH+PZA, RIF+PZA, RIF+EMB with excipients were not observed to interact either.

The only incompatibility detected, whilst testing the active and inactive ingredients of a commercial anti-TB FDC using microcalorimetry at 50°C, was between RIF and INH. Our prior testing at 40°C (with and without 75% RH) did not show this interaction.

Even at 50°C, the heat flow measured did not indicate a large or rapid reaction. Microcalorimetry is the most sensitive method of measuring chemical incompatibility, with units in µW/g or nW/g. Potential chemical interaction between RIF and INH in the absence of water has been theorized, but if it cannot be measured with microcalorimetry at 40°C it is unlikely to be a major contributor to RIF and INH degradation in anti-TB FDCs.

1. Experimental:

Different mixtures consisting of RIF, INH, PZA, EMB and tablet excipients were tested for compound compatibility. All drugs were purchased from DB Fine Chemicals (Johannesburg, South Africa) and had purity values higher than 95.0 %. The ratios in the mixtures were as

per the commercial FDC product (RIF = 150 mg; INH = 75 mg; PZA = 400 mg; EMB = 275 mg). The following excipients were tested: Croscarmellose sodium = 10.5 mg; polyvinyl pyrrolidone = 7 mg; ascorbic acid = 3.5 mg; starch = 113.31 mg; lactose = 210.44 mg; sodium lauryl sulphate = 3.5 mg and magnesium stearate = 5.25 mg and was a kind donation from the Department of Pharmaceutics, School of Pharmacy, North-West University. The tablet mixture was tested in relation to the different excipients that are included in the tablet formulation.

A 2277 Thermal Activity Monitor (TAMIII) (TA Instruments, USA) equipped with an oil bath with a stability of ±100 µK over 24 hours was used during this study. The temperature of the calorimeters was maintained at 50°C (dry). Heat flow was measured for the single components as well as the mixtures. The calorimetric outputs observed for the individual samples are summed to give a theoretical response. This calculated hypothetical response represents a calorimetric output that would be expected if the two or more materials do not interact with each other. If the materials interact the measured calorimetric response will differ from the calculated theoretical response. Samples for testing were prepared by accurately weighing sufficient quantities as outlined in Table 1 providing a total mass of ≅ 100 mg, into glass ampoules. Each ampoule was tightly sealed and used for subsequent microcalorimetric analysis.

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DISCLAIMER

Any opinions, findings and conclusions, or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability in regards thereto.

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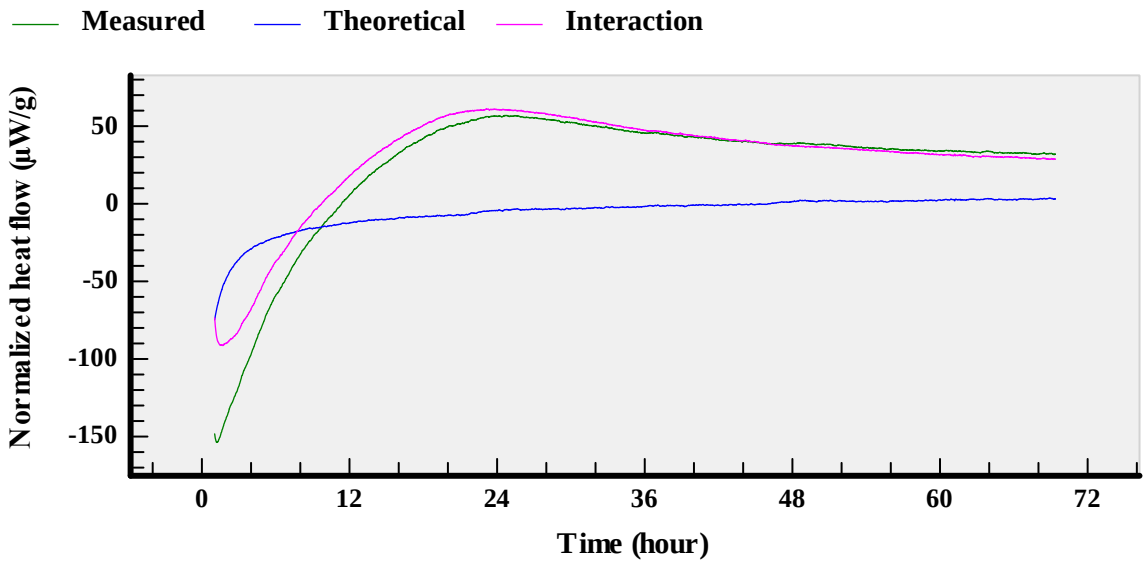

