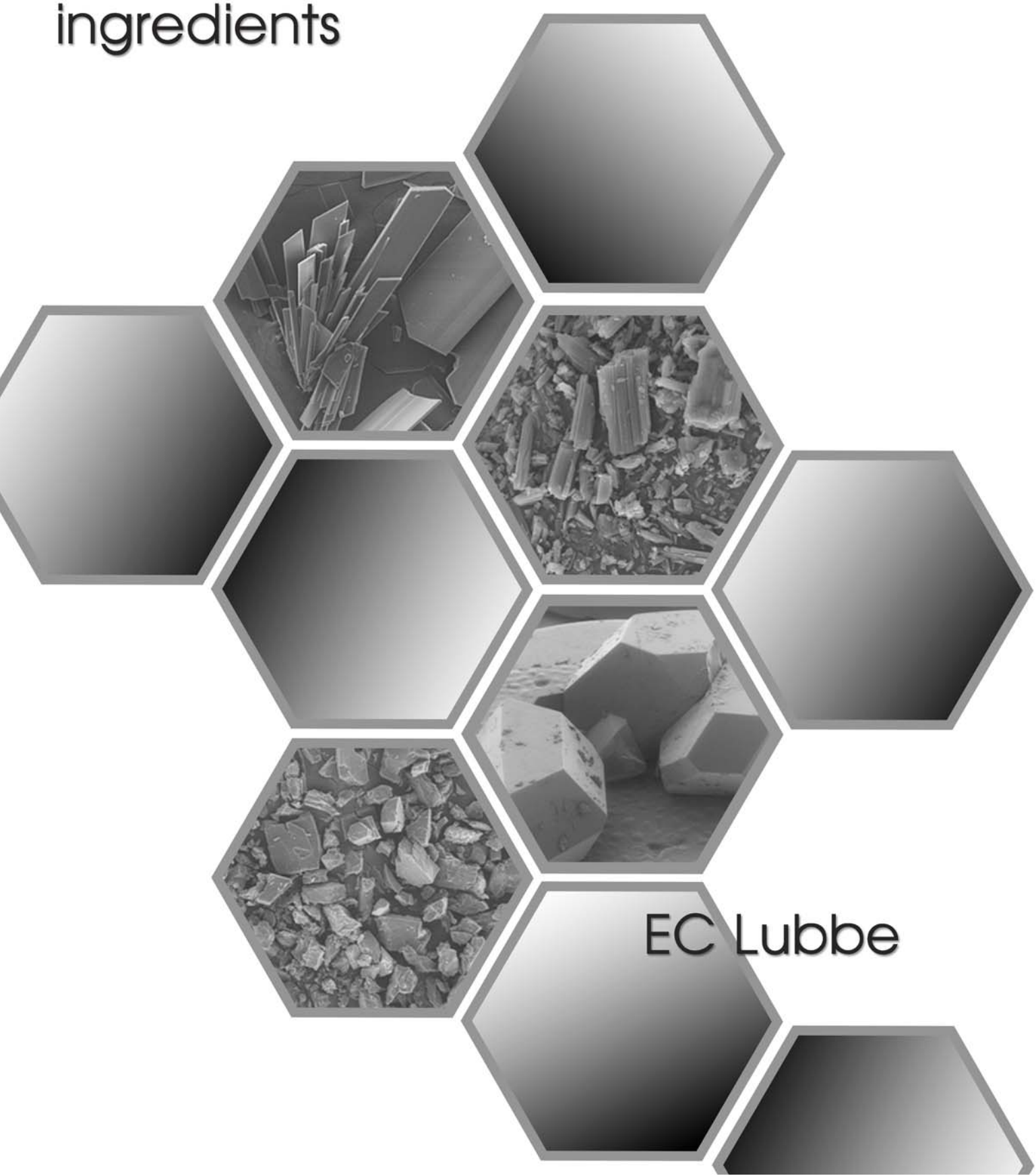
Influence of particle size on solubility of active pharmaceutical ingredients



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Influence of particle size on solubility of active pharmaceutical ingredients

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Abstract

The aqueous solubility of an active pharmaceutical ingredient (API) is an important property that requires evaluation during early development and prior to formulation of the final product. With general, experimental, solubility testing of different APIs, the question always arises as to whether particle size had been determined beforehand or not. All available literature suggests that particle size, for pharmaceutical powders, does not significantly affect equilibrium solubility. The dissolution rate will differ according to different particle sizes, but the overall results should be identical after equilibrium is established.

This study was therefore planned to investigate as to whether different particle size fractions of the same API, dissolving at different rates, would all reach solubility equilibrium within 24 hours. Also, APIs from different solubility classes were investigated, because poorly soluble substances would most likely require a longer period of time to equilibrate. The time period of 24 hours was selected, because many published solubility studies report using that interval and is it the standard for our research group also.

Available APIs were selected to determine the influence (if any) of particle size on their equilibrium solubilities and the time required for attaining that status. For the purpose of this investigation, five APIs were selected from compounds at our disposal in-house, ranging from freely soluble to poorly soluble in the order: chloroquine phosphate > pyrazinamide > mefloquine hydrochloride > closantel sodium > roxithromycin.

Solubility studies were successfully completed on four of the five APIs selected. For closantel sodium, pyrazinamide and roxithromycin it was demonstrated that the 24 hour test period was sufficient for the attainment of equilibrium solubility, regardless of the particle size fractions tested. Surprisingly, the only API in this study for which 24 hours was an insufficient test period was mefloquine HCl,

which was not the least soluble compound tested. Further testing would be required to clarify this anomaly.

What was evident from the outcomes of this investigation was that although the ubiquitous 24 hour solubility test may work well in many cases, its suitability should be reviewed on a case-by-case basis and not just for the most poorly soluble compounds. Researchers testing solubility at temperatures lower than 37°C should be especially cautious of using a standardised test period, because equilibrium solubility would take longer to achieve with less energy available to the system.

KEYWORDS: particle size ; solubility ; closantal sodium ; chloroquine phosphate ; mefloquine hydrochloride ; pyrazinamide ; roxithromycin

Uittreksel

Die wateroplosbaarheid van ‘n aktiewe farmaseutiese bestanddeel (AFB) is ‘n belangrike eienskap, wat alreeds tydens vroeë ontwikkeling van die geneesmiddel, asook voor formulering van die finale produk geëvalueer moet word. In die geval van algemene, eksperimentele oplosbaarheidsbepalings van verskillende geneesmiddels ontstaan die vraag altyd of deeltjiegrootte wel vooraf bepaal is, al dan nie? Alle beskikbare literatuur voer aan dat die deeltjiegrootte van farmaseutiese poeiers nie ‘n noemenswaardige invloed op ewewigsoplosbaarheid het nie. Verskillende deeltjiegroottes sal ‘n invloed op die dissolusie-tempo hê, maar die algehele resultate behoort identies te wees nadat ewewig eers bereik is.

Hierdie studie was dus beplan om ondersoek in te stel of verskillende deeltjiegrootte-fraksies van dieselfde AFB, wat teen verskillende snelhede oplos, almal ewewigsoplosbaarheid binne 24 uur sou kon bereik. Voorts is geneesmiddels van verskillende oplosbaarheidsklasse ondersoek, aangesien swak oplosbare stowwe heel waarskynlik ‘n langer tydperk sou benodig om ewewig te bereik. ‘n Periode van 24 uur is gekies, omdat die meeste gepubliseerde oplosbaarheidstudies van hierdie tydsinterval melding maak en aangesien hierdie interval ook standaardpraktyk vir ons navorsingsgroep is.

Beskikbare AFBe is gekies ten einde die invloed, al dan nie, van deeltjiegrootte op die ewewigsoplosbaarheid van hierdie geneesmiddels vas te stel, asook die nodige tyd om daardie ewewig te bereik. Vir die doel van hierdie ondersoek is vyf geneesmiddels, wat in-huis tot ons beskikking was, gekies wat vanaf vrylik oplosbaar tot swak oplosbaar gewissel het in die orde: chlorokienfosfaat > pirasienamied > meflokienhidrochloried > klosantelnatrium > roksitromisien.

Oplosbaarheidstudies is suksesvol op vier van die vyf gekose geneesmiddels voltooi. In die geval van klosantelnatrium, pirasienamied en roksitromisien is bewys gelewer dat die 24 uur toetsperiode genoegsaam was om ewewigsoplosbaarheid te bereik, ongeag die deeltjiegrootte-fraksies wat

getoets is. Verrassend genoeg was meflokienhidrochloried, wat nie die swakste oplosbare AFB in die groep van vyf geneesmiddels was nie, die enigste geneesmiddel waarvoor 24 uur onvoldoende tyd was om ewewig te bereik. Verdere navorsing sal nodig wees om hierdie sodanige teenstrydigheid te verklaar.

Wat duidelik uit hierdie uitkomstes na vore gekom het, was dat die algemeen aanvaarde 24 uur oplosbaarheidstoets goed mag werk in baie gevalle, maar dat die toepaslikheid daarvan op ‘n individuele basis geëvalueer behoort te word en nie net vir die mees swak oplosbare middels nie. Navorsers wat oplosbaarheid by temperature laer as 37°C toets, moet veral daarteen waak om van standaard toetsperiodes gebruik te maak, aangesien ewewigsoplosbaarheid langer sal neem om bereik te word, as gevolg van minder energie wat vir die sisteem beskikbaar is.

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Chapter 1

Solubility of Pharmaceutical Actives

1.1 Introduction

Any active pharmaceutical ingredient (API) that is presented to the body must normally be in solution, for it to be absorbed into the cells by biological processes (Florence & Attwood, 2006). A *solution* forms when two or more components mix to form a single, homogeneous phase on the molecular level (figure 1.1). The *solvent* is the phase determining component and usually comprises the largest portion of the mixture. *Solutes* constitute the other components and are dispersed in the solvent as molecules or ions. The solutes are therefore dissolved in the solvent to form a solution (Aulton, 1988). The process whereby a solute dissolves in a solvent is called *dissolution* and the rate at which the solute is dissolved is the *dissolution rate* (Florence & Attwood, 2006).

Solutions may be grouped according to their physical state, i.e. a gas, a solid, or a liquid (Aulton, 1988). When the solvent and solute are both liquids, solubility is described by the term *miscibility* (Pharmalabs, 2012a). This study focuses on solutes in solid form and on liquid solvents.

Several factors control the solubility of an API in solution, such as the nature of the API molecule and the crystalline or amorphous form in which it exists, its hydrophobicity, its shape and surface area, among others (Florence & Attwood, 2006).

When any quantity of solute(s) is dissolved in a solvent at certain conditions of temperature and pressure, until a solubility limit is reached at which the solute in solution is in equilibrium with the undissolved solute, the resulting quantity of solute (concentration) in solution is called the *equilibrium solubility* of that solute in that specific solvent. Such a solution is called a *saturated solution*. A

supersaturated solution is formed when the solubility limit is exceeded, but this solution is unstable and the excess solute will precipitate readily (Aulton, 1988; Florence & Attwood, 2006; James, 1986; Lund, 1994).

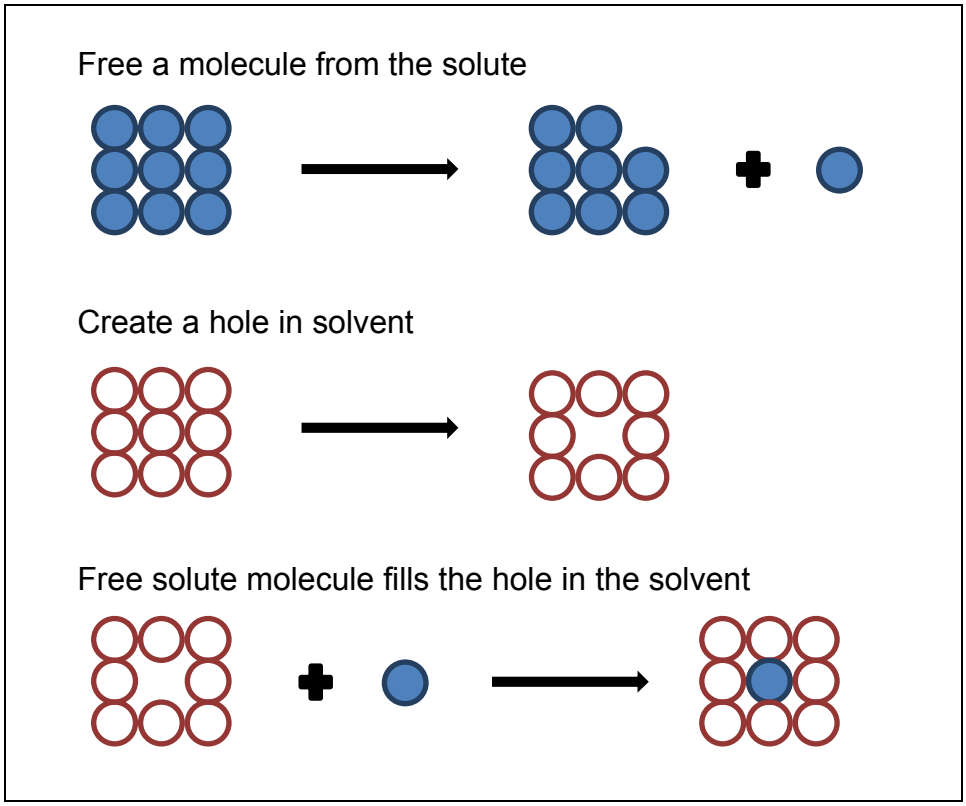


Figure 1.1: The solution process (Adapted from Gong *et al*., 2010).

In this chapter all aspects regarding solubility and factors influencing solubility are discussed.

1.2 Pharmaceutical importance of solubility

The aqueous solubility of an API is an important property that should be evaluated early and prior to formulating any product. At the initial stages of testing a new compound, an insoluble, or poorly soluble active, can affect the outcomes of screening assays, as well as of animal studies. The degree of solubility will affect the potential to develop a compound (Chen *et al*., 2006).

Pharmaceutical solutions may comprise of simple systems, but they can also occur in complex systems. The scientist should always be aware of that during preformulation. The most common type of interaction that occurs in dosage forms is the reaction between the API and water (Carstensen, 2007).

For an API to be absorbed into the systemic circulation to exert a therapeutic effect, it has to be in solution. The difference in solubility between various solid-state forms of a specific API will influence its bioavailability (Lund, 1994; Bernstein, 2002).

Bioavailability is when a substance is available in the body fluids for absorption after the substance has been introduced into the body, as well as the final amount of substance that is absorbed. (Lund, 1994).

The physical properties of an API, such as its solubility and hydrophobicity, can further influence its bioavailability. Also, different polymorphic and amorphic forms of a substance may exhibit different solubility values (Florence & Attwood, 2006).

The absorption rate of a substance, which is generally limited by its dissolution rate, determines the speed of onset of the therapeutic effect of the substance and the duration of the therapeutic response. Some substances are poorly soluble at the pH of body fluids, such as the stomach and intestines, which may pose challenges during formulation (Florence & Attwood 2006).

Amidon *et al.* (1995) proposed a biopharmaceutical, API classification scheme (BCS), whereby the API's *in vitro* dissolution and *in vivo* bioavailability are correlated. With this scheme the API's dissolution and gastrointestinal permeability are the main parameters controlling the rate and extent of absorption. These classes are defined as summarised in table 1.1.

Table 1.1: Classification of API substances according to the biopharmaceutical classification scheme (Amidon *et al*., 1995)

Class I High solubility High permeability	**Class II** Low solubility High permeability
Class III High solubility Low permeability	**Class IV** Low solubility Low permeability

The dissolution rate and solubility, in a solvent medium, are therefore two of the most important characteristics of an API, because these characteristics determine the bioavailability of the API for its intended therapeutic use (Brittain & Grant, 1999).

1.3 Units of solubility or concentration

1.3.1 Parts

Traditionally, solubility has been expressed in *parts,* without indicating any units, for example: Solute A is soluble in 200 parts of solvent B at 20ºC. This means that an amount of solvent, 200 times the volume of the solute, must be added to the solute at 20ºC to create a saturated solution of the solute. Very viscous liquids, however, will not give reliable results when measured in volume, therefore universal rules were set to avoid the problem:

- Mobile liquids are measured by volume; and
- Gases, solids and viscous liquids are measured by weight (James, 1986).

1.3.2 Solubility expressions / descriptions

Expressions or descriptive phrases are sometimes used by the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP) to describe approximate solubility (table 1.2).

Table 1.2: Common expressions used to describe solubility (James, 1986)

Descriptive phrase	Approximate amount of parts by volume (ml) of solvent for 1 part of solute by weight (g)
Very soluble	Less than 1 part
Freely soluble	From 1 - 10 parts
Soluble	From 10 - 30 parts
Sparingly soluble	From 30 - 100 parts
Slightly soluble	From 100 - 1000 parts
Very slightly soluble	From 1000 - 10 000 parts
Practically insoluble	More than 10 000 parts

Other sources, which also use these terms, were found to be the European Pharmacopoeia (EP) and Merck (2001) (table 1.4). These terms are, however, imprecise and not to be used for quantitative applications.

1.3.3 Quantity per quantity

Concentration is most commonly expressed as the weight of the solute in the volume of the solution. Its international system of units (SI) is $kg.m^{-3}$ ($g.dm^{-3}$), but more convenient weights and volumes may be used (Aulton, 1988). In this dissertation, the terms mg/ml or µg/ml were used.

1.3.4 Percentage

Concentration is often expressed as the amount of solute dissolved in 100 equivalent units of the solution:

- % w/w means percentage weight in weight;
- % w/v means percentage weight in volume; and
- % v/v means percentage volume in volume.

The expression is determined by the nature of the solute and the solvent (James, 1986).

1.3.5 Molarity and molality

*Molarity***,** symbolised by M, is the number of moles (molecular weight in gram) of solute in 1 dm^3 (1 litre) of solution. It is expressed as $mol.l^{-1}$ (SI unit: $mol.dm^{-3} = 10^3\ mol.m^{-3}$).

The dissociation of salts by solvation in a solvent like water means the separation of the anions and cations. Ionic substances could contain more moles of ions relative to the number of moles of the dissolved solute, i.e. 1.0 M of sodium sulfate would be 1.0 M in sulfate ions and 2.0 M in sodium ions (Gong *et al*., 2010).

Molality is the number of moles of solute in 1 kg of solvent. It is symbolised by m and the SI unit is $mol.kg^{-1}$. The use of molality is preferred over molarity, as it is not influenced by temperature (Aulton, 1988; James, 1986).

1.3.6 Mole fraction

Mole fraction, as discussed by Aulton (1988), is commonly used in theoretical calculations where the mole fraction of a component (x) in a solution is determined by dividing the number of moles of the component (n) by the total number of moles in the solution.

In the case of a binary solution (solution consisting of two components), the following equations may be used:

- Mole fraction of solvent = $x_1 = n_1/(n_1+n_2)$
- Mole fraction of solute = $x_2 = n_2/(n_1+n_2)$

where:

(n_1+n_2) is the total amount of moles in the solution consisting of a solvent ($_1$) and a solute ($_2$).

