Rifampicin raw materials complexities and approaches to address solubility and *in vitro* permeability

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Abstract

Tuberculosis is classified as a global emergency because it is taking lives at an alarming rate and developing countries with insufficient health care systems are primarily affected. Fixed dose combination drugs (FDCs) were created to improve patient compliance and reduce the appearance of drug resistance, but unfortunately this drug combination has a few shortcomings, where rifampicin is a drug well-known for its problematic outcomes regarding bioavailability in FDCs.

After a thorough literature study, it was found that rifampicin pose with different challenges like poor solubility and an amorphous content which could result in poor bioavailability.

The poor solubility leads to issues or questions regarding the poor wettability of rifampicin. Surfactants are known to improve the wettability of poorly water soluble drugs. By making use of the surfactant sodium lauryl sulphate (SLS), the surface tension of rifampicin could be lowered and possibly result in an improvement of wettability of the drug. This could further lead to an increase in solubility of the drug and drug permeability, and consequently lead to better bioavailability.

This hypothesis was tested by using dissolution studies and permeability studies with the Caco-2 model. Rifampicin powder in water and SLS showed enhanced solubility from the lowest concentration (0.05%) SLS in comparison with rifampicin powder in distilled water alone. Rifampicin powder in 0.20-0.30% SLS showed a significant increase in the solubility profile due to an increase in the wettability of the powder. For rifampicin in 0.1 M HCL the solubility was 2121.39 µg/ml after 360 min. In distilled water a solubility value of 872.05 µg/ml was obtained after 360 min, whereas in the presence of 0.30% SLS rifampicin solubility increased remarkably to 2344.88 µg/ml after 360 min. The 0.30% SLS resulted in the best dissolution profile, even better than the current pharmacopoeia medium, i.e. 0.1 M HCl (2121.39 µg/ml).

With the Caco-2 model it was found that rifampicin in SLS can influence the paracellular permeability and can have an impact on intestinal absorption. SLS has a pronounced effect on tight junctions and the permeation of rifampicin was remarkably increased, but due to the cell toxicity of SLS the permeation studies should be tested on a more robust cell model.

These studies showed that the poor solubility of rifampicin is a result of poor wettability and these issues need to be dealt with before any further steps are taken towards improving the bioavailability of the drug.

Key words: Bioavailability, Caco-2, dissolution, fixed dose combinations, multidrug-resistant tuberculosis, *Mycobacterium tuberculosis*, permeability, rifampicin, sodium lauryl sulphate, solid-state, solubility, spring and parachute, surfactants, tuberculosis, wettability

Aim and objectives

Aim:

The aim of this study is to improve the bioavailability of rifampicin by adding a surface-active ingredient to improve its wettability, thereby improving its solubility and permeability.

Objectives:

The specific objectives for this project are:

1. Determine the polymorphic form of the rifampicin raw material through tests.
2. Determine the lowest percentage surfactant needed to improve the wettability of rifampicin raw material.
3. Perform powder dissolution studies on the raw material and raw material plus surfactant in three different dissolution media.
4. Evaluate the permeation of rifampicin in the presence of various SLS concentrations through *in vitro* permeation studies, on Caco-2 cultured cell Transwell® plates.

Chapter 1

Tuberculosis

1.1 Introduction

Tuberculosis (TB) is an infectious disease, caused in most cases by the pathogenic bacterium called *Mycobacterium tuberculosis*. As declared by the World Health Organization (WHO), TB is classified as a global emergency. Approximately one-third of the world's population is infected by this deadly, bacterial disease, in either its active (symptomatic) or its latent (asymptomatic) form. According to the most recent WHO report, there are 10 million new TB cases per annum, 1.2 million deaths of which 95% of these cases are in developing countries (WHO, 2019). The bacterium is an airborne disease and usually spreads through respiration droplets, it enters the body by being inhaled through the lungs. They spread via the bloodstream from the lungs to other parts of the body (Er *et al*., 2010). The strategy to fight this disease includes making use of anti-TB drugs, which include a fixed dose combination (FDC) containing, rifampicin, pyrazinamide, ethambutol hydrochloride and isoniazid (Bhutani *et al*., 2005). This FDC is the most effective dosage regime against TB and is used, in South Africa, during the start of the intensive phase of TB treatment (Blomberg *et al.,* 2001).

1.2 Epidemiology

In 1882, Robert Koch discovered that the infectious microorganism, *M. tuberculosis,* is the leading cause of TB. Koch's findings explained the possibility of antimicrobial medicine that could be developed to fight against this disease. Today, TB is still a major global health emergency, regardless the accessibility of anti-tuberculosis drugs. Third world countries are mostly affected by TB, causing more cases of this disease to be recorded than ever before. TB epidemiology and the ability to control TB are more complex due to the appearance of drug-resistant bacilli and the synergism of TB with HIV co-infection (Mathema *et al.,* 2006).

The WHO (2019) reported that Asia and Africa have the most estimated incidents of TB, where India has the highest occurrence, which ranges from 2.4-2.9 million followed by China with a 0.9-1.1 million range. In 2018 the mortality of TB was estimated at 1.1-1.3 million deaths which includes 223 000-281 000 HIV-correlated cases (WHO, 2019).

1.3 Socio-economic impact

TB is a chronic disease that causes a concerning impact on morbidity and mortality rates. Even though *M. tuberculosis* was discovered over a century ago and with a significant number of effective regimes available, there has been a minor impact on the enormous problem of tuberculosis. The socio-economic impact of this disease is enormous in developing countries.

TB affects mostly young adults in their most productive years and as result leads to a high economic cost for society (Dheeraj *et al*., 2004; WHO, 2019).

Studies revealed that patients in India with TB mainly face financial obstacles and this impacts their daily tasks like housework and childcare adversely. It was found that patients loss of wages and decrease in earning ability due to TB, were relatively high. The loss of wages were an average of three months, which lead to a decrease in care giving activities in female patients, and further resulted in a fifth of school children that discontinued their studies. Income reduction was reported in more than 20% of patients due to the inability to work (Glaziou *et al*., 2013; Rajeswari *et al*., 1999).

Researchers showed that patients diagnosed with multidrug-resistant tuberculosis (MDR-TB) both physical and mental well-being is negatively impacted. This is due to limited treatment options, long period of treatment and associated drug-toxicity. MDR-TB patients face economic issues due to the long period and complexity of treatment. These patients have increased treatment costs due to the increase in patients' recurring hospitalisation and the number of tests required to diagnose these patients. Inaccessibility of treatment, distance, transport costs and costs incurred during hospitalisation are a few of the socio-economic barriers that appear to these patients (Maciel *et al*., 2010). One study found that 23% of MDR-TB patients had failed on medical treatment due to financial limits (Kaliakbarova *et al*., 2013). Another study reported that 5 out of 10 patients discontinued work after a year of treatment. Work absence lead to a decrease in wages, subsequently impacting the treatment of the illness negatively (Thomas *et al*., 2016).

People in developing countries often have poor housing circumstances such as overcrowding, which increases the risk of disease transferral. Tuberculosis is an airborne disease so when a person coughs or sneezes, aerosol droplets containing tubercle bacilli, are spread into the atmosphere. By decreasing the amount of air space that is shared, overcrowding results in increased exposure to TB (Nazaroff & Hubbard, 2010).

1.4 Drug resistance

MDR-TB occurs due to bacteria that develop a major resistance level to both isoniazid and rifampicin, with or without resistance to other anti-TB drugs. The appearance of MDR-TB has led to a major public health risk worldwide. It is estimated that about 60% of MDR-TB cases occur in India, China, and Russia. Most MDR-TB patients stay undetected and untreated, uncovering their families and community to the danger of getting MDR-TB strains through airborne transmission. Individuals with HIV/AIDS living in overcrowded areas are particularly more susceptible to obtain this strain. Poor patient compliance and non-adherence to the prescribed regimen are primary factors that cause MDR-TB to emerge. Worldwide, the extent of MDR-TB patients who effectively complete treatment stays under 50%. These poor results are

mainly because of the long period of treatment and medication toxicity. Resistance to isoniazid and rifampicin can be explained. Resistance to isoniazid start at one of two main sites namely *katG* or *inhA* genes, this occurs due to mutations that take place on these sites. Mutations in the *rpo* gene in the beta subunit of DNA-dependent RNA polymerase are mostly the cause of resistance to rifampicin. MDR-TB is diagnosed by testing positively for the *M. tuberculosis* organism along with drug susceptibility testing. Rifampicin is used as a reference for MDR-TB, when more than 95% of drug resistance to rifampicin take place then MDR-TB is strongly suspected. Mono-resistance occur when the bacteria is less than 10% resistant to rifampicin. Due to its expanding frequency, resistance just to rifampicin and isoniazid is presently recognised as 'basic' MDR-TB and resistance to one or more additional first- and/or second-line drugs is acknowledged as 'MDR-TB-plus' (Ormerod, 2005; Thomas *et al*., 2016).

Drug resistance is a concern because if mono-resistance takes place, treatment periods with rifampicin and ethambutol hydrochloride extend to 9-12 months. Mono-resistance to rifampicin leads to a 2 month treatment of pyrazinamide followed by treatment with isoniazid and ethambutol hydrochloride for 18 months. Hence, loss of reaction to both rifampicin and isoniazid implies that patients stay infectious for longer, and treatment could be extended to 12-24 months which leads to other unfavourable factors like drug toxicity and financial difficulties. Consequently, the requirement for shortening the length of treatment is vital, as it would have an enormous effect on patient compliance and drug resistance (Mathema *et al.,* 2006; Ormerod, 2005).

1.5 Anti-tuberculosis fixed dose combinations

Combination therapy, using more than one antibiotic, was recommended by investigators to reduce the changes of drug resistance appearance during treatment. Patient's non-adherence has over and again been proposed as one of the vital reasons for the inadequacy of numerous tuberculosis control programs (Blomberg & Fourie, 2012). Therefore, combination therapy was created to increase patient compliance and to make the treatment more manageable (Blomberg *et al.,* 2001).

Rifampicin, pyrazinamide, ethambutol hydrochloride and isoniazid are the anti-TB drugs that are included in South Africa's FDC regime (Bhutani *et al*., 2005). Each antibiotic has different working mechanisms to kill the bacteria and works different to avoid drug resistance. Various tests have indicated that a six-month treatment of rifampicin and isoniazid, enhanced by pyrazinamide and streptomycin or ethambutol hydrochloride for the initial two months, give a cure in more than 95% of cases if the drug is taken effectively (Ormerod, 2005).

1.6 Reported incompatibilities and degradation of FDCs

In contrast to all the positive effects of FDCs there are a few incompatibilities that should not be overlooked. Rifampicin's extreme hydrophobic characteristics makes it difficult to mix with the

other ingredients during pre-formulation and thus causing problems in tableting, mixing, and, grinding of these ingredients. This in part is responsible for variable bioavailability of FDCs. Furthermore, isoniazid and rifampicin are known to interact with each other in the solid-state and therefore in tablets (Aucamp *et al.,* 2019). Previous studies have proven that rifampicin's degradation is accelerated in the presence of isoniazid. Isoniazid causes hydrolysation of rifampicin forming the insoluble, 3-Formyl rifamycin SV (3-FRSV), product. This reaction may be caused by the direct interaction of the amino group of rifampicin with the hydrazine group of isoniazid. This leads to reduction of rifampicin's oral absorption and could result in poor bioavailability of rifampicin in FDCs (Shishoo *et al*., 2001).

Studies have showed that due to ethambutol hydrochloride's hygroscopic characteristic, humidity and high temperatures result in quick degradation of the different components of the FDCs and influence the dosage form stability as such adversely (Singh & Mohan, 2003). Bhutani *et al.* (2004) proved that ethambutol hydrochloride presents physical instability, where the caplets virtually bled inside the package (under speeded restrictions after storage for 7-8 weeks). To avoid these incompatibilities, rifampicin is often incorporated in the outer layers of the tablets (Singh *et al*., 2001).

1.6.1 Bioavailability of rifampicin

Bioavailability is the amount or portion of drug that ends up in the body's circulatory system after oral absorption. Toxic effects are related to the bioavailability of a drug which makes drug bioavailability clinically important (Aungst, 1993).

Rifampicin is a drug known to have poor bioavailability especially when incorporated in FDC products (Doshi *et al*., 1986; Ellard *et al*., 1986; Fox, 1990; Mouton *et al*., 1979). It has been reported that raw material characteristics, variation of crystalline form of rifampicin and gastro-intestinal degradation amplify the causes of poor bioavailability (Panchagnula & Agrawal, 2004). Consequently, the quality and effectiveness of the FDCs are influenced and poor bioavailability of rifampicin could cause faulty treatment and contributed to drug resistance (Singh *et al.*, 2001).

1.7 Conclusion

With tuberculosis taking lives at an alarming rate, it is no surprise that it is classified as a global emergency. Developing countries with inadequate health care systems are mainly affected. Emergence of MDR-TB has a concerning negative impact on not only the world's economy but also impacts TB patient's socio-economic lifestyle. FDCs were created to improve patient compliance and minimise the appearance of drug resistance. This drug combination has a few shortcomings where rifampicin is a drug known for its problematic results regarding bioavailability in FDCs.

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Chapter 2

Solid-state properties of drugs

2.1 Importance of solid-state properties

The solid-state properties of a drug play an important role in pre-formulation of a pharmaceutical product and have an impact on manufacturing, formulation, and quality control of the drug to name a few. Byrn *et al.* (1994) also stated that the solid-state properties of a drug can influence the flow rate of the powder, the tableting performance, and the bulk density of the drug. These properties may affect the compression, solubility, and dissolution of the pharmaceutical active. Therefore, it is important to obtain information about the physical characterisation of a given drug during the pre-formulation stages.

Crystallography studies are used to examine the physical characterisation of drugs and are carried out to determine possible arrangement of atoms of solid drug forms (Brittain, 1997). Polymorphism can be described as different arrangements or conformations of a compound, in its solid-state, which present as different crystalline forms (Bernstein, 2002; Yu *et al*., 2002). As a result, different polymorphic forms can exhibit various physical, mechanical, and chemical properties.

During drug development it is standard and required to choose the most stable polymorphic form of the drug. The characteristics of various solid forms can be defined as compounds that are either polymorphs, solvates, desolvated solvates, or amorphous. Polymorphs are in the solid-state, that can exist in more than one form or crystal structure but they exhibit a matching chemical composition. Solvates/hydrates are solid forms that contain a solvent (water in the case of a hydrate). A desolvated or dehydrated solvate is when the solvent is detached from a solvate. Amorphous are considered a non-crystalline solid, it is a solid that lacks the long-range order characteristics of a crystal (Byrn *et al.,* 1994; Rodriguez & Wu, 1991). Due to these different physico-chemical properties of a drug, it is important to examine and regulate the characteristics of solid compounds to control the impact on the drug substance stability and bioavailability (Brittain, 1997).

2.2 Solid state-properties of rifampicin

Rifampicin, discovered in 1966, is considered one of the most effective and important broad-spectrum antibiotics and is a key component in fighting *Mycobacterium tuberculosis*. The discovery of rifampicin significantly reduced the period of tuberculosis (TB) chemotherapy. Rifampicin is freely distributed into soft tissue, living cells and bacteria, making it exceptionally effective against bacteria like *M. tuberculosis.* Rifampicin works by targeting DNA-dependent RNA polymerase, blocking the translocation step that follows development of the first

phosphodiester bond, inhibiting RNA elongation, and thus inhibiting transcription. However, there are some cases where bacteria developed resistance to rifampicin alone and fixed dose combinations (FDCs) were created to avoid drug resistance (Angiolini *et al.,* 2017; Campbell *et al.,* 2001; Du Toit, 2016).

2.2.1 Rifampicin and polymorphism

Polymorphism is reported for rifampicin, which is due to multiple hydrogen bonding possibilities, conformational changes, and the ionisation grade within this macro molecule. Two polymorphic forms were described in literature for rifampicin, i.e. polymorphic Form I and Form II. The commercially available form which is mainly used in manufacturing is Form II. Rifampicin can also form hydrates and solvates, which eventually transforms at room temperature after desolvation or dehydration into an amorphous form (Agrawal *et al.,* 2004).

Henwood *et al.* (2001) reported that the different polymorphic forms of rifampicin showed different solubility and dissolution properties. Also, batch differences were observed from a single manufacturer. Five different batches were evaluated by means of dissolution and solubility tests. Three of the batches contained Form II, the commercially preferred form and two batches contained a mixture of Form II and amorphous particles.

The solubility values obtained were 1.60 mg/ml for one batch and 1.51 mg/ml for two of the other crystalline batches (Form II). The solubility values of the two batches that contained Form II and an amorphous part were a bit higher and were determined as 1.74 mg/ml (Henwood *et al*., 2001). This could be expected due to the higher solubility normally observed with amorphous material.

With the same study it was interesting to note that the powder dissolution profiles in water were contradicting. The two batches with amorphous powder and Form II displayed much slower dissolution rates (unfortunately no mg/ml values were reported for the dissolution test, only percentage dissolved). According to the authors these results were unexpected, since amorphous powders are usually more soluble than crystals (Henwood *et al*., 2000).

The reason for the higher solubility values of the batches which contain amorphous particles could be because it entails rotation of the test tubes which could improve the wettability of the powders. This phenomenon caused us to question whether rifampicin raw materials may rather have wettability problems, as opposed to poor solubility.

