THE DEVELOPMENT OF AN ORODISPERSIBLE SILDENAFIL CITRATE TABLET INTENDED FOR PAEDIATRIC USE

by

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ABSTRACT

Sildenafil citrate (SC) is a phosphodiesterase-5 inhibitor that is used to treat pulmonary hypertension (PH) in paediatric patients. The purpose of these studies was to develop a formulation and manufacture an orodispersible tablet (ODT) that can be easily administered to neonates and children with PH. The advantages of ODT dosage forms include ease of administration, rapid dissolution of the API, SC. Furthermore the dosage form can be taken without water which is beneficial to patients without immediate access to potable fluids.

A simple, rapid, accurate, precise and selective reversed-phase HPLC method was developed and validated in accordance with International Conference on Harmonization (ICH) guidelines and was successfully used for the analysis of SC as raw material and in SC containing pharmaceutical dosage forms.

Preformulation studies were performed on SC, alone and in combination with potential excipients that could be used to make tablets. Investigations into potential interactions between SC and the excipients were performed using Differential Scanning Calorimetry (DSC) and Infrared Spectroscopy (IR). DSC results revealed that SC was compatible with all potential excipients except mannitol and magnesium stearate. However these interactions were not observed with IR and therefore it was concluded that the interactions were induced by the high temperatures that DSC operates at. Particle size and shape was also established by use of Scanning Electron Microscopy (SEM) and flow properties were monitored by calculating Carr's Index (CI) and the Hausner Ratio (HR).

Direct compression was used as the method of manufacture for SC tablets as this approach is simple and the most economic production approach. The powder blends were assessed for bulk and tapped density and the CI and HR were used to determine the flowability of the blends. The quality attributes of the resultant tablets that were monitored included uniformity of weight, friability, crushing strength, tensile strength, disintegration, wetting and *in vitro* dispersion times.

Design of Experiments is an efficient statistical approach that has become a popular tool used in the pharmaceutical industry to optimize formulation compositions, as it allows for the investigation of several input factors at the same time whilst not using the tedious and traditional " modification of one variable at a time" approach.

A Central composite experimental design was chosen as the most appropriate means to optimize the formulation as it produces more accurate results as opposed to other experimental designs approaches as input factors are investigated at five different levels.

Through the use of mathematical modelling, optimum concentrations of disintegrant(s) and an appropriate blending time were established. Analysis of the data from the experimental design and mathematical modelling studies reveal that no changes in disintegrant concentration or blending time altered the disintegration time of the formulation to any significant extent. This result is most likely due to the fact that the critical disintegrant concentration has been reached and increasing the disintegrant concentration further has no effect on disintegration time. It was also established that a change in the concentration of CMS and CRP altered the wetting time of the tablet significantly. Finally it was noted that there was a linear relationship between blending time and the uniformity of content of the tablets produced in these studies.

The optimized product was a white tablet with a diameter of 7.31 mm with a thickness of 2.80mm. The dosage form had no visible cracks or evidence of picking or sticking. The tablet exhibits suitable friability and tensile strength while exhibiting a disintegration time of only 8s. Therefore an orodispersible tablet containing SC intended for paediatric use has been successfully developed, manufactured and optimized through the use of preformulation studies, appropriate quality control monitoring and mathematical modelling. These formulations require further optimization in respect of addition of flavours and or additional sweetening agents.

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STUDY OBJECTIVES

Sildenafil citrate (SC) is used as an alternative to other treatments such as inhaled nitrous oxide, which is not always effective in the treatment of Pulmonary Hypertension in paediatric patients. The prescribing of SC in paediatric patients is becoming common practice. However since the API is only available in tablet form the only option for the pharmacist is of preparation of extemporaneous preparations. Potential problems may arise with the use of extemporaneous formulations as the stability of SC in these formulations has not been monitored.

Orodispersible tablets (ODT) is a drug delivery technology that has been identified as a potential dosage form for paediatric patients in addition to patients that have difficulty in swallowing. Therefore the development of an ODT of SC may ensure the successful administration of SC to patients for which an age appropriate dosage form is not available.

The objectives of this study were:

i. To develop and validate a high performance liquid chromatographic method for the analysis of SC as raw material and in pharmaceutical dosage forms.
ii. To conduct preformulation studies to ensure the selection of appropriate excipients to produce a SC ODT and establish if any interactions may occur between SC and these excipients.
iii. To develop and optimize a suitable method of manufacture for an SC containing ODT.
iv. To use Response Surface Methodology (RSM) and experimental design to establish relationships between identified independent variables and selected responses
v. To use RSM and experimental design to optimize an ODT for SC.
vi. To evaluate and ensure the quality of the SC ODT by evaluation of the *in vitro* release profiles, as well as friability, crushing and tensile strength, disintegration time, wetting time, in vitro dispersion time and the water absorption ratio of the tablets.

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LIST OF ACRONYMS

ACN	Acetonitrile
ANOVA	Analysis of Variance
AOR	Angle of Repose
API	Active Pharmaceutical Ingredient
BCS	Biopharmaceutics Classification System
BP	British Pharmacopoeia
CI	Carr's Index
cGMP	Cyclic Guanosine Monophosphate
CMS	Croscarmellose Sodium
CRP	Crospovidone
DSC	Differential Scanning Calorimetry
DZ	Diazepam
EC	Exclusion Chromatography
ECMO	Extracorporeal Membrane oxygenation
ED	Erectile Dysfunction
FDA	Food and Drug Authority
GIT	Gastrointestinal Tract
H_2O_2	Hydrogen Peroxide
HCl	Hydrochloric Acid

HPLC	High Performance Liquid Chromatography
i.d.	Internal Diameter
IE	Ion Exchange
ICH	International Conference on Harmonization
iNO	Inhaled Nitric Oxide
IR	Infrared
IS	Internal Standard
LOD	Limit of Detection
LOQ	Limit of Quantitation
MCC	Microcrystalline Cellulose
MeOH	Methanol
NaOH	Sodium Hydroxide
NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide
NP	Normal Phase
ODT	Orodispersible Tablet
PDE	Phosphodiesterase
PH	Pulmonary Hypertension
PHR	Peak Height Ratio
PPHN	Persistent pulmonary hypertension of the newborn
PVP	Polyvinylpyrrolidone
RH	Relative Humidity
ROP	Retinopathy of Prematurity

RP	Reverse Phase
RSD	Relative Standard Deviation
RSM	Response Surface Methodology
R_t	Retention Time
SC	Sildenafil Citrate
SEM	Scanning Electron Microscope
SSG	Sodium Starch Glycolate
TGA	Thermogravimetric Analysis
USP	United States Pharmacopoeia
UV	Ultra Violet

The definition of insanity is doing the same thing over and over again and expecting different results

-Albert Einstein

CHAPTER ONE
SILDENAFIL CITRATE

1.1 INTRODUCTION

Sildenafil citrate (SC), a selective phosphodiesterase 5(PDE5) inhibitor, is the drug of choice for the treatment of erectile dysfunction (ED) (1; 2). It has recently also been shown to be effective in the treatment of Pulmonary Hypertension (PH) in adult and paediatric patients (3-7). In the latter group, PH is regarded as a disease that is characterized by pulmonary vascular resistance (7) and has a mortality rate of approximately 10–20% (8).

Medicines for use in neonates and children are not typically evaluated and doctors have to prescribe medication off-licence and/or off-label for paediatric patients (9-11). This is the case for SC, which is licensed for the treatment of PH in adult patients only.

Because SC is not licensed for paediatric use, it is important to ensure that paediatric patients are not exposed to avoidable risk. Conducting controlled clinical trials (9) in this patient subgroup is therefore a matter of utmost importance. Published reports of SC use in adult patients and in animal models (12-14), prior to its use in newborns, have been documented. More recently, some limited randomized trials on the use of SC, as a treatment for paediatric patients suffering from PH have been undertaken.

There is no cure for pulmonary hypertension of the neonate (PPHN) and treatment is therefore aimed at relieving the symptoms of the disease (5). In the past, the treatment for PPHN included inhalation of nitrous oxide, the use of calcium channel blockers, and extracorporeal membrane oxygenation (ECMO) (5; 8). The disadvantage of this treatment is that it is expensive and, in the case of ECMO, is not readily available, particularly in developing countries (8). The advantage of treating PH with SC relates to the availability of the compound and the lower cost compared to other modes of PH treatment. An additional benefit of SC is that its use in paediatric patients is safe, with only a few side effects having been reported (3).