Figure 1: Heat flow graph obtained with the combination of RIF, INH, PZA and EMB.

The average interaction heat flow obtained during this run was calculated to be: 29.8 µW/g with an interaction error of 44.8 µW/g. This is indicative of an interaction occurring between the different active ingredients that forms the active ingredient component of the FDC tablets.

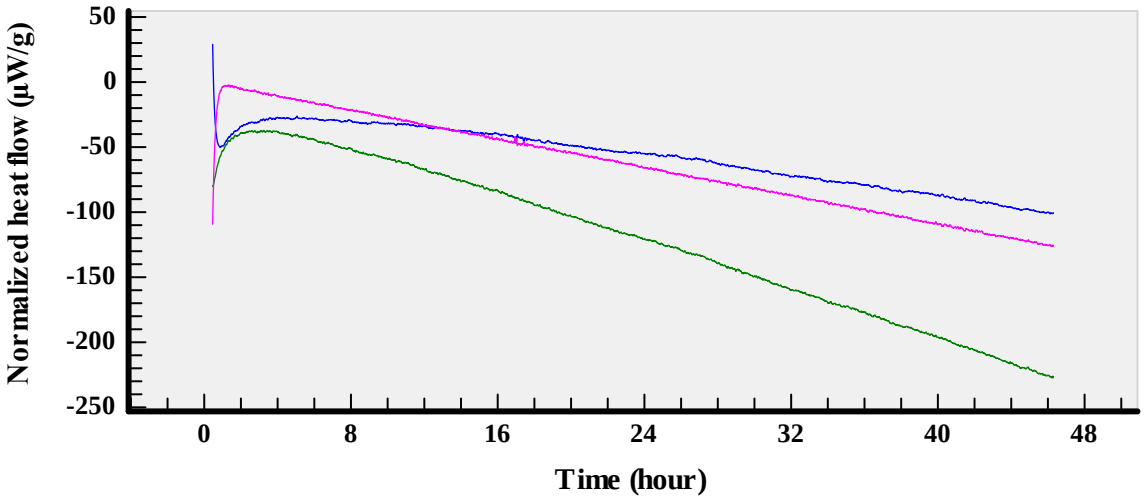

Figure 2: Heat flow graph obtained with the combination of RIF, INH, PZA and EMB.

The heat flow graph obtained for RIF+INH showed an incompatibility as measured at 50°C. An average heat flow of -64.05 µW/g and an interaction error of 73.41 µW/g were calculated. The presence of a distinctive slope and a clear difference in the physically measured and the theoretically calculated heat flow curves are indicative of an incompatibility.

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Annexure B

Title of publication: Anti-Tuberculosis Fixed-Dose Combination Products: A Review on Current Regimes and Future Approaches

Authors: T. Okaecwe, M. Aucamp, W. Liebenberg and N. Stieger.

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Anti-Tuberculosis Fixed-Dose Combination Products: A Review on Current Regimes and Future Approaches

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Abstract

The treatment of tuberculosis (TB) requires multi-drug therapy throughout the day for a long period of time. This may lead to the induction of certain factors such as poor patient compliance, treatment failure and drug resistance. To restrain these factors, the World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease (IUATLD) recommended the use of fixed-dose combination (FDC) tablets for the treatment of TB. FDCs can be defined as formulations of two or more active ingredients in a single product, available in fixed doses. The recommended treatment approach of TB includes rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB) daily for two to three months. RIF and INH are further used for four months, either daily or three times a week or EMB and INH daily for 6 months. This leads to a total number of nine tablets a day as compared to taking three or four FDC tablets daily. Thus, the use of FDCs may simplify treatment and patient compliance especially in patients co-infected with human immunodeficiency virus (HIV).