2.2.2 Description of rifampicin

2.2.2.1 Appearance and colour

According to Nogueira *et al.* (2018) rifampicin can be identified as a red-orange to red-brown even brick red, crystalline powder with no odour or smell.

2.2.2.2 Chemical name

The chemical name for rifampicin is either:

3-[[(4-Methyl-1-piperazinyl)imino]methyl]rifamycin

or

5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22– heptamethyl-8-[N-(4-methyl-1-piperazinyl)formimidoyl]-2,7(epoxypentadeca [1,11,13]trienimino)naphtho[2,1-b]furan-1,11(2H)-dione 21acetate (Nogueira *et al.,* 2018).

It is also known as rifampin.

2.2.2.3 Structural formula

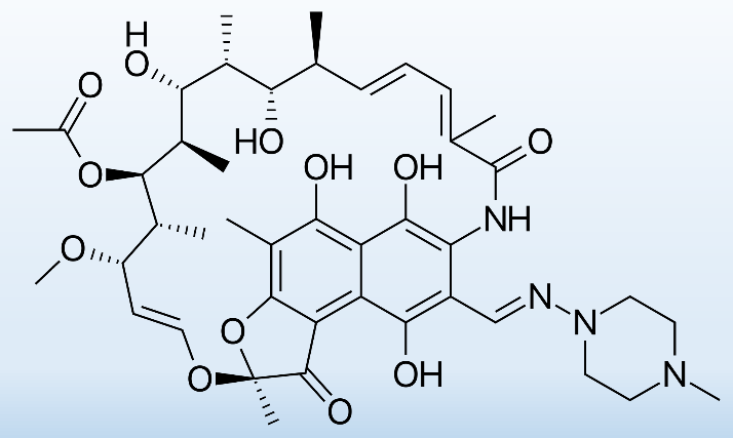

Figure 2.1: Structural formula of rifampicin (Adapted from Chrencik *et al*., 2005).

2.2.2.4 Molecular weight

822.94 g/mol (BP, 2020).

2.2.2.5 Empirical formula

$C_{43}H_{58}N_4O_{12}$ (BP, 2020).

2.2.2.6 Melting point

183 to 188°C (BP, 2020).

2.3 Biopharmaceutics classification system

The Biopharmaceutics Classification System (BCS) is a regulatory mechanism by which drugs are categorised into four classes based on their *in vitro* permeability and water solubility data:

- Class I - High permeability, high solubility. Higher absorption rates than excretion rates are characteristic of the compounds in this class.
- Class II - High permeability, low solubility. These compounds' solvation rate is the limiting factor for their bioavailability.
- Class III - Low permeability, high solubility. These compounds go into solution fast, but the permeability limits their absorption.
- Class IV - Low permeability, low solubility. These compounds have poor bioavailability (Yu *et al.,* 2002).

Rifampicin is the only hydrophobic active of the FDC product and belongs to Class II. Isoniazid, pyrazinamide, and ethambutol hydrochloride belong to Class I of the BCS, i.e. they are highly soluble and highly permeable (Yu *et al.,* 2002).

2.4 Solubility

Solubility equilibrium is that point where a solid is unable to dissolve further and unable to crystallise. Therefore, equilibrium refers to a condition of saturation. When the solution of a solid substance is in equilibrium, solubility can be referred to as the concentration thereof (Byrn *et al.,* 1994). Yu *et al.* (2002) defined solubility as "the amount of a drug that can be dissolved in a solvent".

2.4.1 Solubility of rifampicin

Rifampicin is described as sparingly soluble in water and it is soluble in methanol and in chloroform (Reynolds, 1989). Literature showed solubility values of rifampicin at 1.4 mg/l (25°C) (Yalkowsky *et al.,* 2019) and 1.5-1.74 mg/ml in water (Henwood *et al*., 2001).

As stated previously, rifampicin is a BCS Class II compound as it is slightly soluble in water with high intestinal permeability (Panchagnula & Agrawal, 2004). It is important for a drug to have high solubility rates because improved solubility is usually associated with better bioavailability which further results in a therapeutic effect (Chiappetta & Sosnik, 2007). With rifampicin having poor soluble characteristics the therapeutic effectiveness of the drug is negatively impacted (Theja *et al.,* 2012).

2.5 Stability of rifampicin

Bhutani *et al.* (2004) showed that rifampicin is light sensitive, and that degradation of rifampicin increased in the presence of light. Furthermore, rifampicin is also not very stable in an acidic environment and the degradation is pH dependent. Studies showed that 8.5% to 50% of rifampicin degraded during the gastric emptying time in humans. In the acidic environment of the stomach,

rifampicin hydrolyses to the poorly soluble 3-formyl rifamycin SV (3-FRSV) where isoniazid accelerates the breakdown of rifampicin into this poorly absorbed product. One of the major reasons for the poor bioavailability of rifampicin is due to the degradation of rifampicin in an acidic pH (Rajaram *et al.,* 2014).

Rifampicin also undergoes oxidation in alkaline medium and it involves the autoxidation of the hydroquinone group in the naphthyl core to form inactive quinone derivative (Angiolini *et al.,* 2017).

2.6 Conclusion

The physico-chemical properties of a compound are an essential matter to consider, mainly during pre-formulation and manufacturing of such a product. It is important to understand the impact of different solid-state forms and the implications thereof, on the pharmaceutical manufacturing processes. Investigation of the solid-state characteristics of a product will ensure quality and stability of the end-product.

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BP see British Pharmacopoeia

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Chapter 3

Solubility and wettability

3.1 Introduction

In the pharmaceutical field, wettability describes the interaction between a solid (powder) and a liquid. The degree of hydrophobicity influences the solubility of an active pharmaceutical ingredient (API) in the dissolution media (Yarce *et al*., 2016). In literature, few or minimal mention is made of the wettability properties of rifampicin. The extreme hydrophobicity of rifampicin could be the cause of many poor dissolution and/or solubility results reported for rifampicin over the years.

Previously, Henwood *et al.* (2001) investigated the physico-chemical properties of rifampicin when crystallised from various solvents, and 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, according to the authors, 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). Unfortunately, no wettability studies were performed on the rifampicin powders tested in this particular study.

3.2 Solubility and wettability properties

There are many factors that have an influence on the bioavailability of a drug, including dissolution, solubility, and permeability, all of which are crucial steps in the therapeutic outcome of a drug (Smith, 2015; Song *et al.,* 2004). The solubility of active pharmaceutical ingredients (APIs) is important to determine the performance of a drug (Heng *et al*., 2006). The dissolution rate of a solid can be defined as “The rate at which the solute is broken down to individual ions, atoms or molecules to finally form a homogenous phase with the solvent” (Smith, 2015).

This definition is described by the Noyes-Whitney equation as:

$$\frac{dC}{dt} = k(C_s - C_b) \quad (1)$$

Where:

- dC/dt refers to drug dissolution rate,
- k as the constant for dissolution rate,
- C_s represents the drug concentration in the stagnant layer,
- C_b the drug concentration in bulk of the solution at a certain time (Smith, 2015).

Dissolution is only described by Noyes-Whitney through a single equation. Dissolution for tablets and capsules can be divided into three steps, i.e. disintegration, disaggregation and dissolution.

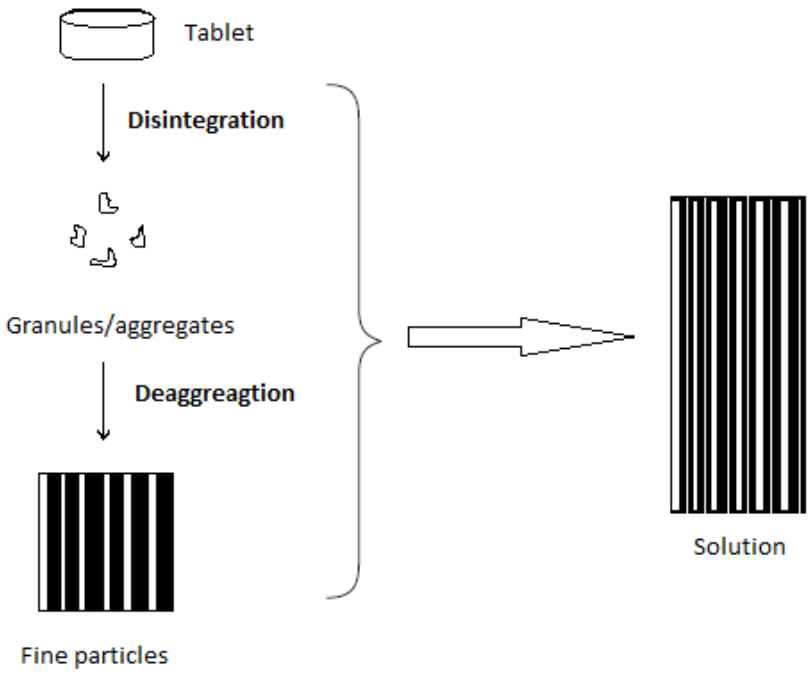

Figure 3.1: The steps for tablet dissolution (adapted from Smith, 2015).

The Noyes-Whitney equation focuses on the most important step of dissolution namely the movement of fine particles into solution. However, for the fine particles to be able to form from granules or aggregates, wetting needs to occur. Wetting is the ability of a liquid to relocate air and expand on a solid surface. The process of fine particle dissolution takes place in three different steps. The first step is wetting, followed by immersion whereas the final step is diffusion of particles of solute, into a bulk solution.

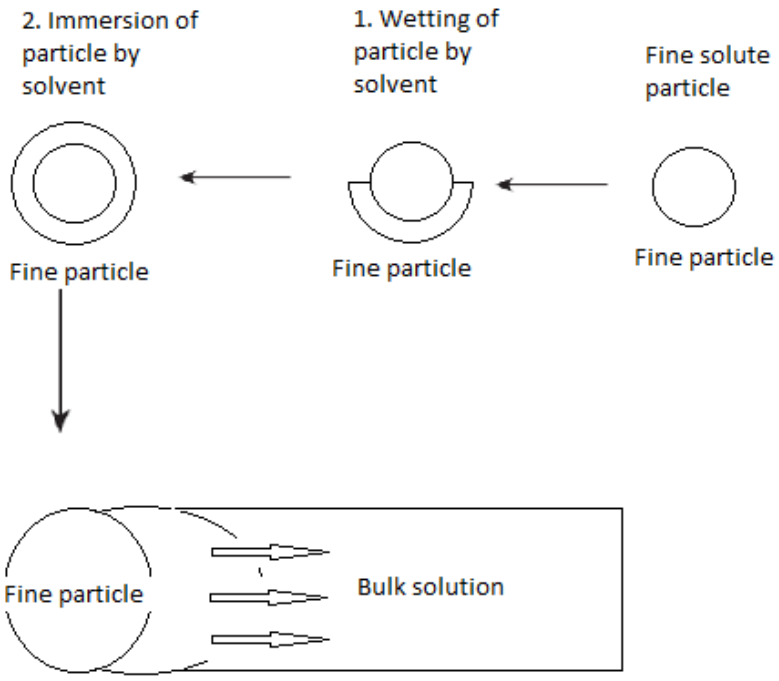

Figure 3.2: Three steps of fine solute particle dissolution (adapted from Smith, 2015).

As a result, the dissolution rate of a drug is primarily dependent on the wettability of a drug. Without wettability the other steps will not be able to take place. Wettability of powders is usually determined by using a solid-liquid contact angle, which are indicative of the drug's hydrophobicity.

Effective wetting occurs when the solid/liquid adhesive forces are greater than the liquid cohesive and solid/gas adhesive forces (Lazghab *et al.,* 2005). Contact angle measurements (Figure 3.3) can be a useful tool for analysing qualitative differences in wetting of drug substances (Luner *et al*., 1996).

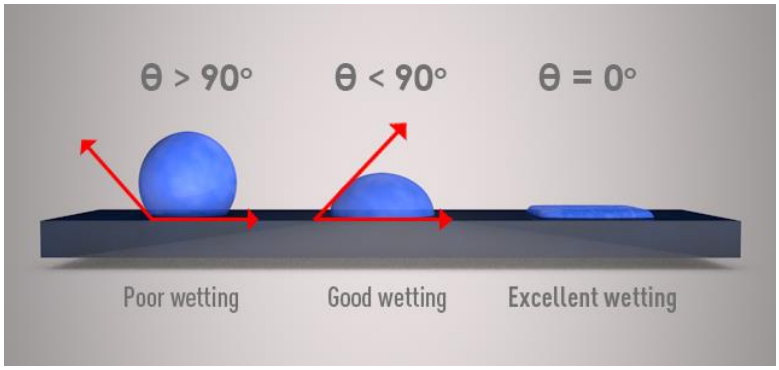

Figure 3.3: Different contact angles explaining wettability (Anon, 2019).

It can therefore be concluded that poor wetting properties could lead to a poor dissolution rate and subsequently lead to reduced bioavailability of the drug. Enhancing the wettability and solubility of rifampicin could potentially result in improved therapeutic outcomes (Onoue *et al.,* 2012; Patil & Suresh, 2009).

3.3 Enhancing rifampicin wettability

The poor wettability and solubility of rifampicin, in aqueous solutions, are main issues that need to be dealt with before any further steps are taken towards improving the other inadequacies of the drug. Surfactants like sodium lauryl sulphate (SLS) are commonly used in dissolution media for water insoluble drugs and therefore could improve the wettability of rifampicin (Zhao *et al*., 2004).

3.3.1 Use of surfactants in the pharmaceutical industry

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 possessing two distinct regions (Figure 3.4), namely hydrophilic (polar) and hydrophobic (non-polar) (Sekhon, 2013).

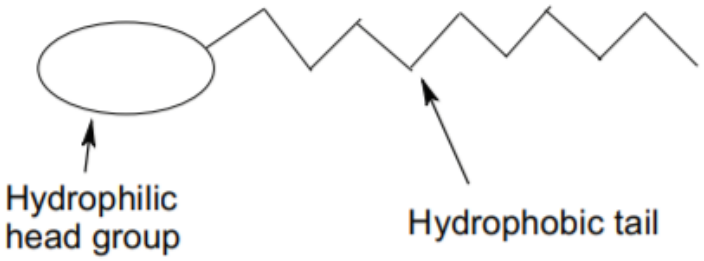

Figure 3.4: Illustration of a surfactant with two regions (adapted from Sekhon, 2013).

The hydrophilic group of surfactants can be grouped into four classes namely: anionic (negatively charged), cationic (positively charged), non-ionic surfactants (uncharged) and zwitterionic (both positively and negatively charged) (Sekhon, 2013).

3.3.2 Surface area tension

Surface area tension plays an important role for the ability for a solvent to wet. In a liquid the surface tension is caused by the attraction of the particle in the surface layer by the bulk of the liquid, the ultimate goal is to minimise surface area. This explains why liquids try to reach as small as surface area as possible, because energy is required to increase the surface. The same principle applies to solid surface tension, except that it is difficult to measure the quantity work/energy necessary to increase the surface. The reason is because it is difficult to separate

the bulk phase. By using contact angles, the wettability of the solid can be measured (Thomsen, 2008).

As illustrated in Figure 3.5, surfactants are compounds that can be used to decrease the surface tension between the liquid medium and the drug, making it ideal for compounds that are poorly soluble in water. Consequently, surfactants also improve solubility and make it suitable to overcome the poor wettability property of an API (Sekhon, 2013).

Surfactants may perform as wetting agents, foaming agents, emulsifiers, detergents, or dispersants. During manufacturing of self-care products like soaps, facial cleaners, shower gels and detergents, surfactants are commonly added (Cross, 1998).

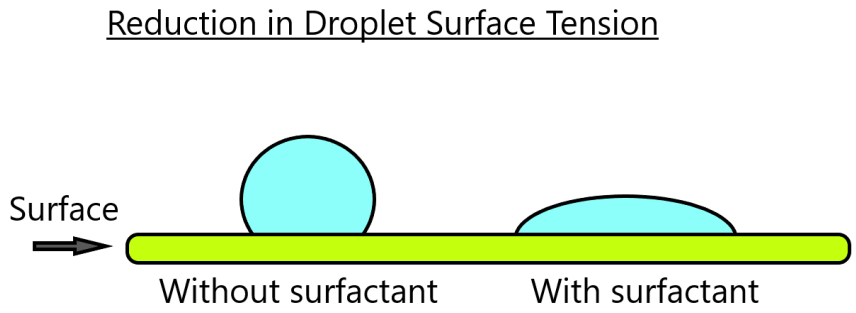

Figure 3.5: Illustration of reduction in droplet surface tension with and without surfactant (adapted from Lee, 2008).

In a previous study, the wettability of rifampicin powder was investigated by using various concentrations of surfactants and polyhydroxy compounds. A surfactant (polysorbate 80) was added as a wetting agent in a rifampicin suspension to increase the dissolution rate of the drug (Lubna *et al.,* 2006). Prasanthi *et al*. (2014) studied the solubility and stability of the FDC in different aqueous media containing SLS and ascorbic acid. It was found that high concentrations of SLS tend to reduce the surface area tension of the poorly water-soluble drugs (Prasanthi *et al*., 2014). However, these studies were conducted on the FDC tablet and a rifampicin suspension and not on the rifampicin raw material itself. In this study, the surfactant SLS will be used as a wetting agent to try and enhance the wettability of rifampicin powder.