Although there are many published reports on the benefits of using SC in paediatric patients, there have not been many clinical trials conducted and the few that have been undertaken have used very small cohort sizes (3; 6). This clearly highlights the need for more research on the use of SC in paediatric patients. Available research is however promising and results so far suggest that SC is an acceptable alternative to ECMO for the treatment of PH in paediatric patients.

1.2. PHYSICOCHEMICAL PROPERTIES

1.2.1. Description

SC is an odourless white to off-white crystalline powder (15). The chemical name for SC is 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulphonyl]-4-methylpiperazine citrate. The empirical formula for SC is $C_{22}H_{30}N_6O_4S{\cdot}C_6H_8O_7$ and the molecular weight of SC is 666.71. The chemical structure of SC is depicted in Figure 1.1.

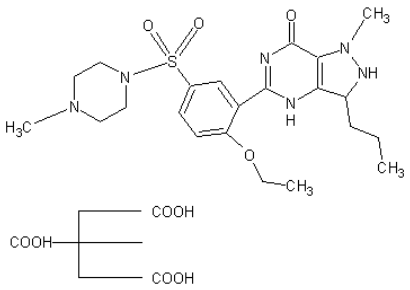


Figure 1.1 The chemical structure of sildenafil citrate (15)

1.2.2. Dissociation Constant (pKa)

Sildenafil has two pKa values, viz. pK_{a1}=6.78 and pK_{a2}=9.12. This indicates that sildenafil is an ordinary ampholyte with mild basicity and weak acidity. The compound is therefore typically neutral at physiological pH (16). A depiction of the different ionization states of SC is given in Figure 1.2.

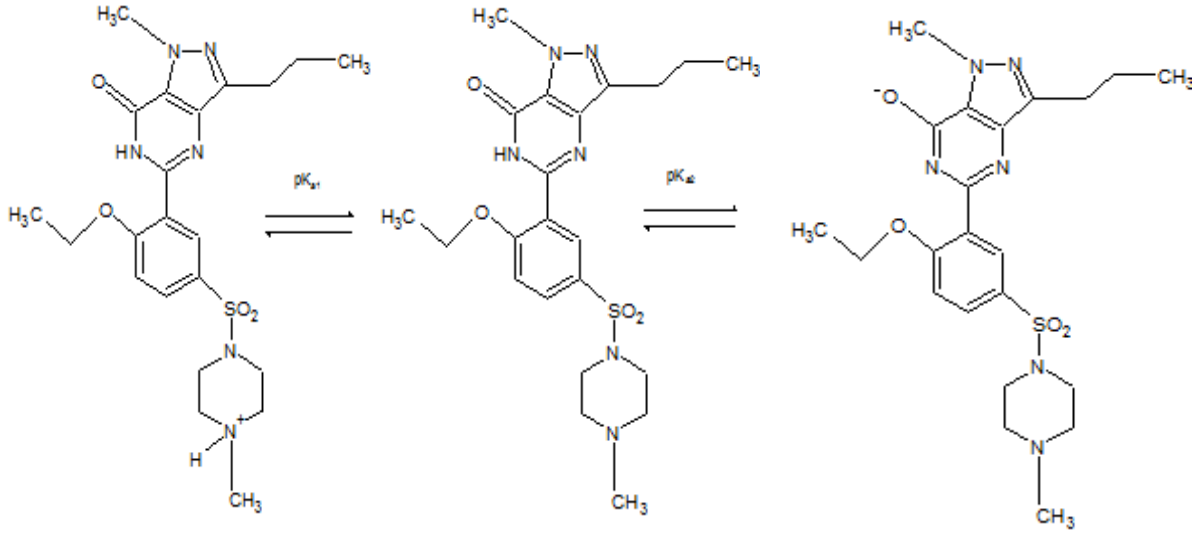


Figure 1.2 Ionization equilibria of sildenafil (16)

1.2.3. Solubility

The solubility of SC, determined in different solvents, is summarized in Table 1.1 (15).

Table 1.1 Solubility of SC in various solvents (15)

Solvent	Solubility (mM)
Methanol	1.2
Water	0.6
Ethanol	0.17
Dichloromethane	0.005
Diethyl ether	0.005
n-Hexane	0.0001

1.2.4 Partition coefficient

The partition coefficient is the equilibrium distribution of a molecule between a hydrophilic phase (aqueous) and a hydrophobic phase (oil). The hydrophilic solvent used is usually water while the hydrophobic solvent in these experiments is usually octanol as this solvent has the ability to mimic the aqueous/membrane interface, due to its chemical characteristics (17).

The partition coefficient is an important physicochemical property of a compound since it provides an indication of the hydrophobicity of a drug molecule and hence the ability of the molecule to cross membranes. Hydrophobic drugs are preferentially concentrated in the hyprophobic compartments of membranes, viz. the lipid bilayers, whereas hydrophilic drugs favour hydrophilic compartments such as blood serum (18).

The value of the partition coefficient (log P) of SC is 2.7–3.18 (16; 19) indicating a relatively lipophilic molecule.

1.2.5. Melting range

SC melts within a range of 194-199°C (15) . Lower melting points within the range 182°C –186°C have however been reported with regard to SC however this is attributed to the incomplete extraction process of the API from the dosage form (20).

1.2.6 Ultraviolet absorption spectrum

An ultraviolet scan, generated using a Lamba 25 UV/VIS Spectrometer (Perkin Elmer® Ltd, Beaconsfield, England) at a scan speed of 480 nm.min^{-1}, is depicted in Figure 1.3

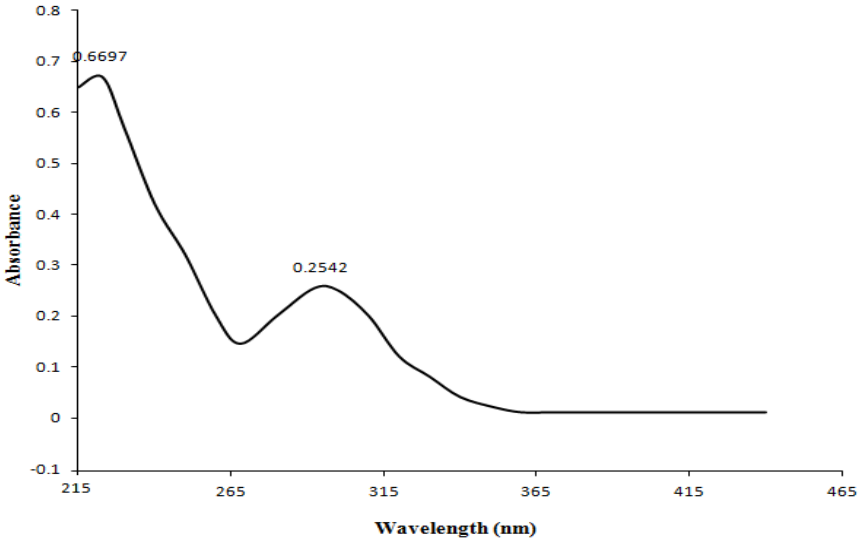


Figure1.3 Ultraviolet absorption spectrum of SC

1.2.7 Infra-red spectrum

The Infrared (IR) absorption spectrum of SC powder was generated using a Spectrum 100 Fourier transform-infrared attenuated total reflectance spectrometer (Perkin Elmer® Ltd Beaconsfied, England). The spectrum, obtained at a wave-number range of 4000–650cm^{-1} and a resolution of 4cm^{-1}, is depicted in Figure 1.4. Band assignments for the resultant spectrum are summarized in Table 1.2.