Key words: tuberculosis, fixed-dose combination, rifampicin, isoniazid, ethambutol, pyrazinamide, layer-by-layer

Tuberculosis is an infectious respiratory disease that has claimed the lives of humankind throughout history. Mycobacterium tuberculosis (M. tuberculosis) is the causative agent of TB, whose principal host is humans [1]. Approximately one-third of the world's population (2 billion) is infected with tuberculosis. Annually, more than 8.8 million patients develop active TB, 1.6 million deaths are estimated to occur of which 95% of those deaths occur in developing countries. There are 22 countries that have been labeled "high-burden" countries and among the 15 countries with the highest incidence rates of TB, 12 are in Africa [2]. TB now ranks alongside HIV as the leading cause of death globally. The 2015 WHO report estimated that 9.6 million people had fallen ill with TB in 2014, 5.4 million were men and 3.2 million were women. 1.5 million of these were killed by TB; 1.1 million were HIV negative and 0.4 million were HIV positive. However, the occurrence of TB has fallen by

approximately 1.5 % per year since the year 2000. From 2016, the goal is to end the global TB epidemic by implementing the End TB Strategy. This strategy was implemented by the World Health Assembly in 2014. The goal is to reduce the number of TB deaths by 90 % by 2030, cut new cases by 80 % and decrease the financial costs in cured by families due to TB [3].

The recommended treatment approach of TB includes RIF, INH, PZA, and EMB daily for approximately three months during the initial intensive phase of treatment. RIF and INH are further used for four months, either daily or three times a week during the continuation phase of treatment. In cases where ethambutol is contraindicated, streptomycin may be used. However, streptomycin is generally recommended because of its higher resistance than ethambutol and it requires the parenteral route of administration [4]. Where there is resistance of INH or RIF during the continuation phase, EMB and PZA may be used daily for 6 months. In certain cases where there is a resistance of INH in the absence of RIF resistance, levofloxacin (third generation flouroquinoline) may be used or moxifloxacin (fourth generation) [5]. Successful treatment of TB requires multiple doses per day, for a long period of time (6 - 8 months). This may lead to problems such as poor patient compliance, treatment failure and drug resistance. To minimize these factors, the WHO and the IUATLD recommend the use of FDC tablets for the treatment of TB. FDCs can be defined as formulations of two or more active ingredients in a single product, available in fixed doses [6-8].

HIV and tuberculosis

TB and HIV are inseparably linked. In certain communities, TB infection is seen as a sign of being infected with HIV. This stigma associated with TB and HIV then leads

to delayed health-seeking by TB infected persons [9]. In 2009, 12% of over 9 million new TB cases that were reported to the WHO were HIV positive [10]. Countries such as South Africa and Zimbabwe carry most of this HIV-associated TB burden, with at least 50% of new TB cases being attributed to HIV. The lifetime risk of developing active TB in persons infected with HIV exceeds 10% in one year alone. This risk is lesser in persons infected with TB alone [11-12].

TB is one of the opportunistic infections that mark the progression of HIV infection to acquired immunodeficiency syndrome (AIDS). It is also the leading cause of death amongst persons living with AIDS. TB accelerates the course of HIV infection by increasing the viral load in some patients, thus making TB the number one cause of death in HIV infected persons. Due to the HIV mortality rates, life expectancy in sub-Saharan Africa has decreased to 45 years [13-14].

Co-infection of TB and HIV resulted in major public health problems such as the emergence of multi-drug resistant TB (MDR TB) and extensively drug-resistant TB (XDR TB). The presence of MDR TB in persons with HIV makes therapy almost impossible resulting in patients having a decreased survival rate. Medication used in TB therapy may have overlapping toxicities with antiretroviral drugs. Rifampicin, the mainstay of TB therapy for instance, is a cytochrome P450 enzyme inducer. This enzyme metabolizes all three major classes of antiretroviral. Co-administration of rifampicin with antiretroviral can lead to reduced levels of HIV medication in the systemic system, which in turn leads to more resistant strains of *M. tuberculosis* [14].