3.4 Sodium lauryl sulphate

3.4.1 Appearance and colour

Sodium lauryl sulphate (SLS) consists of white/cream to light yellow coloured crystals, flakes, or powder having a smooth/soapy feel with a bitter taste (Rowe *et al*., 2006).

3.4.2 Chemical name

Sulfuric acid monododecyl ester sodium salt (Rowe *et al*., 2006).

3.4.3 Structural formula

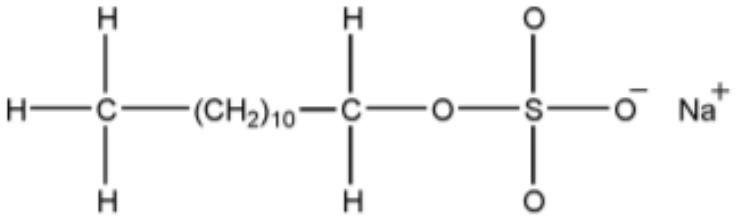

Figure 3.6: Structural formula of SLS (Adapted from Rowe *et al*., 2006).

3.4.4 Molecular weight

288.38 g/mol (Rowe *et al*., 2006).

3.4.5 Empirical formula

$C_{12}H_{25}NaO_4S$ (Rowe *et al*., 2006).

3.4.6 Melting point

204 to 207°C (Rowe *et al*., 2006).

3.5 Uses of sodium lauryl sulphate

Sodium lauryl sulphate (SLS), or sodium dodecyl sulphate, is an anionic surfactant which is Generally Regarded as Safe (GRAS). SLS is commonly used as an emulsifying cleaning agent, penetration enhancer, solubilising agent, tablet, and capsule lubricant (Bondi *et al*., 2015). Although there are no reported adverse reactions with SLS used in tablets and capsules, there are safety limits for the use of SLS in skin products such as creams, ointments, gels, and shampoos (Lee & Maibach, 1995).

Table 3.1: Uses of SLS and the concentrations (Rowe *et al*., 2006)

Use	Concentration
Skin cleanser in topical products	1% $^{w}/_{v}$
Tablet lubricant	0.5 - 2% $^{w}/_{w}$
Wetting agent in toothpastes	1 - 2% $^{w}/_{w}$
Releasing agent in suppositories	0.4 - 1% $^{w}/_{w}$
Wetting agent in solid oral dosage forms	0.2 - 1.5% $^{w}/_{w}$
Foaming agent in shampoos	10 - 25% $^{w}/_{w}$

3.6 Conclusion

The poor wettability and solubility of rifampicin, in aqueous solutions, are two separate issues which could be interlinked with each other. The poor solubility could be a result of poor wettability. These issues need to be dealt with before any further steps are taken towards improving the bioavailability of the drug. SLS could be used for poorly water-soluble drugs and therefore could lead to the improvement of the wettability, solubility, and bioavailability of rifampicin.

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Chapter 4

In vitro drug permeation

4.1 Introduction

Oral drug delivery is the most suitable route of administration for patients. However, drug delivery must conquer several biological obstacles including, the acidic gastric environment and the constant secretion of mucus that protects the gastrointestinal tract. The drug itself needs to overcome obstacles like, poor solubility, stability, and bioavailability which make achieving therapeutic levels via the gastrointestinal tract challenging. Drug solubility and permeability are two important rate-limiting steps following oral drug administration (Bergström, 2004; Ensign *et al*., 2012; Pidgeon *et al.,* 1994; Sastry *et al*., 2000).

Drug absorption is the process where drug molecules are moved from the intestine into the bloodstream. Numerous variables impact this process, including a medication's physico-chemical properties, drug formulation and drug administration route. Several physico-chemical properties have been associated with poor membrane permeability, for example: the presence of strongly charged functional groups, high molecular weight, many hydrogen-bonding functional groups, and high polarity of the drug surface area (Aungst, 2000). Drugs that are orally administered normally need to cross the intestinal epithelial layer before entering the blood. Drugs which are administered via intravenous treatment, intramuscular infusion, and enteral nutrition, are directly available in the bloodstream. Regardless of the administration route, it is important for a drug to be dissolved, contain high permeation characteristics and to be absorbed to achieve a therapeutic effect (Paine *et al*., 2004).

Oral drug delivery stays the desired route of drug delivery. This makes intestinal absorption one of the first properties to be studied on a molecular level for new drugs during drug development (Sastry *et al*., 2000).

4.2 Drug permeation

Intestinal permeation can be referred to as the movement of drug molecules across the cell membranes and the depth of the substance penetration per time unit (Dahan & Miller, 2012).

The permeation of a drug across the epithelial cell membrane of the intestine can occur through different mechanisms, such as phagocytosis, facilitated transport, active transport, and passive diffusion (Hunter & Hirst, 1997).

In Equation 2, it can be seen that drug permeation (Pm), is directly proportional to the mass transfer coefficient (Km). Passive drug diffusion through cell membranes not only depends on Km, but also on the membrane diffusion coefficient of the solute (Dm). Molecular particle size and molecular mass are important factors which will influence the Dm. When molecular mass

increases, Dm intensely decreases. This leaves the quantity of drugs, left for absorption into the bloodstream, low (Yamashita *et al.,* 2000).

$$Pm = \frac{DmKm}{L} \quad (2)$$

Where:

- Pm refers to drug permeation,
- Dm represents the membrane diffusion coefficient of the solute,
- L the membrane thickness and
- Km the mass transfer coefficient (Yamashita *et al.,* 2000).

The combined outcomes of passive diffusion and multiple parallel transport impact the effective permeation of drugs (Lennernäs, 2013).

4.3 Permeation enhancers

There is an interest and a medical necessity for improving the bioavailability of oral drugs. Increasing oral bioavailability is therapeutically essential because the amount of drug bioavailability directly impacts the plasma concentrations, as well as the therapeutic and toxic effects, after oral drug administration. As seen in Table 4.1, there are different methods that can be used to enhance drug permeation (Anilkumar *et al.,* 2011).

Table 4.1: Intestinal permeation enhancers and classification

Enhancers	Classification
Surfactants	**Ionic:** Sodium lauryl sulphate, sodium dodecyl sulphate & dioctyl sodium sulfosuccinate. **Nonionic:** Polysorbitate, nonylphenoxypolyoxetyylenes & Tween 80.
Bile salts & its derivative	Sodium glycholate, sodium deoxycholate, sodium taurocholate, sodium dihydrofusidate & sodium glycodihdrofusidate.
Fatty acids & its derivatives	Oleic acid, caprylic acid, lauric acids, sodium caprate, acyl carnites & acyl choline.
Chelating agents	EDTA, citric acid & salicylates.
Chitosans & derivatives	N-sulfanto-N,O-carboxymethylchitosan, N-trimethylated chloride(TMC) & chitosan glutamate.
Other enhancers	Zonula occludens toxin (Zot) & polycarbophyl-cysteine conjugate (PCP-Cys).

4.3.1 Surfactants and permeation

As explained in the previous chapter surfactants can be described as agents that lower the surface tension of drugs. Studies have shown that surfactants are important in drug development because of their affinity for membranes and their ability to increase the permeability of membranes (Florence & Gillan 1975). To achieve a high level of bioavailability there should be an increase in drug absorption, and this is usually a result of an increase in drug permeability, whereas surfactants are commonly used to achieve permeability by lowering the surface area tension (Sekhon, 2013). Boulernc *et al.* (1995) showed that sodium lauryl sulphate (SLS) can increase the paracellular transport route. SLS could therefore potentially be used to improve rifampicin's permeability.

4.4 *In vitro* methods for studying drug permeability

Drug absorption studies can be carried out by using artificial membranes such as epithelial cell cultures, immobilised phospholipids, or liposomes, or cultured cells (Anilkumar *et al.,* 2011). We will be using a Caco-2 cultured cell technique in this study.

4.4.1 Caco-2 permeability

Cultured mammalian cells have been applied to estimate intestinal drug permeability across cell membranes. The Caco-2 cell line, originally obtained from human colon carcinoma, is the cell model most frequently used for *in vitro* intestinal permeation studies. Caco-2 cells express uptake and efflux transporters, which are important for predicting drug absorption in humans. Like the human small intestine, this cell monolayer presents with tight junctions, microvilli, and increased brush border hydrolytic enzymes. Drug permeation studies are performed using this cell line due to its similarity to human small intestinal epithelium, when cultured as an intact monolayer (Küblbeck *et al*., 2016; Shah *et al*., 2006). Recently, pharmaceutical companies have increasingly started to culture Caco-2 cell lines as intestinal epithelial models. Caco-2 cell monolayers have been broadly acknowledged as a powerful *in vitro* model to assist in drug development, as it is an efficient way to evaluate characteristics like drug absorption and metabolism (Yamashita *et al.,* 2000).

Drugs can be transported across the intestinal epithelium by a minimum of one out of four different routes, namely the passive transcellular, paracellular, carrier mediated and transcytosis routes. All four routes can be studied via Caco-2 monolayers as illustrated in Figure 4.1. Most drugs given orally are transported by the passive transcellular route across the intestinal mucosa (Artursson *et al.,* 1996).

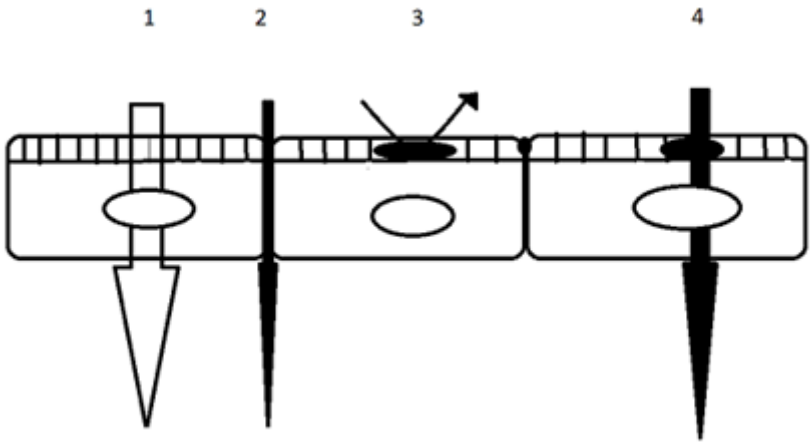

Figure 4.1: Mechanisms of intestinal drug permeability (adapted from Artursson *et al.,* 1996). 1. Passive transcellular and 2. paracellular transport routes; 3. carrier mediated and 4. transcytosis transport routes.

4.5 Conclusion

By making use of the surfactant SLS, the surface tension of rifampicin could be lowered and resulted in an improvement of wettability of the drug. This could further lead to an increase in drug permeability, and consequently lead to the increased bioavailability of the drug. This hypothesis will be tested using the Caco-2 model.

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Chapter 5

Materials and methods

5.1 Introduction

Rifampicin raw material was purchased from DB Fine chemicals (Johannesburg, South Africa) and after polymorph screening, it was determined to be identical to that of the commercially available form (Form II) used in tablets. This study was conducted in two parts: firstly to determine the wettability of rifampicin with different concentrations of a surface active reagent, and secondly to test the best combinations for their *in vitro* drug permeation abilities using an *in vitro* Caco-2 cell culture model.

5.2 Materials

The various raw materials and consumables used for the dissolution and cell culture maintenance and experiments, are listed in Tables 5.1 and 5.2 respectively.

Table 5.1: List of materials used in the dissolution experiments

Materials	Manufacturer / Supplier	Batch number
Rifampicin raw material	DB Fine Chemicals	RMP/XP/003-12
SLS (sodium lauryl sulphate)	Ace	151-21-3
Methanol HPLC grade	Merck	1.06007.2500

Table 5.2: List of consumables used for the cell culture maintenance and experiments

Materials	Manufacturer / Supplier	Catalogue number
Dulbecco's Modified Eagle's Medium (DMEM)	HyClone	SH30243.01
Phosphate buffered saline (PBS)	HyClone	SH30256.01
Penicillin/Streptomycin	Lonza	DE17-602E
Non-essential amino acids (NEAAs)	Lonza	BE13-114E
L-glutamine	Lonza	BE17-605E
Foetal bovine serum (FBS)	Gibco Life technologies	10270-106
Trypsin EDTA	Lonza	BE17-161E
Trypan Blue	Merck, Sigma-Aldrich	T8154
HEPES Buffer	Corning	25-060-Cl

5.3 Methods

5.3.1 Powder dissolution studies

A LABINDIA DS8000 (Labindia Equipments, Maharashtra, India) dissolution bath was used for dissolution testing. USP apparatus 2 (paddle) was set up at 37°C ± 2°C with a rotational speed of 75 rpm, 900 ml dissolution media (distilled water and various concentrations of SLS) was added to each dissolution vessel, respectively. A rifampicin powder mass (2 g), which was determined experimentally from the preceding solubility studies, was used. Five ml of solution was withdrawn from each dissolution vessel at predetermined time intervals. The dissolution medium was not replaced after each withdrawal since a supersaturated solution is required to observe solution-mediated transformations. After withdrawal, the samples were filtered through a 0.45 µm PVDF filter into a vial and suitably diluted to fit into the calibration curve. The diluted solutions were analysed with a UV-VIS Shimadzu spectrophotometer 1800 (Kyoto, Japan). For this study rifampicin samples were measured at a wavelength of 336 nm in a 1 cm quarts cuvette. The method was validated in-house for suitability by preparing a five point standard curve for each of the dissolution media.

5.3.2 *In vitro* drug permeation studies

5.3.2.1 Culturing of Caco-2 cells

The Caco-2 cell line was obtained from the European Collection of Cell Cultures (ECACC) through Sigma-Aldrich, Johannesburg, South Africa. The cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) (Separations, Randburg, South Africa), supplemented with 10% foetal bovine serum (FBS) (The Scientific Group, Johannesburg, South Africa), 1% non-essential amino acids (NEAA) (Whitehead Scientific, Cape Town, South Africa), 1% Penicillin/Streptomycin (Whitehead Scientific, Cape Town, South Africa) and 2 mM L-glutamine solution (Whitehead Scientific, Cape Town, South Africa). The cells were cultured at 37°C in a 95% humidified atmosphere with 5% CO_2 in a Galaxy 170R incubator (Eppendorf Company, Stevenage, United Kingdom). The growth medium was exchanged every second day under sterile conditions, and sub-cultured through trypsinisation at 50-60% cell confluency. Before each media change, the cells were inspected under an inverted light microscope for any unwanted contamination.

5.3.2.2 Sub-culturing Caco-2 cells

The culture media was removed, and the cells were washed twice with 5 ml PBS. 1 ml Trypsin was added and the cells were incubated for 4-5 min. Following detachment of the cells, 5 ml media was added and the cell suspension transferred to a 15 ml tube. The cells were centrifuged for 5 min at 140 x *g*. The media was removed by decanting and 5 ml culture media was added to resuspend the cell pellet. The cell suspension was then aliquoted into new flasks and growth medium added accordingly.

5.3.2.3 Seeding Caco-2 cells in 6-well Transwell® plates

A cell suspension was prepared and the viable cell count determined with a Trypan blue (Sigma Aldrich) exclusion method using a haemocytometer and an inverted light microscope (Nikon Eclipse TS100/TS100F, Nikon Instruments, Tokyo, Japan). The cell suspension was subsequently diluted to 20 000 cells per ml in culture media and then seeded onto Transwell® 6-well plate membranes (Corning Costar® Corporation, Tewksbury, MA, USA) with a pore diameter of 0.4 µm and a surface area of 4.67 cm^2. Cells were seeded by pipetting 2.5 ml of the final cell suspension into each apical chamber of the membrane filter plate wells, and 2.5 ml growth media was dispensed into each basolateral chamber. The Caco-2 cells were maintained for 21 to 24 days to ensure the formation of intact epithelial cell monolayers. Growth medium was replaced every second day under sterile conditions.

5.3.2.4 Permeability studies

Prior to and during the 120 min transport studies, the integrity of the monolayers was confirmed by measuring the transepithelial electrical resistance (TEER) of each cell monolayer using a Millicell ERS II meter (Millipore, Billerica, MA, USA). A measured resistance of higher than 150 Ω (700.5 Ω·cm^2) was acceptable (Alqahtani *et al.,* 2013). The integrity of the model was also confirmed with Lucifer yellow permeation (50 µg/ml in non-additive culture media) (Bhushani *et al.,* 2016). Lucifer yellow is an exclusion marker molecule and a transport percentage of less than 2% (Wahlang *et al.,* 2011) or an apparent permeability coefficient (P_{app}) of less than 0.66-0.75 x 10^{-6} cm/s (Bhushani *et al.,* 2016) are indicative of an intact cell monolayer.

Mono-directional permeation of rifampicin was studied in the apical-to-basolateral (AP-BL) direction (uptake). The culture medium was removed from the basolateral compartments of the Transwell® plates, and a volume of 2.5 ml pre-heated DMEM buffered with HEPES (2-[4-(2-hydroxyethel)piperazin-1-yl] ethanesulfonic acid) was added to the basolateral compartments. Then the plate was placed in a CO_2 incubator to equilibrate for 30 min at 37°C. After this, the culture medium was removed from the apical compartment and replaced with 2.5 ml of each test solution in DMEM only. A sample (200 µl) was withdrawn from the basolateral compartment at time intervals of 20, 40, 60, 80, 100 and 120 min, and replaced with 200 µl of pre-heated DMEM buffered with HEPES. Samples withdrawn at the time intervals described above was analysed by means of a Spectramax® Paradigm plate reader (Molecular Devices®, Separations, Gauteng, South Africa) at an absorbance wavelength of 336 nm and 450 nm. The Lucifer yellow concentrations were quantified with fluorescence excitation and emission wavelengths of 485 and 535 nm, respectively (Wahlang *et al.,* 2011).