The sum of the mole fractions of all the components of the solution equals unity (1), thus for a binary solution: $x_1+x_2=1$.

1.3.7 Milli-equivalents

Milli-equivalent (mEq) is a clinical unit expressing the number of millimoles of an ion of a solute in a litre of solution. It is commonly used to refer to body fluids and the solutions used to replace body fluids, for example electrolytes (Aulton, 1988).

Equivalent weight (Eq) for monovalent ions expresses the molecular weight of the ion in gram or mole. For multivalent ions, the valency must be taken into account (Florence & Attwood, 2006).

The term, *normal solution*, or *normality* (N), is an analytical chemistry term and refers to a solution that contains the equivalent weight (Eq) of the solute in gram, dissolved in 1 litre of solution (1 $Eq.dm^{-3}$). For example: 1 N NaCl consists of 5.8 g NaCl in 100 ml (molecular mass of NaCl = 58.44 g/mol). Care should be taken not to confuse normality (N) with the term, *normal saline solution*, which in medical terms is a general phrase referring to a solution of 0.9 g NaCl in 100 ml of water (Aulton, 1988; Florence & Attwood, 2006). The normality of this 0.9 g/100 ml NaCl solution would then be 0.015 N.

1.4 Determining solubility

To determine the solubility of an API in a solvent, an excess thereof is dissolved in the solvent through shaking or stirring over a certain period of time at a set temperature until equilibrium is reached and a saturated solution is formed. To reach equilibrium more quickly, the solvent with an excess of solute may be heated and the resulting solution then allowed to cool to the required temperature. Care should be taken though if an API exhibits polymorphism, because the heating and subsequent cooling could induce or promote the growth of another crystalline form having a different solubility. The detailed method (shake-flask) for determining solubility in this study is discussed in chapter 2.

A quantity of the saturated solution is then filtered to separate the solution from the undissolved substance, prior to analysis. This is done at the temperature at which solubility is determined, to prevent a change in equilibrium between solvent and solute.

A suitable method of analysis is chosen to determine the concentration, for example ultraviolet (UV) spectrophotometry or high performance liquid chromatography (HPLC). The properties of the solvent and solute determine which method to use (Aulton, 1988).

1.5 Factors influencing solubility

The factors that may influence the solubility of a solid in a liquid include nature of the solute, nature of the solvent, temperature and additives. Each is discussed in detail next.

1.5.1 Nature of the solute

1.5.1.1 Molecular structure of the solute

The nature of the solute has a large influence on the solubility of the solute in a solvent. Even a small change in the molecular structure of a compound may result in a significant effect on the solubility of the compound in a specific solvent (Aulton, 1988).

Normally, the assumption is made that all molecules will dissolve either in water or in an organic solvent. If a molecule dissolves completely in water, the term *hydrophilic* is used, or it is said that the molecule has a hydrophilic character. The following terms explain the nature of molecules in relation to their water- or lipid "loving" or -"hating" characters:

- Hydrophilic Water loving
- Lipophobic Lipid hating
- Lipophilic Lipid loving
- Hydrophobic Water hating

The key to solubility is whether the chemical or molecule and its functional groups can bind to water- or lipid solvent molecules. Water is an important solvent in the pharmaceutical industry and to predict water solubility, the number of hydrophilic- and lipophilic groups in a molecule should be calculated. A molecule with mostly hydrophilic groups and interaction with water through hydrogen bonding, or ion-dipole attraction can be expected to dissolve in water. Contrary, a molecule with a lipophilic character and capable of Van der Waals attraction would probably dissolve in nonaqueous- or lipophilic media (Lemke, 1995).

A lipophilic group normally includes molecules with a large hydrocarbon moiety, like the alkane group, $CH_3(CH_2)_n$ with $n > 4$. Hydrophilic groups are normally charged groups, such as phosphates, sulfates, sulfonates and amine groups (Lemke, 1995).

The conversion of a weak acid into its sodium salt leads to a much higher degree of ionic dissociation when it dissolves in water. Salicylic acid and its sodium salt are excellent examples of this effect, in which case the solubility of the salicylic acid in water is 1 in 550 and the solubility of the salt is 1 in 1 (Aulton, 1988).

The altering or modification of the molecular structure of a specific API could also be used to mask taste, or to protect it against degradation in the stomach. Chloramphenicol palmitate is less soluble than the chloramphenicol base, but this esterification of the base is used to mask the taste of the parent API. The palmitate is used in paediatric suspensions to mask the bitter taste of the base. Similarly, erythromycin propionate is less soluble than erythromycin, but the propionate is used to protect the API against degradation in the stomach (Aulton, 1988).

The chloroquine base, for example, is less soluble than its diphosphate salt, with the solubility of the diphosphate in water being reported as 50 mg/ml and that of the base as 10.6 mg/ml (Sciencelab, 2011; Drugbank, 2010b).

1.5.1.2 Crystal characteristics / various solid-state forms

McCrone's (1965) definition of polymorphism is still the most accurate description found in literature, stating that "The polymorphism of any element or compound is its ability to crystallise as more than one distinct crystal species." (McCrone, 1965).

Polymorphism could impact on the solubility of an API. Mebendazole, for example, occurs as three known polymorphs, with their solubilities in physiological media differing markedly and in the order of polymorph B > polymorph C > polymorph A (Brits *et al*., 2010).

Solvatomorphism is the ability of a substance to exist in two or more crystalline phases, whilst differing in their elemental compositions through the inclusion of water (hydrates), or other solvent molecules (solvates) (Gong *et al*., 2010).

Phase changes can occur in solid-state hydrated or solvated systems, as a result of environmental changes, such as temperature and humidity. Hydrated and solvated compounds can convert into their amorphous phases upon dehydration or desolvation. Alternatively, some compounds may convert from a lower to a higher state of hydration, yielding forms with lower solubilities. A kinetically favoured, but thermodynamically unstable form may convert into a more stable, but less soluble form during pharmaceutical processing (Vippagunta *et al*., 2001). Van Tonder *et al*. (2004) investigated the solubility of several solvates of niclosamide in water. The solubilities of those solvates were lower than that of the anhydrous form, because the solvates had transformed into the least soluble hydrate. Furthermore, two solvates are reported for glibenclamide, i.e. pentanol and toluene, with both these solvates having higher solubility values than those of the two non-solvated polymorphs (Suleiman & Najib, 1989).

The different internal energies of these different solid-state forms are manifested in different magnitudes of lattice energy, which lead to different solubilities for these different forms mentioned (Gong *et al*., 2010).

1.5.1.3 Amorphism

Some excipients and pharmaceutical actives have no long-range order of molecular packing, like the crystalline solids and they are referred to as amorphous (glass) solids (Yu, 2001). Amorphous solids are disordered in nature and are thermodynamically less stable than their corresponding, crystalline forms (Gong *et al*., 2010). An amorphous form represents the most highly energetic solid-state form of a material (Hancock & Zografi, 1997), and therefore amorphous materials exhibit the highest degree of solubility for a given substance (Gong *et al*., 2010).

Aucamp *et al.* (2010) had prepared an amorphous glass form of roxithromycin, after which a solubility study was conducted on the material. This amorphous form was, at the time of testing, stable enough to show a significant

improvement in its solubility in water compared to the more crystalline raw material.

It should be noted that the higher solubility of amorphous forms (or any other metastable forms) *versus* their crystalline counterparts (stable forms) can be demonstrated by means of dissolution studies, showing concentration against time. Theoretically, however, it is impossible to determine the equilibrium solubility of most organic amorphous materials, since progressive transformation of the amorphous solid into a more stable crystalline form (lower energy state) has the effect that no equilibrium is reached, until the material has completely transformed, at which time the measured equilibrium solubility will be that of the more stable form.

1.5.1.4 Particle size of the solid

The Ostwald-Freundlich equation defines the effects of particle radius (r), molar volume (v), density (ρ) and interfacial tension (γ) on solubility (S) at temperature T. By reducing the particles size, the API solubility will increase, all other factors being constant (Kipp, 2010).

$$\ln \frac{S}{So} = \frac{2v\gamma}{rRT} = \frac{2M\gamma}{\rho rRT'}$$

where:

S_0 is the solubility of a flat solid sheet ($r \rightarrow \infty$),

M is the molecular weight of the solid, and

R is the ideal gas constant.

A reduction in particle size increases the surface area of a substance that is exposed to the solvent and this tends to increase its dissolution rate. It is necessary to control the particle size during production, since powders consisting of different particle sizes may alter the volume of powder that is

encapsulated or compressed during solid dosage form production (Aulton, 1988). Particle size control during the formulation of dosage forms can even prove beneficial to controlling bioavailability, as it is sometimes necessary that substance absorption is prolonged to have a prolonged therapeutic effect (Florence & Attwood, 2006).

Measuring particle size is the first necessary step in particle size control. However, because particle shapes are irregular, it is impractical and difficult to measure more than one dimension and therefore a particle is considered an approximate sphere. The approximate diameter measurement of particles is referred to as the equivalent diameter.

Since powders contain particles of different equivalent diameters, the data is presented by generating a histogram. The histogram can be used to compare the particle size distribution of different powders (Aulton, 1988).

Aulton (1988) describes the sieve-, microscopic-, coulter counter-, laser light scattering- and sedimentation methods through which to determine particle size.

The biopharmaceutical importance of particle size is a highly discussed topic. The absorption rate of a poorly water soluble API is limited by its dissolution rate and its permeability. The particle size of such a poorly soluble API is thus very important (Florence & Attwood, 2011). Poor permeability characteristics, however, could also be responsible for poor bioavailability data, and is solubility and particle size hence not the only factors involved (Lindenberg *et al*., 2004).

The dissolution rate increases as the particle size is reduced (refer to the Noyes-Whitney equation as discussed in section 1.6).

The relevance of solid-state properties to this equation lies in the fact that it is determined by particle size. The effect on the dissolution rate will be increased if the area of the solid being exposed to the solvent is also increased by micronisation and further by amorphous APIs (Florence & Attwood, 2011).

It is generally accepted that the dissolution rate of a substance will increase with a decrease in particle size, but the influence thereof on solubility is very small, unless the particle size is reduced to less than a micron (Aulton, 1988). Aulton (1988) also refers to the findings of Buckley, which states that with particles having a very small radius, the electrical charge on the particles will become more important as the size of the particle decreases. The resultant effect is that with particles having a very small radius, solubility will cease to increase indefinitely as the particle size continues to decrease, because of the fact that the particles tend to aggregate. Florence and Attwood (2006), however, state that the intrinsic solubility of a substance may be influenced by a particle size reduction of below 0.1 μm, since very small particles have a very high surface/bulk ratio, which increases the interaction with solvent.

Although the general rule is that amorphous materials do have a higher solubility than crystalline material, the opposite was found in a study by Henwood *et al*. (2000). In solubility and dissolution studies of generic rifampicin materials, it was found that the batches with amorphous content tended to demonstrate a much lower dissolution rate than those of the more crystalline samples. Also, the solubility of the amorphous samples was much lower than that of the crystalline samples. This behaviour was attributed to the electrostatic properties of the fine amorphous particles, which resulted in lump formation (Henwood *et al*., 2000).

Particles must also be kinetically stabilised in suspensions, for example, to prevent aggregation. Another problem that manifests in suspensions is referred to as Ostwald ripening. During this phenomenon, smaller particles, being more soluble, will dissolve preferentially and deposit onto the surface of larger particles, which then results in particle growth over time.

To conclude, particle size reduction, unless on nano scale, will lead to an increase in the dissolution rate of an API, but it will only yield a very small increase in solubility, if any.

1.5.2 Nature of the solvent

In an ideal solution the forces among the molecules of the solvent, solute and solvent-solute are theoretically equal, but in a real solution, however, the forces are not the same (Aulton, 1988; Gong *et al*., 2010).

The intermolecular forces of a solute may be broken if the dielectric constant (δ) of the solvent is high enough, resulting in electrolytes. This process is known as ionisation, or dissociation (Aulton, 1988). The dielectric constant of a substance is a term used to indicate the polarity of the substance (Pharmalabs, 2012b). This is also a measure of the energy required for separating molecules with opposite charges (Persky & Hughes, 2006).

Solvents are grouped, depending on the dielectric constant, as polar (δ > 50), semi-polar (δ = 20), or non-polar (δ = 1 - 20) (Pharmalabs, 2012b).

Water, one of the most common and important solvents used in pharmaceutical solutions, has a dipole molecular structure with highly organised hydrogen bonds. This type of bonding results in polar molecules and thus a high dielectric constant (80.4 at 20°C). Water is an effective solvent for sodium chloride (NaCl), in which the intermolecular forces are also polar. The general rule of 'like dissolves like' can be followed where polarity is used to describe a solvent, meaning that when a polar solute must be dissolved, a polar solvent should be used and when a non-polar solute needs dissolving, a non-polar solvent should be used (Persky & Hughes, 2006).

1.5.2.1 Ions and electrolytes

The following equation is used to demonstrate the equilibrium between the sparingly soluble salt in solution with the undissolved solid of the salt:

$$AB \leftrightarrow A^{+} + B^{-}$$

where:

AB is the solid and A^{+} and B^{-} are the ions in solution.

If either of the ions is added to the solution, the solid will precipitate and the solubility of the solute will decrease.

In a solution containing a sparingly soluble electrolyte, the addition of a second electrolyte, not containing the same ions as the first electrolyte, will increase the solubility of the first electrolyte (Aulton, 1988).

The solubility of a non-electrolyte in water depends on the formation of weak intermolecular bonds between the molecules of the non-electrolyte and the water molecules. The addition of a very soluble electrolyte to the solution will decrease the solubility of the non-electrolyte, because the molecules of the electrolyte compete with the molecules of the non-electrolyte and break the intermolecular bonds between the molecules of the non-electrolyte and the water molecules (Aulton, 1988).

1.5.2.2 pH

For ionisable substances, pH may influence the solubility. If the pH of a solution containing a weakly acidic solute is decreased, the proportion of unionised acid molecules increases. The solute may precipitate, because the solubility of the unionised form is less than that of the ionised form. If the pH of a solution containing a weakly acidic solute increases, the solubility of the solute will increase. For solutions of weakly basic solutes, precipitation will occur when the pH is increased, but by lowering the pH, the solubility will improve (Aulton, 1988).

For substances that are non-ionisable, pH will not significantly affect solubility. In such instance, solubility may be improved by adding co-solvents, as discussed in section 1.5.4.1 (Persky & Hughes, 2006).

1.5.3 Temperature

During the process of dissolution, energy is required to break intermolecular forces to separate the molecules. It is often observed that by adding heat, solubility increases, as the heat provides energy to break the intermolecular forces (Persky & Hughes, 2006).

It must, however, also be taken into account that certain solutes may decompose at higher temperatures (James, 1986).

With non-polar compounds, the intermolecular forces are small and heat will not have a significant impact on their solubility. With polar compounds, where the intermolecular forces are greater, the adding of heat supplies the required energy to break the forces, which usually has a cooling effect that is called an endothermic reaction. Most dissolution processes of APIs are endothermic. The opposite, where there is an interaction between the solute and solvent that releases heat, the reaction is called exothermic (Persky & Hughes, 2006).

For endothermic reactions, solubility increases with an increase in temperature, whilst the reverse is true for exothermic reactions. With $Na_2SO_4.10H_2O$ an endothermic reaction will occur during dissolution below 32.5ºC, but with an increase in temperature, the decahydrate will transform into the anhydrous form, resulting in an exothermic reaction (Aulton, 1988).

1.5.4 Additives

1.5.4.1 Co-solvents

A co-solvent is another solvent in which the solute dissolves more easily than in the primary solvent. Co-solvents are often added to a pharmaceutical solution to increase the solubility of a solute in water, such as the use of ethanol or propylene glycol, for example (Aulton, 1988). This is achieved because of the co-solvent that decreases the dielectric constant of the solvent (Persky & Hughes, 2006).

1.5.4.2 Complex formation

When a third substance is added to a solution containing a solute that is dissolved in a solvent, an intermolecular bond may form between the molecules of the third substance and the original solute. The solubility of the resulting complex may increase or decrease the apparent solubility of the original solute (Aulton, 1988).

1.5.4.3 Solubilising agents

Solubilisation is when a solubilising agent, such as a surface active agent (surfactant) is added to a solvent containing a poorly soluble or insoluble solute. A surfactant has a polar- and a non-polar end. When a surfactant is added to a solution containing a non-polar solute, the solute will bind to the non-polar end of the surfactant and form a *micelle*, or *aggregate*, as illustrated in figure 1.2. The centre of the micelle has a different organic phase than the solvent. The outer part of the micelle will interact with the solvent, resulting in an increase in apparent solubility of the solute (Aulton, 1988; Persky & Hughes, 2006).

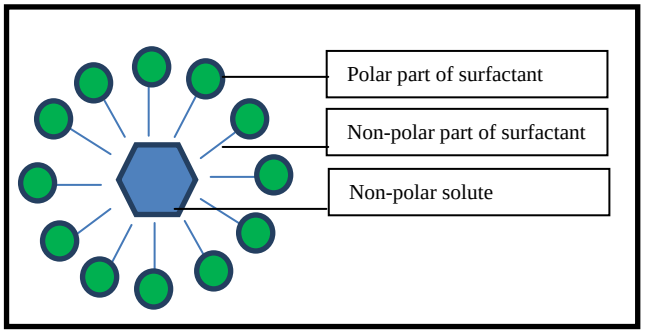


Figure 1.2: Formation of a micelle (Adapted from Persky & Hughes, 2006).

1.6 Dissolution rate and the factors influencing it

The rate at which the solute is dissolved in the solvent is called the dissolution rate (Florence & Attwood, 2006). According to James (1986), the dissolution rate of a solid in a liquid was quantified by Noyes and Whitney into an equation, known as the Noyes-Whitney equation:

$$\frac{dm}{dt} = \frac{AD(Cs - C)}{d}$$

The equation parameters and the factors that influence them are summarised in table 1.3.

Table 1.3: Noyes-Whitney equation parameters and factors affecting them (Aulton, 1988; Florence & Attwood, 2006)

Equation parameter	Description of parameter	Factors affecting the parameter
dm/dt or d*w*/d*t*	The **dissolution rate**, where *m* or *w* is the mass of solute that has passed into solution in time (*t*).	C_s, C, D, A, d
C_s	The **solubility** of the solute.	Refer to section 1.5
C	The **concentration** of the solute in solution at a certain time (*t*).	Volume of solvent
k	The (intrinsic) **dissolution rate constant**.	D, A, d
D	**Diffusion coefficient** of the solute in the solvent.	Viscosity of solvent. Size of diffusing molecules.
A	The **surface area** of the undissolved solute that is in contact with the solvent.	Particle size of solute particles. Dispersibility of solute. Porosity of solute particles.
d = h = δ	**Thickness** of the diffusion layer.	Degree and method of agitation.

Variations of the equation were found in Aulton (1988):

$$d\mathit{m}/d\mathit{t} = kA\ (C_s\text{-}C),\ \text{where } k = D/V\mathit{h}$$

and Florence & Attwood (2006):

$$d\mathit{w}/d\mathit{t} = k\ (C_s\text{-}C),\ \text{where } k = DA/\delta.$$

These equations are in essence the same, with the only difference being the V (volume of dissolution medium), which is added to Aulton's (1988) equation.