Problems regarding resistance

MDR TB is defined as *M*. *tuberculosis* that is resistant to at least isoniazid and rifampicin. MDR TB does not occur because of horizontal gene transfer but rather, through the natural selection of rare drug-resistant strains in the human body. Therefore, MDR TB is a man-made problem and it can only be controlled by immediate diagnosis and effective TB treatment [15]. In 2008, the WHO reported 440 000 cases of MDR TB. 360 000 of these cases were new and relapse cases (maybe due to an MDR strain), and 94 000 were persons previously treated for TB. The global epidemic of MDR TB is mainly caused by a combination of acquired and primary resistance. The latter is defined as the transmission of drug-resistant strains of *M*. *tuberculosis*, giving rise to MDR TB in individuals never previously exposed to anti-tuberculosis drugs [16].

Problems regarding MDR TB can be caused by the following factors; (1) inconsistent or interrupted treatment (public health systems and/ patient factors), (2) when wrong drugs are prescribed for a wrong period, (3) when drugs of poorer quality are prescribed, or (4) when there's an interrupted supply of drugs. While MDR TB is generally treatable, it may take up to two years to complete the regime and the program may use more toxic and expensive drugs than when treating a drug susceptible case. Co-infection of TB and HIV may also lead to an increased rate of spontaneous resistance-conferring mutations. Persons with HIV-associated TB may have a decreased compliance due to increased pill burden, overlapping of adverse reactions or fragmentation of TB from HIV/AIDS in healthcare systems. These problems are a direct result of the lack of infection control [14].

In 2005, another phenomenon was described, extensively drug-resistant TB (XDR TB). XDR TB is defined as resistance to any flouroquinoline and one of the three second-line injectable drugs (amikacin, kanamycin or capreomycin). XDR TB has been reported all over the world, however Asia and Eastern Europe, carry the greatest burden. In 2006 mortality rates of XDR TB infected persons who are co-infected with HIV were as high as 98%. These alarming results were documented from a high prevalence setting in rural KwaZulu Natal, South Africa. Failing TB programs and lack of support from government has created fertile conditions for a "perfect storm" of drug-resistant TB [17].

FDCs: simplifying treatment and prevention of drug resistance

One of the main constraints of TB chemotherapy is that the treatment regimens include multi-drug therapy, thereby leading to frequent drug administration of drug doses, causing a significant "pill-burden" on the patient. The whole rationale behind the development and use of FDC products is to reduce this "pill-burden". Using FDCs reduces the number of dosage forms that needs to be taken per day for the whole duration of treatment. This decrease can make treatment easier and even prevent monotherapy, especially in persons infected with HIV. For each of the anti-TB medication there is a fixed recommended dose per kg body weight (Table 1) [8]. The number of tablets/capsules required is calculated according to the patient's body weight. The recommended doses of the first line anti-TB drugs are INH 5 mg/kg, RIF 10 mg/kg, PZA 25 mg/kg, EMB 15 mg/kg and streptomycin 15 mg/kg. The use of streptomycin is normally not recommended in children and the elderly because of the pain and other side effects such as auditory nerve damage. The dose-to-body-weight relationships in FDCs are correctly balanced to ensure adequate drug delivery of all

anti-TB medication [4]. Problems that arise when using single drug formulations occur because of three main reasons: absence of stock, delays in receiving stock and lack of replacements when expiry dates are reached. These situations may lead to other drugs being given in isolation (monotherapy) while new stock is being waited upon. The use of FDCs does prevent the occurrence of such instances. The use of FDC's has a number of advantages over single drug therapy, namely: (1) prevention of monotherapy, thus the risk of selection of drug resistant bacilli is reduced (2) drug product prescription and administration is simplified (3) better product stock management shipping and distribution (4) the risk of misuse of rifampicin for conditions other than TB (such as chlamydia) is reduced, thereby reducing the risk of the development of MDR-TB [18]. Also, a study done on acquired drug resistance postulated that the use of INH and RIF FDC may decrease the rate of resistance. Among the 4000 participants that were involved in this trial, only 8 participants acquired drug resistance. The study then concluded that the use of INH and RIF FDC may result in minimal acquired drug resistance [19-20].