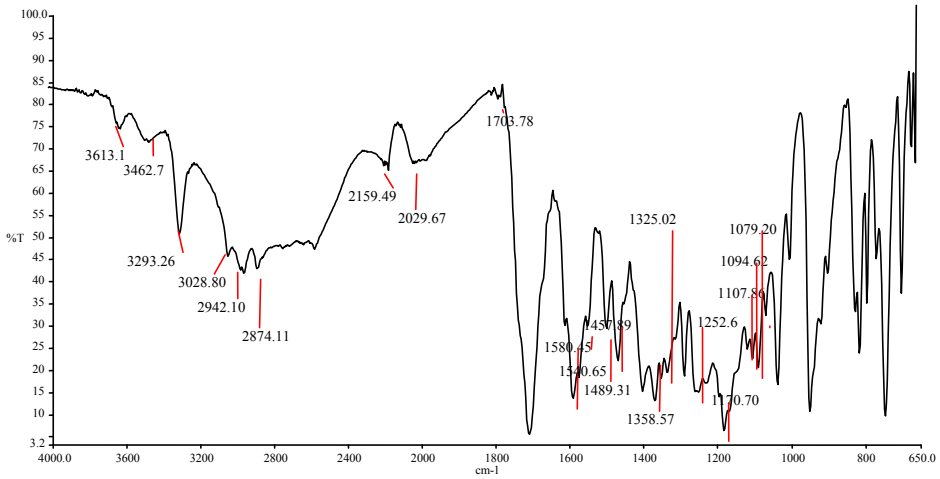


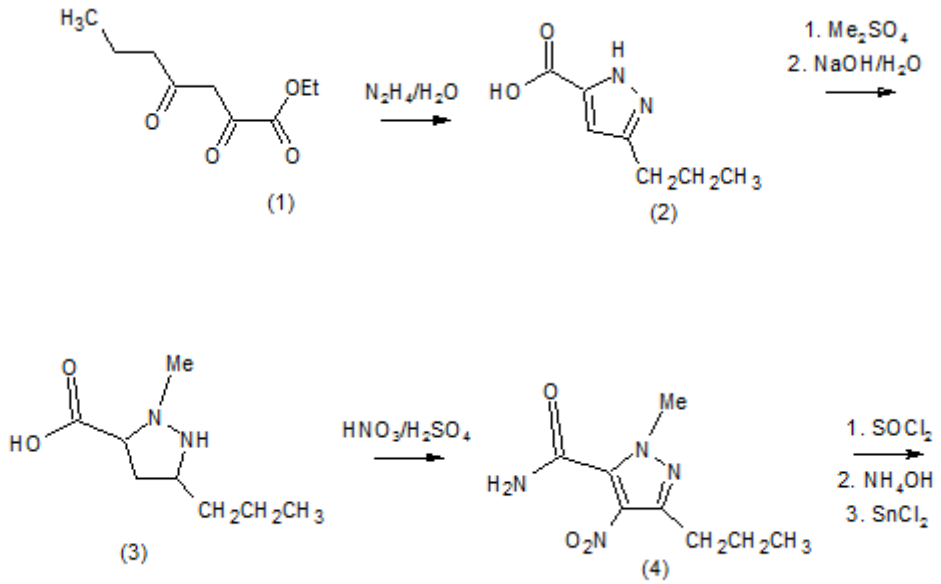
Figure 1.4 IR absorption spectrum of SC

Table 1.2 IR absorption spectrum band assignments

Bands	Assignment
3617	OH (Stretching)
3300	NH (Stretching)
3028	CH (Stretching Aromatic)
3000–2270	CH (Stretching aliphatic)
1700	C=O (Stretching)
1600-1500	C=C (Stretching)
1358, 1170	SO_2 (Stretching)
1240	C-N (Stretching)

1.2.8 Synthesis

SC is synthesized as follows: an initial reaction between a diketoester (Figure 1.5, **1)** and hydralazine results in the formation of a pyrazole ring, which is then subjected to N-methylation and hydrolysis to produce a carboxylic acid (Figure 1.5, **3**). The compound is then nitrated, resulting in carboxamide formation. Nitro group reduction is then performed to produce a pyrazole intermediate (Figure 1.5, **5**). The amine group is then acylated with 2-substituted benzoyl chloride (Figure 1.2, **6**), followed by cyclization under basic conditions to produce the pyrazolopyrimidinone (Figure 1.5, **7**). Chlorosulfonylation at the 5' position of the phenyl ring occurs (Figure 1.5, **8**) allowing for ready coupling with piperazine to yield the product, sildenafil, which reacts with citric acid to produce sildenafil citrate. The synthesis of SC is depicted in Figure 1.5



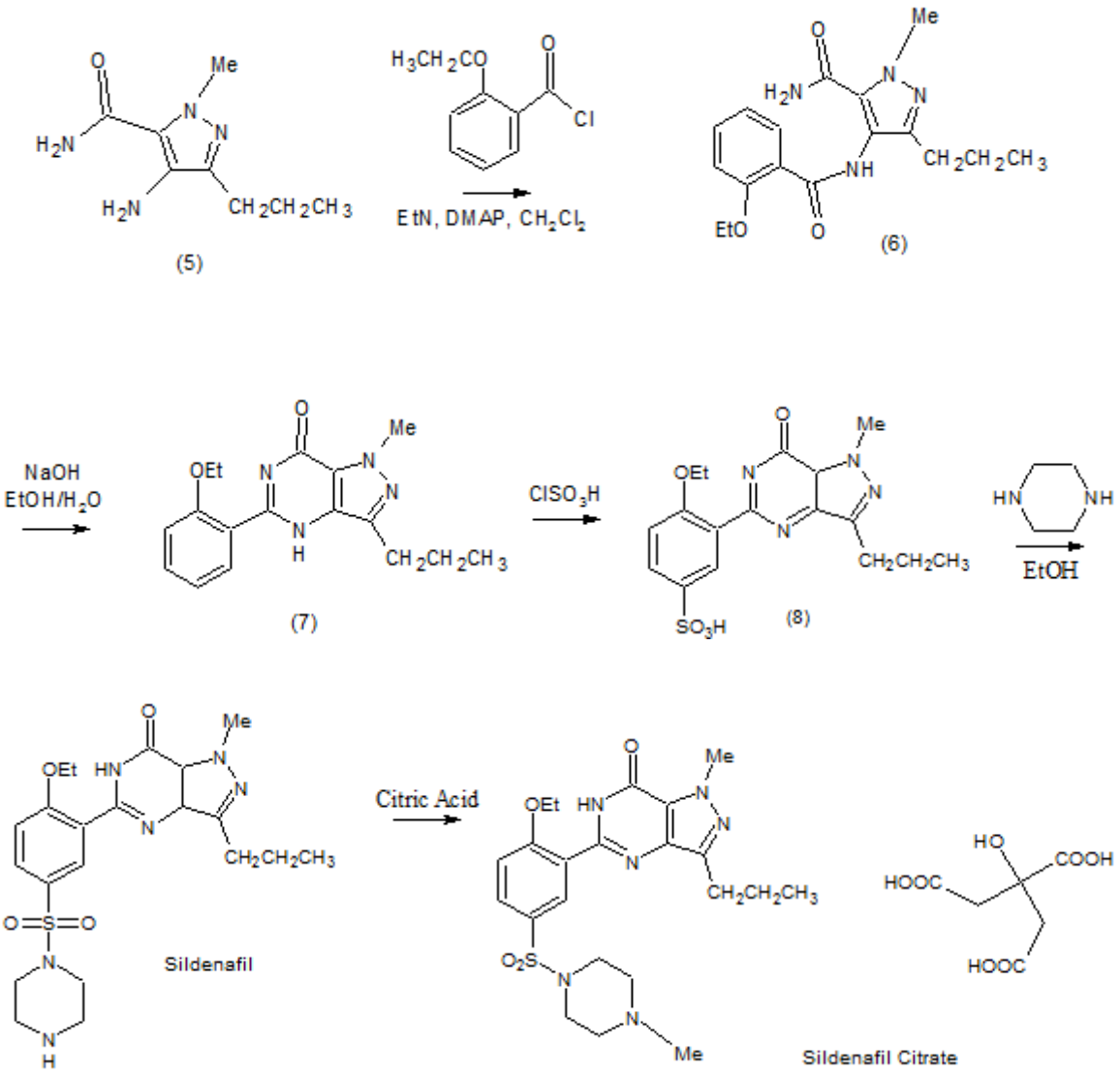


Figure 1.5 The synthesis of SC (21)

1.2.9 Structure Activity Relationships

SC is an inhibitor of the PDE type 5 enzyme. It has a similar structure to the enzyme, which facilitates its interaction with the enzyme substrate. A major problem relating to the use of this compound is that several different types of PDE enzymes are found in the human body (22; 23). When investigating PDE inhibitors it is therefore important to isolate a molecule that is not only potent, but also exhibits a high degree of selectivity for the PDE type 5 enzymes.