To summarise, factors which influence the dissolution rate, according to the Noyes-Whitney equation, are the diffusion coefficient, the surface area of the solute particle, the concentration of the solute particles at the boundary layer and the height or thickness of the boundary layer (Anon, 2012).

1.6.1 Solubility (Cs) / Concentration (C)

If the solubility of a solute in a solvent is high, the dissolution rate will also be high (Florence & Attwood, 2006).

When the concentration reaches the point where it is the same as the solubility, the net dissolution rate is zero, because of the dissolution medium being saturated with solute at that point (Aulton, 1988). This means that the solute dissolves at the same rate as the rate at which it recrystallises.

Concentration is influenced by the volume of the solvent. When the volume increases, the dissolution rate will also increase. Also, when the concentration is reduced by absorption into the cells of the body, the dissolution rate will increase (Florence & Attwood, 2006).

1.6.2 Diffusion coefficient

Diffusion is when a substance is transferred spontaneously from a region where it has a high chemical potential to a region where it has a low chemical potential. The *diffusion coefficient* (D) has a constant value for a specific

system at a certain temperature. D will be higher for solutes with smaller molecules and for solvents with lower viscosity (Aulton, 1988).

1.6.3 Surface area: Wettability

The Noyes-Whitney equation indicates that the dissolution rate is proportional to the surface area of the solute being exposed to the solvent (James, 1986). When particle size is reduced, the area exposed to solvent increases, which may increase the dissolution rate (Florence & Attwood, 2006). This is highly significant for solutes that are poorly soluble whether in a preparation, or in biological fluids. Where particle size is very small the solute may, however, be difficult to wet, which will reduce the dissolution rate. This may happen when the solute particles aggregate and trap air, hence preventing contact between the particles and the solvent (Parsons *et al*., 1992).

Wetting occurs when the solvent penetrates the powders, tablets or granules before dissolution. The wettability of a powder is measured by the contact angle (θ) between the solid and solvent. If the contact angle is zero, the substance will be completely wettable. This happens when the forces of attraction between the liquid and solid particles are equal or larger than the forces between the liquid particles. The wettability of a solute with poor wettability may be improved by adding surfactants. This also improves the dispersibility of the solute, which in turn increases the dissolution rate (Florence & Attwood, 2006).

1.6.4 Thickness of diffusion layer / aqueous boundary layer

The diffusion layer is the boundary layer surrounding the undissolved solute. The thickness of the diffusion layer will decrease when agitation increases (James, 1986).

The higher the value of the diffusion coefficient, the larger the surface area and the more concentrated the solute particles at the boundary layers are, and

hence the higher the dissolution rate. According to the Noyes-Whitney equation, the thickness of the boundary layer is indirectly proportional to the dissolution rate, hence the lower the thickness, the faster the dissolution rate (Anon, 2012).

1.7 Aims, objectives and experimental design

With general experimental solubility testing of different APIs, the question always arises as to whether particle size had been determined before or not. All available literature suggests that particle size, for pharmaceutical powders, does not significantly affect equilibrium solubility. The dissolution rate will differ according to different particle sizes, but the overall results should be identical after equilibrium is established. This study was therefore planned to investigate whether different particle size fractions of the same API, dissolving at different rates, would all reach solubility equilibrium within 24 hours.

Furthermore, APIs from different solubility classes were investigated, because poorly soluble substances would most likely require a longer period of time to equilibrate. A period of 24 hours was selected, because many published solubility studies used this time interval and is it the standard of our research group also. A comprehensive literature study was conducted beforehand with the aim of selecting a series of APIs ranging from freely soluble to poorly water soluble, in order to investigate their experimental solubilities and to determine the influence (if any) of particle size on their equilibrium solubilities and on the time required for that status to be attained.

The solubility values of a variety of APIs available in-house are listed in table 1.4, from which five pharmaceutical actives were chosen for this study (table 1.5). The selection criterion was to cover the whole spectrum of pharmaceutical actives from very soluble to poorly soluble.

Table 1.4: Solubility data of various APIs

API	Solubility
Abacavir	In water (25°C): > 80 mM (pH 7) (Merck, 2001).
Amodiaquine dihydrochloride dihydrate	Soluble in water; sparingly soluble in alcohol; very slightly soluble in benzene, chloroform, ether. pH of 1% aqueous solution 4.0 – 4.8 (Merck, 2001).
Artemether	Practically insoluble in water; very soluble in dichloromethane & acetone; freely soluble in ethyl acetate & dehydrated ethanol (Merck, 2001; Artepal, 2010).
Artesunate sodium	Poor stability in aqueous solutions (Merck, 2001).
Azithromycin	Experimental water solubility: slightly. Predicted water solubility: 5.14e-01 mg/ml (Merck, 2001; Drugbank, 2010a).
Chloroquine diphosphate	Freely soluble in water; pH of 1% solution about 4.5; less soluble at neutral and alkaline pH. Stable to heat in solution of pH 4.0 – 6.5. Practically insoluble in alcohol, benzene, chloroform, ether (Merck, 2001). Easily soluble in cold water. Solubility in water: 50 mg/ml (Sciencelab, 2011).
Chloroquine	Experimental water solubility 10.6 mg/L. Predicted water solubility 1.75e-02 mg/ml (Merck, 2001; Drugbank, 2010b).
Closantel sodium	No reliable data available.
Didanosine	Solubility at 23°C (mg/ml): acetone <1; acetonitrile <1; *t*-butanol <1; chloroform <1; dimethylacetamide 45; DMSO 200; ethanol 1; ethyl acetate <1; hexane <1; methanol 6; methylene chloride <1; polyethylene glycol-300 1; 1-propanol <1; 2-propanol <1; polyethylene glycol 8 (Merck, 2001). Partially soluble in cold water (USP, 2011). Experimental water solubility 15.8 mg/ml. Predicted water solubility 6.58e+00 g/L (Drugbank, 2011a).
Dihydroartemisinin	Practically insoluble in water; slightly soluble in acetonitrile R, ethanol (~750 g/L) TS, dichloromethane R (Artepal, 2010).
Doxycycline HCl hemiethanolate hemihydrate	Soluble in water (Merck, 2001).
Efavirenz	Practically insoluble in water at 9.2 µg/ml (pH 8.7) at 25°C. The aqueous solubility increases as the pH increases above 9.0, consistent with the loss of the proton on the amine of the carbamate. The solubility of efavirenz increases in Miglyol 810, soybean oil and safflower oil to 150 mg/ml, 82 mg/ml,

	and 77 mg/ml, respectively. The solubility is further increased in polyethylene glycol 400 (PEG 400), propylene glycol and Tween 80 to concentrations of 420 mg/ml, 368 mg/ml and 150 mg/ml, respectively. Alcohols afford dramatic improvements in solubility with values of 725 mg/ml, 663 mg/ml and 598 mg/ml for methanol, ethanol and isopropanol, respectively (Merck, 2001; Rowe, 1999).
Erythromycin estolate	Practically insoluble in water; freely soluble in alcohol; soluble in acetone. Practically insoluble in dilute hydrochloric acid (EP, 2011). Experimental water solubility: Slightly soluble (1.44 mg/L). Predicated water solubility 4.59e-01 g/L (Drugbank, 2011b).
Erythromycin stearate	Practically insoluble (USP, 2009).
Ethambutol dihydrochloride	Soluble in water, DMSO; sparingly soluble in ethanol; poorly soluble in acetone, chloroform (Merck, 2001).
Isoniazid	Solubility in water at 25° about 14%; at 40° about 26%; in alcohol at 25° about 2%; in boiling alcohol about 10%; in chloroform about 0.1%. Practically insoluble in ether, benzene. pH of 1% aqueous solution 5.5 - 6.5 (Merck, 2001).
Lamivudine	Solubility in water (20°C): ~ 70 mg/ml (Merck, 2001).
Lopinavir	Freely soluble in methanol and ethanol; soluble in isopropanol; practically insoluble in water (Merck, 2001; Aidsinfo, 2010).
Lumifantrine	Poorly soluble in water, oil and most organic solvents. Soluble in unsaturated fatty acids (Merck, 2001).
Mefloquine hydrochloride	Soluble in ethanol, ethyl acetate; slightly soluble in water (Merck, 2001). Experimental water solubility 5000 mg/L (HCl salt). Predicted water solubility 3.80e-02 g/L (Drugbank, 2011c).
Nevirapine	Lipophilic. Solubility in water ~0.1 mg/ml at neutral pH; highly soluble at pH <3 (Merck, 2001).
Pyrazinamide	Solubility (mg/ml): water 15; methanol 13.8; ethanol (absolute) 5.7; isopropanol 3.8; ether 1.0; isooctane 0.01; chloroform 7.4. Aqueous solutions are neutral (Merck, 2001).
Quinine	1 g dissolves in 1900 ml water, 760 ml boiling water, 0.8 ml alcohol, 80 ml benzene (in 18 ml benzene at 50°C), in 1.2 ml chloroform, 250 ml dry ether, 20 ml glycerol, 1900 ml of 10% ammonia water. Almost insoluble in petroleum ether (Merck, 2001).

Quinine dihydrochloride	1 g dissolves in about 0.6 ml water, in about 12 ml alcohol. Slightly soluble in chloroform; very slightly soluble in ether (Merck, 2001).
Rifampicin	Freely soluble in chloroform, DMSO; soluble in ethyl acetate, methanol, tetrahydrofuran; slightly soluble in water (pH <6), acetone, carbon tetrachloride (Merck, 2001).
Ritonavir	Freely soluble in methanol, ethanol; soluble in isopropanol; practically insoluble in water (Merck, 2001; Aidsinfo, 2010).
Roxithromycin	Experimental water solubility 0.0189 mg/L at 25°C. Predicted water solubility 1.87e-01 mg/ml (Merck, 2001; Drugbank, 2010c).
Stavudine	Experimental water solubility: 5 - 10 g/100 ml at 21°C (Merck, 2001; Drugbank, 2010d).
Streptomycin sulfate	Predicted water solubility 1.28e+01 g/L (Drugbank, 2011d).
Zidovudine	Solubility in water (25°C): 25 mg/ml (Merck, 2001).

From this original list of available pharmaceutical actives, a short list of actives was selected for conducting this study (table 1.5). The BCS classification (table 1.1) of the APIs chosen for this study, as well as the preferred methods of analysis are also shown in table 1.5.

Table 1.5: APIs chosen for this solubility study

API	Summary of solubility	BCS classification	Summary of BCS classification	Test method
Closantel sodium	No data available	No data available	-	HPLC
Roxithromycin	Practically insoluble in water	[b]Class IV	Low solubility and low permeability	HPLC
Mefloquine HCl	Slightly soluble in water	[a]Class II / IV	Low solubility and high permeability / Low solubility and low permeability	UV
Pyrazinamide	Sparingly soluble in water	[a]Class I	High solubility and high permeability	UV
Chloroquine phosphate	Easily soluble in cold water	[a]Class I	High solubility and high permeability	UV

a Lindenberg *et al*., 2004.

b Benet *et al*., 2011.

1.8 Conclusion

In this chapter, the solubility of pharmaceutical actives and factors influencing the solubility thereof were discussed. The focus of this study, however, was to determine whether or not 24 hours would allow sufficient time for establishing equilibrium solubility, irrespective of the influence that particle size has on the dissolution rate of various APIs, ranging from virtually insoluble to freely soluble in water. Temperature and time were kept at constant values throughout the study. APIs on which solubility studies were performed were chosen, depending on their known solubility values, as obtained from the literature. The solubility values of the chosen APIs ranged from freely soluble to practically insoluble in water. The results of the different API solubility studies are discussed in the following chapters.

References

Aidsinfo. 2010. Aidsinfo Drug Database: Lopinavir / Ritonavir. http://www.aidsinfo.nih.gov/DrugsNew/DrugDetailT.aspx?int_id=316 Date of access: 03 February 2010.

Amidon, G.L., Lennernäs, H., Shah, V.P. & Crison, J.R. 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharmaceutical Research*, 12:413-420.

Anon. 2012. http://pharmacyclassroom.blogspot.com/2011/01/noyes-whitney-equation.html Date of access: 10 October 2012.

Artepal. 2010. The portal of information and orientation on malaria and its treatments with ACT - Monographs. http://www.artepal.org/index.php?option=com_content&task=blogcategory&id=66&Itemid=132 Date of access: 03 February 2010.

Aucamp, M., Liebenberg, W. & De Villiers, M.M. 2010. Preparation method of a medicament. PCT application: PCT/1B2010/055841.

Aulton, M.E., *ed*. 1988. Pharmaceutics: the science of dosage form design. United Kingdom: Churchill Livingston, 734 pp.

Benet, L.Z., Broccatelli, F. & Oprea, T.I. 2011. BDDCS applied to over 900 drugs. *The AAPS Journal*, 13:519-547.

Bernstein, J. 2002. Polymorphism in molecular crystals. Oxford: Oxford University Press, 410 pp.

Brits, M., Liebenberg, W. & De Villiers, M.M. 2010. Characterisation of polymorph transformations that decrease the stability of tablets containing the WHO essential drug mebendazole. *Journal of Pharmaceutical sciences*, 99:1138-1151.

Brittain, H.G. & Grant, D.J.W. 1999. Effects of polymorphism and solid-state solvation on solubility and dissolution rate. (*In* Brittain. H.G. *ed*. Polymorphism in pharmaceutical solids. New York: Marcel Dekker Inc., 427pp).

Carstensen, J.T. 2007. Solid-state stability. (*In* Carstensen, J.T. & Rhodes, C.T. *eds*. Drug stability: principles and practices. Informa Healthcare: New York, 773pp).

Chen, X., Antman, M.D., Gesenberg, C. & Gudmundsson, O.S. 2006. Discovery pharmaceutics: challenges and opportunities. *The AAPS Journal*, 8:E402-E408.

Drugbank. 2010a. Azithromycin. http://www.drugbank.ca/drugs/DB00207 Date of access: 03 February 2010.

Drugbank. 2010b. Chloroquine. http://www.drugbank.ca/drugs/DB00608 Date of access: 03 February 2010.

Drugbank. 2010c. Roxithromycin. http://www.drugbank.ca/drugs/DB00778 Date of access: 03 February 2010.

Drugbank. 2010d. Stavudine. http://www.drugbank.ca/drugs/DB0064 Date of access: 06 February 2010.

Drugbank. 2011a. Didanosine. http://www.drugbank.ca/drugs/DB0090 Date of access: 21 June 2011.

Drugbank. 2011b. Erythromycin estolate. http://www.drugbank.ca/drugs/ DB00199 Date of access: 21 June 2011.

Drugbank. 2011c. Mefloquine HCl. http://www.drugbank.ca/drugs/DB00358 Date of access: 21 June 2011.

Drugbank. 2011d. Streptomycin sulfate. http://www.drugbank.ca/drugs /DB01082 Date of access: 20 June 2011.

EP see European Phamacopoeia.

European Phamacopoeia. 2011. Erythromycin estolate monograph. 5.0:1520-1521. http://lib.njutcm.edu.cn/yaodian/ep/EP5.0/16_monographs/monographs_d-k/Erythromycin%20estolate.pdf Date of access: 21 June 2011.

Florence, A.T. & Attwood, D. 2006. Physicochemical principles of pharmacy. 4th ed. Pharmaceutical Press: London, 492 pp.

Gong, Y., Grant, D.J.W. & Brittain, H.G. 2010. Principles of solubility. (*In* Augustijns, P. & Brewster, M.E. *eds*. Solvent systems and their selection in pharmaceutics and biopharmaceutics. Belgium: Springer, AAPS Press, 459pp).

Hancock, B.C. & Zografi, G. 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. *Journal of Pharmaceutical Sciences*, 86:1-12.

Henwood, S.Q., De Villiers, M.M., Liebenberg, W. & Lötter, A.P. 2000. Solubility and dissolution properties of generic rifampicin raw materials. *Drug Development and Industrial Pharmacy*, 26:403-408.

James, K.C. 1986. Solubility and related properties. United States of America: Marcel Dekker Inc., 425 pp.

Kipp, J.E. 2010. Solubilizing systems for parenteral formulation development: small molecules. (*In* Augustijns, P. & Brewster, M.E. *eds*. Solvent systems and their selection in pharmaceutics and biopharmaceutics. Belgium: Springer, AAPS Press, 459pp).

Lemke, T.L. 1995. Review of organic functional groups. 3rd ed. Philadelphia: Lea and Febiger, 148 pp.

Lindenberg, M., Kopp, S. & Dressman, J.B. 2004. Classification of orally administered drugs on the World Health Organization model list of essential medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics*, 58:265-278.

Lund, W. *ed.* 1994. The pharmaceutical codex. 12th ed. London: The Pharmaceutical Press, 1117 pp.

McCrone, W.C. 1965. Polymorphism: physics and chemistry of the organic solid-state, 2:725.

Merck & Co., Inc. 2001. The Merck Index: an encyclopedia of chemicals, drugs and biologicals. 13th ed. Whitehouse Station: NJ, 1818 pp.

Parsons, G., Bucton, G. & Chatham, S.M. 1992. The use of surface energy and polarity determinations to predict physical stability of non-polar, non-aqueous suspensions. *International Journal of Pharmaceutics*, 83:163-70.

Persky, A.M. & Hughes, J.A. 2006. Solutions and solubility. http:www.cop.ufl.edu/safezone/prokai/pha5100/solubility.htm Date of access: 31 March 2006.

Pharmalabs. 2012a. Factors influencing the solubility of drugs: introduction. http://pharmalabs.unc.edu/labs/solubility/intro.htm Date of access: 28 June 2012.

Pharmalabs. 2012b. Factors influencing the solubility of drugs: solute and solvent structure. http://pharmalabs.unc.edu/labs/solubility/structure.htm Date of access: 28 June 2012.

Rowe, S., Fontalbert, L., Rabel, S. & Maurin, M. 1999. Physical chemical properties of efavirenz. *The AAPS Journal*: Annual Meeting Abstracts, S1(1):1005. http://www.aapsj.org/abstracts/AM_1999/1005.htm Date of access: 03 February 2010.

Sciencelab. 2011. Material safety data sheet for chloroquine phosphate. http://www.sciencelab.com/MSDS Date of access: 20 June 2011.

Suleiman, M.S. & Najib, N.M. 1989. Isolation and physicochemical characterisation of solid glibenclamide. *International Journal of Pharmaceutics*, 50:103-109.

United States Phamacopoeia. 2009. Material safety data sheet: erythromycin stearate. http://www.usp.org/pdf/EN/referenceStandards/msds/1248006.pdf Date of access: 09 March 2009.

United States Pharmacopoeia. 2011. Material safety data sheet: didanosine. Revision date: March 31, 2006. http://www.usp.org/pdf/EN/referenceStandards /msds/1191204.pdf Date of access: 20 June 2011.

USP see United States Phamacopoeia.