5.3.2.5 Data analysis of the permeability findings

The quantified rifampicin or Lucifer yellow concentrations obtained with the microplate reader, were corrected for the withdrawal and dilution during the experiments. This was followed by

determining the accumulative percentage transport at each time point with respect to the initial administered concentration added to each well.

The P_{app} values were then calculated using the following equation (Kotzé *et al.,* 1998):

$$Papp = \left(\frac{\frac{dQ}{dt}}{A * C0 * 60} \right) \quad (3)$$

Where:

- P_{app} represents the apparent permeability coefficient ($cm \cdot s^{-1}$),
- dQ/dt portrays the permeability rate,
- C_0 is the initial administered drug concentration and the 60 corrects for the expression of the P_{app} values as $cm \cdot s^{-1}$ and
- A shows the surface area of the membrane (cm^2).

All the transport experiments were conducted in triplicates.

5.4 Conclusion

All the experiments were performed according to the methods described in this chapter, and the results of the experiments will be discussed in Chapter 6 and Chapter 7.

5.5 References

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Chapter 6

Dissolution studies

6.1 Introduction

Literature in previous chapters shows clearly that rifampicin is poorly soluble in water. Dissolution testing is developed to guarantee product performance, product development and quality control of the product (Kulkarni *et al*., 2012; Sharma *et al*., 2012).

Dissolution testing with rifampicin was performed to determine the solubility of the drug in water, and in the different concentrations of a surface active agent i.e., sodium lauryl sulphate (SLS). To understand the results found during the dissolution studies, background of the term “spring and parachute” will be explained.

Guzmán *et al.* (2007) explained the “spring and parachute” phenomenon shown in Figure 6.1. Metastable compounds like amorphous compounds initially display peak solubility but rapidly drop to the low solubility of the crystalline form. Thus, causing the hydrophobic drug particles to become supersaturated in the aqueous medium. This is known as the “spring” phenomenon and the particles immediately precipitates and forms loosely gathered clusters. The “parachute” phenomenon is when this supersaturated state of the drug particles is maintained for an adequate period of time (Figure 6.1).

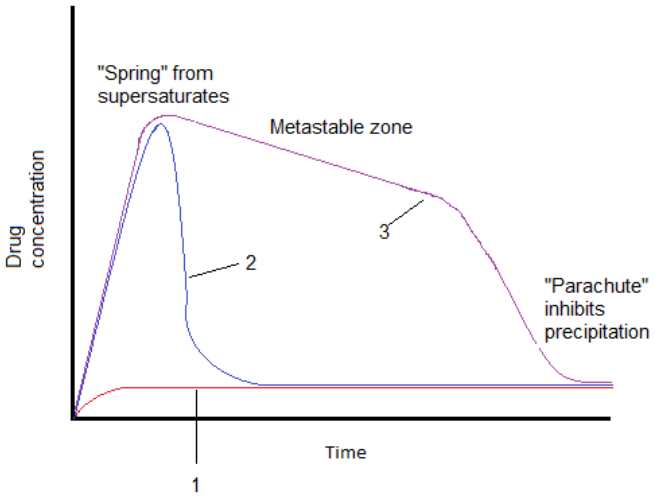


Figure 6.1: ‘‘Spring and Parachute’’ method to uphold and maintain supersaturation (Bavishi & Borkhataria, 2016).

1. The stable crystalline form has low solubility. 2. An amorphous phase displays peak solubility but immediately drops to the low solubility of the crystalline form. 3. Highly soluble drug forms are sustained for a long period of time in the metastable zone.

Solubility class limits are based on the highest dose strength of an instant release drug. A drug is considered as highly soluble when the highest dose strength is soluble in ≤250 ml of water over a pH range of 1.0-7.5. Drugs that are low in solubility are soluble in ≥250 ml water over the equal pH range. The volume estimate of 250 ml is consequent from distinctive bioequivalence study protocols that recommend drinking the drug with a glass of water.

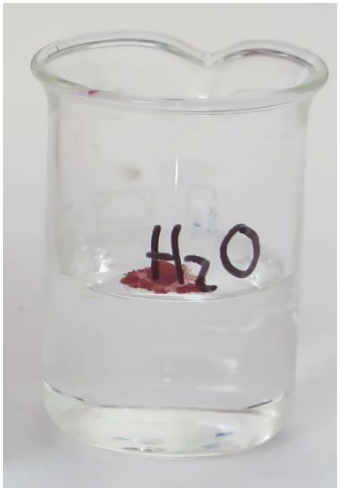
A drug can be determined as rapidly dissolving when a minimum of 85% of the drug substance dissolves within 30 min in 900 ml of three distinct buffers (0.1 M HCl, pH 4.5 buffer, pH 6.8 buffer) (Volpe, 2008).

6.1.1 Dissolution study results

For this study, the powder dissolution of rifampicin in water and a series of rifampicin with different SLS concentrations were evaluated. The dissolution in water was used as control. Before starting the dissolution in water experiments, a series of tests were performed to make sure that the optimum saturation concentration for rifampicin was achieved in 900 ml water. After several experiments and visual observations, 2 g was decided as the optimum quantity to use for the powder dissolution and SLS experiments.

6.1.1.1 Pre-screening of the SLS concentrations

The SLS concentrations of 0.01%-2% in water were screened beforehand with a series of in-house experiments. An amount (± 54 mg) of rifampicin powder was put into a small glass beaker containing either water or the different concentrations of SLS. Pictures were taken every 15 seconds. No mechanical stirring was involved. The results of the visual screening tests are presented below.



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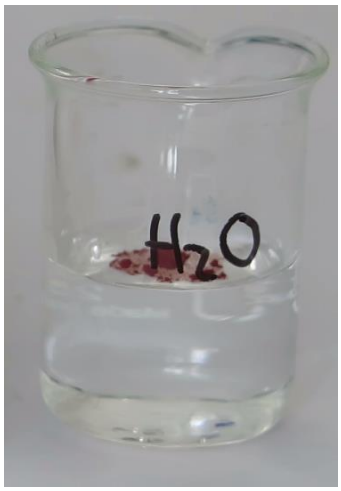


Time = 15 sec

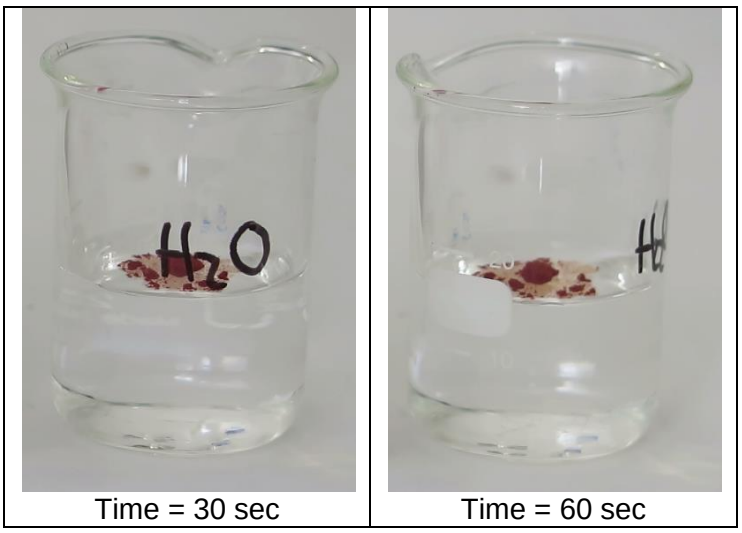


Figure 6.2: Pre-screening test of rifampicin powder in water.

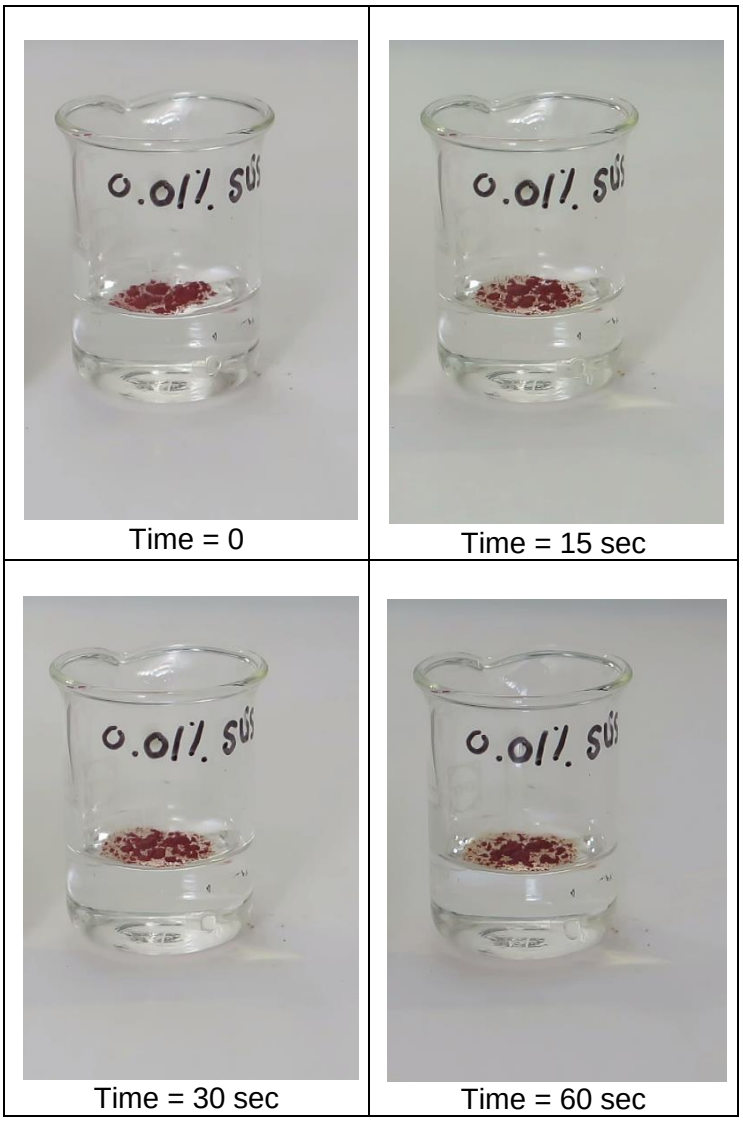


Figure 6.3: Pre-screening test of rifampicin powder in 0.01% SLS and water.

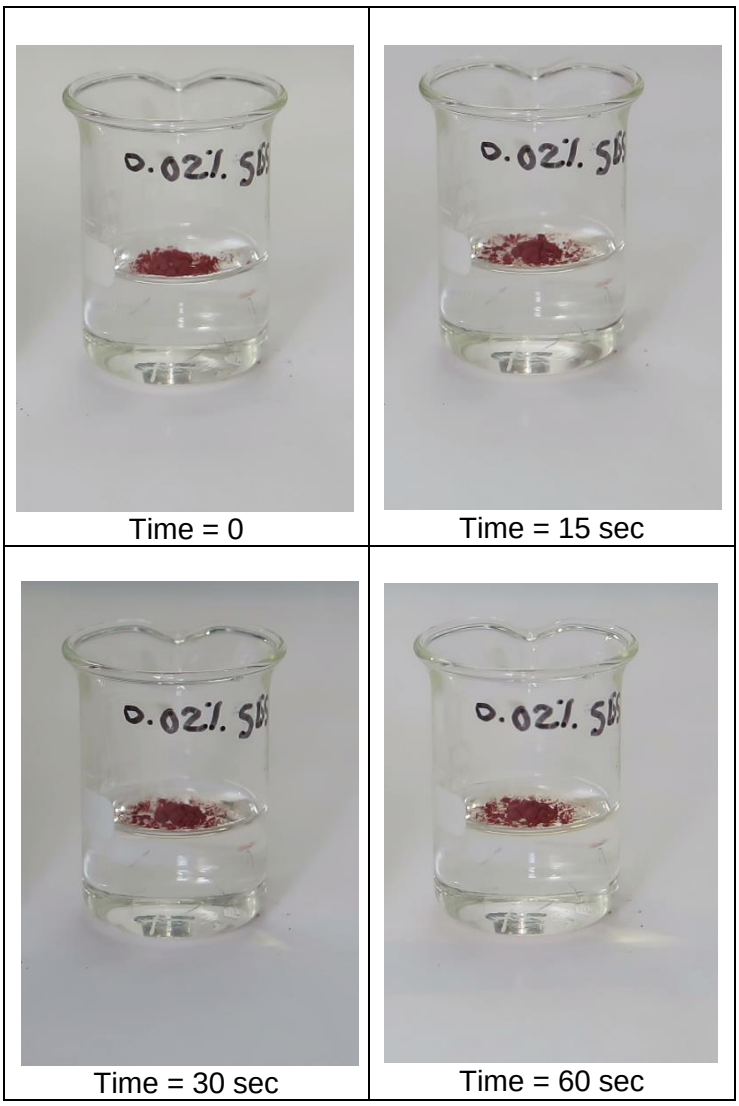
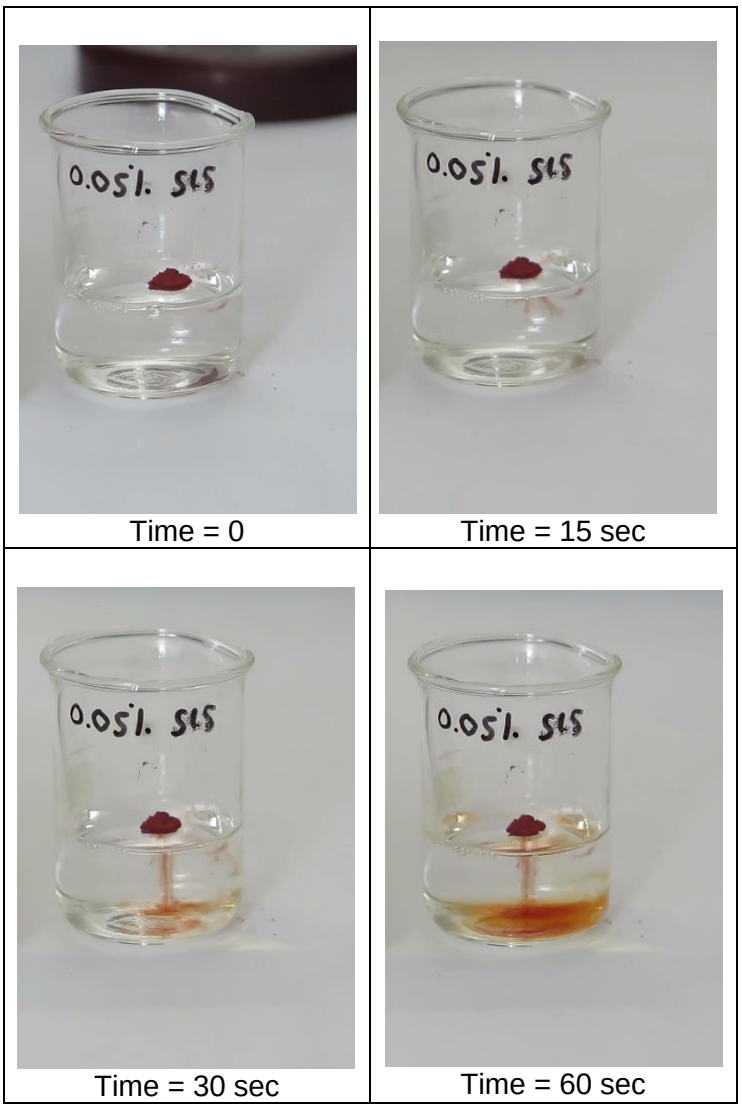


Figure 6.4: Pre-screening test of rifampicin powder in 0.02% SLS and water.

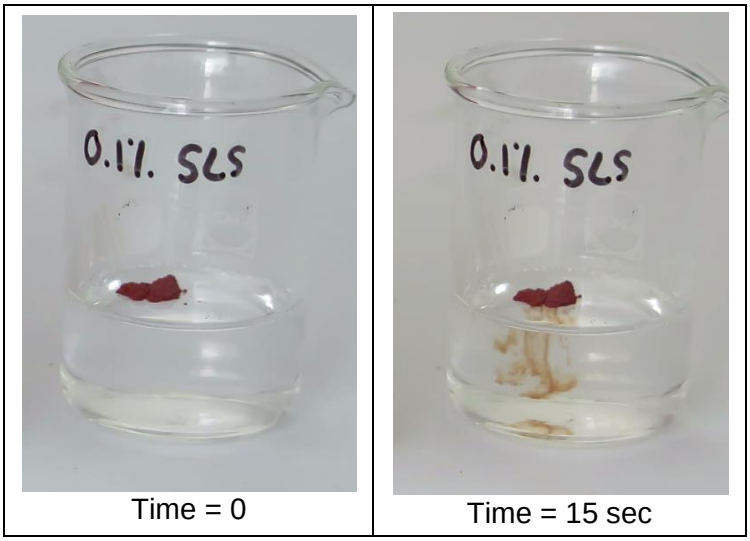
Figure 6.5: Pre-screening test of rifampicin in 0.02% SLS in water after 147 sec.



Time = 0 | Time = 15 sec

Time = 30 sec | Time = 60 sec

Figure 6.6: Pre-screening test of rifampicin powder in 0.05% SLS and water.