Initially investigations into the selectivity and potency for PDE type 5 enzymes involved exploring a range of 2-alkoxyphenyl-substituted heterocyclic systems. Results indicated that pyrazolo [4,3-d]pyrimidin-7-one derivatives showed potent cGMP (cyclic guanosine monophosphate) PDE type 5 enzyme inhibition. Modelling studies suggested that the nucleus of the pyrazolo [4,3-d] pyrimidin-7-one molecule could mimic the guanosine base of cGMP, as these molecules have a similar shape, size and dipole moment (21). The similarity of cGMP and pyrazolo [4,3-d] pyrimidin-7-one is depicted in Figure 1.6.

By changing the substitute at position 3 from a methyl to a propyl group, the potency of the compound was increased significantly. It is surmised that, by extending chain length of the substitute, the compound would be able to fill a space on the enzyme site that is normally occupied by ribose (21). Removing the methyl group at Position 1from the pyrazole decreased the selectivity of the compound for the PDE type 5 enzyme (21).

The 5'-substituent on the 2-ethoxyphenyl ring has the potential to fill a space occupied by the phosphate of cGMP in the PDE active site. It was shown that the addition of sulphones or sulphonamides increased the affinity of the compound for this enzyme (21). These functional groups have the added benefit of being less lipophilic, which improves the low solubility of the compound (21).

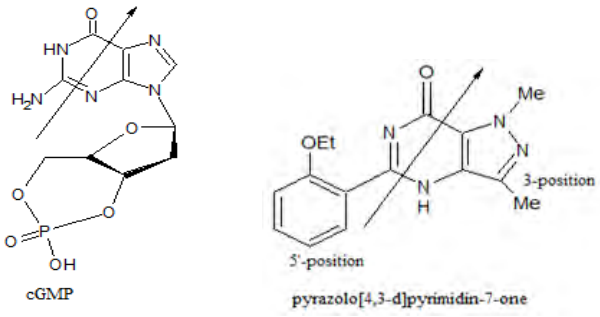


Figure 1.6 Relationship between pharmacophoric sub-structures of cyclic GMP and pyrazolo [4,3-d]pyrimidin-7-one (21)

1.3. STABILITY

The stability of pharmaceutical compounds depends on two factors *viz.,* the chemical and physical properties of the molecule and the impact of environmental factors such as temperature and light on the active pharmaceutical ingredient (API) (24).

In a study conducted by Badwan *et al*., SC showed a high degree of stability when stored under different climatic conditions (15). Following storage for two months at 40°C, 75% relative humidity (RH) and later at 50°C and 75% RH, the compound remained stable with little degradation being observed. Similar results were noted when SC was exposed to ultraviolet (UV) light for a period of two months.

The pH stability profile of SC was characterised over a pH range of 1–13. The profile was studied under storage conditions of 65°C for two weeks. Results indicated that minimal degradation of SC occurred under these conditions (15) .

SC has been shown to be stable after undergoing three freeze-thaw cycles involving storage at –20°C for one month followed by thawing at room temperature for 24 hours (25).

1.4 CLINICAL PHARMACOLOGY

1.4.1 PH and paediatric patients

PH is diagnosed when patients have a mean arterial pressure of > 25mm Hg at rest or > 30mm Hg following exercise, irrespective of the age (26). Many paediatric cardiologists agree that a more appropriate definition of PH in paediatric patients would be a systolic pulmonary artery pressure that exceeds 50 % of the systolic systemic pressure at rest (27).

There are conflicting records of the incidence of PH in newborns. Farrows *et al.,* report that the incidence of PPHN is 0.2% (28) whereas another study reports that PPHN occurs in 0.43– 6.8 patients per 1000 live births (8; 29). The incidence is likely to be greater in developing countries where reporting is poor and very little information is available (8). The mortality rate of PPHN has remained stationary at approximately 10–20 % over the last decade (27).

1.4.2 Persistent Pulmonary Hypertension of the Newborn (PPHN)

Prior to birth, the pulmonary circulation of the foetus is characterized by high pulmonary vascular resistance and low pulmonary blood flow, since the lungs receive less than 8% of the ventricular output (30) as they are filled with fluid (31) and gaseous exchange occurs in the placenta. At birth

the pulmonary circulation undergoes significant vasodilatation to facilitate rapid and increased flow of blood to the lungs of the neonate (32). This does not occur in all neonates, as some fail to achieve a decrease in pulmonary vascular resistance, which leads to respiratory distress and hypoxia, a condition known as PPHN (33).

The mechanism by which altered lung vascular structure and function arises is still poorly understood, but there is increasing evidence suggesting that oxidative stress plays an important role in the pathogenesis of PPHN (33). A ductal ligation model of PPHN has also confirmed that increased levels of reactive oxygen species promote vasoconstriction (34).

There are a number of predisposing factors associated with the development of PPHN and, with an increasing knowledge of the condition, the list of such factors keeps increasing. Some of the common predisposing factors associated with PPHN are listed in Table 1.3.

Table1.3 Predisposing factors for PPHN (31)

Predisposing factors	Remarks
Birth asphyxia	Both intrauterine and perinatal hypoxia
Early onset sepsis/pneumonia	Active constriction of pulmonary vessels possibly due to increased thromboxane
Pulmonary hypoplasia due to causes such as congenital diaphragmatic hernia, amniotic fluid leak, pleural effusion	Reduction in the number of intralobar arteries; their increased muscularity
Maternal drug intake of non steroidal anti-inflammatory drugs such as ibuprofen, aspirin, indomethacin) and/or use of selective serotonin reuptake inhibitors such as fluoxetine)	Use of Non steroidal anti-inflammatories (NSAID) leads to in-utero constriction of the ductus arteriousus and pulmonary vasculature remodelling; the use of selective serotonin reuptake inhibitors (SSRI) in the late third trimester is associated with PPHN
Familial occurrence	Familial occurrence is uncommon
Idiopathic	Quite common however unrecognised hypoxia or ductal closure may account for a large proportion of idiopathic cases

PPHN typically occurs in full-term neonates that are frequently born through meconium-stained amniotic fluids (31).

Infants with PPHN that is related to asphyxia or idiopathic causes may exhibit severe cyanosis and tachypnoea, although respiratory distress may not initially be noticeable (31). Infants with PPHN related to meconium aspiration, pneumonia, diaphragmatic hernia or pulmonary hypoplasia present with grunting, nasal flaring, chest retractions, tachycardia and shock (31).

1.4.3. Use of SC in PPHN

SC is a selective inhibitor of the phosphodiesterase type 5 enzyme that is found in high concentrations in the pulmonary vascular smooth muscle (34). PDE5 enzymes are responsible for the hydrolysis of (cGMP) to form guanosine 5'-cyclic phosphate (4). cGMP is a secondary messenger of nitric oxide (NO). NO is a vasodilator and hence an increase in cGMP will lead to an increase in NO, thereby promoting vascular smooth muscle relaxation, as represented in Figure 1.7.