Van Tonder, E.C., Mahlatji, M.D., Malan, S.F., Liebenberg, W., Caira, M.R., Song, M. & De Villiers, M.M. 2004. Preparation and physicochemical properties of five niclosamide solvates and one hemisolvate. *AAPS PharmSciTech*, 5:1-10.

Vippagunta, S.R., Brittain, H.G. & Grant, D.J.W. 2001. Crystalline solids. *Advanced Drug Delivery Reviews*, 48:3-26.

Yu, L. 2001. Amorphous pharmaceutical solids: preparation, characterization and stabilization. *Advanced Drug Delivery Reviews*, 48:27-42.

Chapter 2

Materials and Methods

2.1 Introduction

Solubility is one of the most significant considerations in the drug formulation process and its accurate measurement is therefore of utmost importance. Since this study mainly focused on the equilibrium solubility of pharmaceutical actives, the solubility testing parameters and the methods used in determining concentration, i.e. UV and HPLC, are discussed in this chapter.

2.2 Solubility determination

Solubility determinations in this study were conducted according to a modified saturation, shake-flask method. This method was based on the technique that had been developed by Higuchi and Connors (1965).

The steps involved in this method include sample preparation, time to equilibrate the samples in solution, collection of samples, data analysis and -interpretation.

2.2.1 Sample preparation

An excess amount of sample is added to a test tube with a screw-on cap. If a theoretical or solubility value for a given API is available, it can be tested beforehand to estimate what the excess would be. Accurate measurement of the amount to be used is unnecessary. It is important to add adequate sample to ensure a suspension, but it is equally important not to add too much of the sample, as the latter can alter the properties of the solubility medium and its pH (Tong, 2010).

2.2.2 Equilibration

Equilibration times can vary, because of wettability issues of the API and the tendency of the powder to float. Poorly water soluble drugs may take even longer to reach equilibrium. If values from replicate samples differ, it may be due to the system not yet having reached equilibrium. The opposite is not necessarily true though, since equilibrium is reached when dissolution and recrystallisation/precipitation occur at an equal rate and at the point that no further increase in concentration is observed.

2.2.3 Sample collection or separation of phases

Filtration and centrifugation are the most common methods used to separate the saturated solution from the solid phase. In this study, filtration was the method of choice, taking care to avoid, where possible, filter sorption. To overcome the risk of filter sorption, filters were pre-rinsed with the saturated solution. It was furthermore important for the solid to always be separated from the solution at the same temperature at which equilibrium took place (Tong, 2010).

2.2.4 Analysis and data interpretation

HPLC (discussed in section 2.4) and UV (discussed in 2.3) are the most commonly used analytical tools to determine the concentration of a given API.

2.2.5 Solubility equipment and methodology

The apparatus used in this study, to achieve equilibrium solubility of an API, consisted of a solubility bath equipped with a horisontal rotating axis (54 rpm) that was submerged in water. The temperature of the water bath was maintained at 37°C ± 2°C.

The following experimental procedure was followed in preparing the solubility samples: A surplus of sample powder was added to each test tube. Solvent (20 ml) was added to each tube and the tubes sealed with a lining of Parafilm® inside the screw-on cap to prevent leaking. The test tubes were submerged in the solubility bath for 24 hours. The samples were collected and the contents of each tube filtered, using a pre-rinsed, PVDF (0.45 µm), disposable filter.

2.3 Ultraviolet-visible spectroscopy

Spectrophotometry involves the measurement of chemical species using light energy. When light of a specific wavelength passes through a solution in a quartz cell, the difference between the incident and the emerging beams of light is measured. This is known as absorption. Spectroscopic analysis is usually carried out in solutions and the absorption value is used in the quantitative and qualitative analyses of pharmaceutical materials (Raghavan & Joseph, 2007).

Wavelength selection: Usually, from a spectra scan, the wavelength corresponding to the absorption maximum is selected (Raghavan & Joseph, 2007).

Solvents: Not every organic solvent is suitable for use in UV spectroscopy. In aqueous solution, pH and temperature can alter the position and intensity of the observed maxima. Polar solvents can degrade the spectrum into broad bands, because of molecular, electronic and hydrogen bond interactions (Raghavan & Joseph, 2007).

Cells (cuvettes): The cells used in this study were of the regular square, quartz type, with inside measurements of 1 cm each. Since glass and plastic materials absorb in the UV region, quartz cells are used in measurements below 340 nm (Raghavan & Joseph, 2007).

Beer Lambert's Law: The absorbance of a solution is directly proportional to the concentration of the absorbing species in solution and the path length. This direct proportionality between absorbance and concentration must be

established experimentally for a given instrument, as well as for the test compound. If this relationship is linear, the system is said to obey Beer's Law. For a fixed path length, UV-vis spectroscopy can be used to determine the concentration of the test material in solution. This value can be taken from a predetermined calibration curve (Raghavan & Joseph, 2007).

The instrument used in this study was a Shimadzu UV-1800 (Shimadzu, Japan) UV spectrophotometer. All the determinations were done manually with the said quarts cuvettes. Also, before a solubility study was performed, a calibration curve for each pharmaceutical active was generated.

2.4 High performance liquid chromatography (HPLC)

HPLC is a chromatographic technique used to separate a mixture of components, with its main purpose the quantification of an individual component. This separation is based on a forced transport of the liquid (mobile phase) through a column (stationary phase). The components (analytes) are dispersed in the mobile phase and are separated from each other as a result of the interaction between the analyte and the stationary phase (Kazakevich & LoBrutto, 2007).

There are four main types of HPLC techniques, i.e. normal-phase (NP), reversed-phase (RS), ion-exchange chromatography (IEX) and size-exclusion chromatography (SEC).

Reversed-phase HPLC, which was applied during this study, uses dispersive forces (hydrophobic, or van der Waals interactions). The stationary phase is a porous, rigid material with a hydrophobic surface (for example chemically modified silica), while the mobile phase is polar and usually consists of water-based solutions. The popularity of this technique is due to the fact that closely related compounds may be separated easily through retention variation and because low concentrations can be detected. In most cases of NP, RS and IEX techniques, when the analyte is retained for longer, separation is better.

HPLC is a key analytical instrument that is used in all stages of drug development and research in the pharmaceutical industry. A HPLC system consists of the following main components:

- Solvent reservoirs: They keep adequate volumes of HPLC solvents for the system to operate uninterruptedly over the estimated time.
- Pump: It provides a constant and continuous flow of the mobile phase through the system.
- Injector: It injects the analyte into the mobile phase.
- Column: This most important component of the HPLC system enables the separation of the analytes in a mixture, or quantifies a single component.
- Detector: The most commonly used detector in pharmaceutical analysis is the UV detector.
- Data acquisition and control: A computer based program controls the whole system and is responsible for the data acquisition (Kazakevich & LoBrutto, 2006).

For this study the HPLC configuration used was a Shimadzu Prominence (Shimadzu, Japan) series HPLC, equipped with:

- A quaternary pump (LC-20AD);
- An auto-sampler (SIL-20AC);
- A diode array detector (SPD-M20A), and
- Data acquisition and -analysis software (Shimadzu LCSolution).

2.5 Particle size fraction collections

A sieve shaker is the most common and widely used method to separate particles into different fractions. The Fritsch Pulverisette (from Labotec, South Africa, type 03.502 no. 6331), 220 Volt, set at an amplitude of '6', shaking for 30 - 35 minutes and equipped with Madison test sieves, were used for the particle size fraction collections. Five sieves with sizes of 45, 90, 106, 180, and 250 µm were utilised in this study, with the smallest 45 µm sieve being placed at the bottom, just above the collecting pan and the 250 µm sieve at the top, just below the closure.

2.6 Scanning electron microscopy (SEM)

A SEM is used to examine samples at much higher magnification and resolution than is possible using a light microscope. A SEM produces images that show excellent detail. A SEM uses an upper magnification of about 250 000X, compared to about 1000X for a light microscope (Nichols *et al*., 2011).

Samples were analysed during this study by covering the carbon tape on the SEM pin with an amount of sample, which was then covered with a gold-palladium film (Eiko engineering ion coater IB-2, Japan) in a vacuum. The samples were placed in the microscope sample holder and analysed using a FEI Quanta 200 ESEM & Oxford INCA 400 EDS microscope system (FEI Corporation, Hillsboro OR, USA).

2.7 Materials

2.7.1 Chemicals and reagents

1) Water was prepared using a Millipore™ MilliQ® Ultrapure water purification system (USA);

2) The methanol used was gradient grade for liquid chromatography from Merck, Germany; and

3) Glass beads for dissolution were acid washed and from Sigma G4649 B/N 088K5313, and ≤ 106 µm (-140 US sieve).

2.7.2 APIs tested during this study

The APIs that were tested during this study were closantel sodium, chloroquine phosphate, mefloquine hydrochloride, pyrazinamide and roxithromycin.

The instruments, methods, chemicals and reagents used for testing each specific API are discussed in the relevant chapters that follow.

References

Higuchi, T. & Connors, K.A. 1965. Phase solubility techniques. *Advances in Analytical Chemistry and Instrumentation*, 4:117-212.

Kazakevich, Y. & LoBrutto, R. 2006. HPLC theory and practice. (*In* Kazakevich, Y. & Lobrutto, R. *eds*. HPLC for Pharmaceutical Scientists. Wiley & Sons: New Jersey, USA., 1104pp).

Nichols, G., Luk, S. & Roberts, C. 2011. Microscopy. (*In* Storey, R.A. & Ymén, I. *eds*. Solid-state characterisation of pharmaceuticals. Wiley & Sons: West Sussex, UK. 506pp).

Raghavan, R. & Joseph, J.C. 2007. Spectroscopic methods of analysis: ultraviolet and visible spectrophotometry. (*In* Swarbrick, J. *ed*. Enyclopedia of Pharmaceutical Technology. p. 3460- 3475).

Tong, W. 2010. Practical aspects of solubility determination in pharmaceutical preformulation. (*In* Augustijns, P. & Brewster, M.E. *eds*. Solvent systems and their selection in pharmaceutics and biopharmaceutics. Springer: New York. 459pp).

Chapter 3

Closantel Sodium

3.1 Introduction

Closantel sodium is a broad-spectrum, salicylanilide, veterinary anthelmintic (Merck, 2006). The halogenated salicylanides are mainly used for their antiparisitic activity in animals. Closantel sodium and rafoxanide are the two most well-known drugs in this group (Sakhaee & Derakhshanfar, 2010). Closantel is usually used in combination with benzimidazole anthelmintics, such as mebendazole. Scientists have recently discovered a potentially novel use for closantel also, namely to combat river blindness, a tropical disease that causes blindness in humans (Gloeckner *et al*., 2010).

3.2 Physicochemical properties

Closantel sodium is a fine, slightly yellowish powder and had shown minor static tendencies during the process of sieving into different particle size fractions in this study. It has the molecular formula $C_{23}H_{15}Cl_2I_2N_2NaO_4$, of which the chemical structure is illustrated in figure 3.1. It has a molecular weight of 731.08091 g/mol (Lookchem, 2011).

No reliable sources were found to confirm the solubility of either closantel base, or its sodium salt. It is, however, well-known that the salicylanilides are very poorly water soluble (Terada *et al*., 1988). It is similarly commonplace for salt derivatives of poorly soluble pharmaceutical compounds to be prepared for the express purpose of improving their solubilities (Stahl & Wermuth, 2002). The only conclusion one can safely draw from this is that closantel sodium is likely to be more soluble than its base, although the exact difference in solubility is unknown. Also, no data was available on its BCS classification due to a lack of solubility and permeability data (table 1.1).

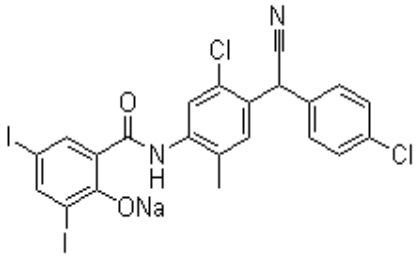


Figure 3.1: Chemical structure of closantel sodium (Lookchem, 2011).

3.3 Pharmaceutical active used for testing

Closantel sodium, batch number 20051231, from Jiang Shan Tai G CHE Co. Ltd. was used during this investigation.

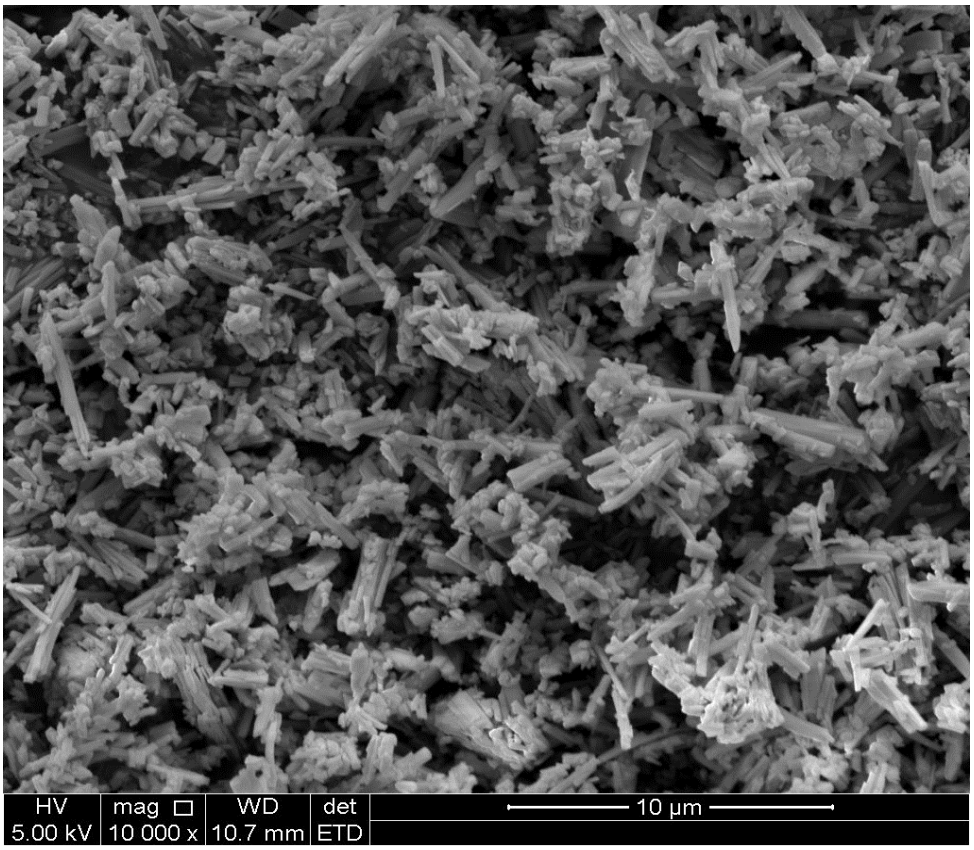


Figure 3.2: SEM micrograph illustrating the morphology of closantel sodium raw material.

The appearance of the powder was slightly yellow. The solubility test was performed on this API as purchased. The morphology of the sample, as determined with the SEM, is shown in figure 3.2.

3.4 Solubility method development

Since the exact solubility of closantel sodium was unknown, the appropriate amount needed to obtain a saturated solution, whilst still leaving an undissolved excess, had to be determined by trial-and-error. Following the solubility determination procedure, as described in section 2.2, using a solubility bath, a surplus (± 8 mg/20 ml) of unsieved closantel sodium was added to 5 test tubes and water added to each sample.

The samples were removed from the solubility bath after 24 hours. A small volume from each sample was filtered and transferred directly into HPLC sample vials.

These samples were analysed on an Agilent 1100 HPLC system, equipped with an isocratic pump, variable wavelength detector, autosampler system controller and a Novapak C_{18} (150 x 4.0 mm) HPLC column. The mobile phase consisted of HPLC grade acetonitrile and water (80:20 v/v) and the pH was adjusted to 3.1 with phosphoric acid. The flow rate of the mobile phase was set at 1.5 ml/min, with an injection volume of 50 µl and wavelengths of detection set at 264 nm and 336 nm. The column temperature was ambient. All sample- and standard solutions were filtered through pre-rinsed 0.45 micron PVDF filters before analysis (Malan *et al.,* 1996).

A standard curve in the analytical range of 20 - 103 µg/ml was generated and used to determine the solubility values of the samples. The linear equation obtained at 264 nm was y = 6.0071x – 0.9356 and the regression coefficient (r^2) was 1.0. The linear equation obtained at 336 nm was y = 17.391x – 11.571 and the regression coefficient (r^2) was 0.9998.

The outcomes of the solubility testing are presented in tables 3.1 and 3.2.

Table 3.1: Concentration of unsieved closantel sodium samples at λ = 264 nm in water

	Closantel sodium concentration in H_2O (µg/ml)
Sample 1	268.3
Sample 2	276.8
Sample 3	276.4
Sample 4	267.0
Sample 5	252.0
Average	**268.1**

Table 3.2: Concentration of unsieved closantel sodium samples at λ = 336 nm in water

	Closantel sodium concentration in H_2O (µg/ml)
Sample 1	256.0
Sample 2	267.8
Sample 3	259.8
Sample 4	255.0
Sample 5	242.1
Average	**255.9**

All the results obtained were outside of the calibration range of 20 - 103 µg/ml, because of the higher than anticipated solubility of this material.

Since the instrument used originally was no longer available for further analysis and the samples had already being disposed of, it was not possible to dilute the samples for re-injection.

A Shimadzu (Kyoto Japan) UFLC (LC-20AD) chromatographic system, consisting of a SIL-20AC auto-sampler, fitted with a sample cooler, a UV-vis

Photodiode Array detector (SPD-M20A) and a LC-20AD solvent delivery module, was used in the remaining experimental work. The system was further equipped with a Luna C_{18} (150 x 4.6 mm) HPLC column.

The mobile phase consisted of HPLC grade acetonitrile and water (80:20 v/v) and the pH was adjusted to 3.1 with phosphoric acid. The flow rate of the mobile phase was set at 2.0 ml/min and an injection volume of 10 µl was used. The column temperature was maintained at 37°C. All sample and standard solutions were filtered through pre-rinsed 0.45 micron PVDF filters prior to analysis. Apart from the HPLC system having changed, the only true difference was the use of a Luna C_{18}, instead of a NovaPak C_{18} column.

Due to the small peaks obtained with the initial analysis, it was decided to investigate alternative wavelengths of detection. It was concluded from the UV spectrum and contour view applications of the LCSolution software that a wavelength of 195 nm would result in the optimum detection of closantel sodium. Figure 3.3 shows the absorbance of closantel sodium at various wavelengths. The magenta colour represents the wavelengths of no absorbance. The dark blue colour represents a lower degree of absorbance in comparison with the turquoise area. The contour view application can assist largely with deciding on which wavelength to use during analysis of a specific compound. Since the UV spectrum view obtained with the above method showed higher absorbance in the area of 190 – 195 nm, it was decided to analyse closantel sodium, using a detection wavelength of 195 nm.