The main disadvantages of current TB FDC products are the large size of the tablets resulting from the combination of the four anti-TB drugs plus excipients, and the chemical incompatibilities of the drugs. However, a study conducted in Hong Kong noted that only 1% of 312 patients on FDCs complained about the quantity and/or size of tablets to be ingested or difficulty with swallowing, in contrast to the 5% of 308 patients receiving single drug formulations [8]. In addition, a study conducted in Indonesia on the anti-TB side-effects, complaints and treatment, suggested that FDC-based products were associated with fewer side-effects compared to single-drug-short-course regimen [21]. Thus, the correct use of FDC tablets of can ensure adequate drug delivery and may prevent resistance when given in correct doses.

Quality of anti-TB FDC products

The major quality issue of using FDCs is the bioavailability of RIF. Dr Acocella of the University of Pavia showed that the bioavailability of RIF can be compromised when given as FDC tablets, especially the three-drug combination. Thus far, bioavailability problems were only identified with RIF and no problems with INH, PZA and EMB when formulated together in FDC products [22]. The high aqueous solubility of INH, PZA and EMB has been proposed as a possible reason for no bioavailability problems associated with these three drugs. Several reasons have been proposed for the poor or high bioavailability. The reasons that have been hypothesized for the compromised RIF bioavailability include raw material characteristics, changes in the crystalline structure of rifampicin RIF, excipients, manufacturing variables, degradation in the gastrointestinal tract (GIT) and inherent variability in absorption and metabolism. The versatile behavior of rifampicin can be best described by physiological, physico-chemical, pharmaceutical and manufacturing factors [23-26]. Furthermore, a dissolution test, which is a simple and robust mechanism of judging the quality of formulations, cannot guarantee acceptable RIF bioavailability [25]. To ensure that FDC tablets of good quality are used, WHO and the IUATLD have developed a simplified, effective protocol for the assessment of RIF bioavailability from FDC formulations. A worldwide mechanism for pre-qualification of FDCs has been proposed to ensure that only FDCs of good quality are used in TB therapy [10].

Incompatibilities of the first line anti-TB drugs

The four first line anti-TB drugs, discussed in this review, belong to two different classes of the biopharmaceutical classification system (BCS) according to differences in their aqueous solubility and bioavailability. INH, PZA and EMB

hydrochloride belong to class I (highly soluble and highly permeable) of the BCS. RIF on the other hand, a zwitterionic molecule with two pKa values (1.7 and 7.9), is the only hydrophobic ingredient of the FDCs and belongs to BCS class II. In addition, RIF exhibits a highly pH-dependent solubility and lipophilicity profile which affects its absorption in the GIT [27]. When RIF is taken on an empty stomach, peak serum concentrations are achieved within two hours, however if ingested with food, absorption may be delayed and incomplete [28].

It has been postulated that below pH 2, RIF is converted to its inactive metabolite 3-formylrifampicin. The reaction then proceeds to form HYD (isonicotinyl hydrazone) when RIF is combined with INH in formulation [29]. Furthermore, it has been observed that PZA and EMB hydrochloride are catalytic towards the reaction between RIF and INH as FDC's containing 4-drug combinations have shown far more chemical instability than 2-drug FDC's containing only RIF and INH [30]. The catalytic role of EMB hydrochloride may be attributed to its hygroscopic and acidic nature (pKa value 6.6). It is further postulated that both PZA and EMB hydrochloride exhibit catalytic roles through the involvement of intra-molecular proton transfer during the reaction between RIF and INH, which is believed to occur through a base-catalysed transhydrazone formation process entailing a tetrahedral mechanism [30-31]. Nonetheless, it has been suggested in a study that even under non-acidic formulation conditions a direct reaction (although much slower), between RIF and INH, yielding HYD as a product is still possible [32].