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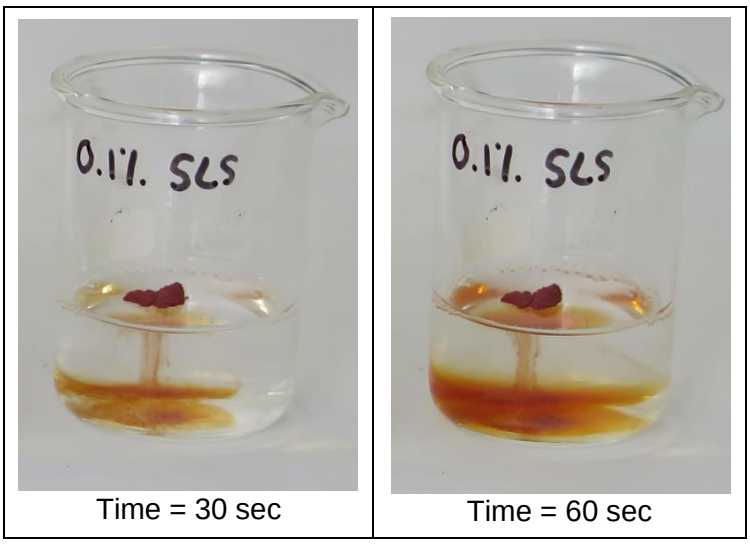


Figure 6.7: Pre-screening test of rifampicin powder in 0.1% SLS and water.

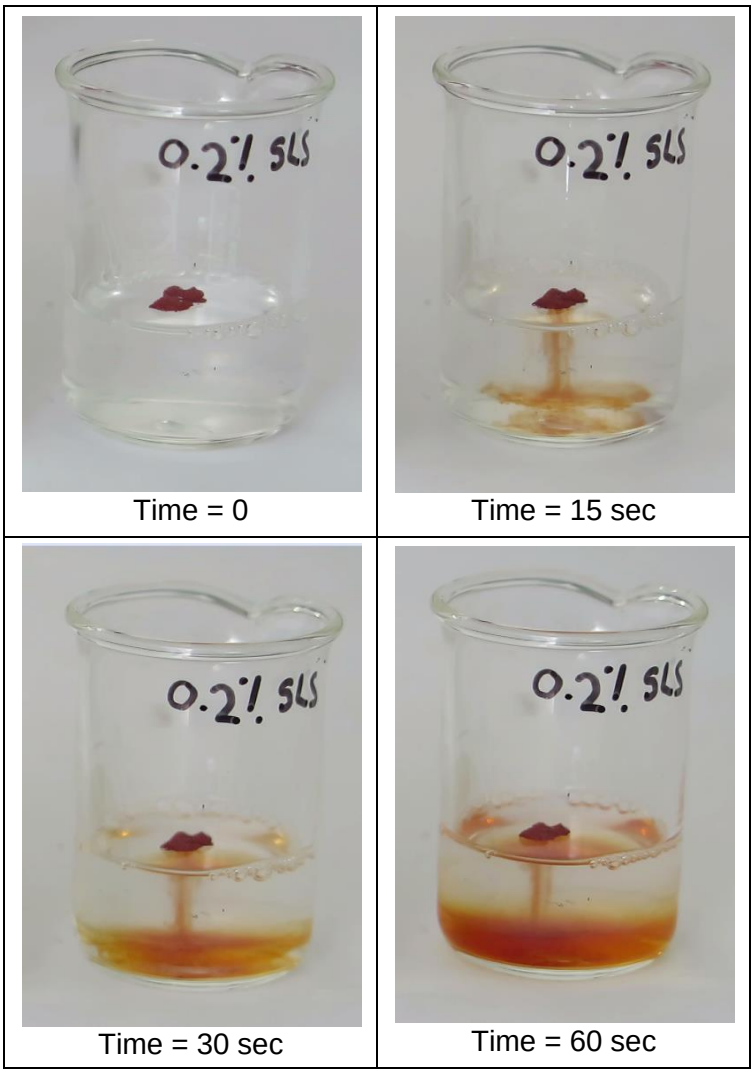


Figure 6.8: Pre-screening test of rifampicin powder in 0.2% SLS and water.

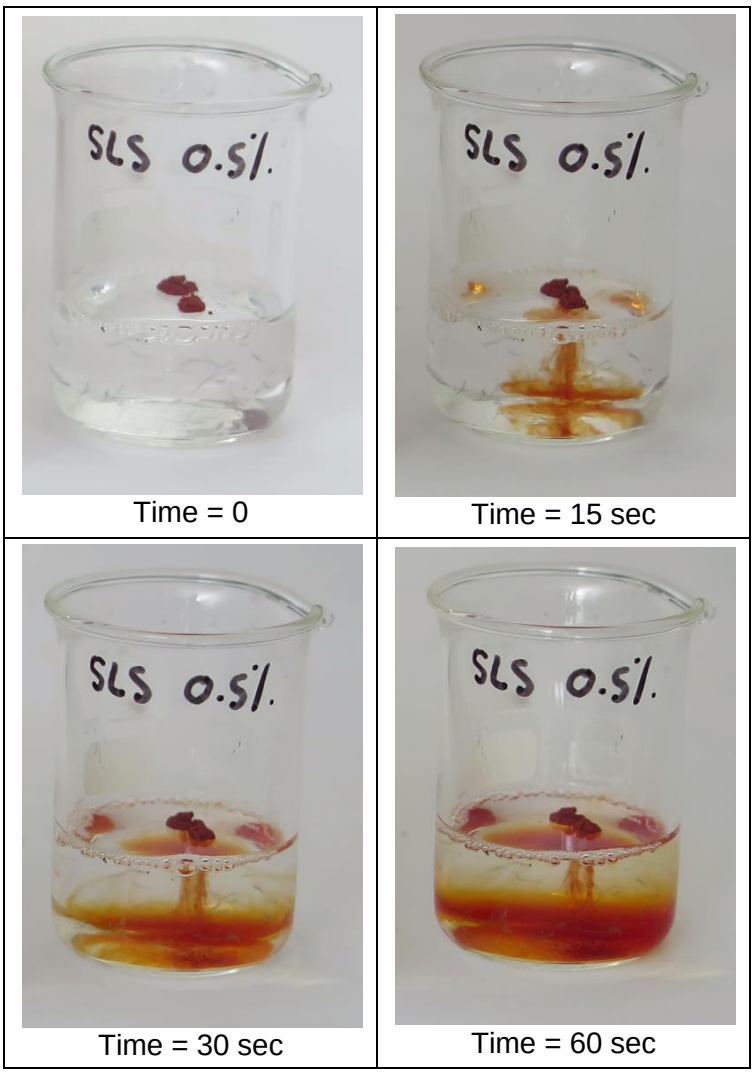
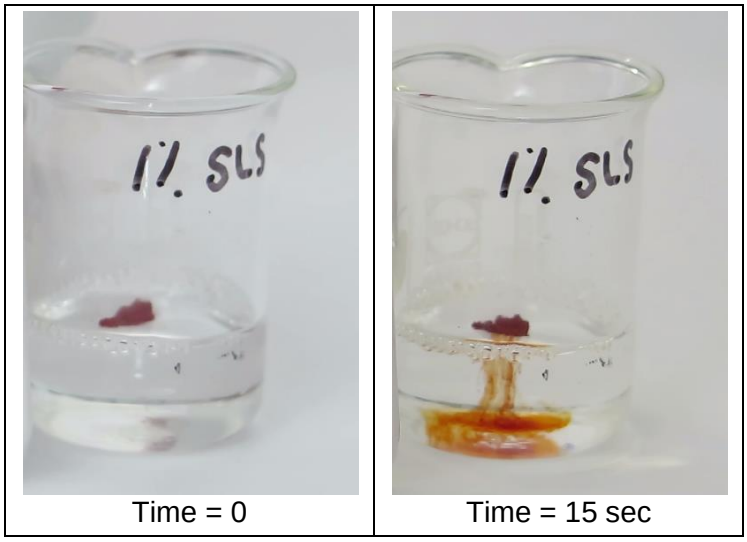
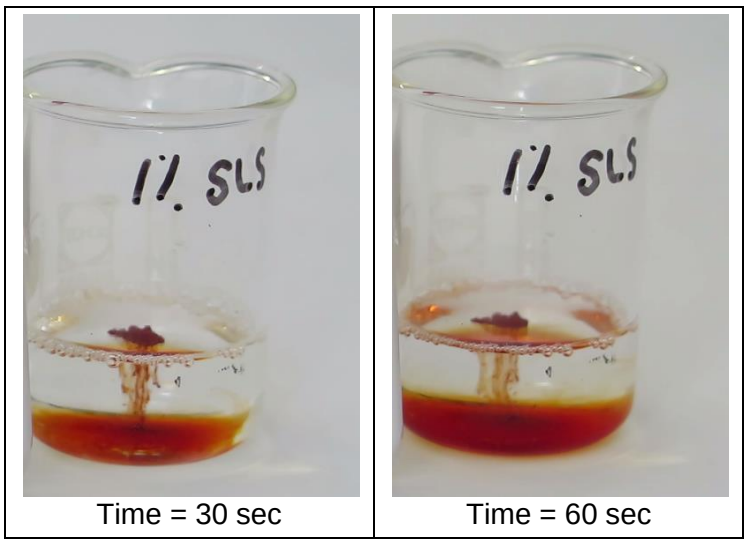


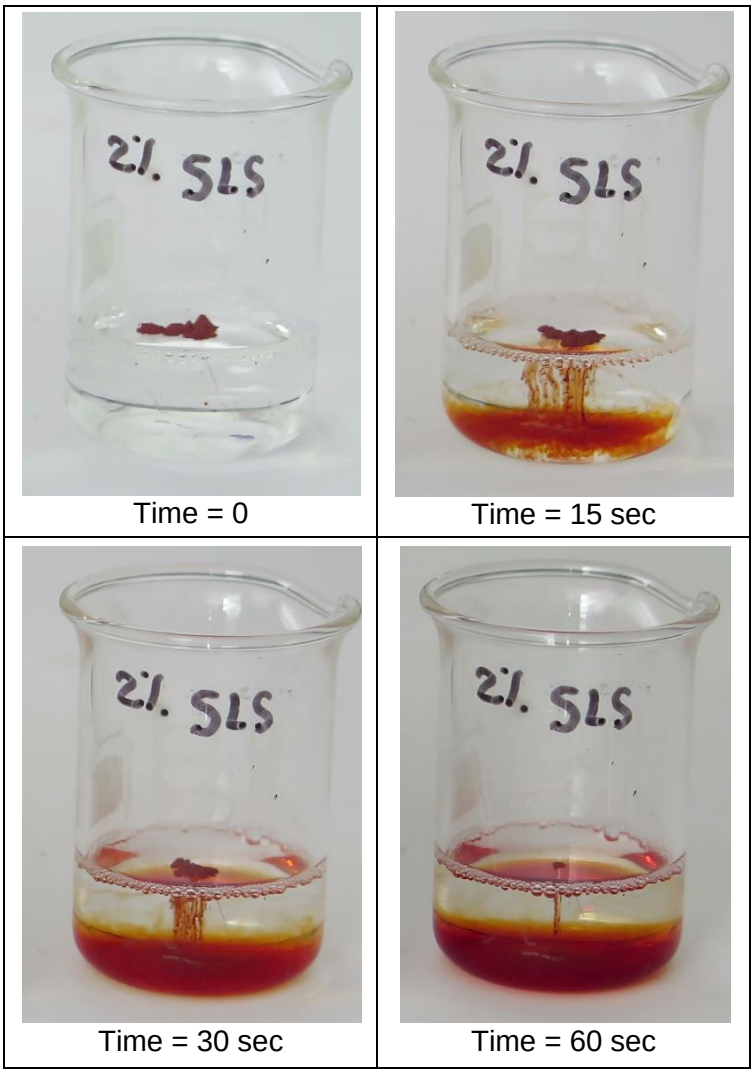
Figure 6.9: Pre-screening test of rifampicin powder in 0.5% SLS and water.





Time = 30 sec | Time = 60 sec

Figure 6.10: Pre-screening test of rifampicin powder in 1% SLS and water.



Time = 0 | Time = 15 sec

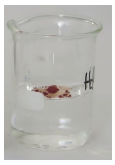
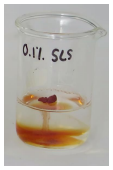
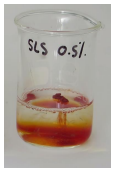
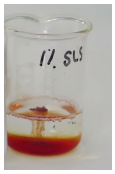
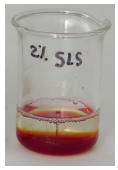
Time = 30 sec | Time = 60 sec

Figure 6.11: Pre-screening test of rifampicin powder in 2% SLS and water.

6.1.1.2 Discussion

The wettability of rifampicin was excellent with SLS concentrations from 0.1% and higher. A concentration of 0.05% was also efficient, though not as profound as the other concentrations. The dividing line was at a concentration of 0.02% and lower, the SLS at these low concentrations does not improve the wettability of rifampicin powder (Table 6.1). The rifampicin powder in water alone, 0.02% and 0.01% SLS remained on the surface of the water. The 0.02% SLS experiment also showed that after a longer period, more than 2 minutes, the rifampicin powder remained on the surface of the liquid even after (Figure 6.4). A summary of the results are presented in Table 6.1.

Table 6.1: A summary of the wettability influence of the different SLS concentrations tested on rifampicin powder after 60 seconds

0.0%	0.01%	0.02%	0.05%	0.1%	0.2%	0.5%	1%	2%
								

Based on the results obtained in these pre-screening tests, it was decided to use 6 different SLS concentrations between 0.05% and 0.30% for the dissolution experiments.

Since 0.1 M HCl is prescribed by the USP (USP, 2020) as dissolution medium it was included in the series of experiments. Water as dissolution medium was included as reference for the SLS media.

6.1.2 Powder dissolution studies and results

The dissolution experiments were conducted over a period of 6 hours in 6 dissolution vessels, at a paddle rotating speed of 100 rpm at 37°C ± 0.5°C. The UV spectrometer was set to 336 nm (BP, 2020).

6.1.2.1 Rifampicin powder dissolution study in water

When the 2 g of rifampicin powder was added to the distilled water, plenty of rifampicin lumps where observed floating on the surface of the water, which are indicative of poor wettability. The powder particles agglomerate on the surface of the water, which results in water-insoluble lumps. At 60 min, 673.03 µg/ml of rifampicin dissolved. After 120 min of powder dissolution testing, only 836.42 µg/ml of the powder was dissolved. After 180 min it seemed that a plateau was reached with the dissolved value of rifampicin at 895.42 µg/ml.

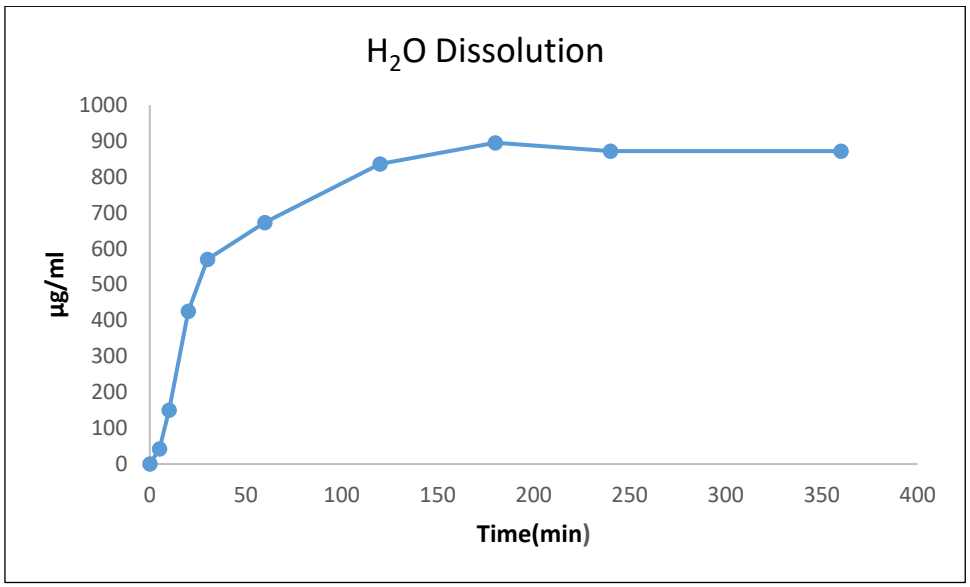


Figure 6.12: The dissolution profile of rifampicin dissolved in 900 ml distilled water.

6.1.2.2 Rifampicin powder dissolution study in water and 0.05% SLS

Less agglomeration of rifampicin was observed, which may be a result of the SLS, which starts to improve the wettability at this low concentration. As seen in Figure 6.13, 1108.77 µg/ml of rifampicin dissolved in the first 60 min of the dissolution. The solubility almost doubled over the first 60 min in 0.05% SLS compared to distilled water. Solubility obtained after 360 min equalled 1188.44 µg/ml.