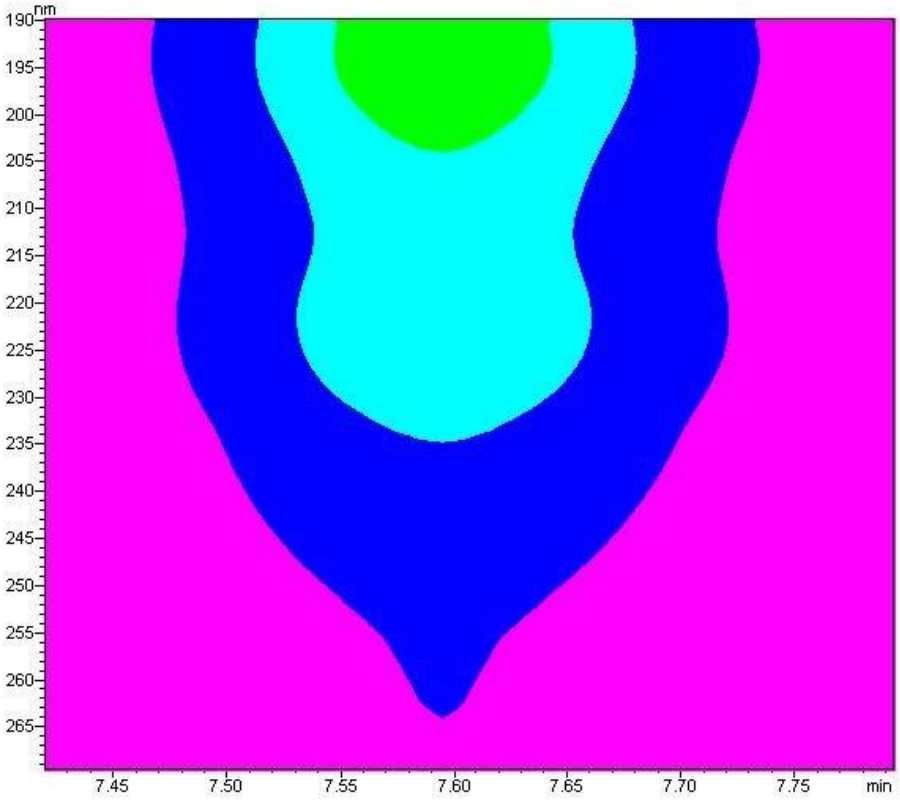


Figure 3.3: A UV spectrum and contour view generated with LCSolution software. Y-axis: Wavelength (nm) and X-axis: Retention time.

It was decided to obtain different particle size fractions to determine whether particle size of a very poorly soluble API would affect the attainment of equilibrium solubility after 24 hours. Particle size fractions collected through sieving were (1) particles > 106 µm and (2) particles 45 - 106 µm in size.

Following the solubility determination procedure, as described in section 2.2, using a water bath, a surplus (± 8 mg/20 ml) of each of the two particle size fractions of closantel sodium and of the unsieved API was each added to a set of five test tubes and 20 ml of water was added to each as solvent. The samples were removed from the solubility bath after 24 hours. A small volume from each test tube was filtered and transferred directly into HPLC sample vials.

A standard curve in the analytical range of 20 - 400 µg/ml was generated and used to determine the solubility values of the samples. The linear equation

obtained at 195 nm was y = 55600x – 12460 and the regression coefficient (r^2) was 1.0. The standard curve is presented in figure 3.4. The solubility results are summarised in table 3.3.

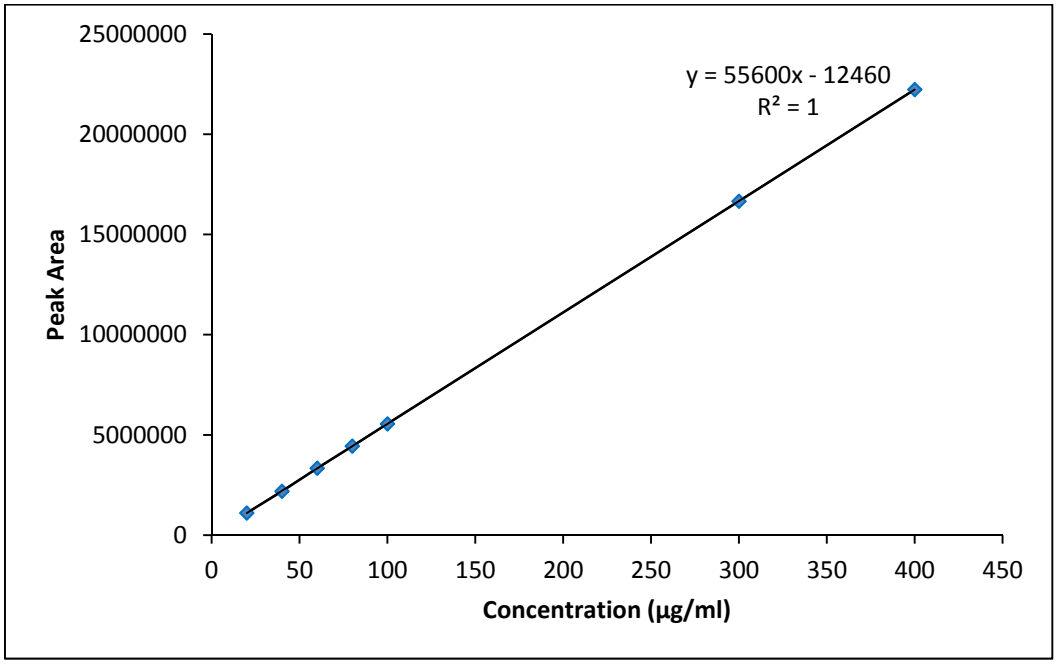


Figure 3.4: HPLC calibration curve of closantel sodium at λ = 195 nm.

Table 3.3: Concentration of unsieved and sieve fractions of closantel sodium samples at at λ = 195 nm in water

	Closantel sodium concentration (µg/ml)		
	Unsieved closantel sodium	**Particles > 106 µm**	**Particles 45 - 106 µm**
Sample 1	291.5	296.5	149.0
Sample 2	Experimental error	252.6	158.2
Sample 3	Experimental error	339.6	105.9
Sample 4	232.4	244.4	156.3
Sample 5	346.5	337.0	147.4
Average	**290.1**	**294.0**	**143.4**
% RSD	**19.7**	**15.3**	**15.0**

The difference in solubilities (P value) between the unsieved closantel sodium and the sieve > 106 µm fraction was 0.9386, which was statistically insignificant. The P value of the unsieved API and sieve 45 - 106 µm fraction was 0.0032, which was statistically very significant. The difference in P value calculated for the two sieve fractions was 0.0005, which was statistically extremely significant (GraphPad© Software).

It was noted that the percentage relative standard deviation (% RSD) of this solubility experiment was high for all three sample groups (table 3.3). The reason for the high % RSD values obtained may have resulted from the samples not having reached equilibrium solubility. In order to determine whether 24 hours allowed for adequate time to reach solubility equilibrium, the test was conducted over a period of 48 hours. It is well-known that poorly soluble APIs may require a longer period of time to reach equilibrium solubility than their freely soluble counterparts (Tong, 2010). The results, however, showed that a 24 hour period was sufficient time for this API to reach solubility equilibrium. The difference in solubility values after 24 and 48 hours was statistically insignificantly (P value = 0.0982). The mean values of the solubility tests after 24 hours and 48 hours were 290.13 µg/ml and 286 µg/ml, respectively.

Thorough investigation revealed that the difference between the test samples at room temperature (21°C) and the experimental setup (37°C) caused recrystallisation/precipitation of the API from the solution, following filtration for HPLC analysis. This emphasised the significance of temperature during solubility determinations. Exposure of the samples to different temperatures, i.e. test temperature conditions and room temperature, may result in variable results and may thus have been the reason for the continued high variability in the second sample set. The possible impact of a variation in temperature conditions was especially clear from the sample collection of the 45 - 106 µm fraction, as the precipitation that had occurred in the test vials resulted in much lower solubility values. The samples with a longer queuing time during HPLC

analysis also showed lower solubility values, i.e. particle size fraction of 45 - 106 µm.

Taking into consideration the above observations, a final set of solubility tests was conducted. During these tests the test tubes remained in the water bath (37°C) until all undissolved particles had moved to the surface of the solution. A tube fitted with a syringe was carefully inserted into the solution, taking care that no undissolved particles adhered to the tube. The clear solution was then withdrawn from the test tube into the syringe. This solution was then filtered through a pre-rinsed 0.45 µm PVDF filter into another test tube that was also kept in the water bath at 37°C. 2 ml of this filtrate was subsequently diluted to 20 ml with HPLC grade methanol. The diluted samples were then filtered using a 0.45 µm PVDF filter and analysed (HPLC column at 37°C). The results are presented in table 3.4.

Table 3.4: Concentration of unsieved and sieve fractions of closantel sodium samples at λ = 195 nm in water at 37°C

	Closantel sodium concentration (µg/ml)		
	Unsieved closantel sodium	**Particles > 106 µm**	**Particles 45 - 106 µm**
Sample 1	294.7	251.5	262.3
Sample 2	273.9	274.8	291.3
Sample 3	256.0	282.4	234.3
Sample 4	265.7	235.2	255.7
Sample 5	233.8	303.7	263.5
Sample 6	278.6	293.9	259.1
Sample 7	308.7	No sample	No sample
Sample 8	269.2	No sample	No sample
Sample 9	279.2	No sample	No sample
Average	**273.3**	**273.6**	**261.0**
% RSD	**7.9**	**9.5**	**7.0**

From these results it could be concluded that particle size did not play a significant role in the time required for equilibrium solubility to be reached and that 24 hours was a sufficiently long testing period, even for this poorly soluble API. The % RSD for all the fractions were well within experimental limits for this kind of study (< 10%). The solubility values of closantel sodium API and that of the sieve > 106 µm fraction were almost identical. The P-values for the comparison between the closantel API and the 45 – 106 µm fraction was 0.2728 and was considered to be statistically insignificant (GraphPad© Software).

3.5 Conclusion

It was established during this project that the wavelength of analysis for closantel sodium by means of HPLC should be 195 nm, rather than the 264 nm and 336 nm, as suggested by Malan *et al.* (1996).

From the outcomes of these tests it was concluded that a change between the temperature at which solubility testing is performed and the temperature at which the samples are kept during analysis significantly affect solubility results, because of precipitation resulting from the varying temperatures. This emphasised the importance of temperature control during all stages of a solubility experiment. The temperature difference between testing conditions (37°C) and room temperature (21°C) may thus influence the outcomes of a solubility study by giving misleading results. Without temperature control during the analysis stage, the results were random with high %RSD values.

This study demonstrated that particle size did not influence the mean solubility values of closantel sodium over a period of 24 hours at 37°C. Therefore, even for this poorly water soluble API, a period of 24 hours was deemed sufficient to accurately determine its equilibrium solubility.

References

Gloeckner, C., Garner, A.L., Mersha, F., Oksov, Y., Tricoche, N., Eubanks, L.M., Lustigman, S., Kaufmann, G.F. & Janda, K.D. 2010. Repositioning of an existing drug for the neglected tropical disease Onchocerciasis. *Proceedings of the National Academy of Sciences of the United States of America*, 107(8):3424-3429.

GraphPad© Software. 2012. http://www.graphpad.com/quickcalcs//ttest2.cfm Date of access: 18 June 2012.

Lookchem. 2011. Closantal sodium. http://www.lookchem.com/closantal sodium/ Date of access: 20 July 2011.

Malan, C.E.P., de Villiers, M.M., Handford, J.S. & Swanepoel, E. 1996. HPLC determination of closantel in multi-component anthelmintic suspension and tablets. *Pharmazie,* 51:250.

Merck & Co., Inc. 2006. The Merck Index: an encyclopedia of chemicals, drugs and biologicals. 14th ed. Whitehouse Station: NJ, 1756 pp.

Sakhaee, E. & Derakhshanfar, A. 2010. Polioencephalomalacia associated with closantel overdosage in a goat. *Journal of the South African Veterinary Association*, 70:61-70.

Stahl, P.H. & Wermuth, C.G. *eds*. 2002. Handbook of pharmaceutical salts: properties, selection, and use. John Wiley & Sons, Weinheim, 388 pp.

Terada, H., Goto, S., Yamamoto, K., Takeuchi, I., Hamada, Y. & Miyake, K. 1988. Structural requirements of salicylanilides for uncoupling activity in mitochondria: quantitative analysis of structure-uncoupling relationships. *Biochimica et Biophysica Acta: Bioenergetics*, 936(7):504-512.

Tong, W. 2010. Practical aspects of solubility determination in pharmaceutical preformulation. (*In* Augustijns, P & Brewster, M.E. *eds* Solvent systems and their selection in pharmaceutics and biopharmaceutics, Belgium: Springer, AAPS Press. 459pp).

Chapter 4

Chloroquine Phosphate

4.1 Introduction

Chloroquine phosphate is a 4-aminoquinoline compound that is primarily used in the treatment and prevention of malaria (Peters, 1970). Plasmodium resistance to antimalarial medicines is a major obstacle in the fight against malaria. Ineffective treatment, substandard- and counterfeit medicines promote the spread of drug resistance. Despite recommendations by the World Health Organization since 2001 (WHO) to use artemisinin based actives as the first-line treatment of uncomplicated *Plasmodium falciparum*, to help prevent the development of drug resistance, chloroquine is still being used as a first-line treatment in some countries (WHO, 2010).

Chloroquine had been used for the treatment and/or prevention of malaria since 1947, but due to mass drug administrations, the malaria parasite *P. falciparum* had developed resistance to this active. It, however, still remains the treatment of choice for *P. vivax* malaria in some areas (WHO, 2010). Other treatment applications of chloroquine include autoimmune disorders (Fox, 1996), retroviral- and viral infections (Savarino *et al.*, 2003) and radiosensitising / chemosensitising in cancer treatment (Savarino *et al.*, 2006).

4.2 Physicochemical properties

Chloroquine phosphate is a white to yellowish crystalline powder that is freely soluble in cold water, having a solubility of 100 mg/ml (Kasim *et al*., 2004). It has the molecular formula $C_{18}H_{26}ClN_3.2H_3PO_4$, of which the chemical structure is illustrated in figure 4.1. It has a molecular weight of 515.87 g/mol (Sigma Aldrich, 2011).

According to the BCS classification system, chloroquine phosphate is a class I drug, i.e. highly soluble and highly permeable (Lindenberg *et al*., 2004).

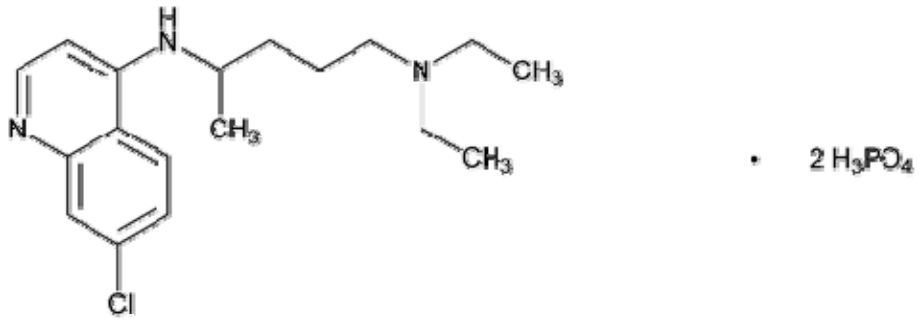


Figure 4.1: Chemical structure of chloroquine phosphate (USP, 2011).

4.3 Pharmaceutical active used for testing

Chloroquine phosphate, batch number 0602026, from Sinoway Industrial Co. Ltd. (Xiamen, China) was used during this investigation. Particle size fractions collected through sieving were (1) 106 - 250 µm and (2) 45 - 106 µm in size.

4.4 Experimental and results

4.4.1 Generation of a calibration curve

Two chloroquine phosphate standard stock solutions were prepared:

- Stock A: 20 mg/100 ml in water with a theoretical concentration of 0.2 mg/ml.
- Stock B: 15 mg/100 ml in water with a theoretical concentration of 0.15 mg/ml.

A set of dilutions was made from each of these two stock solutions, as indicated in table 4.1.

According to the USP official monograph (2011), two peaks of maximum absorbance for chloroquine phosphate are detected by UV analysis at 343 nm

and 329 nm respectively, using a solution with a concentration of 0.01 mg/ml. A standard absorption spectrum was generated with the UV spectrophotometer, using standard solution (9) (table 4.1) to determine the exact wavelength to use in the analysis of the chloroquine phosphate standard- and sample preparations.

The standard solutions were analysed using the photometric function of the Shimadzu UV spectrophotometer at λ = 342.9 nm. The absorbance readings are summarised in table 4.1.

A standard curve was generated, as illustrated in figure 4.2, for use in the determination of the solubilities of the chloroquine phosphate samples.

Table 4.1: Concentrations and absorbances of chloroquine phosphate standard solutions at λ = 342.9 nm in water

	Dilutions	**Concentration (µg/ml)**	**Absorbance at 342.9 nm**
(1)	Dilute 4 ml of A in 10 ml of water	80.1	2.835
(2)	Dilute 10 ml of B in 25 ml of water	60.4	2.278
(3)	Dilute 5 ml of A in 20 ml of water	50.1	1.870
(4)	Dilute 2 ml of A in 10 ml of water	40.1	1.519
(5)	Dilute 2 ml of B in 10 ml of water	30.2	1.156
(6)	Dilute 1 ml of A in 10 ml of water	20.0	0.728
(7)	Dilute 1 ml of B in 10 ml of water	15.1	0.540
(8)	Dilute 3 ml of A in 50 ml of water	12.0	0.441
(9)	Dilute 5 ml of A in 100 ml of water	10.0	0.368
(10)	Dilute 2 ml of A in 50 ml of water	8.0	0.305
(11)	Dilute 1 ml of B in 25 ml of water	6.0	0.223

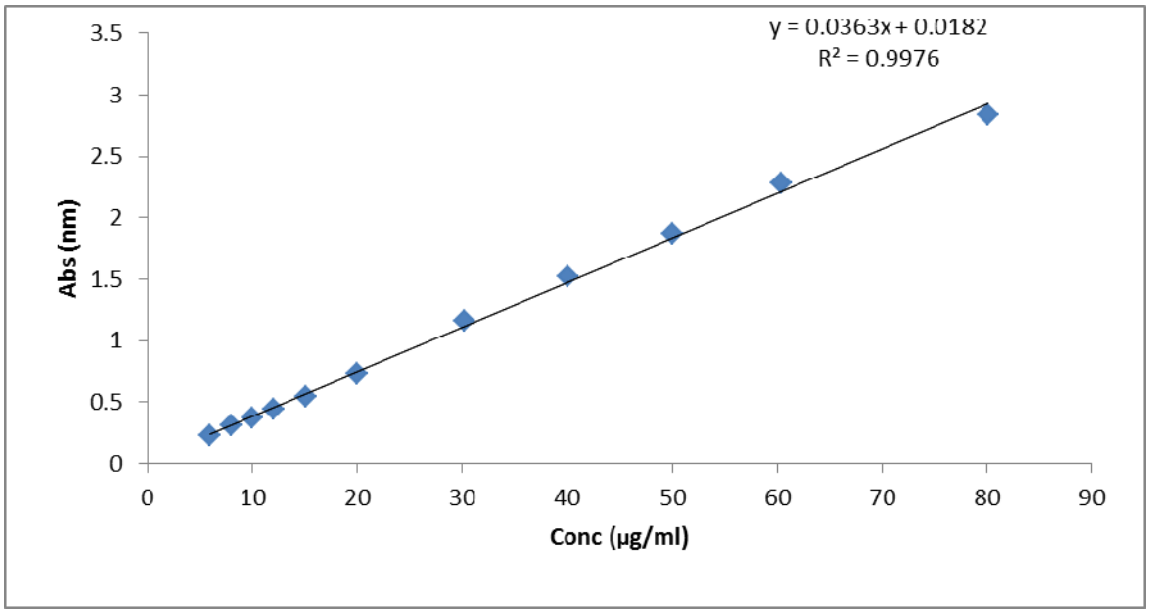


Figure 4.2: UV calibration curve of chloroquine phosphate standard solutions in water at λ = 342.9 nm.