This direct interaction is said to be best explained through a transhydrazone formation, via nucleophilic attack on the imine group of RIF by the amino group of INH, following a tetrahedral mechanism. Since these reactions are inherently

reversible [30], the overall decomposition leading to HYD depends on the following factors; the relative nucleophilic property of the amino group of INH and that of the 1-amino-4-methyl piperazine and the relative leaving group ability of the terminal amide anion of INH and that of 1-amino-4-methyl piperazine. The nitrogen lone pair that is adjacent to the amino group of 1-amino-4-methyl piperazine makes it α-nucleophilic, which has a stronger nucleophilic property than that of INH. However, the carbonyl group of INH withdraws the lone pair of electrons from the nitrogen atom that is adjacent to the amino group, making the corresponding amide anion a better leaving group, compared to the amide anion of 1-amino-4-methyl piperazine. Thus, the overall equilibrium should not favor decomposition under non-acidic conditions [31].

Stability studies done by Sankar *et al*. (2003) on the behavior of RIF in the presence of INH showed that 34 % of RIF is decomposed, and approximately 10% of INH is decomposed in acidic medium (stomach fasting condition). The decomposition of marketed FDC samples were also tested in fasted-state pH conditions (0.01 M HCL solution) and the degradation values ranged from 13-35% and 4-11% for RIF and INH respectively [32]. Unsuitable storage and packing conditions may also cause stability problems in FDC products. Prior to Sankar's study, Mohan (2001) had conducted a comparative stability indicating assay on marketed FDC products. The FDC products were stored unpacked in light and humidity chambers set at 40 °C/ 75% relative humidity (RH). The products that were stored in the light chambers showed more physical changes than those stored in dark chambers [33]. Singh and co-workers (2002) later explored this phenomenon and concluded that if packaging is of a low quality, EMB, due to its hygroscopic nature absorbs moisture from the atmosphere and gives rise to "bleeding" of API's i.e. the API leaks out of the

formulation [34]. EMB thus dissolves in the absorbed moisture and may even act as a solvent for the other API's in formulation. This results in poor bioavailability of all the anti-TB drugs in formulation and eventually therapeutic failure [30, 34].

The incompatibility of the four anti-TB dugs has led to two major problems, namely; (1) the decrease in bioavailability of RIF upon oral administration and (2) instability of drugs within the formulation environment [30].

Current drug delivery approaches as a solution to the discussed incompatibilities

Certain drug delivery and formulation approaches have been suggested to solve the problems associated with the decomposition of RIF in the presence of INH as well as RIF's overall incompatibility with the other anti-TB drugs. It has been hypothesized that the following approaches may serve as solutions: (1) enteric coating of solid formulations or drug granules/powders; (2) using an alkalinizer at the time of administration of FDC formulations, the use of sodium bicarbonate during the administration of RIF containing FDC formulations may be advantageous as there will be less decomposition of RIF when it is in insoluble form ; and (3) exploitation of formulation factors, by including additives (pH sensitive polymers) in the formulation and also (4) segregating the method of delivery of RIF and INH [34].

Since one of the problems associated with anti-TB FDC products is the hygroscopic nature of EMB hydrochloride, Bhutani and co-workers suggested that film coating of the tablets with water-resistant polymers may be essential in preventing moisture entry in the dosage form [35]. Whilst film coating will prevent moisture entry, it cannot preclude the non-acid catalyzed reaction that will take place due to direct contact between the individual drugs. An analogous solution, which could be used to