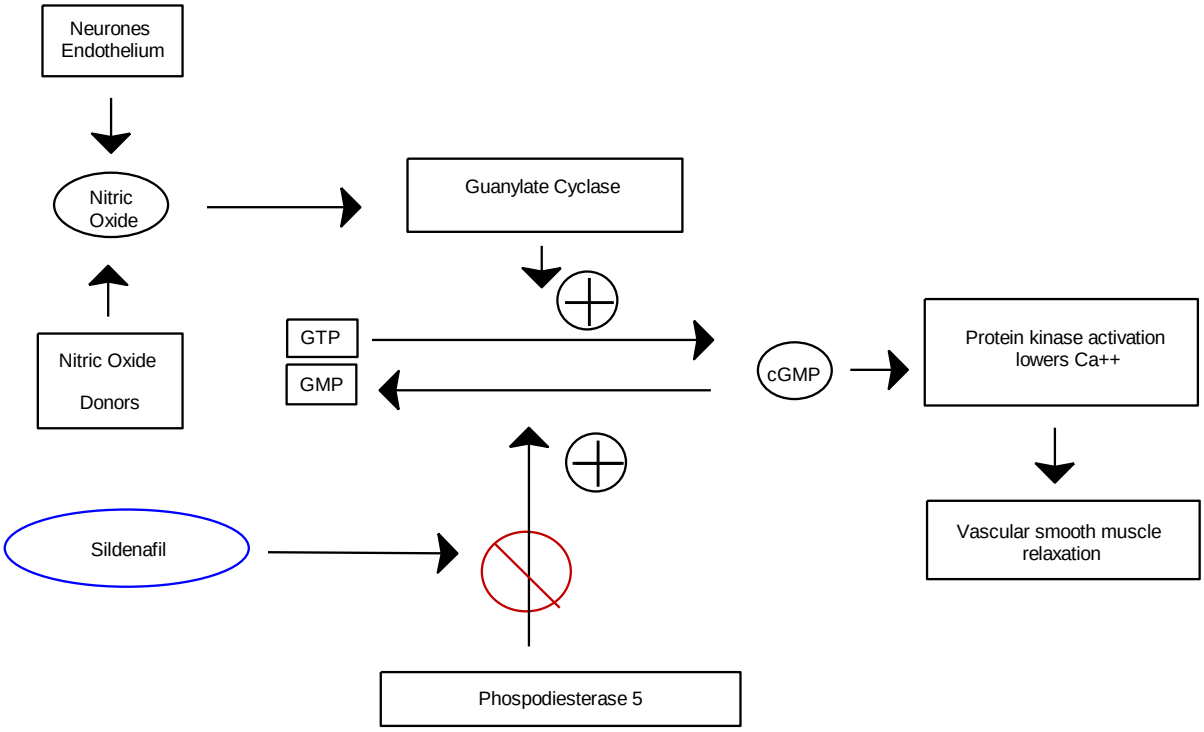


Figure 1.7 A schematic representation of the mechanism of action of SC (35)

Knowing when to administer SC to a paediatric patient can be a difficult decision for a prescriber as there is always the risk of an untoward response when administering medicines off-license. Sola *et al.,* have attempted to provide direction in this regard by stating that potential candidates would most likely be term or near-term infants with severe PPHN and refractory hypoxemia (36).

SC administration is also an appropriate alternative when other treatments fail. Inhaled nitric oxide (iNO) is used frequently for the treatment of PH, but there are reports that up to 30% of infants fail to improve after administration of iNO (8). SC is also an alternate choice in developing countries, where iNO is not readily available for use (37).

SC is available in tablet form. This provides a challenge for the safe and efficacious administration of the compound to paediatric patients who are unable to ingest tablets. SC tablets are therefore crushed, dissolved in water and administered either orally or via a nasogastric tube (6).

1.4.4. Indications

SC is indicated primarily for the treatment of erectile dysfunction (ED) and is marketed by Pfizer under the trade name Viagra®. Recently SC has been approved by the Food and Drug Administration (FDA) for the treatment of PH in adults and is marketed as Revatio® (38).

Besides the approved use of SC in ED and PH, research has shown that SC may be beneficial in a number of other conditions, including the treatment of pain in animals and humans. SC has been shown to produce an antinociceptive effect in animal models for pain following local, peripheral and systemic administration.

Neurogenesis is the 'birth' of new neuronal cells and is essential for synaptic plasticity and formation of memory (2). Neuronal growth decreases with age, primarily due to decreased production of cGMP (39). The use of SC, which increases cGMP levels, may therefore be valuable for the improvement of brain function.

Multiple sclerosis is a potentially debilitating inflammatory disease of the central nervous system (2). SC has been shown to protect multiple sclerosis patients from neuro-degeneration through increased gray matter perfusion in the brain (2).

1.4.5 Dose

An appropriate dose of SC for paediatric patients has not yet been defined. A suitable paediatric dose for oral administration has however been determined by extrapolation from the adult dose range (3). A dose range of 0.5–2 mg/kg every 6 hours was initially considered appropriate but it was noted that, although there was an improvement in the clinical status of the patient, the effect was not found to be dose-dependent. The major limitation of this study was that only three patients were used and therefore a definitive conclusion for appropriate neonate dosing was not achieved.

A slightly larger study, using a sample size of 14, investigated the effect of SC in children suffering from pulmonary arterial hypertension. In this case an initial oral SC dose of 0.25 mg/kg every six hours (6) was administered to all patients. The dose, which was increased to 0.5mg/kg every six hours, was well tolerated (6).

In an intravenous dose-response study, SC was administered to piglets in increasing doses of 0.4, 1 and 3mg/kg. SC caused a decrease in the mean pulmonary artery pressure and a reduction in the pulmonary vasculature by 30%. This effect was achieved at the lowest dose level with no further change being noted following administration of higher doses (40).

Based on case reports and studies conducted on paediatric patients, the optimal initial dose to administer is 0.25–0.5mg/kg every 4–8 hours, together with a dose trituration of 1–2 mg/kg every six hours, if necessary (34).

1.4.6 Overdose

There is limited information concerning overdose of SC, particularly in paediatric patients.

In one case report a 19-month male weighing 10.4 kilograms ingested six 50 mg SC tablets. The patient presented with facial flushing and priapism. The patient also experienced mild tachycardia for approximately 24 hours post-ingestion. No hypotension was observed and the patient was discharged the following day having been treated with maintenance IV fluids only (41).

In another case report a two year old male paediatric patient ingested 75mg SC. The patient presented with facial flushing, priapism and diarrhoea. No cardiovascular effects were noted but the patient did complain of pain in the penile region, one day after treatment (42).

1.4.7 Adverse drug reactions of SC in paediatric patients

Typical adverse reactions of SC in adult patients include flushing, headache and dyspepsia (38). Studies conducted in neonates and children with pulmonary hypertension suggest however that there is a low incidence of adverse effects and that those that have been reported are considered to be of minor importance (7).

In a limited study, designed to evaluate the safety of oral SC in children, results showed that no adverse reactions were observed following administration of the compound (3).

Despite reports suggesting a low incidence of adverse reactions following administration of SC to paediatric patients, there are special precautions that need to taken into account before administering SC. Intravenous SC has been shown to significantly reduce the systemic blood pressure (43; 44). This can be minimised with the concomitant administration of plasma volume expanders (45). This decrease in blood pressure was not however significant in all paediatric patients and no further

treatment was required (6). Another important consideration is that SC can cause deterioration in arterial oxygenation and an increase in the alveolar arterial gradient. This is most likely due to the increase in intrapulmonary shunting (13; 43). In premature babies, retinopathy of prematurity (ROP) may be of concern; however, only one case of SC-induced ROP has been reported (46).

1.4.8. Contraindications

SC is well tolerated and does not alter the biochemical and physiological effects of most other drugs (47). The use of organic nitrates is however contraindicated with SC use, as sildenafil enhances the vasodilatory effects of the nitrates, which can lead to excessive vasodilation followed by a significant decrease of blood pressure (34; 47).

1.4.9. Drug Interactions

SC metabolism is primarily via the CYP 3A4 system and, to a slightly lesser extent, via the CYP 2C9 system. Consequently, any inhibitor or inducer of these enzyme systems may alter the clearance of SC (5). Protease inhibitors such as indinavir, saquinavir and ritonavir are CYP3A4 inhibitors. When co-administered with SC, the plasma levels of these drugs were not affected, yet the level of SC was significantly increased (48; 49). Similar results were observed when erythromycin was co-administered with SC but the same response was not observed when azithromycin was used concomitantly (50).

Any drug that acts as an inducer of CYP 3A4 and CYP 2C9 is also likely to have an effect on the disposition of SC. Bosentan, an endothelin receptor antagonist used for the treatment of pulmonary arterial hypertension, induces the activity of CYP 3A4; co-administration of bosentan with SC resulted in an increase in bosentan levels, while SC levels decreased (51).

1.5 PHARMACOKINETICS

1.5.1. Absorption

SC is rapidly absorbed from the gastrointestinal tract (GIT) (3). Despite rapid absorption, SC exhibits a low bioavailability of approximately 40%, with maximum observed plasma concentrations being reached within one hour of administration (3). Absorption studies using ^{14}C labelled SC revealed that approximately 92% of orally administered SC is absorbed, suggesting that the relatively low oral bioavailability of SC is not due to incomplete absorption, but rather extensive hepatic first-pass metabolism (52).