4.4.2 Solubility of chloroquine phosphate in water after 24 hours

Following the solubility determination procedure, as described in section 2.2, using a solubility bath, amounts considered an excess (based on published solubility values) of the unsieved chloroquine phosphate and of the two different particle size fractions, were added to three sets of six tests tubes each. The samples were removed from the solubility bath after 24 hours and filtered.

Since the ideal concentration for UV analysis is 0.01 mg/ml (USP, 2011), each filtered portion was suitably diluted before analysis on the Shimadzu UV spectrophotometer. The results for the different sample solutions are shown in table 4.2.

The average concentration of chloroquine phosphate raw material in water was 318.1 mg/ml. For the particle size fraction between 106 - 250 µm, the concentration was 340.6 mg/ml and for the 45 - 106 µm fraction it was 327.1 mg/ml.

Table 4.2 Concentration of chloroquine phosphate samples in water at λ = 342.9 nm

	Chloroquine phosphate concentration (mg/ml)		
	Unsieved chloroquine phosphate	**Particles 106 - 250 µm**	**Particles 45 - 106 µm**
Sample 1	309.13	373.96	322.06
Sample 2	327.02	315.32	341.47
Sample 3	No sample	332.53	326.74
Sample 4	No sample	No sample	330.19
Sample 5	No sample	No sample	317.52
Sample 6	No sample	No sample	324.54
Average	**318.07**	**340.60**	**327.09**
% RSD	**3.98**	**8.85**	**2.52**

According to GraphPad© software, the P value for the raw material and the 106 - 250 µm fraction was calculated as 0.4073, which was considered as statistically insignificant. The calculated P value for the raw material and the 45 – 106 µm fraction was 0.2719, which was also statistically insignificant. The calculated P value for the two sieve fractions was 0.3124 and thus statistically insignificant also.

4.5 Conclusion

The solubility values generated for chloroquine phosphate during this investigation far exceeded the solubility value of 100 mg/ml, as reported by Kasim *et al*. (2004). Unfortunately, no definite temperature was stipulated for that study. This investigation, which was performed at 37°C, resulted in values approximately three times higher than that of the reported value. The experimental setup was furthermore complex, because of the large amount of

powder needed to achieve a saturated solution, since the samples kept on dissolving completely and more powder had to be added to each solution.

Given the fact that the solubility of chloroquine phosphate at 37°C far exceeded published solubility values, all powder used in the solubility testing did in fact dissolve, leaving no excess at the time of UV analysis. The high % RSD values were most likely due to variations in the amount of powder (> 1 g) used for each sample. Unfortunately, an insufficient amount of chloroquine phosphate powder was available, following the first sample preparation, with which to repeat this experiment aimed at establishing as to whether saturated solutions had indeed been prepared.

The only definitive conclusion that could thus be made from the above outcomes was that the aqueous solubility of chloroquine phosphate is much higher than previously thought. It is also likely that the aqueous solubility of this API is particularly sensitive to temperature. This would explain the discrepancy with previously published data, had those tests been conducted at a lower temperature.

References

Fox, R. 1996. Anti-malarial drugs: possible mechanisms of action in autoimmune disease and prospects for drug development. *Lupus*, 5(1):S4-S10.

GraphPad© Software. 2012. http://www.graphpad.com/quickcalcs//ttest2.cfm Date of access: 18 June 2012.

Kasim, N.A., Whitehouse, M., Ramachandran, C., Bermejo, M., Lennernäs, H., Hussain, A.S., Junginger, H.E., Stavchansky, S.A., Midha, K.K., Shah, V.P. & Amidon, G.L. 2004. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. *Molecular Pharmaceutics*, 1:86-96.

Lindenberg, M., Kopp, S. & Dressman, J.B. 2004. Classification of orally administered drugs on the World Health Organization model list of essential medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics*, 58:265-278.

Peters, W. 1970. Chemotherapy and drug resistance in malaria. Academic Press: London, 876 pp.

Savarino, A., Boelaert, J.R., Cassone, A., Majori, G. & Cauda, R. 2003. Effects of chloroquine on viral infections: an old drug against today's diseases. *The Lancet*, 3(11):722-727.

Savarino, A., Lucia, M.B., Giordano, F. & Cauda, R. 2006. Risks and benefits of chloroquine use in anticancer strategies. *The Lancet Oncology*, 7(10):792-793.

Sigma-Aldrich. 2011. Chloroquine phosphate. http://www.sigmaaldrich.com/catalog/DisplayMSDSContent.do Date of access: 20 June 2011.

United States Pharmacopoeia. 2011. Chloroquine phosphate monograph. http://www.uspnf.com/uspnf/pub/data/v33282/usp33nf28s2_m16080.xml Date of access: 24 February 2011.

USP see United States Pharmacopoeia.

WHO see World Health Organization.

World Health Organization. 2010. Global report on antimalarial drug efficacy and drug resistance: 2000-2010. WHO Press: Switzerland, 115 pp.

Chapter 5

Mefloquine Hydrochloride

5.1 Introduction

Mefloquine hydrochloride was developed in the 1970s as a synthetic analogue of quinine for use in the prevention and treatment of malaria, among the US combat troops especially, following the end of the Vietnam War (Croft, 2007). Mefloquine is highly effective against chloroquine-resistant *Plasmodium vivax* malaria (Maguire, 2006). Resistance to this drug was first observed at the Cambodia-Thailand border, a few years after its introduction (Boudreau, 1982).

5.2 Physicochemical properties

Mefloquine is a 4-quinolinemethanol derivative and belongs to the amino-alcohol family, to which quinine, quinidine, halofantrine and lumefantrine also belong (WHO, 2010). Mefloquine HCl has a molecular formula of $C_{17}H_{16}F_6N_2O{\cdot}HCl$ and its chemical structure is illustrated in figure 5.1 (USP, 2011). Mefloquine HCl has a molecular weight of 414.77 g/mol. This substance is a fine, white powder and had shown a pronounced static tendency during the process of sieving into different particle size fractions in this study. According to Strauch *et al*. (2011), mefloquine has an experimental water solubility of 1.806 mg/ml at 37°C as the HCl salt.

According to Lindenberg *et al*. (2004) and Strauch *et al*. (2011), mefloquine belongs to either Class II (low solubility and high permeability), or Class IV (low solubility and low permeability) of the BCS classification. Mefloquine further belongs to a group of APIs of which the bioavailability is < 90%, but this lower value could have been due to either a low solubility, or a poor permeability, or both. Lindenberg *et al*. (2004) and Strauch *et al*. (2011) report that insufficient

data for either solubility, or permeability, or both were found for this drug, resulting in its classification into two groups, classes II and IV.

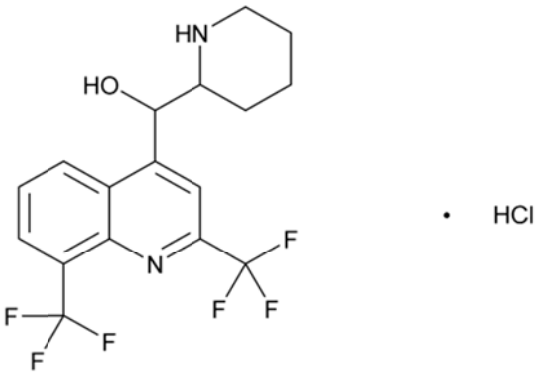


Figure 5.1: Chemical structure of mefloquine HCl (USP, 2011).

5.3 Pharmaceutical active used for testing

Mefloquine HCl, batch number IF-ME-080830, from DB Fine Chemicals was used during this investigation. Three particle size ranges were identified for testing, i.e. (1) > 250 µm, (2) 45 - 106 µm and (3) < 45 µm in size. Figure 5.2 illustrates the morphology of the samples, i.e. small, rod-like particles.

5.4 Experimental and results

5.4.1 Generation of a calibration curve

Using the method, as described by Wessels (2010), a standard stock solution was prepared, from which a set of dilutions were made in water, as summarised in table 5.1. The standard solutions were analysed, using the photometric function of the Shimadzu UV spectrophotometer at λ = 283 nm (table 5.1).

A standard curve was generated (figure 5.3) for use in the determination of the solubilities of the mefloquine HCl samples.

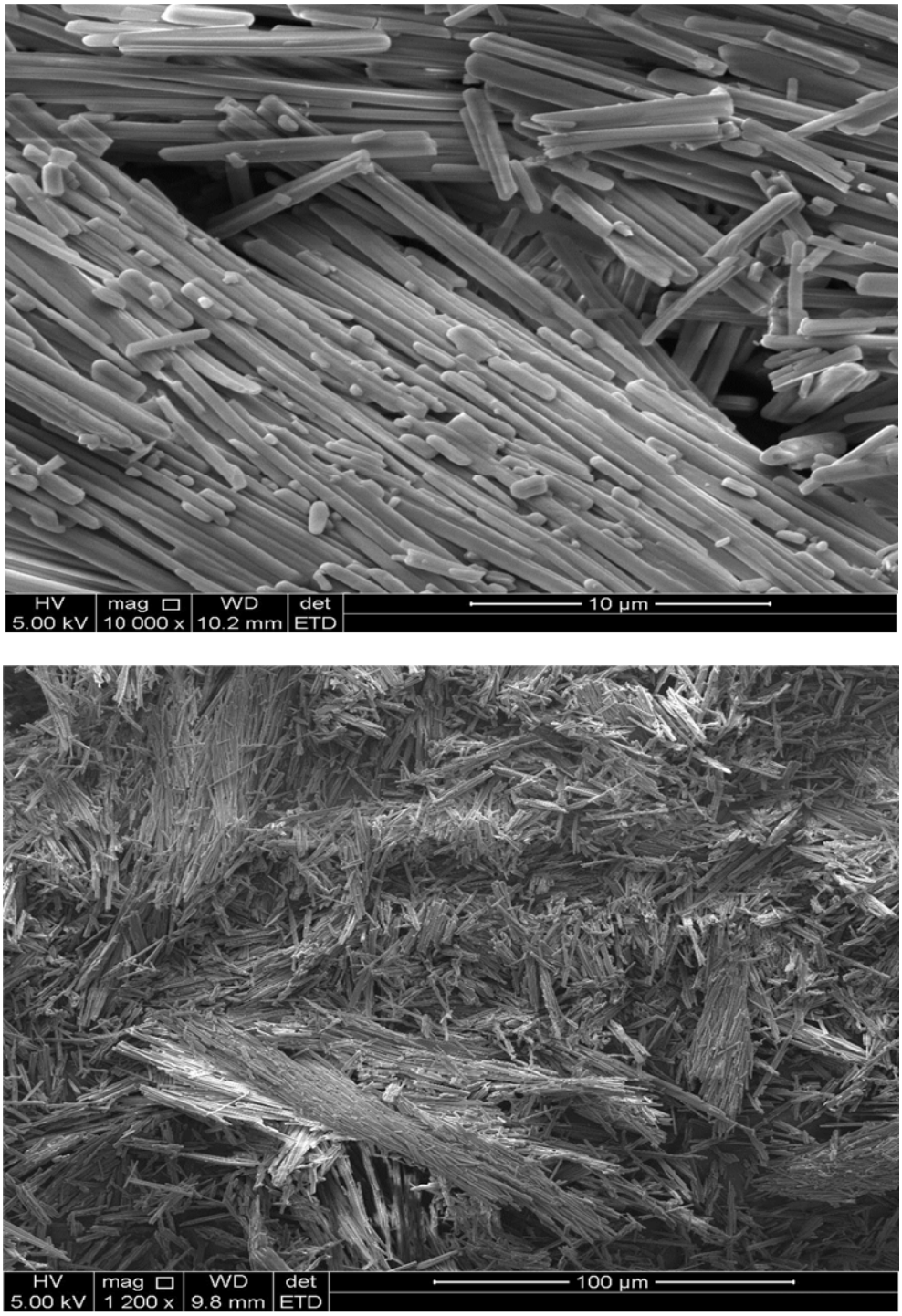


Figure 5.2: SEM micrographs of mefloquine HCl raw material.

Table 5.1: Concentrations and absorbances of mefloquine HCl standard solutions in water at λ = 283 nm

	Dilutions in water	Concentration (mg/ml)	Absorbance at 283 nm
(1)	2 ml/50 ml	0.0101	0.152
(2)	2 ml/20 ml	0.0251	0.355
(3)	4 ml/25 ml	0.0402	0.556
(4)	2 ml/10 ml	0.0503	0.707
(5)	3 ml/10 ml	0.0754	1.060

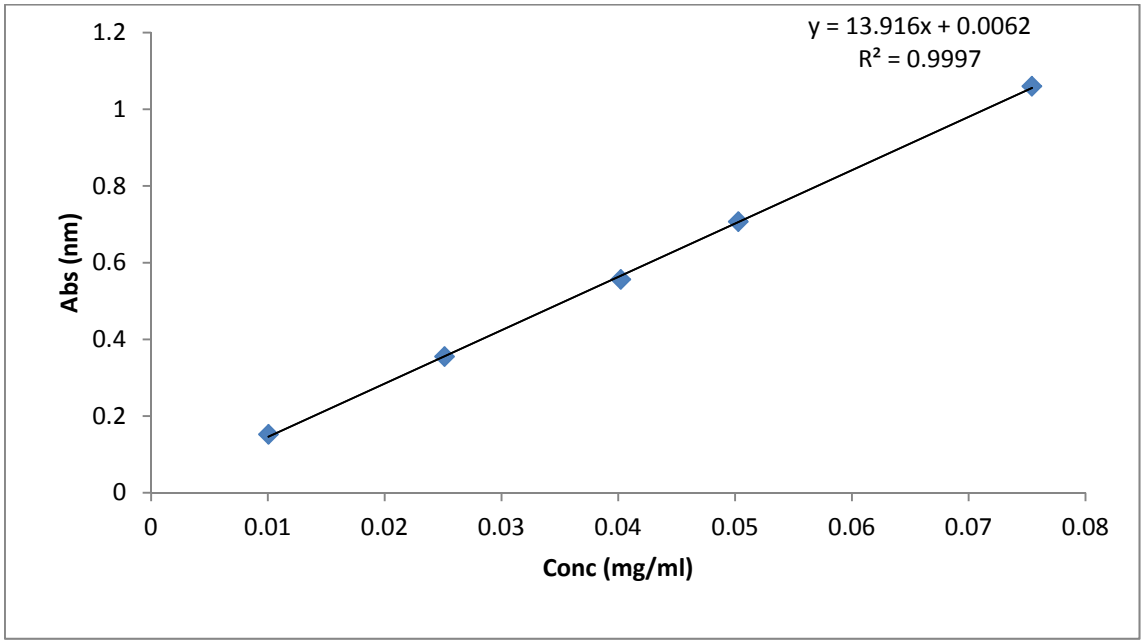


Figure 5.3: UV calibration curve of mefloquine HCl standard solutions in water at λ = 283 nm.

5.4.2 Solubility of mefloquine HCl in water after 24 hours

Following the solubility determination procedure, as described in section 2.2, a surplus (at least 70 mg) of each of the three particle size fractions of mefloquine HCl and of the unsieved raw material was added to each set of 5 test tubes. Approximately 10 ml water was added to each sample as solvent.

The samples were removed from the solubility bath after 24 hours and filtered. Following filtration, 1 ml from each sample was diluted to 100 ml with water, for analysis on the Shimadzu UV spectrophotometer.

The results are summarised in table 5.2 and illustrated in figure 5.4.

Table 5.2: Concentrations of mefloquine HCl (MFQHCL) samples in water at λ = 283 nm

	Mefloquine HCl concentration (mg/ml)			
	Unsieved MFQHCL	**Particles > 250 µm**	**Particles 45 - 106 µm**	**Particles < 45 µm**
Sample 1	3.84	3.88	3.93	4.28
Sample 2	3.84	3.88	3.95	4.25
Sample 3	3.86	3.84	3.92	4.21
Sample 4	3.96	3.91	3.97	4.21
Sample 5	3.91	3.91	3.98	4.19
Average	**3.88**	**3.88**	**3.95**	**4.23**
% RSD	**1.34**	**0.74**	**0.65**	**0.86**

The % RSD of the raw material and the sieve fractions was ≤ 1.5, which was demonstrative of excellent precision and repeatability among the different samples.

The average water solubility value of the particle size fraction of < 45 µm was calculated as 4.23 mg/ml, that of the particle size fraction between 45 – 106 µm was 3.95 mg/ml and of the unsieved API and particle size fraction of > 250 µm were both 3.88 mg/ml (figure 5.4).

The P values, according to the GraphPad© Software were as follows, with the mefloquine HCl raw material (unsieved) applied as reference:

- Unsieved mefloquine HCl *vs* > 250 µm fraction = 0.9420 (statistically insignificant);
- Unsieved mefloquine HCl *vs* 45 - 106 µm fraction = 0.0307 (statistically significant); and
- Unsieved mefloquine HCl *vs* < 45 µm fraction = less than 0.0001 (statistically extremely significant).

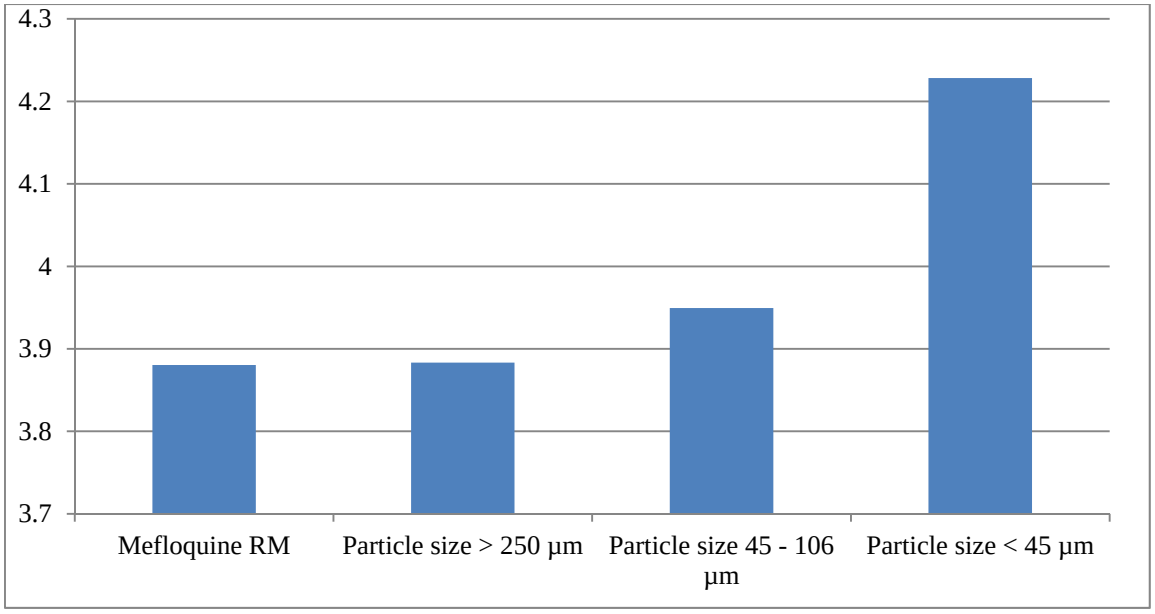


Figure 5.4: Graphic comparison of the solubilities of the unsieved mefloquine HCl and three different sieve fractions.