prevent both water entry and chemical interactions, is the application of polyelectrolyte nano-coatings using the layer-by-layer (LbL) self-assembly technique [36]. This technique has previously been used successfully to protect photosensitive pharmaceutical products to increase their photostability. Nifedipine microcrystals were previously coated with charged poly(diallyldimethylammonium chloride) (PDDA) and negatively charged titanium dioxide (TiO_2) particles to increase their photostability [37]. Advantages of this process include an increased dissolution rate and bioavailability of poorly water-soluble drugs. The LbL-technique has also been used successfully in the stabilization of dexamethasone nanoparticles. This technique has proven to also protect micron sized particles from aggregation, resulting in a controlled drug release of certain poorly soluble drugs [38-39]. The use of the LbL-technique was developed when Decher and colleagues (1992) used synthetic polyelectrolytes with ionizable surface groups to form polyions that were successfully layered onto a substrate by electrostasis [40-41]. For pharmaceutical applications, LbL self-assembly may be used to: 1) directly apply coats of oppositely charged polyelectrolytes onto drug micro/nanoparticles or, 2) produce hollow polyelectrolytes capsules which may be loaded with drug molecules [42]. The LbL self-assembly technique is based on alternate adsorption of oppositely charged materials. A charged substrate is immersed in a solution of an oppositely charged coating material to adsorb the first monolayer. The coated substrate is subsequently submerged in a second coating solution. Repetition of this process then leads to an ordered multi-layer assembly on the solid substrate surface [43-47].

In recent times *in vitro* release studies were conducted on TB drugs entrapped in niosomes and liposomes in an effort to improve the stability and bioavailability. Niosomes are structurally related to liposomes, both consist of bilayers. The only

difference is that the bilayer of niosomes consists of non-ionic surface agents. In liposomes it consists of phospholipids [48].

Co-encapsulation of RIF and INH in liposomes is an alternative to protect sensitive APIs from interacting adversely. INH (water soluble) and RIF (lipid soluble) were successfully co-encapsulated in the same liposome formulation by loading RIF in the lipid layer and INH in the aqueous phase. This technique could be a good alternative for combination therapy [49].

RIF was encapsulated in Triton niosomes (Fig. 1) and showed no degradation or photo instability after a 4 week test period. INH as well as PZA was successfully entrapped in the hydrophylic region [50].

A study was done to investigate the feasibility of formulating segregated delivery of rifampicin and isoniazid from FDC bilayer tablets. The tablet contained an immediate release layer composed of both RIF and PZA and a retarded release layer composed of only INH. This tablet would allow the segregated delivery of RIF and INH. This proved to be a suitable strategy to prevent the contact of RIF and INH under acidic conditions [51]. In another study, enteric minitablets of INH were formulated using a cold extrusion method. The minitablets were coated using hydroxypropylmethylcellulose phthalate (polymer) and dibasic calcium phosphate. The use of enteric polymer in the minitablet core ensured the release of INH in alkaline medium. Capsules that contained RIF powder and enteric isoniazid minitablets were reported to show complete drug release in acidic and alkaline media respectively. By incorporating this method in anti-TB FDC formulations, the degradation of RIF can be retarded to a certain extent in FDC formulations containing RIF and enteric coated INH [52].

A study done on the comparison of conventional and novel FDC of RIF and INH to improve bioavailability of RIF for treatment of TB proved that there is no difference in the clinical efficacy of RIF and INH drug levels of the novel FDC formulation as compared to the conventional FDC formulation [53].

A recent novel anti-TB FDC formulation was approved in India which included piperin in the formulation to augment the effect of rifampicin. Based on this product a study was conducted to test the effect that piperin has on RIF and other anti-TB drugs. The results from this study suggested that the combination of RIF and piperin yields a synergistic effect. Piperin has no activity against *M tuberculosis* however it increases the bioavailability of RIF. This means that a lower dosage of RIF would be needed and thus a reduction of the adverse effects experienced with the use of RIF [54].

Therefore using conventional methods such as layer-by-layer nanocoating, niosomes and segragated drug delivery may not influence the bioavailability of the first-line anti-TB drugs if the appropriate excipients are used.

Conclusion

Approaches like LbL, nanomedicines and encapsulating of drugs in niosomes or liposomes are possible efforts to improve the treatment of TB. The challenge will be to deliver or formulate FDCs effectively in these new regimes for drug delivery, keeping in mind all the physico-chemical and stability issues mentioned earlier.