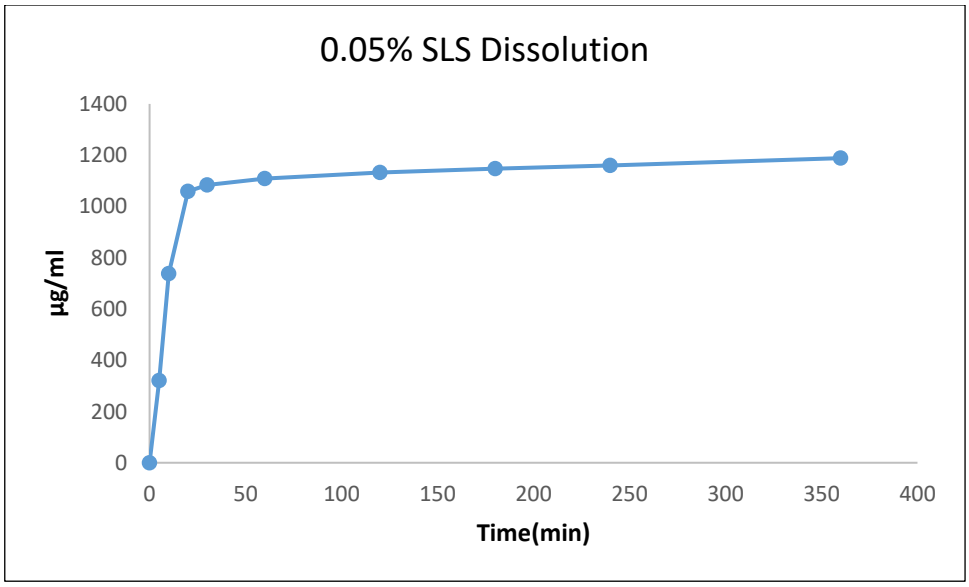


Figure 6.13: The dissolution profile of rifampicin dissolved in water and 0.05% SLS.

6.1.2.3 Rifampicin powder dissolution study in water and 0.10% SLS

With this dissolution medium the “spring and parachute” effect as discussed in par.6.1 was observed (Figure 6.14). The “spring” effect was optimal after 20 min with a solubility value of 1437.28 µg/ml. Thereafter the solubility “parachute” to a low value of 718.859 µg/ml after 360 min**.**

It seems that the concentration of SLS were just enough to enhance the wettability to a point where the amorphous part dissolved rapidly, but then after 20 min the recrystallisation of the amorphous part of the powder started which resulted in a much lower solubility value.

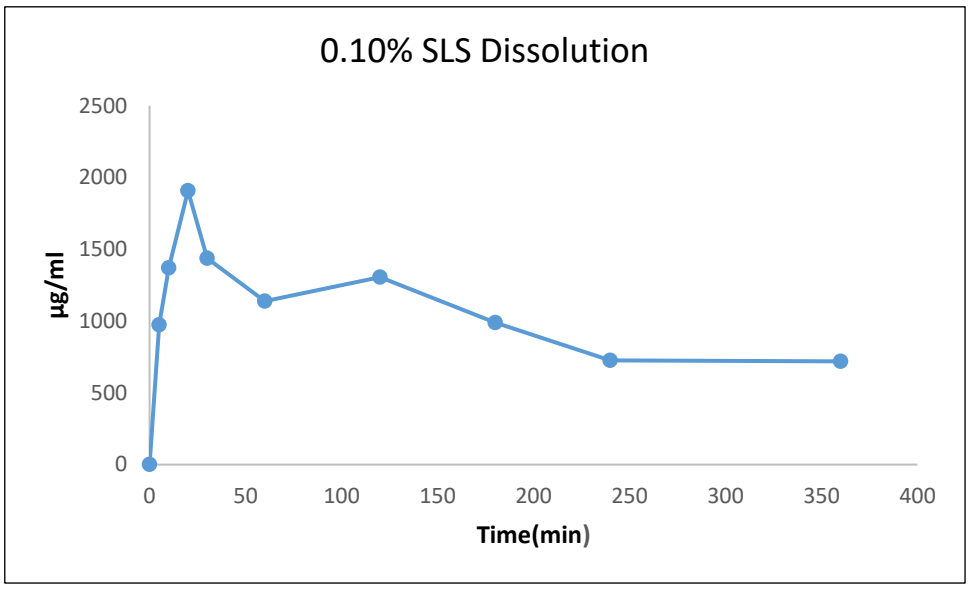


Figure 6.14: The dissolution profile of rifampicin dissolved in water and 0.10% SLS.

6.1.2.4 Rifampicin powder dissolution study in water and 0.15% SLS

This was the first dissolution experiment where no agglomeration of rifampicin powder and no floating on the surface of the dissolution medium were observed.

Once again the “spring and parachute” effect was visible (Figure 6.15). After 20 min of dissolution, a peak concentration of 2402.89 µg/ml was achieved. Thereafter it dropped to a much lower value of 794.11 µg/ml after 360 min.

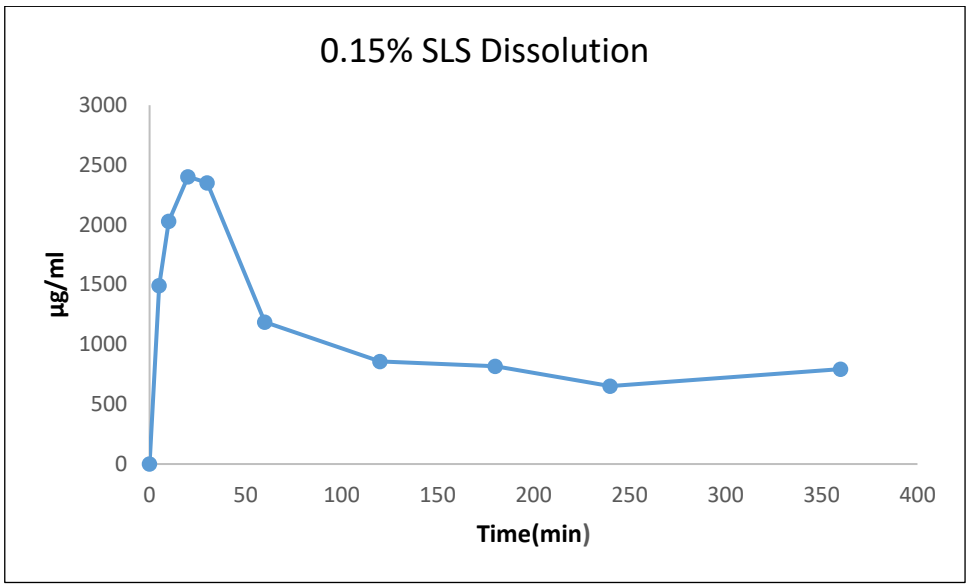


Figure 6.15: The dissolution profile of rifampicin dissolved in water and 0.15% SLS.

6.1.2.5 Rifampicin powder dissolution study in water and 0.20% SLS

No agglomeration of rifampicin and no floating rifampicin powder were observed. The "spring and parachute" effect was again visible (Figure 6.16). The maximum solubility was again measured after 20 min (2597.89 µg/ml). With this dissolution study the enhanced solubility effect remained longer in comparison with the other two dissolutions where the "spring and parachute" effect was also visible.

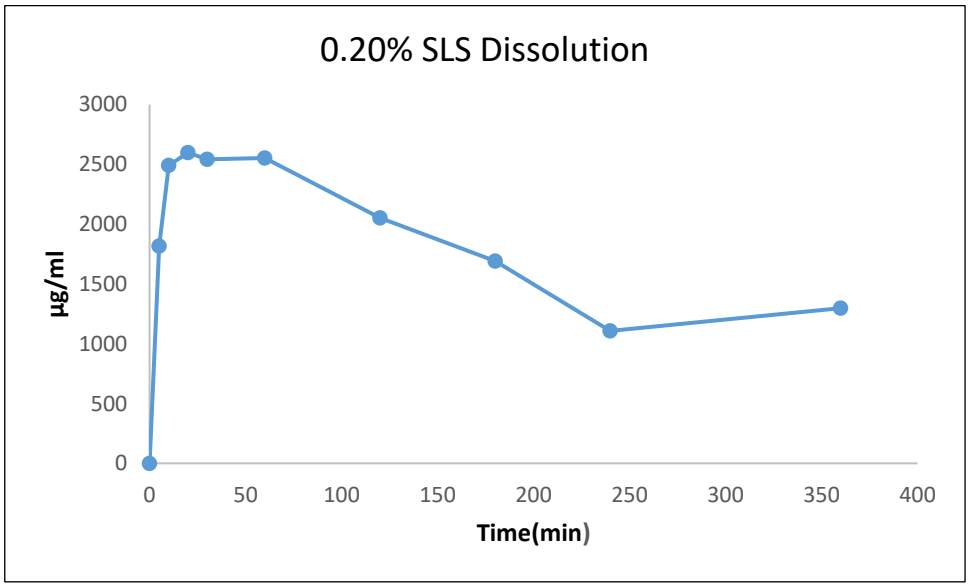


Figure 6.16: The dissolution profile of rifampicin dissolved in water and 0.20% SLS.

6.1.2.6 Rifampicin powder dissolution study in water and 0.25% SLS

No agglomeration of rifampicin and no floating of the rifampicin powder on the surface of the dissolution medium were observed. The “spring and parachute” effect was not that pronounced with this concentration of SLS (Figure 6.17). Again the optimum solubility value was obtained after 20 min (2643.55 µg/ml). The solubility value at 360 min dropped to 1649.10 µg/ml.

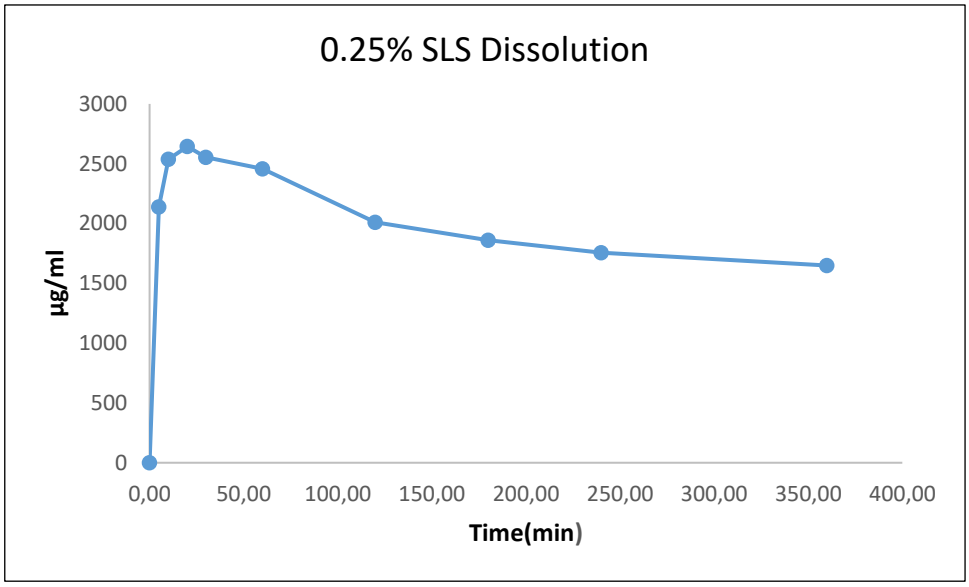


Figure 6.17: The dissolution profile of rifampicin dissolved in water and 0.25% SLS.

6.1.2.7 Rifampicin powder dissolution study in water and 0.30% SLS

No agglomeration of rifampicin and no floating of rifampicin powder were observed on the surface of the dissolution medium. No “spring and parachute” effect was visible (Figure 6.18). The peak concentration of dissolved rifampicin powder was reached in this instance after 60 min (2356.22 µg/ml). The dissolution profile indicates that the concentration remained at this level until the end of the dissolution at 360 min.

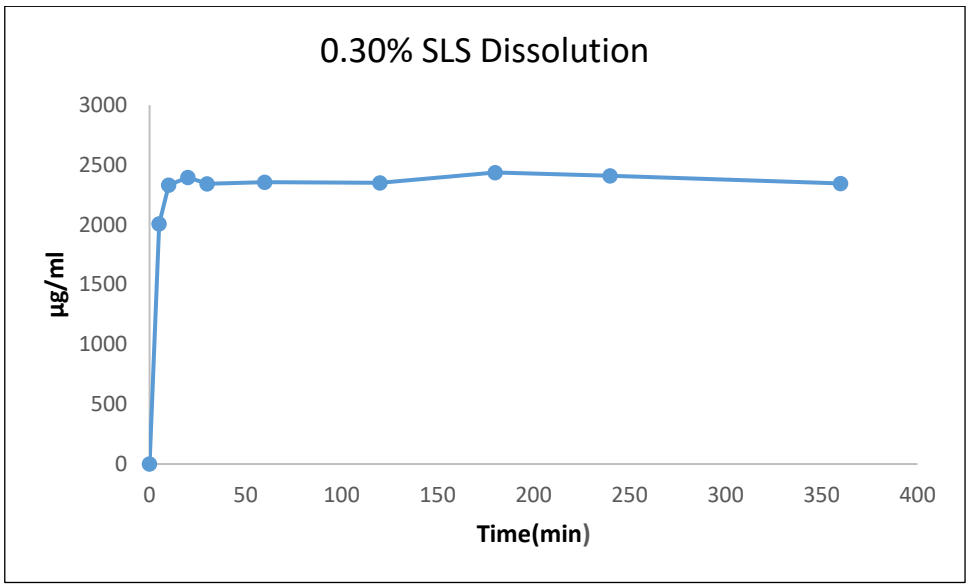


Figure 6.18: The dissolution profile of rifampicin dissolved in water and 0.30% SLS.

A summary of results are presented in Table 6.2.

Table 6.2: A summary of the solubility values obtained in µg/ml over time for various SLS concentrations

Time (min)	Rifampicin and water	Rifampicin in water and 0.05% SLS	Rifampicin in water and 0.10% SLS	Rifampicin in water and 0.15% SLS	Rifampicin in water and 0.20% SLS	Rifampicin in water and 0.25% SLS	Rifampicin in water and 0.30% SLS
5	42.033	320.60	973.11	1493.33	1815.97	2137.77	2007.33
10	150.56	737.67	1369.98	2030.00	2491.72	2537.33	2333.77
20	425.93	1058.55	1907.38	2402.89	2597.89	2643.55	2396.44
30	570.27	1084.28	1437.28	2350.72	2542.55	2553.33	2344.44
60	673.03	1108.77	1139.53	1185.99	2552.22	2456.66	2356.22
120	836.42	1132.53	1305.18	857.19	2053.55	2010.66	2351.11
180	895.42	1147.53	989.78	818.86	1691.88	1860.66	2437.55
240	871.95	1159.53	725.65	651.84	1108.61	1756.88	2410.88
360	872.05	1188.44	718.86	794.11	1297.27	1649.10	2344.88

After this series of dissolution testing the following observations were made:

The water, 0.05% and 0.30% SLS dissolution medium did not show the “spring and parachute” effect. With the water, 0.05% and 0.30% SLS dissolution media the optimum solubility was reached after 60 min remaining at the plateau for the remainder of the dissolution. This dissolution of rifampicin in water displayed almost the perfect graph, but the solubility values

were much lower in comparison to the dissolution in the 0.05% SLS. The 0.30% SLS dissolution test results resulted in the highest solubility values with results double that of 0.05% SLS.

The dissolution studies in 0.10%, 0.15%, 0.20% and 0.25% SLS demonstrated a clear “spring and parachute effect” with optimum solubility values already reached after 20 min.

6.1.2.8 Rifampicin powder dissolution study in 0.1 M HCl

At the 20 min withdrawal time, a value of 2324.55 µg/ml was obtained. Thereafter the concentrations remained more or less identical (Figure 6.19).

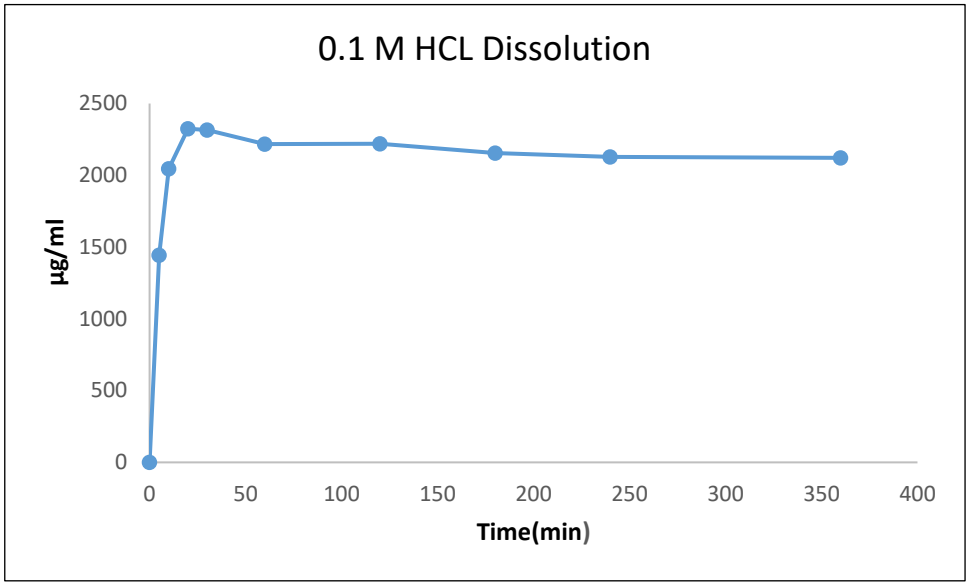


Figure 6.19: The dissolution profile of rifampicin dissolved in 0.1 M HCl.