A study investigating the pharmacokinetics of SC in hemodynamically challenged neonate patients showed that levels of SC 30 minutes after administration via a naso-gastric tube were unpredictable. Results showed that SC levels were undetectable in half of the children (53). Possible explanations for these finding included general anaesthesia, opiate analgesia and transient neuromuscular blockade, all of which have the ability to modify gastric emptying and therefore the absorption from the GIT (53).

1.5.2. Distribution

SC and its major active metabolite, N-desmethyl sildenafil, are approximately 96% protein bound. Such binding is independent of the concentration of the drug in the plasma. The volume of distribution of SC at steady state is 105 L (54), which is far greater than the total volume of body water (approximately 42L), indicating that SC is distributed into different tissues (52). A population pharmacokinetic analysis, conducted following oral administration of SC to patients with ED, revealed a volume of distribution of approximately 310L for SC (55).

1.5.3. Metabolism

SC is predominantly metabolized by the cytochrome P-450 isozyme CYP3A4 and to a lesser degree by CYP2C9 (56). At least 16 different metabolites of SC have been identified, with the primary routes of metabolism being N-desmethylation, oxidation, and aliphatic hydroxylation (57). The major metabolite is N-desmethyl sildenafil (UK-103, 320), which has been shown to have 50% of the activity of SC (3; 57). N-desmethyl sildenafil makes up 40% of the serum concentration and is therefore responsible for 20% of the pharmacological activity of SC (5).

The second most important metabolite is UK-150 564, which is the product of a 2-carbon fragment loss from the piperazine ring of SC (52). This metabolite constitutes 25% of the serum concentration

and has 10% of the pharmacological activity of SC (52). Therefore, UK-150 564 does not contribute significantly to the inhibition of PDE type 5 enzymes.

1.5.4. Elimination

Approximately 80% of an oral dose of SC is excreted as metabolites, in the faeces and 13% in the urine (57). The systemic clearance of SC was 41L/hr, following intravenous administration to healthy volunteers (58). SC can therefore be regarded as having an intermediary hepatic extraction ratio of approximately 50% (52). Plasma concentrations of SC and N-desmethyl sildenafil are reported to decline bi-exponentially with a mean terminal half-life of 3–5 hours (58). Sildenafil is primarily eliminated via the hepatic route. Neonates with hepatic dysfunction may therefore exhibit reduced SC clearance and the administered dose should be adjusted accordingly (36).

1.6 CONCLUSION

SC is a versatile molecule that has been approved for the treatment of ED and PH, with current research evaluating its use for the treatment of pain, neurogenesis and multiple sclerosis (2). Although SC has not yet been approved for the treatment of PH in paediatric patients, it is fast becoming the treatment of choice for neonates and infants that exhibit the condition.

SC is readily available and well tolerated in paediatric patients and is cheaper than other treatment options, making it a favourable choice in developing countries (37). The use of SC is only contraindicated when administered together with organic nitrates, due to potential synergistic hypotensive effects that the combination could produce.

SC has an excellent side-effect profile and the drug is generally well tolerated in patients. These data have however been generated from small studies or single reports and larger clinical trials are therefore required to confirm the safety and efficacy of SC in paediatric patients. The challenge of administering SC to paediatric patients is a result of no appropriate dosage form having been registered for use in this population group. Thus, SC tablets are typically crushed, dissolved in water, and administered either orally or via a nasogastric tube. It is therefore vital that further development on the formulation of SC be carried out, to develop an age-appropriate dosage form, which will ensure that the patient receives the correct dose of SC in a safe and efficacious manner. The objective of this project, therefore, is to formulate SC as an orodispersible tablet, which allows for easy administration to paediatric patients.

CHAPTER TWO
DEVELOPMENT AND VALIDATION OF AN HPLC METHOD FOR THE DETERMINATION OF SILDENAFIL CITRATE

2.1 INTRODUCTION

Chromatography is defined as the separation of mixtures by distribution between two or more immiscible phases (59). The term "chromatography", which was first described in 1901 by the Russian botanist Mikhail Tsweet, is translated from Greek to "colour writing"(60). Chromatography involves the use of an adsorbent or stationary phase e.g. silica packed into a column and eluted with a suitable liquid or mobile phase. The sample mixture, from which the components are intended to be separated, is then introduced onto the top of the column.

A component of the mixture (solute) that is adsorbed weakly by the stationary phase will move through the column faster than other solutes that are more strongly adsorbed.

Liquid chromatography therefore allows for solutes to be separated based on differences in their polarities.

High Performance Liquid Chromatography (HPLC) is an analytical technique that was developed in the 1960's. As with all chromatographic techniques, its function is the separation of different chemical species from each other. The primary advantage of HPLC is its ability to fulfil this function with speed, sensitivity and precision (61).

2.1.1 Overview of HPLC

The main advantage of selecting liquid chromatography as a preferred analytical method is the fact that a number of different mechanisms to achieve a chromatographic separation, exist.
These separations can be classified into separate categories *viz*., normal phase (NP), reversed-phase (RP), ion-exchange (IE) and size exclusion chromatography (EC).

2.1.1.1 Normal-Phase (NP) Chromatography

In NP chromatography , which is used to separate polar compounds (62), the stationary phase is more polar than the mobile phase (63), with the mobile phase being typically comprised of a mixture of organic solvents, with the exclusion of water. The organic solvents in the mobile phase are non-polar

and include solvents such as hexane or benzene. The column packing in NP chromatography is usually an inorganic adsorbent for example silica.

Alternatively NP chromatography can also be used for the separation of polar solutes that are generally poorly retained in Reversed-Phase chromatography (64).

2.1.1.2 Reversed-Phase (RP) Chromatography

RP chromatography is a popular choice for HPLC analysis for the following reasons:

i. it allows for the separation of samples in which components have a wide range of polarities.
ii. bonded non-polar stationary phases are relatively stable and tend to be more robust and reproducible than for other HPLC modes.
iii. many biochemical samples are aqueous solutions that can be injected directly onto the column without the need for sample preparation by extraction (65).

In RP chromatography the stationary phase is non-polar in nature and in most cases silica base is covered with chemically-bonded *n*-alkane chains or other groups such as $C_{18}H_{37}$ (octadecyl) , C_8H_{17} (octyl) or C_6H_5 (phenyl) phases (66).

The mobile phases used in RP chromatography are usually extremely polar and normally comprised of HPLC grade water in combination with methanol (MeOH), acetonitrile (ACN) or isopropanol; this facilitates elution of polar rather than non-polar analytes.

2.1.1.3 Ion-Exchange HPLC

Ion-exchange chromatography involves the substitution of one ionic species located on the stationary phase for another ion in the sample (62). The stationary phase is either acidic, often containing carbonic or sulphonic acid groups that act as cation exchangers, or alternatively, it may be comprised of a basic stationary phase composed of amine groups that facilitate anion exchange. In the case of anionic exchange, the surface carries a net positive charge, designated as R^+. If a mobile phase (in which anions are present) is used the exchange site will attract and hold a negative counter-ion or Y^-. Anions in the sample to be analyzed, X^-, can then be exchanged with the counter-ion, Y^- as the mobile phase and sample to be analyzed flows through the column (62).

In contrast the exchange of cations can occur where the surface of the matrix carries a net negative charge, R^- and the counter-ions are cations, Y^+ that will be exchanged for cations, X^+, in the sample matrix (67).

Anion and cation exchanges are represented by Equation 2.1 and Equation 2.2 respectively.

$$R^+Y^- + X^- \rightleftharpoons R^+X^- + Y^- \qquad \textbf{Equation 2.1}$$

$$R^-Y^+ + X^+ \rightleftharpoons R^-X^+ + Y^+ \qquad \textbf{Equation 2.2}$$

2.1.1.4 Size Exclusion Chromatography

Exclusion chromatography is a chromatographic technique in which the separations that occur are due to differences in the molecular weights of the solutes that are to be separated (68). Solutes with larger molecular weights normally elute at an early stage, as the stationary phase is usually a material with small pore widths. Analytes of smaller dimension will have a larger space available for transport through the column than the larger molecules that are first eluted (66; 69).