The solubility values obtained during this study (3.88 - 4.23 mg/ml in water at 37°C after a period of 24 hours) differed significantly from the solubility (1.806 mg/ml in water at 37°C after an unknown period) reported by Strauch *et al*. (2011). According to their experimental description, Strauch *et al*. (2011) also used the shake-flask method, but no mention is made of either the particle size distribution, the fraction of the powder tested, or of the time allowed for reaching solubility equilibrium.

5.5 Conclusion

Mefloquine HCl was the first API in this study in which particle size directly affected its solubility values, as generated after a 24 hour equilibration period. This lends credence to the argument that 24 hours, as a standard period, may not be enough time for all API powders to reach equilibrium solubility. It also shows that particle size could have a significant influence on the time required for a particular API to reach its solubility equilibrium. It thus became evident that the period used for equilibrium solubility testing would need to be evaluated and decided upon on a case-by-case basis through scientific experimental design.

It was also found that mefloquine HCl was more soluble in water than previously reported by Strauch *et al*. (2011), i.e. more than twice as soluble. The highest solubility value measured in this study (4.23 mg/ml in water at 37°C after a period of 24 hours for the < 45 µm sieve fraction) should have theoretically been closer to the true, but still unknown equilibrium solubility of mefloquine HCl, since the smaller particles would dissolve faster and would thus have been closer to the equilibrium point than the larger sieve fractions.

References

Boudreau, E.F., Webster, H.K., Pavanand, K. & Thosinga, I. 1982. Type II mefloquine resistance in Thailand. *Lancet*, ii:1335.

Croft, A.M. 2007. A lesson learnt: the rise and fall of Lariam and Halfan. *Journal of the Royal Society of Medicine*, 4:170–4.

GraphPad© Software. 2012. http://www.graphpad.com/quickcalcs//ttest2.cfm Date of access: 13 September 2012.

Lindenberg, M., Kopp, S. & Dressman, J.B. 2004. Classification of orally administered drugs on the World Health Organization model list of essential medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics*, 58:265-278.

Maguire, J.D., Marwoto, K.H., Richie, T.L., Fryauff, D.J. & Baird, J.K. 2006. Mefloquine is highly efficacious against chloroquine-resistant *Plasmodium vivax* malaria and *Plasmodium falciparum* malaria in Papua, Indonesia. *Clinical and Infectious Diseases,* 42:1067–1072.

Strauch, S., Jantratid, E., Dressman, J.B., Junginger, H.E., Kopp, S., Midha, K.K., Shah, V.P., Stavchansky, S. & Barends, D.M. 2011. Biowaiver monographs for immediate release solid oral dosage forms: mefloquine hydrochloride. *Journal of Pharmaceutical Sciences*, 100(1):11-21.

United States Pharmacopoeia. 2011. Mefloquine Hydrochloride monograph. http://www.uspnf.com/uspnf/pub/data/v34290/usp34nf29s0_m48040.xml Date of access: 20 June 2011.

USP see United States Pharmacopoeia.

Wessels, J.C. 2010. International Phamacopoeia Monographs: antimalarial dosage forms. PhD Thesis, North-West University, Potchefstroom, 204pp.

WHO see World Health Organization.

World Health Organization. 2010. Global report on antimalarial drug efficacy and drug resistance: 2000-2010. 115p. http://www.who.int/malaria /publications/atoz/9789241500470/en/ Date of access: 25 June 2012.

Chapter 6

Pyrazinamide

6.1 Introduction

The use of pyrazinamide in the treatment of pulmonary tuberculosis was first documented as far back as in 1952 by Yeager *et al*. (as cited by WHO, 2010). Today it is still one of the four drugs used in the standard treatment of tuberculosis (TB) (WHO, 2010). Pyrazinamide is a synthetic analogue of nicotinamide, which is only weakly bactericidal against *Mycobacterium tuberculosis*. It has sterilising activity and is highly effective during the first two months of treatment in cases of acute TB (WHO, 2012). This antibacterial (tuberculostatic) drug is always used in combination with ethambutol, isoniazid and rifampicin and is the recommended treatment for tuberculosis by the WHO (2010). Pyrazinamide plays an important and unique role in shortening TB therapy, normally lasting between 9 and 12 months, to only 6 months, as it kills a population of semidormant tubercle bacilli residing in an acidic environment, which are not killed by the other TB drugs (Mitchison, 1985; Heifets & Lindholm-Levy, 1992).

6.2 Physicochemical properties

Pyrazinamide has the molecular formula $C_5H_5N_3O$, of which the chemical structure is illustrated in figure 6.1. Pyrazinamide has a molecular weight of 123.113 g/mol. The substance is a fine, white, crystalline powder. Pyrazinamide has an experimental water solubility of 15 mg/ml, 13.8 mg/ml in methanol, 5.7 mg/ml in ethanol, 3.8 mg/ml in isopropanol, 1.0 mg/ml in ether and 7.4 mg/ml in chloroform (Merck, 2006).

Pyrazinamide is a Class I drug, according to the BCS classification system (highly soluble and highly permeable) (Lindenberg *et al*., 2004).

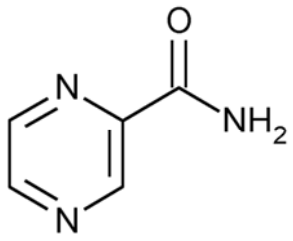


Figure 6.1: Chemical structure of pyrazinamide (USP, 2011).

6.3 Pharmaceutical active used for testing

Pyrazinamide, batch number PYR/P-228/06, from Linaria Chemicals (Thailand) LTD was used during this investigation. Particle size fractions collected through sieving were (1) > 106 µm, (2) 45 - 106 µm and (3) < 45 µm in size.

6.4 Experimental and results

6.4.1 Generation of a calibration curve

Two pyrazinamide standard stock solutions were prepared:

- Stock A: 20 mg/100 ml in water having a theoretical concentration of 0.2 mg/ml; and
- Stock B: 15 mg/100 ml in water having a theoretical concentration of 0.15 mg/ml.

10 ml of methanol was added to first dissolve the standard before diluting the solution to 100 ml with water. A set of dilutions was made from each of the stock solutions, as summarised in table 6.1.

Table 6.1 Concentrations and absorbances of pyrazinamide standard solutions at λ = 268.8 nm in water

	Dilutions	Actual concentration (mg/ml)	Absorbance at 268.8 nm
(1)	Dilute 2 ml of A in 10 ml of water	0.0404	2.632
(2)	Dilute 2 ml of B in 10 ml of water	0.0308	2.052
(3)	Dilute 1 ml of A in 10 ml of water	0.0202	1.351
(4)	Dilute 1 ml of B in 10 ml of water	0.0154	1.019
(5)	Dilute 3 ml of A in 50 ml of water	0.0121	0.803
(6)	Dilute 5 ml of A in 100 ml of water	0.0101	0.666
(7)	Dilute 4 ml of A in 100 ml of water	0.0081	0.537
(8)	Dilute 1 ml of B in 25 ml of water	0.0062	0.403

According to the USP official monograph of pyrazinamide (USP, 2011), a solution of 10 µg/ml should be detected by UV analysis at 268 nm, but Moffat *et al.* (2004) state that the specific absorption for pyrazinamide is A ¦ = 659 at 269 nm, meaning that a concentration of 15 µg/ml should be used for UV analysis. A standard absorption spectrum was generated on the UV spectrophotometer, using standard solution (4) to determine the exact wavelength for use in the analysis of the pyrazinamide standard- and sample preparations.

The standard solutions were analysed, using the photometric function of the Shimadzu UV spectrophotometer at λ = 268.8 nm. The absorbances are given in table 6.1.

A standard curve was generated (figure 6.2) for use in the determination of the solubilities of the pyrazinamide samples.

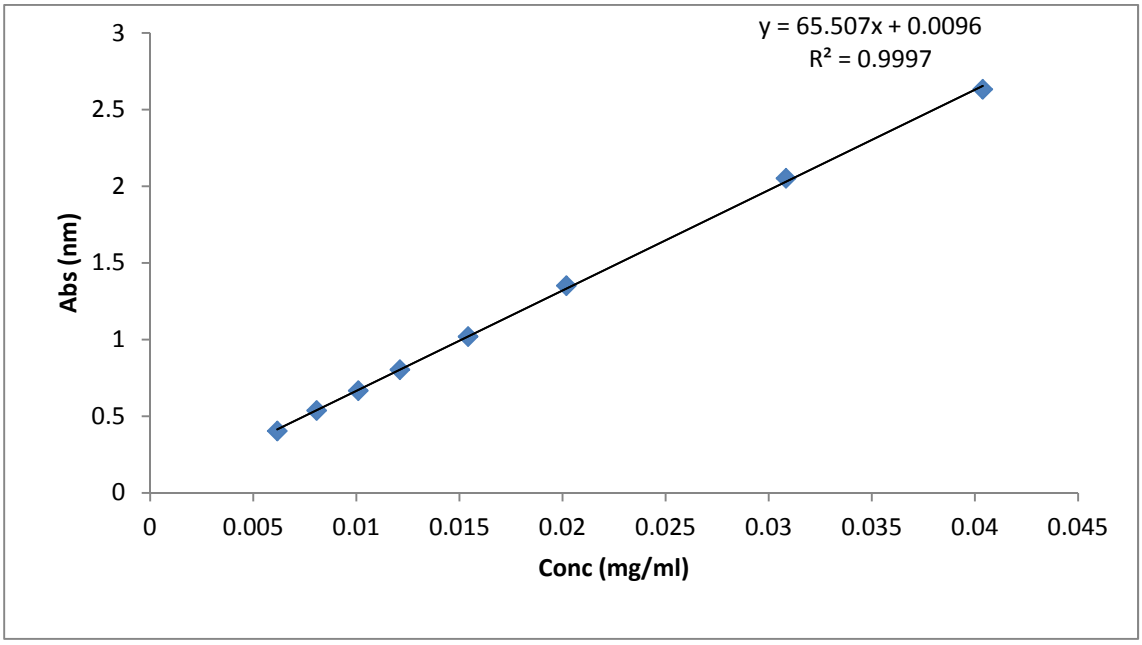


Figure 6.2: UV calibration curve of pyrazinamide standard solutions in water at λ = 268.8 nm.

6.4.2 Solubility of pyrazinamide in water after 24 hours

Following the solubility determination procedure, as described in section 2.2, a surplus (at least 90 mg) of each of the three particle size fractions of pyrazinamide and of the original API were added to each set of 5 test tubes. Approximately 5 ml of water was added to each as solvent.

It was observed that the pyrazinamide fractions of 45 - 106 µm and of < 45 µm had dissolved rapidly. According to Merck (2006), the water solubility of pyrazinamide at room temperature (25°C) is 15 mg/ml. It was deduced that the solubility of pyrazinamide was markedly increased by the higher temperature (37°C), as applied during this study. More powder from the two larger sieve fractions was added to each relevant test tube, but there was insufficient powder left for the smallest sieve fraction of < 45 µm to continue with the testing thereof.

The samples were removed from the solubility bath at 37°C after 24 hours and filtered. Needle-like crystals started to form rapidly on cooling of the filtered portions.

The filtered portions were returned to the solubility bath, until the crystals were again dissolved. 1 ml of methanol was added to each filtered portion upon removal from the solubility bath, to ensure that the pyrazinamide stayed in solution.

As the solubility of pyrazinamide was expected to be around 15 mg/ml (Merck, 2006) and the ideal concentration for UV analysis being 0.01 mg/ml (USP, 2011), each filtered portion was diluted before analysis on the Shimadzu UV spectrophotometer as follows:

Theoretical concentration:

15 mg/ml

Dilution

↳ 1ml/100 ml (concentration: 0.15 mg/ml)

Dilution

↳ 1 ml/15 ml (concentration: 0.01 mg/ml)

The results for the sample solutions are shown in table 6.2.

The average concentration of unsieved pyrazinamide in water at 268.8 nm was determined as 18.63 mg/ml. For the particle size fraction of > 106 µm the average concentration was 18.83 mg/ml and for the 45 - 106 µm fraction it was 18.69 mg/ml.

Table 6.2: Concentration of pyrazinamide samples at λ = 268.8 nm in water

	Pyrazinamide concentration (mg/ml)		
	Unsieved pyrazinamide	**Particles > 106 µm**	**Particles 45 - 106 µm**
S1	18.79	18.30	21.65
S2	18.12	18.03	17.43
S3	18.03	17.96	17.71
S4	20.23	20.00	19.29
S5	17.98	19.86	17.39
Average	**18.63**	**18.83**	**18.69**
%RSD	**5.11**	**5.38**	**9.78**

The P value obtained when comparing the solubility values of the unsieved pyrazinamide and the particle size fraction of > 106 µm was 0.7540, for the unsieved pyrazinamide raw material and the particle size fraction of 45 - 106 µm it was 0.9462 and for the two sieve fractions it was 0.8867. None of these P values was considered statistically significant (GraphPad©).

6.5 Conclusion

It could be concluded that the different particle size fractions did not influence the solubility of the pyrazinamide samples. It was further evident that 24 hours was a sufficiently long period to establish equilibrium solubility for this API and its various sieve fractions. This confirmed the known theory that highly soluble APIs tend to reach their equilibrium solubility quickly.

The solubility of pyrazinamide at 37°C was found to be almost 25% higher than that of a previously reported (Merck, 2006) solubility value determined at 25°C.

References

GraphPad© Software. 2012. http://www.graphpad.com/quickcalcs//ttest2.cfm Date of access: 18 June 2012.

Heifets, L. & Lindholm-Levy, P. 1992. Pyrazinamide sterilizing activity *in vitro* against semidormant *Mycobacterium tubercolosis* bacterial populations. *The American Review of Repiratory Disease*, 145:1223-1225.

Lindenberg, M., Kopp, S. & Dressman, J.B. 2004. Classification of orally administered drugs on the World Health Organization model list of essential medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics*, 58:265-278.

Merck & Co., Inc. 2006. The Merck Index: an encyclopedia of chemicals, drugs and biologicals. 14th ed. Whitehouse Station: NJ, 1818 pp.

Mitchison, D.A. 1985. The action of antituberculosis drugs in short-course chemoterapy. *Tubercle*, 66:219-225.

Moffat, A.C., Osselton, M.D. & Widdop, B. *eds*. 2004. Clarke's analysis of drugs and poisons. 3rd ed. Volume 2. London: Pharmaceutical Press, 1117pp.

United States Phamacopoeia. 2011. Pyrazinamide monograph. http://www.uspnf.com/uspnf/pub/getPFDocument?usp=24&nf29&supp=0&collection Date of access: 20 June 2011.

USP see United States Phamacopoeia.

WHO see World Health Organization.

World Health Organization. 2010. Treatment of Tuberculosis guidelines, 4the edition. http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf Date of access: 25 September 2012.

World Health Organization. 2012. WHO model prescribing information: drugs used in mycobacterial diseases. http://apps.who.int/medicinedocs/es/d/Js5511e/2.5.html Date of access: 15 May 2012.

Yeager, R.L., Munroe, W.G. & Dessau, F.I. 1952. Pyrazinamide (aldinamide) in the treatment of pulmonary tuberculosis. *American Review of Tuberculosis*, 65(5):523-546.

Chapter 7

Roxithromycin

7.1 Introduction

Roxithromycin is a semi-synthetic, macrolide antibiotic, derived from erythromycin. A macrolide antibiotic contains a macrolide ring, responsible for the activity of the antibiotic (Chan & Luft, 1986). The ring consists of a large macrocyclic lactone ring to which one or more deoxy sugars may be attached. Roxithromycin contains a 14-membered lactone ring, but differs from erythromycin in that an N-oxime side chain is attached to the lactone ring, as shown in figure 7.1 (Kirst & Sides, 1989).

The mechanism of action of macrolides is to interfere with bacterial protein synthesis by binding to the subunit 50S of the bacterial ribosome, responsible for inhibiting the translocation of bacterial peptides, which prevents the growth of the bacteria (Tenson *et al*., 2003).

Roxithromycin has activity against a variety of bacteria, including some *Staphylococcus* spp., many *Streptococcus* spp., *Moraxella (Branhamella) catarrhalis*, *Mycoplasma pneumoniae* and *Chlamydia trachomatis*. It is more effective against certain gram-negative bacteria, than erythromycin, particularly against *Legionella pneumophila*. It has also been shown to have *in vitro* activity against *Haemophilus influenzae* (Markham & Faulds, 1994).

Roxithromycin is clinically effective against infections of the upper and lower respiratory tract, skin and soft tissues, urogenital- and orodental infections (Markham & Faulds, 1994). *In vitro* studies have been performed to determine the effectiveness of roxithromycin in combination therapies with other antimalarial drugs (Min *et al.,* 2007).

7.2 Physicochemical properties

Roxithromycin is a white, crystalline powder and has the molecular formula $C_{41}H_{76}N_2O_{15}$, of which the chemical structure is shown in figure 7.1. Its molecular weight is 837.047 g/mol (Merck, 2006). Reported solubility values for roxithromycin cover a wide range, i.e. 0.0189 µg/ml (Venkatesh *et al*., 2009), 1.74 µg/ml (Aucamp, 2010), 44 µg/ml (Aucamp *et al*., 2012) and 283.23 µg/ml (Biradar *et al*., 2006).

According to the BCS classification system, roxithromycin is classified as a Class IV drug (low solubility and low permeability) (Benet *et al*., 2004).