6.2 Discussion and conclusion

After this series of dissolution testing the following observations were made:

The SLS did improve the solubility of rifampicin powder by improving the wettability of the powder. This effect was already visible at the lowest concentration of SLS (0.05%), where less agglomeration and clumping of the powder was visible on the surface of the dissolution medium. The 0.10%, 0.15%, 0.20% and 0.25% SLS concentrations showed the “spring and parachute” effect, with a high solubility value after 20 min, where after it gradually decreased to a lower value. With the higher concentrations of SLS it can be observed that the drug tends to be highly soluble because the drug form is maintained for a longer time in the metastable zone.

The 0.1 M HCl, water and 0.30% SLS medium did not show any “spring and parachute” effect, and the 0.30% SLS also demonstrated better solubility values than the 0.1 M HCl.

Results for these 3 experiments are summarised in Table 6.3.

Table 6.3: A summary of the dissolution rate in µg/ml over time for water, 0.1 M HCl and 0.30% SLS

Time (min):	Rifampicin and 0.1 M HCl	Rifampicin and water	Rifampicin in water and 0.30% SLS
5	1444.63	42.033	2007.33
10	2046.05	150.56	2333.77
20	2324.55	425.93	2396.44
30	2315.72	570.27	2344.44
60	2218.55	673.03	2356.22
120	2218.72	836.42	2351.11
180	2156.05	895.42	2437.55
240	2129.22	871.95	2410.88
360	2121.39	872.05	2344.88

Figure 6.20 illustrates a summary of the dissolution study and a comparison at withdrawal times 20 min and 60 min. It also showed the effect of the "spring and parachute phenomenon with high solubility values and then significantly lower values. It also shows that 0.30% SLS in water maintain the solubility advantage over the dissolution testing period.

To conclude then: rifampicin powder in the water and SLS dissolution medium showed enhanced solubility from the lowest concentration (0.05%) SLS in comparison with rifampicin powder in distilled water. The "spring and parachute" effect were visible with SLS concentrations from 0.10% to 0.25% SLS in water. The 0.30% SLS resulted in the best dissolution profile with solubility values of >2300 µg/ml. Even better than the current pharmacopoeia medium, i.e. 0.1 M HCl (Figure 6.21).

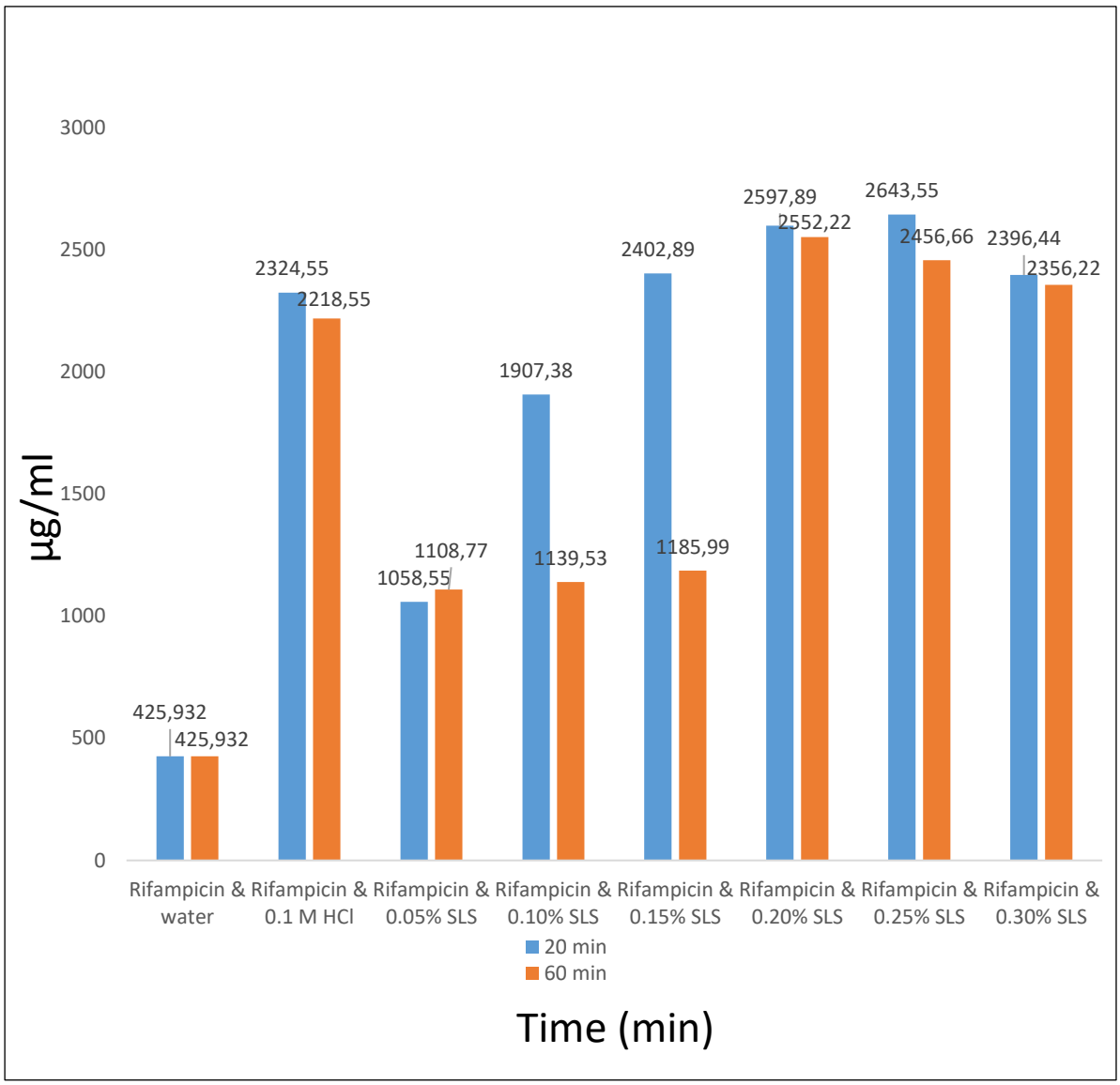


Figure 6.20: Summary of the solubility values at withdrawal times 20 and 60 min.

In Figure 6.21 all the dissolution profiles are presented in one graph.

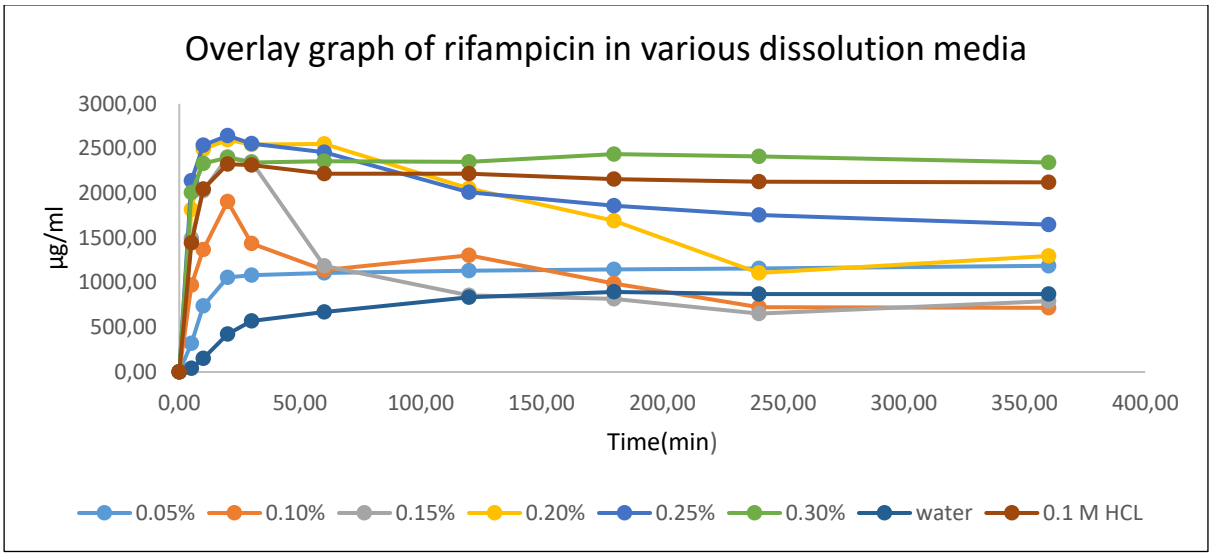


Figure 6.21: An overlay of the dissolution results of rifampicin in 0.05-0.30% SLS, 0.1 M HCl and water.

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Chapter 7

Permeation studies

7.1 Introduction

As mentioned in Chapter 4, the Caco-2 cell line is frequently used to study passive drug absorption (Küblbeck *et al*., 2016; Shah *et al*., 2006). Results from Artursson (1989), showed that oral drug absorption in humans and the absorption obtained with Caco-2 cell models are well correlated, and Caco-2 monolayers can therefore be used as a model to study intestinal drug absorption.

7.2 Results obtained from the permeation studies

During the drug development process, *in vitro* cell permeation experiments can be used to monitor the intestinal absorption potential of drug compounds. A transcellular transport study typically consists of a donor side where compounds are added and a receiver side from which samples are withdrawn. The apparent permeability coefficient (P_{app}) of a drug compound can be calculated from the slope of the concentration-time profile on the receiver side. Assuming that the amount of compound accumulated on the receiver side is proportional to time (Ozeki *et al.,* 2015).

Permeation studies of rifampicin were performed in the absence and presence of sodium lauryl sulphate (SLS), at concentrations of 0.05% and 0.15%. Lucifer yellow was also used to validate the Caco-2 cell model as an intact monolayer.

As explained in Chapter 4, mature Caco-2 cell monolayers form tight junctions between the cells. The presence of these tight junctions can be confirmed with transepithelial electrical resistance (TEER) measurements, which are measured in ohm (Ω). The TEER results can indicate whether the cell monolayer is complete, mature and intact. A TEER value higher than 250 Ω per well usually indicates the presence of good tight junctions with little membrane leakage (Haasbroek *et al*., 2019). In this study, the TEER of all the experimental wells were measured before, during and after the experiment.

7.2.1 Validation of the model with Lucifer yellow

Lucifer yellow is an exclusion marker since this hydrophilic molecule has very low permeability across intact biological membranes. The percentage transport of Lucifer yellow was monitored over a period of 120 min. The percentage transport of Lucifer yellow across the Caco-2 cell monolayer should be < 2% (Haasbroek *et al*., 2019). As shown in Figure 7.1, the permeation never exceeded 2%, suggesting that membrane integrity was maintained and the monolayer was intact.

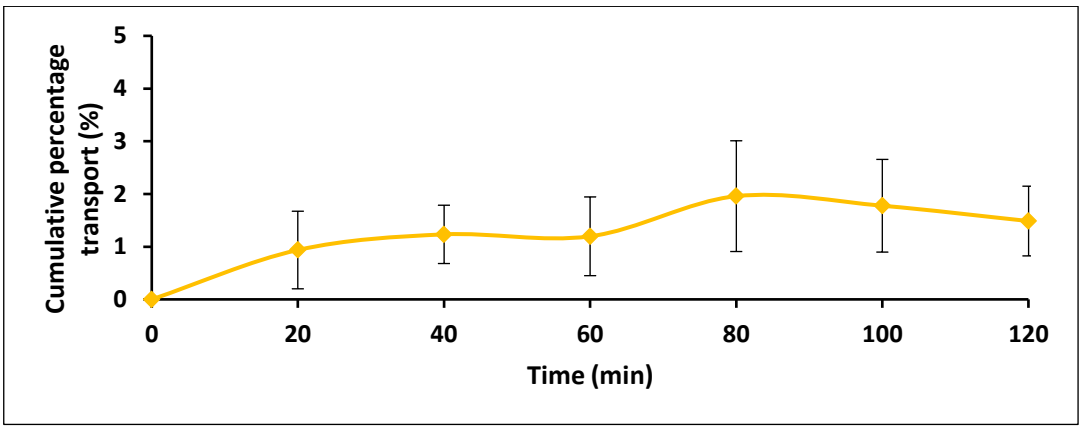

Figure 7.1: Cumulative percentage transport of Lucifer yellow across Caco-2 monolayers in the AP-BL direction. Results are reported as *n* = 3.

The average P_{app} value (cm/s) for Lucifer yellow across the Caco-2 cell monolayer was 4.38×10^{-7}, which indicated poor permeability (Calatayud *et al.,* 2011). As seen in Table 7.1 all cell monolayers exhibited TEER above 250 Ω per well. There was however, a decrease in the TEER (209.83 Ω) observed after the test solution was added, but the TEER increased again to 289.67 Ω by the end of the study. This also confirmed that the cell monolayers stayed intact for the duration of the transport experiments.

Table 7.1: Average TEER measurements for six Caco-2 monolayers exposed to Lucifer yellow

Experimental Group	Average initial TEER value (Ω) (0 min)	Average TEER value after addition of transport buffer (Ω)	Average TEER value after addition of test solutions (Ω)	Average TEER value at the end of the incubation time (Ω) (120 min)
Lucifer yellow	387.33	375.17	209.83	289.67

From these results, it was concluded that the model was suitable to be used for the permeability studies over a duration of 120 min.

7.2.2 Rifampicin permeation in the presence of 0.05% SLS

Permeation of rifampicin alone and in the presence of 0.05% SLS was measured across an intact Caco-2 monolayer over a period of 120 min, to determine if the permeation of rifampicin would be influenced with this SLS concentration. The cumulative percentage transport of rifampicin measured in the acceptor chamber (basolateral side) is shown in Figure 7.2. The cumulative percentage transport for rifampicin in the presence of 0.05% SLS was 113.48% after 120 min, relative to that of rifampicin alone (62.97%).

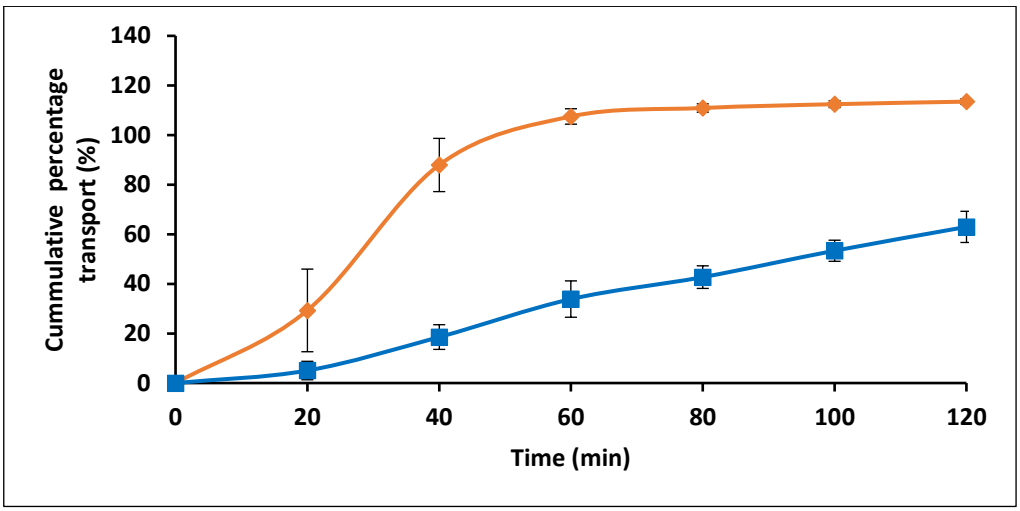

Figure 7.2: The cumulative percentage transport of rifampicin (2 mg/ml) across a Caco-2 monolayer in the absence (blue line) and presence (orange line) of 0.05% SLS (*n* = 3).

The permeation data was used to calculate the P_{app} values for the two experimental groups, and the data are shown in Figure 7.3. The P_{app} value (cm/s) for rifampicin in the presence of 0.05% SLS was 33.76 x 10^{-6} cm/s, compared to rifampicin alone being only 19.74 x 10^{-6} cm/s. There was an increase in the P_{app} in the presence of 0.05% SLS, indicating an increase in permeability of rifampicin at this SLS concentration.

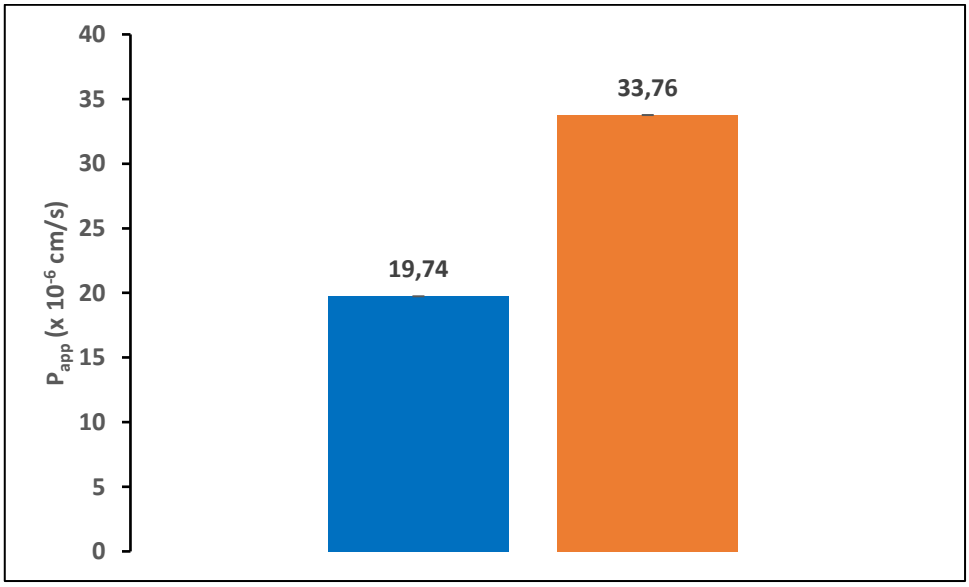

Figure 7.3: The apparent permeability coefficient (P_{app}) values for rifampicin in the absence (blue) and presence (orange) of 0.05% SLS.