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TABLE 1: THE NUMBER OF TABLETS TO BE TAKEN DAILY IN THE INITIAL PHASE OF TREATMENT BY A 50-KG PATIENT AS FDC AS RECOMMENDED BY WHO.

Pre-treatment body weight	**Intensive Phase 7 days a week for 2 months**	**Continuation phase 7 days a week for 4 months**	
	RIF,INH,PZA,EMB (150,75, 400,275)	RH (150,75)	RH (300,150)
30-37 kg	2 tabs	2 tabs	
38-54 kg	3 tabs	3 tabs	
55-70 kg	4 tabs		2 tabs
>70kg	5 tabs		2 tabs

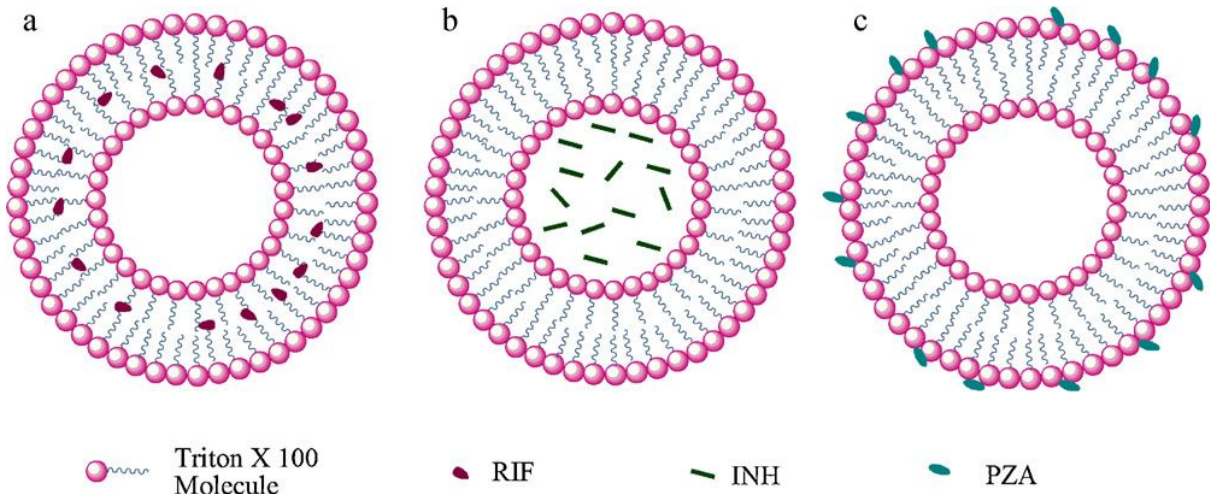

Fig. 1: Location of rifampicin, isoniazid and pyrazinamide in niosomes. Reprinted from [50] copyright © 2016, with permission from Elserivier.

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TABLE 1: THE NUMBER OF TABLETS TO BE TAKEN DAILY IN THE INITIAL PHASE OF TREATMENT BY A 50-KG PATIENT EITHER AS A SINGLE DOSE OR AS FDC

Fig. 1: Location of rifampicin, isoniazid and pyrazinamide in niosomes

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Anti-Tuberculosis Fixed-Dose Combination Products: A Review on Current Regimes and Future Approaches

--Manuscript Draft--

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Abstract:	The treatment of tuberculosis (TB) requires multi-drug therapy throughout the day for a long period of time. This may lead to the induction of certain factors such as poor patient compliance, treatment failure and drug resistance. To restrain these factors, the World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease (IUATLD) recommended the use of fixed-dose combination (FDC) tablets for the treatment of TB. FDCs can be defined as formulations of two or more active ingredients in a single product, available in fixed doses. The recommended treatment approach of TB includes rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB) daily for two to three months. RIF and INH are further used for four months, either daily or three times a week or EMB and INH daily for 6 months. This leads to a total number of nine tablets a day as compared to taking three or four FDC tablets daily. Thus, the use of FDCs may simplify treatment and patient compliance especially in patients co-infected with human immunodeficiency virus (HIV).
Suggested Reviewers:	
Opposed Reviewers:	