Exclusion chromatography was initially used to fractionate naturally occurring polymers in aqueous solution but has more recently been applied to the analysis of synthetic polymers (70).

2.2 REVIEW OF HPLC METHODS USED FOR THE ANALYSIS OF SC

Prior to the development of an HPLC method for the analysis of SC, a review of the literature was undertaken. The relevant aspects of the HPLC methods published for the analysis of SC is summarized in Table 2.1.

The analysis of SC in human plasma, pharmaceutical dosage forms and raw material has been typically achieved by the use of octadecyl or C_{18} reversed-phase columns and UV detection has been the preferred choice for analysis (25; 71-76).

The organic modifier of choice is ACN. This is due the fact that ACN exhibits favourable UV transmittance at low wavelengths, is polar, and is less viscous than methanol (67). The organic modifier content is generally high, with the %ACN v/v ranging from 45-70% v/v.

All methods described in the literature review have run times of less than 10 minutes as well as many of the methods reporting the successful use of an internal standard (25; 71; 72; 77).

Table 2.1 Summary of analytical methods developed for the analysis of SC in different matrices

Stationary Phase	Sample Matrix	Mobile Phase	Flow Rate (ml/min)	Detection	Retention Time (min)	Internal Standard	Reference
RP-C_{18} (Waters) 5µm, 3.9 i.d. x300mm	Raw Material and Dosage Forms	MeOH:water:ACN (60:20:20, v/v/v) adjusted with 0.1% glacial acetic acid	0.5	UV, 290nm	4.2	Cinnarizine	(71)
Phenomenex® Luna C18 5µm, 150x4.6mm i.d.	Human Plasma	ACN: water (45:55, v/v)	1	UV, 220nm	7.2	Phenobarbital Sodium	(72)
Nucleosil® C_{18}® 5µm, 50x4.6mm i.d.	Human Plasma	MeOH:10mM ammonium acetate pH7.0 (85:15, v/v)	0.7	--	1.63	Diazepam	(25)
ChromLith® C_{18} (Merk) 5µm, 100x4.6mm i.d.	Dosage Forms	ACN: water (60:40, v/v)	2	UV, 292nm	0.69	--	(73)
Inertsil® C_{18}, 5µm, 150x4.6mm i.d.	Dosage Forms	ACN: Phosphate Buffer (70:30, v/v, pH 7.0)	0.8	UV, 228nm	4.087	--	(74)
RP-C_{18}, 5µm, 250x4.6mm i.d.	Dosage Forms	ACN: Phosphate Buffer (60:40, v/v)	1	UV, 225nm	6.083	--	(75)
RP-C_{18}, 5µm, 250x4.6mm i.d.	Raw Material	ACN:0.05M Phosphate Buffer (70:30, v/v)	1	UV, 230nm	5.29	--	(76)
LiChrospher® C_{18}, 5µm, 250x4.6mm i.d.	Raw Material and Dosage Forms	ACN: water (52:48, v/v)	1	UV, 245nm	7.17	Piroxicam	(77)

2.3 EXPERIMENTAL

2.3.1 Materials and reagents

SC was purchased from MTT Pharma & Bio-technology Co., Ltd (Shanghai, China) and diazepam was purchased from Sigma Aldrich (Missouri, United States of America).

ACN (UV cutoff 200nm) and MeOH (UV cutoff 215nm) were purchased from Romnil Ltd (Waterbeach, United Kingdom). Sodium hydroxide pellets and potassium dihydrogen orthophosphate were purchased from Merck Chemicals Ltd (Gauteng, South Africa). Hydrogen peroxide 30% v/v was purchased from a local pharmacy.

HPLC grade water for mobile phase and sample preparation was purified using a Milli-RO®15 water purification system (Millipore Co., Massachusetts, United States of America) that consisted of a Super C® carbon cartridge, two ion-X® exchange cartridges and an Organex-Q® cartridge. Prior to use, the water was filtered through a 0.22µm Millipak® filter (Millipore Co., Milford, Massachusetts, United States of America).

2.3.2 HPLC system

The modular HPLC system consisted of a Model P100 dual piston solvent delivery module (Thermo Separation Products, San Jose, California, USA), a Model AS100 autosampler (Thermo Separation Products, San Jose, California, USA) equipped with a Rheodyne® Model 7010 injector (Rheodyne, Reno, Nevada, USA) with a fixed volume 20 µl loop and a Gastight® 250 µl Model 1725 syringe (Hamilton Co., Reno, Nevada, United States of America).

A Linear Model 6200-9060 UV/VIS-500 detectors (Linear Instrument Co., Irvine, California, USA) was used for detection and data collection was achieved using a Spectra Physics SP 4600 integrator (Thermo Separation Products, San Jose, California, USA). Separation was achieved on a Phenomenex® Luna C_{18} (2) 5µm, 150 x 4.6mm i.d. maintained at 22 ± 0.5 °C

2.3.2.1 Column Selection

The stationary phase or column can be described as the "heart" of an HPLC separation as the column is the location where the separation occurs .It is essential that, for the development of a reproducible and robust analytical method, an appropriate stationary phase is selected for use (67).

The selection of a suitable column for RP-HPLC analysis is dependent on the physico-chemical properties of the analyte of interest as these determine the magnitude of the interactive forces between the analyte and the stationary phase during the separation process (67; 70).

It is clear that all stationary phases reported for HPLC analysis of SC are most likely to be C_{18} based, as they are stable and due to the fact that they are hydrophobic. Consequently they are versatile and can be applied to the analysis of a broad range of molecules (65; 78).

2.3.2.2 Internal Standard

The precision of an HPLC method is dependent on a number of factors, including the accuracy with which samples are prepared, instrumental precision and method robustness (79). A common approach used to ensure the accuracy of a method is the inclusion and use of an internal standard (IS) (59).

An IS must be:

i. well resolved from the compound of interest and any other peaks that may appear on the chromatogram.
ii. of similar retention to that of the compound of interest.
iii. not be present in the original sample.
iv. similar and mimic the analyte in sample preparation steps.
v. stable and unreactive with the compound of interest as well as with the mobile phase (67).

The peak heights or areas are responses that are measured in quantitative chromatography. Equation 2.3 depicts by way of example a proportional relationship between peak height and a factor such as injection volume. By use of an IS, errors due to column changes can be negated (79).

$$H = \left(\sqrt{\frac{N}{2\tau}}\right)\frac{C\ Vinj}{t\circ(1+k\,)F} \qquad \textbf{Equation 2.3}$$

Where,

H = peak height
$t_{o\,=}$ the dead column time
τ = the injection time determined by the ratio of the volume of injection (V_{inj})
F = flow rate
C° = the solute concentration
N = column efficiency
k' = the retention factor.

If an IS is used, one can calculate a ratio of the height of the peak of interest and that of the IS peak (79) using Equation 2.4.

$$PHR = \frac{Hj}{Hi} = \frac{C\ j}{c\ i}\left(\frac{1+k\,i}{1+k\,j}\right) \qquad \textbf{Equation 2.4}$$

Where,

H_j = peak height of solute of interest
H_i = peak height of internal standard
$C°_j$ = concentration of solute of interest
$C°_i$ = concentration of internal standard
k'_j = retention factor of solute of interest
k'_i = retention factor of internal standard

As defined in Equation 2.4, the PHR is the quotient of concentration of solute and the IS corrected by the retention factor. The use of the IS technique for the calculation of PHR eliminates the possibility of a change in flow rate or injection volume affecting the separation, and therefore produces more accurate and reproducible data.

Diazepam (DZ) was selected as the internal standard as it does not elute at a retention time similar to that of SC, is readily available and has been successfully applied to methods for the quantitation of SC (25).

2.3.2.3 Preparation of Stock Solutions

Stock solutions of SC (100µg/ml) and IS (100µg/ml) were prepared by accurately weighing approximately 10 mg of each compound using a Model AG135 Mettler Toledo (Mettler Instruments, Zurich, Switzerland) top-loading analytical balance and quantitatively transferred into separate 100ml A-grade volumetric flasks. The compounds were dissolved in an ACN: water mixture in a ratio of 55:45 and sonicated for two minutes using a Model B-12 Ultrasonic bath (Branson Cleaning Equipment Co., Shelton, Connecticut, United States of America). Aliquots of 20, 30, 70, 120, 150, 170 and 200µl of SC stock solution were transferred to separate 10 ml A-grade volumetric flasks in which 25µl of the IS solution had been added. The resultant solutions were diluted serially, using a 55:45 ACN: water mixture to produce solutions that had concentrations of SC of 0.2, 0.3, 0.7, 1.2, 1.5, 1.7 and 2µg/ml and that for the IS of 0.25µg/ml.