The initial TEER values for both experimental groups was above 250 Ω per well, which indicated the presence of an intact monolayer (Table 7.2). The initial TEER measurements for rifampicin alone was 429.67 Ω per well, and in the presence of 0.05% SLS it was 453.00 Ω per well. There was a decrease in the TEER measurements observed for both treatment groups

after the test solutions were added. While the TEER for the rifampicin treatment group recovered to some extent by the end of the experiment, the TEER only continued to decrease in the presence of 0.05% SLS. The presence of 0.05% SLS resulted in a decrease in TEER to 88.17 Ω.

Table 7.2: TEER measurements for three Caco-2 monolayers exposed to rifampicin in the absence and presence of 0.05% SLS

Experimental Group	Average initial TEER value (Ω) (0 min)	Average TEER value after addition of transport buffer (Ω)	Average TEER value after addition of test solutions (Ω)	Average TEER value at the end of the incubation time (Ω) (120 min)
Rifampicin	429.67	409.33	230.00	250.17
Rifampicin in 0.05% SLS	453.00	425.50	108.67	88.17

7.2.3 Rifampicin permeation in the presence of 0.15% SLS

Permeation of rifampicin was measured across an intact Caco-2 monolayer over a period of 120 min, in the absence and presence of 0.15% SLS to determine if SLS at this concentration would influence the rifampicin's permeability. The cumulative percentage rifampicin measured in the acceptor chamber (basolateral side) is shown in Figure 7.4. The cumulative percentage transport for rifampicin in the presence of 0.15% SLS was 110% after 120 min, compared to that of rifampicin alone being only 62.97%.

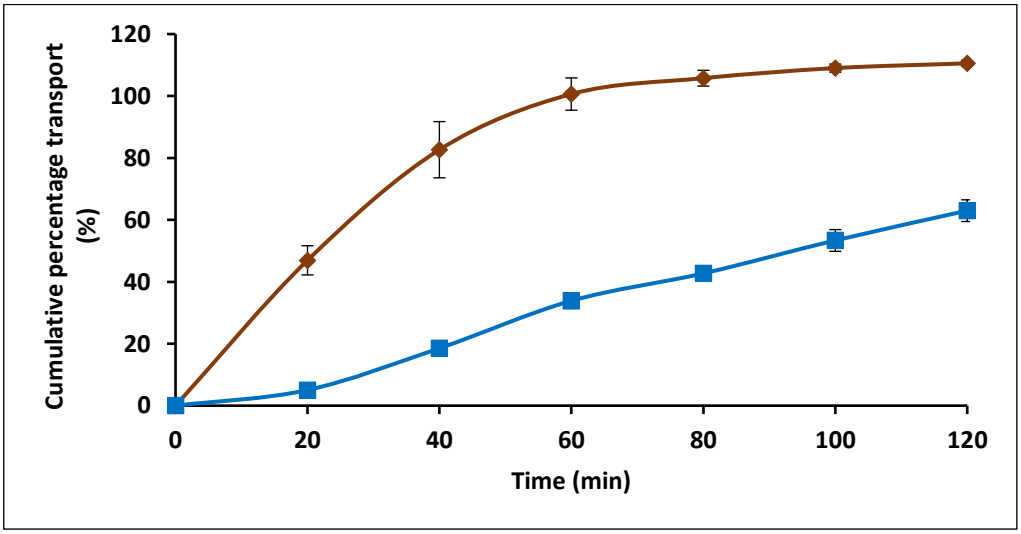

Figure 7.4: The cumulative percentage transport of rifampicin (2 mg/ml) across a Caco-2 monolayer in the absence (blue) and presence (orange) of 0.15% SLS (*n* = 3).

The permeation data was used to calculate the P_{app} values for the two experimental groups, and these values are shown in Figure 7.5. The P_{app} value (cm/s) for rifampicin in the presence of 0.15% SLS was 30.52 x 10^{-6} cm/s, compared to rifampicin alone being only 19.74 x 10^{-6} cm/s. There was a marked increase in the P_{app} in the presence of 0.15% SLS, indicating an increase in permeability of rifampicin at this SLS concentration.

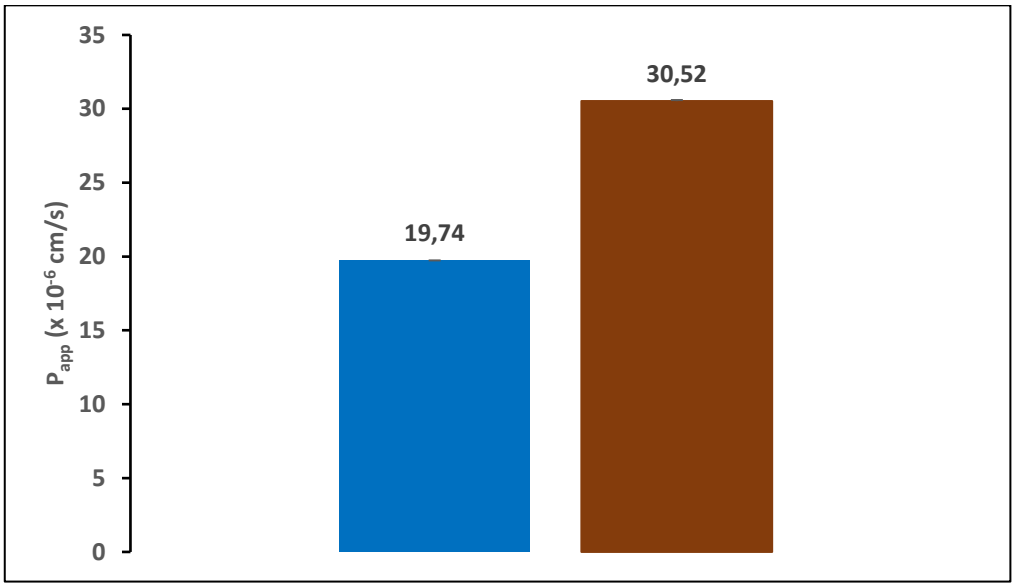

Figure 7.5: The apparent permeability coefficient (P_{app}) values for rifampicin in the absence (blue) and presence (orange) of 0.15% SLS.

The initial TEER values for both experimental groups were above 250 Ω per well, which indicated the presence of an intact monolayer. Following addition of the test solutions, a decrease in the measured TEER values for both groups was observed (Table 7.3). While the TEER measurement for the rifampicin treatment group recovered to some extent by the end of the experiment (250.17 Ω), the TEER measurements in the presence of 0.15% SLS only continued to decrease. The presence of 0.15% SLS resulted in a decrease from 447.33 to 93.67 Ω per well.

Table 7.3: TEER measurements for three Caco-2 monolayers exposed to rifampicin in the absence and presence of 0.15% SLS

Experimental Group	Average initial TEER value (Ω) (0 min)	Average TEER value after addition of transport buffer (Ω)	Average TEER value after addition of test solutions (Ω)	Average TEER value at the end of the incubation time (Ω) (120 min)
Rifampicin	429.67	409.33	230.00	250.17
Rifampicin in 0.15% SLS	447.33	523.83	104.67	93.67

7.2.4 Cytotoxicity assessment of sodium lauryl sulphate

Due to the rapid decrease in the TEER measurements in the presence of SLS, the possibility of cytotoxicity of the compound was considered. To investigate this aspect, the effect of a concentration range of 0.05 to 0.30% SLS (the same concentrations used in the dissolution studies) on the viability of cultured Caco-2 cells were studied using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay.

As seen in Figure 7.6, provided below, viability relative to the untreated control was significantly decreased for all concentrations evaluated, with the remaining cell viability % being less than 10%.

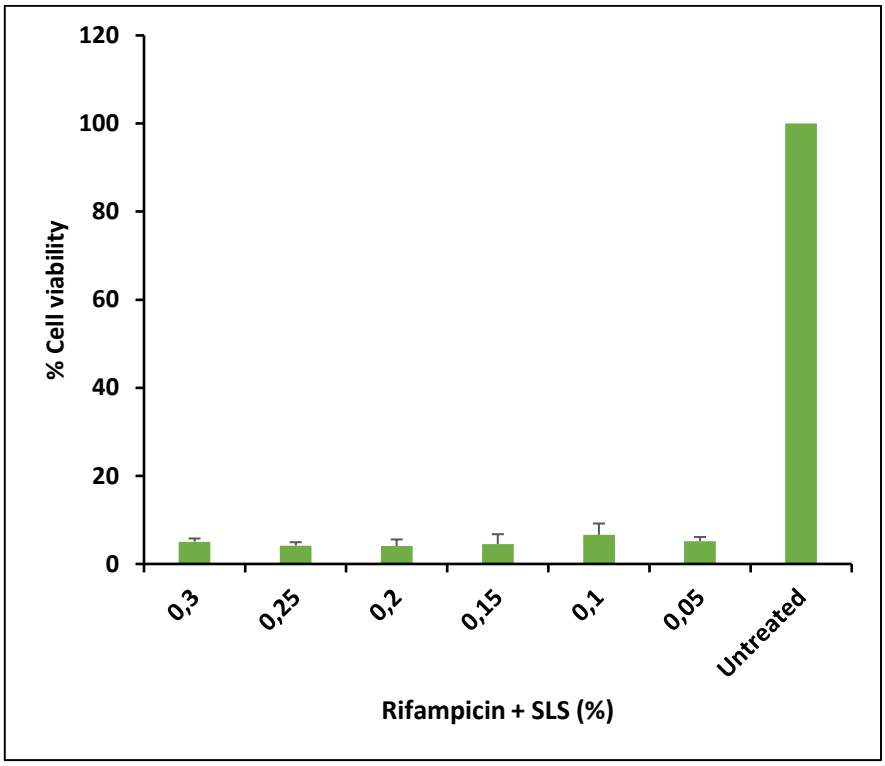

Figure 7.6: Average percentage cell viability relative to an untreated control of Caco-2 cells in the presence of rifampicin (2 mg/ml) and a concentration range of SLS (*n* = 3).

7.3 Discussion

Microscopy, TEER measurements and Lucifer yellow permeation were used to confirm the physical integrity of the confluent monolayers with functional tight junctions. According to literature, P_{app} values of Lucifer yellow ≤ 0.2 x 10^{-6} cm/s (Calatayud *et al.,* 2011) or 0.66–0.75 x 10^{-6} cm/s (Bhushani *et al.,* 2016), were considered indicative of the formation of intact Caco-2 cell monolayers. To evaluate the importance of the P_{app} values obtained, the classification suggested by Yee (1997) was used, in which permeabilities < 10^{-6} cm/s resemble substances with low absorption (< 30%), permeabilities between 10^{-6} cm/s and 10^{-5} cm/s resemble substances with moderate absorption (30–70%), and permeabilities > 10^{-5} cm/s correspond to substances with high absorption (> 70%). The average P_{app} value (cm/s) for Lucifer yellow was

4.38 x 10^{-7} and the average TEER value was 289.67 Ω, confirming that the monolayer integrity was intact at the end of the experiment.

7.3.1 Permeability of rifampicin

Caffeine and atenolol are two substances used as standards to determine high or low transport. According to Heyns *et al.* (2018), caffeine measured a P_{app} (x 10^{-6} cm/s) of 17.059 ± 0.636 which is an indication of a good permeation; whereas atenolol measured a P_{app} (x 10^{-6} cm/s) value of 4.836 ± 1.574. This is an indication of a poor permeation.

For rifampicin alone, the cumulative percentage transport was 62.97%, and measured a P_{app} value of 19.73 x 10^{-6} cm/s. In comparison with Yee (1997) and Heyns *et al.* (2018), this indicated that rifampicin alone can be identified as a moderately absorbed substance.

7.3.2 Permeability of rifampicin in the presence of SLS

Permeation studies were only performed for rifampicin in the presence of 0.05% SLS and 0.15% SLS, to investigate the potential of SLS to increase the transport of rifampicin across the intestinal epithelial barrier. For both concentrations SLS, the cumulative percentage transport as well as the P_{app} values for rifampicin increased markedly. There was no notable difference between that obtained with 0.15% SLS and 0.05%. Both SLS concentrations resulted in decreased TEER measurements. A decrease in TEER measurements could be indicative of opening of the tight junctions between the cells, but a previous study has shown that surfactants can influence Caco-2 cell monolayer integrity and can cause intestinal membrane damage, which results in a change in intestinal membrane barrier function (Ujhelyi *et al.,* 2012).

7.3.3 Cytotoxic potential of SLS

Since the TEER measurements in the presence of SLS did not recover at the end of the experiment, the effect of rifampicin in the presence of both SLS concentrations on cell viability was investigated. According to an *in vitro* study on skin toxicity it was found that SLS produced dose related cell death in a concentration range of 5 x 10^{-5} M to 5 x 10^{-3} M, but these studies were done on JB6 cells (Jain *et al.,* 1992). Two studies did toxicity testing of SLS on gingival cells and it was reported that for SLS, the initial toxicity occurred at 0.006% and 0.009% (Babich & Babich, 1997). According to a human gingival fibroblast *in vitro* wound healing model with 0.05% SLS, by day 4, a large percentage of confluent cells had detached (Chuang *et al.,* 2019). However, in a study on SLS and paracellular transport using Caco-2 monolayers, it was found that SLS concentrations up to 80 mg/l did not affect mitochondrial dehydrogenase and cytosolic lactate dehydrogenase activities; an increase in paracellular transport and tight junction opening were observed (Boulenc *et al*., 1995).

Cell viability is the measurement of the sum of live cells and is usually expressed as a percentage of the untreated control (Kroemer *et al*., 2009). All concentrations SLS decreased

viability of the Caco-2 cells to less than 10% relative to the untreated control, indicating that the SLS concentrations were cytotoxic. It can therefore be assumed that the SLS caused damage to the cell monolayers, making the permeability enhanced results unreliable.

7.4 Conclusion

In conclusion, rifampicin has moderate permeability and therefore moderate intestinal absorption. SLS had a pronounced effect on the permeability of rifampicin, but the subsequent cytotoxicity evaluation made the results unreliable.

The concentrations SLS that were used were based on concentrations previously used in commercial tablets and capsules, and it may be that the cytotoxic effect was because of the monolayer structure of the model used.

Therefore, the permeation studies should be tested on a more robust model such as excised tissue in the future, when testing for drug permeability with rifampicin and SLS.

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Chapter 8

Conclusion

Tuberculosis is classified as a global emergency because it is taking lives at an alarming rate and developing countries with insufficient health care systems are primarily affected. Multidrug resistant TB (MDR-TB) can occur when patient drug compliance is not met. MDR-TB has a concerning adverse impact on the world's economy and the socio-economic lifestyle of TB patients. Fixed dose combination drugs (FDCs) were created to improve patient compliance and reduce the appearance of drug resistance, but unfortunately this drug combination has a few shortcomings, for example rifampicin is a drug well-known for its problematic outcomes regarding bioavailability in FDCs.

During the pharmaceutical development processes, and pre-formulation, is it important to know the physico-chemical properties of an API and to understand the effect and implications of different solid-state forms of a given API. Investigation of the solid-state characteristics of an API is necessary to ensure quality and stability of the end-product.

After a thorough literature study, it was found that rifampicin raw material pose with different challenges like poor solubility and an amorphous content which could result in poor bioavailability.

The poor solubility lead to questions regarding the poor wettability of rifampicin. Surfactants are known to improve the wettability of poorly water soluble drugs. By making use of the surfactant sodium lauryl sulphate (SLS), the surface tension of rifampicin could be lowered and possibly result in an improvement of the wettability of the drug. This could further lead to an increase in solubility of the drug and drug permeability, and consequently lead to better bioavailability.

This hypothesis was tested by using dissolution studies and permeability studies with the Caco-2 model. Rifampicin powder in water and SLS showed enhanced solubility from the lowest SLS concentration (0.05%) in comparison with rifampicin powder in distilled water alone. Rifampicin powder in 0.20-0.30% SLS showed a significant increase in the solubility due to an increase in the wettability of the powder. For rifampicin in 0.1 M HCl the solubility was 2121.39 µg/ml after 360 min. In distilled water a solubility value of 872.05 µg/ml was obtained after 360 min, whereas in the presence of 0.30% SLS rifampicin solubility increased remarkably to 2344.88 µg/ml after 360 min. The 0.3% SLS resulted in the best dissolution results, even better than the current pharmacopoeia medium, i.e. 0.1 M HCl (2121.39 µg/ml).

With the Caco-2 model it was found that rifampicin in SLS can influence the paracellular permeability and can have an impact on intestinal absorption. SLS has a pronounced effect on tight junctions, but due to the cell toxicity of SLS the permeation studies should be tested on a more robust cell model.

These studies showed that the poor solubility of rifampicin is ultimately a result of poor wettability and these issues need to be dealt with before any further steps are taken towards improving the bioavailability of the drug.