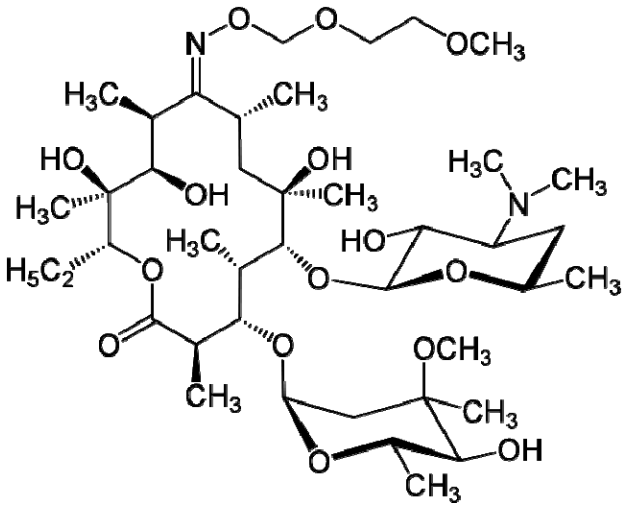
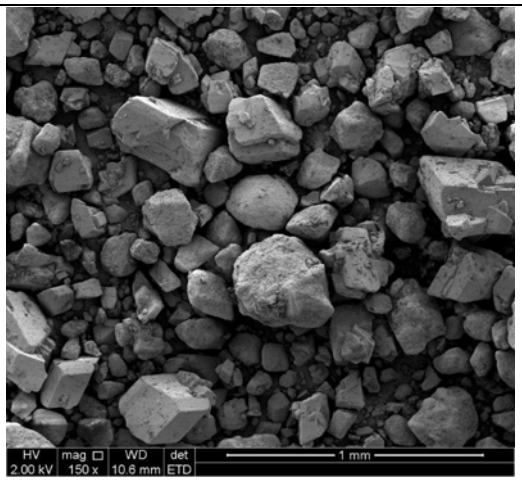


Figure 7.1: Chemical structure of roxithromycin base (Kirst & Sides, 1989).

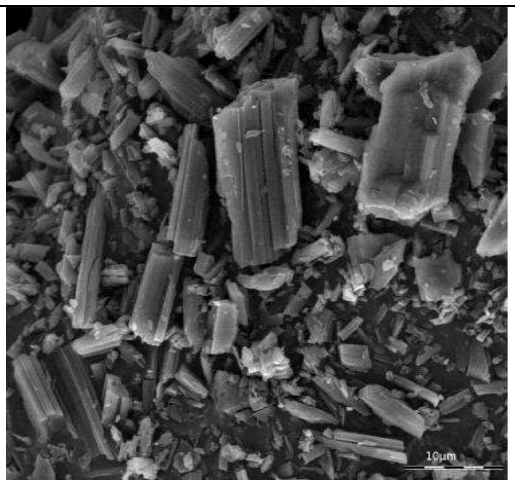
7.3 Pharmaceutical active used for testing

Two forms of roxithromycin are available on the market, i.e. the monohydrate and the anhydrate. Roxithromycin monohydrate, with batch number 110210, from Ria International LLCs, and the anhydrate, with batch number IF-RO-081116, from Iffect Chemphar (HK) Company Limited (China) were used during this investigation. The solubility tests of the monohydrate, as well as the anhydrate were performed on the unsieved raw material and on two sieve fractions of 45 - 90 µm and 90 – 180 µm. The morphologies of the

monohydrate and of the anhydrate are illustrated in figure 7.2. As mentioned in section 1.5.1.2, the crystal characteristics of various solid-state forms may impact on their solubilities. With regards to this API, the effects of the monohydrate and the anhydrate on the solubility properties of roxithromycin were also taken into account during this study.



Roxithromycin anhydrate



Roxithromycin monohydrate

Figure 7.2: SEM micrographs of roxithromycin monohydrate and -anhydrate.

7.4 Experimental and results

7.4.1 Generation of a calibration curve

A standard stock solution was prepared from the anhydrous roxithromycin, from which a set of dilutions was prepared, as indicated in table 7.1.

The actual mass weighed and dissolved in 100 ml of mobile phase was 20.16 mg, giving an actual concentration of 0.2016 mg/ml.

The standard solutions were analysed, according to the BP monograph method (2012), using a Shimadzu (Kyoto Japan) UFLC (LC-20AD) chromatographic system, equipped with a SIL-20AC auto-sampler fitted with a sampler cooler, a UV/VIS Photodiode Array detector (SPD-M20A) and a LC-20AD solvent

delivery module. The HPLC system was equipped with a Luna C_{18} (250 x 4.6 mm, 5 µm) HPLC column.

The mobile phase consisted of a 30 g/L solution of ammonium dihydrogen phosphate, with the pH adjusted to 5.3 with sodium hydroxide, and HPLC grade acetonitrile, mixed in a ratio of 60:40 v/v. The flow rate of the mobile phase was set at 1.0 ml/min and an injection volume of 20 µl was used. The column temperature was 25.2°C. All sample and standard solutions were filtered through pre-rinsed 0.45 micron PVDF filters prior to analysis. The analysis was done at a wavelength of 205 nm.

The peak area results are summarised in table 7.1. A standard curve was constructed, as illustrated in figure 7.3, for use in the determination of the solubilities of the roxithromycin samples.

Table 7.1: Concentrations and peak areas of roxithromycin standard solutions at λ = 205 nm in water

	Dilutions in mobile phase	**Concentration (µg/ml)**	**Area (205 nm)**
(1)	25 ml/50 ml	100.8	604371.5
(2)	20 ml/50 ml	80.6	468991.0
(3)	15 ml/50 ml	60.5	355098.5
(4)	10 ml/50 ml	40.3	232477.5
(5)	5 ml/50 ml	20.2	116080.5
(6)	0.5 ml/100 ml	2.0	5744.5

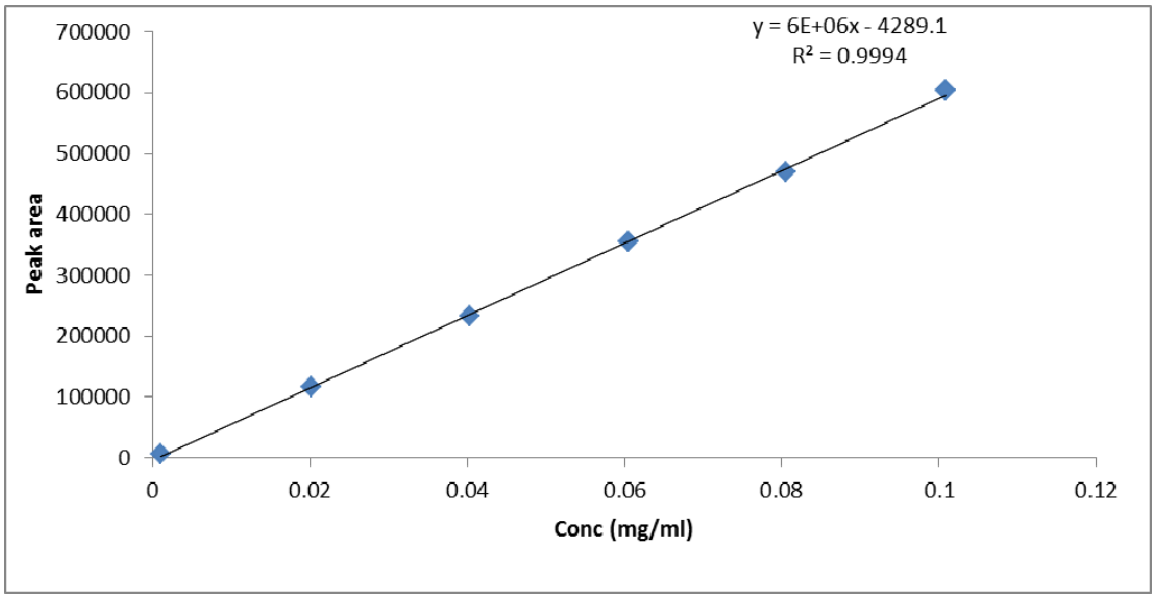


Figure 7.3: HPLC calibration curve for roxithromycin standard solutions at λ = 205 nm.

7.4.2 Solubility of roxithromycin in water after 24 hours

Following the procedure for solubility determinations as described in section 2.2, a surplus (at least 30 mg) of the two particle size fractions of roxithromycin and of the original, unsieved raw material was added to each set of six test tubes. 10 ml of water was added to each sample as solvent.

The samples were removed from the solubility bath after 24 hours and filtered.

1 ml of each filtered portion was diluted to 10 ml with mobile phase, prior to analysis on the HPLC. It is worth mentioning that no distinction between solubility values of the anhydrate and monohydrate forms is made in the available literature. Solubility values could therefore differ (Aucamp *et al*., 2012).

The solubility results for the sample solutions are summarised in tables 7.2 and 7.3. The solubility values obtained for the roxithromycin monohydrate and anhydrate (unsieved) were 42.66 and 56.63 µg/ml, respectively.

Table 7.2: Concentrations of roxithromycin monohydrate and sieve fractions at λ = 205 nm in water after 24 hours

	Roxithromycin monohydrate concentration (µg/ml)		
	Unsieved roxithromycin monohydrate	**Particles 90 - 180 µm**	**Particles 45 - 90 µm**
S1	43.73	53.68	39.41
S2	40.90	62.39	39.94
S3	42.42	59.33	40.99
S4	42.58	61.91	33.87
S5	43.06	63.03	33.62
S6	43.28	58.10	-
Average	**42.66**	**59.74**	**37.57**
%RSD	**2.31**	**5.90**	**9.41**

Table 7.3: Concentrations of roxithromycin anhydrate and sieve fractions at λ = 205 nm in water after 24 hours

	Roxithromycin anhydrate concentration (µg/ml)		
	Unsieved roxithromycin anhydrate	**Particles 90-180 µm**	**Particles 45-90 µm**
S1	54.87	56.87	53.52
S2	57.61	56.23	53.13
S3	55.30	61.83	65.47
S4	56.63	63.04	63.49
S5	58.75	53.37	62.41
Average	**56.63**	**58.27**	**59.60**
%RSD	**2.84**	**6.95**	**9.79**

The calculated P values, according to GraphPad© Software (2012), for the two API forms and the sieve fractions were as follows:

- Unsieved roxithromycin anhydrate *vs* unsieved roxithromycin monohydrate was P = 0.0001 (difference was statistically extremely significant).

The solubility of the two roxithromycin solid-state forms differed significantly.

- Unsieved roxithromycin anhydrate *vs* roxithromycin anhydrate fraction of 90 - 180 µm was P = 0.4253 (difference was statistically insignificant);
- Unsieved roxithromycin anhydrate *vs* roxithromycin anhydrate fraction of 45 - 90 µm was P = 0.3043 (difference was statistically insignificant); and
- Unsieved roxithromycin anhydrate 90 - 180 µm *vs* roxithromycin anhydrate fraction of 45 - 90 µm was P = 0.6852 (difference was statistically insignificant).

The solubilities of the three anhydrate sieve fractions tested did not differ significantly.

- Unsieved roxithromycin monohydrate *vs* roxithromycin monohydrate fraction of 90 - 180 µm was P = 0.0001 (difference was statistically extremely significant);
- Unsieved roxithromycin monohydrate *vs* roxithromycin monohydrate fraction of 45 - 90 µm was P = 0.0164 (difference was statistically extremely significant); and
- Roxithromycin monohydrate fraction of 90 - 180 µm *vs* roxithromycin monohydrate fraction of 45 - 90 µm was P = 0.0001 (difference was statistically extremely significant).

The solubilities of the three roxithromycin monohydrate sieve fractions tested differed significantly from each other.

7.5 Discussion and conclusion

The solubility values obtained for the roxithromycin monohydrate and -anhydrate forms during this study were 42.66 µg/ml and 56.63 µg/ml, respectively. According to literature, incorporation of water molecules into the crystal lattice of an anhydrate changes the physicochemical properties of the unit cell. Also, the thermodynamic activity of an anhydrate, due to hydration, changes its pharmaceutical properties, such as solubility (Khankari & Grant, 1995). A general rule applying to solubility behaviour is that the anhydrous form of an API is always more water soluble than the corresponding hydrate, at identical temperatures.

Since the hydrate has already interacted with the water molecules, the free energy released is less for the hydrate than for the anhydrate (Khankari & Grant, 1995). This statement correlated well with the results obtained during this study for the two unsieved forms of roxithromycin. Interestingly, the solubility concentrations obtained for the 90 – 180 µm sieve fractions for both the monohydrate and the anhydrate were almost identical and therefore seemed to have contradicted the above statement, i.e. that an anhydrate is more soluble in water than the hydrate. However, a very recent study by Aucamp *et al.* (2012) showed that the commercial roxithromycin monohydrate, which was of the same batch as used in this study, actually consisted of both monohydrate and anhydrate fractions. The significant differences obtained in the solubilities of the roxithromycin monohydrate raw material and its sieve fractions could be explained by the fact that it is indeed very likely that two different crystal forms would have different particle sizes and crystal habits.

In the case of roxithromycin, one could conclude that the 24 hour testing period was long enough for equilibrium solubility to be reached, regardless of particle size, as demonstrated by the results obtained for commercial roxithromycin anhydrate.

The results obtained for commercial roxithromycin monohydrate, which proved to be a mixture of the monohydrate and anhydrate forms (Aucamp *et al*., 2012),

demonstrated the importance of having to confirm the crystal identity of commercial raw materials prior to formulation. The above data seemed to have indicated that particle size has a significant effect on solubility, or at least on the time needed to reach solubility equilibrium, when in fact the sieve fractions represented mixtures, each dominated by a different crystal form.

These findings and those of Aucamp *et al*. (2012) explain the large variations in published solubility values of roxithromycin. Not only have previous authors failed to distinguish between the anhydrous and monohydrate forms of roxithromycin, which differ in their solubilities, but polymorph control during commercial production had clearly been overlooked by some, if not by all manufacturers.

References

Aucamp, M.E. 2010. The polymorphism and pseudopolymorphism of roxithromycin. Ph.D thesis, North-West University, Potchefstroom, 229 pp.

Aucamp, M.E., Stieger, N., Barnard, N. & Liebenberg, W. 2012. Solution mediated transformation of roxithromycin and the pharmaceutical significance thereof. *Manuscript in preparation.*

Benet, L.Z., Broccatelli, F. & Oprea, T.I. 2011. BDDCS applied to over 900 drugs. *The AAPS Journal*, 13:519-547.

Biradar, S.V., Patil, A.R., Sudarsan, G.V. & Pokharkar, V.B. 2006. A comparative study of approaches used to improve solubility of roxithromycin. *Powder Technology*, 169(1):22-32.

BP see British Pharmacopoeia.

British Pharmacopoeia. 2012. Roxithromycin. http://www.pharmacopoeia.co.uk/bp2012/ixbin/bp.cgi?a=display&r=pxG4ZY2Vifc&id=6775&tab=search Date of access: 26 June 2012.

Chan, J. & Luft, B.J. 1986. Activity of roxithromycin (RU 28965), a macrolide, against *toxoplasma gondii* infection in mice. *Antimicrobial Agents and Chemotherapy,* 30(2):323-324.

GraphPad© Software. 2012. http://www.graphpad.com/quickcalcs//ttest2.cfm Date of access: 18 June 2012.

Khankari, R.K. & Grant, D.J.W. 1995. Pharmaceutical hydrates. *Thermochimica Acta*, 248:61-79.

Kirst, H.A. & Sides, G.D. 1989. New directions for macrolide antibiotics: structural modifications and *in vitro* activity. *Antimicrobial Agents and Chemotherapy*, 33(9):1413-1418.

Markham, A. & Faulds, D. 1994 Roxithromycin: an update of its antimicrobial activity, pharmacokinetic properties and therapeutic use. New Zealand: Adis International Limited. *Drugs* 48(2):297-326. http://ukpmc.ac.uk/abstract/MED/7527329 Date of access: 26 June 2012.

Merck & Co., Inc. 2006. The Merck Index: an encyclopedia of chemicals, drugs and biologicals. 14th ed. Whitehouse Station: NJ, 1818 pp.

Min, T.H., Khairul, M.F.M., Low, J.H., Che Nasriyyah, C.H., Noor A'shikin, A., Norazmi, M.N., Ravichandran, M. &, Raju, S.S. 2007. Roxithromycin potentiates the effects of chloroquine and mefloquine on multidrug-resistant *Plasmodium falciparum in vitro*. *Experimental Parasitology*, 115:387-392.

Tenson, T., Lovmar, M. & Ehrenberg, M. 2003. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. *Journal of Molecular Biology*, 330(5):1005-1014.

Venkatesh, D.N., Karthick, S., Umesh, M., Vivek, G., Valliappan, R.M., Uthayakumar, B., Deepak, S.V., Ramesh, N., Samanta, M.K. & Suresh, B. 2009. Studies on the preparation, characterization and solubility of β-cyclodextrin-roxithromycin inclusion complexes. *International Journal of Pharmaceutical Sciences and Nanotechnology*, 2(2):523-530.

Chapter 8

Conclusion

In testing the equilibrium solubility of pharmaceutical actives, 24 hours is the period most often used, as it is widely considered to allow for sufficient time for equilibration to be established between the dissolved API and its excess, undissolved component. There are a number of experimental factors that influence the speed at which an API will reach its equilibrium, such as solubility and wettability of the solid, its particle size, the solvent used, temperature, solid-state transformations, agitation and agitation rate.

In this study, the role of particle size on equilibrium solubility was investigated by keeping other factors constant. The aim of this study was furthermore to establish whether or not 24 hours indeed represented a suitable and universally applicable period of time for solubility testing in water at 37°C, using a rotation rate of 54 rpm. For the purpose of this investigation, five APIs were selected from compounds available in-house, ranging from freely soluble to poorly soluble in the order: chloroquine phosphate > pyrazinamide > mefloquine hydrochloride > closantel sodium > roxithromycin.

During method development it was demonstrated that solubility samples should be kept at a constant temperature at all times, from sample preparation through to analysis, and that they should not be allowed to cool for even a brief moment. It is known that the solubility of APIs, especially of poorly soluble compounds, is affected by temperature and that cooling may lead to unexpected, rapid recrystallisation. This in turn would lead to large variations in the data generated. The dilution of samples with a solvent in which the API is freely soluble will prevent precipitation.

No published solubility values were found for closantel sodium, other than that salicylanilides are generally very poorly soluble. Of the four APIs for which published solubility values were found, three proved to be more soluble than

previously reported, when tested using the newly optimised method, as developed during this investigation. Unfortunately, the solubility of chloroquine phosphate proved to be so much higher than anticipated that testing could not be completed successfully with the amount of powder available. Previous solubility values for roxithromycin were extremely inconsistent, likely due to polymorphism not having been taken into account.

Solubility studies were thus successfully completed on four of the five APIs selected. For three of these it was confirmed that the 24 hour test period was sufficient for the attainment of equilibrium solubility, regardless of the particle size fractions tested. The fact that poorly soluble APIs reach equilibrium slower, due to slower dissolution, is also well-known. Surprisingly, the only API in this study for which 24 hours was an insufficient test period was mefloquine HCl, which was not the least soluble compound amongst the chosen materials. Further testing would be required to clarify this anomaly.

What was evident from the outcomes of this investigation was that although the ubiquitous 24 hour solubility test period may work well in many cases, its suitability should be reviewed on a case-by-case basis and not just for the most poorly soluble compounds. Researchers testing solubility at temperatures lower than 37°C should be especially cautious of using a standardised test period, because equilibrium solubility would take longer to achieve with less energy being available to the system.

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