2.3.2.4 Selection of mobile phase

The mobile phase was selected by varying the concentration of ACN used in the method and determining the time that was taken for the peak to appear on the chromatogram. A suitable time required was a time that did not exceed 10 minutes but a time after three minutes after injection of the sample so as not to interfere with the solvent front.

2.3.2.5 Preparation of mobile phase

The mobile phase was comprised of ACN: water in a ratio of 55:45 % v/v. Prior to use the mobile phase was degassed under a vacuum using a Model A-25 Eyela Aspirator (Tokyo Rikakikai Co., Tokyo, Japan) and filtered through a 0.45µm Durapore ® HVLP membrane filter (Millipore Co., Milford, Massachusetts, United States of America).

2.4 RESULTS AND DISCUSSION

2.4.1 Effect of Organic Solvent Composition

The initial mobile phase composition, selected for evaluation was ACN: water in a ratio of 45:55 v/v, which was based on literature data (72). As the content of the organic solvent is increased there was a decrease in the retention times of both SC and the IS (Figure 2.1). At lower concentrations of ACN there was need for a longer analytical run time; this problem was resolved by increasing the amount of ACN in the mobile phase. Shortening the analytical run time means that more samples can be analysed in a specified time period, thus increasing the efficiency of the method.

The shorter retention time (R_t) for both analytes is due to the fact that an increase in the % v/v of ACN in the mobile phase decreases the polarity of the mobile phase, therefore leading to preferential partitioning of the compound into this phase as opposed to interacting with the stationary phase. The organic solvent competes with the bonded phase molecules to attract the analyte and in general the higher the proportion of organic modifier in a mobile phase, the shorter the R_t of the analyte(s) of interest (67).

A mobile phase composition of 45 % v/v ACN produced a separation for which the retention times for SC and the IS were 6 and 12.8 minutes, respectively. This was not deemed suitable as methods with run times of < 10 minutes are considered appropriate for use in our laboratory. When the % v/v ACN was > 60% the retention time for SC was short and the peak was not clearly resolved from the solvent front. Following a series of experiments, a mobile phase of ACN: water in a ratio of 55:45% v/v was selected for use; the R_t of SC and the IS were 3.6 and 6 min, respectively.

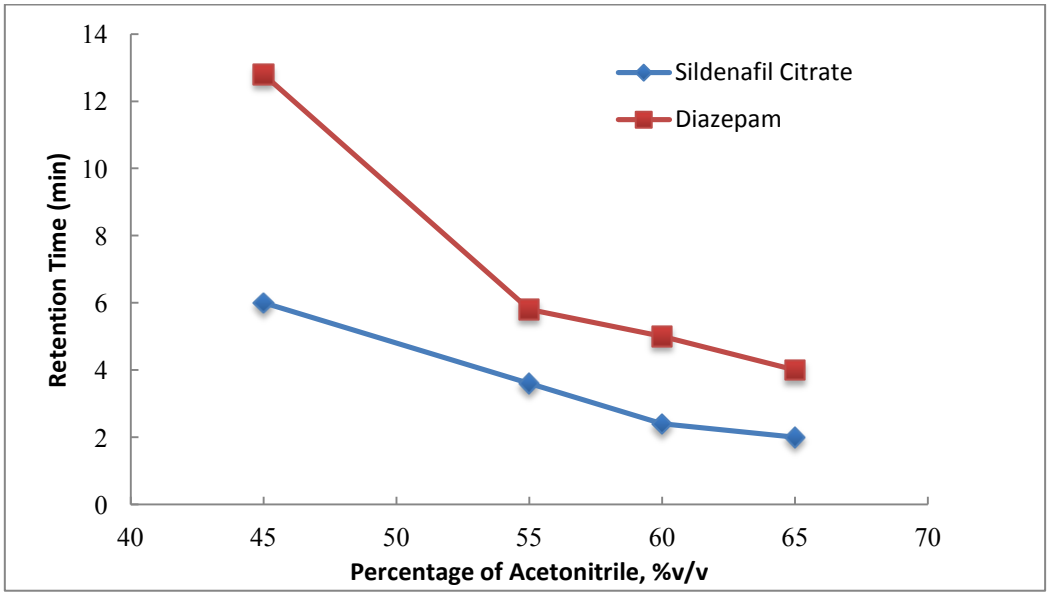


Figure 2.1 The effect of organic solvent content on the R_t time of SC and the IS

2.4.2 Effect of Flow Rate

As expected, an increase in flow rate resulted in a shorter R_t for SC (Figure 2.2). Obviously as the rate of movement of sample increases, it's retention time in the column decreases.

The primary disadvantage of using an elevated flow rate is that the back pressure of the pump is increased which in turn may result in a shorter column life and increased wear and tear on the HPLC system. Furthermore the use of increased flow rates result in increased mobile phase consumption which is, in itself, not cost effective.

A flow rate of 1ml/min was selected for use as this provides sufficient operating pressure as well as suitable R_t with both peaks of interest eluting in less than 10 minutes. Increasing the flow rates above 1 ml/min did not appear to further reduce the R_t to any significant extent.

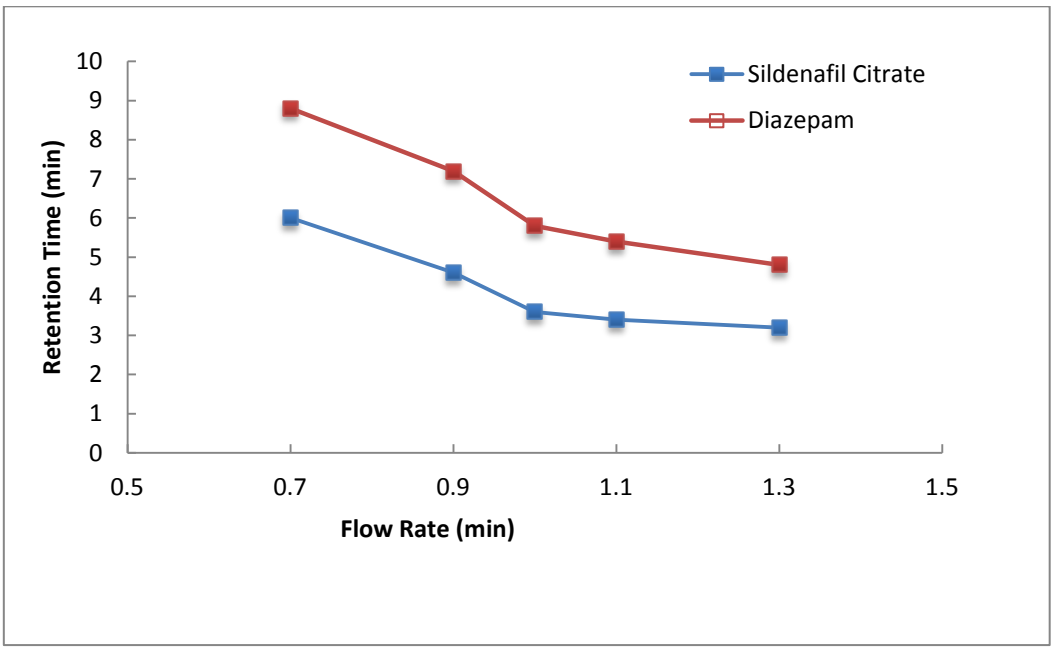


Figure 2.2 The effect of flow rate on the R_t of SC and the IS

2.4.3 Chromatographic Conditions

The final chromatographic conditions selected for use for the analysis of SC are summarised in Table 2.2.

As can be seen from Figure 2.3, the resulting chromatogram shows a clear separation of SC from the IS as well as the solvent front. The SC peak also showed minimal tailing thus ensuring accurate results for measurement of peak height or area were obtained.

Table 2.2 Chromatographic conditions for the analysis of SC.

Column	Phenomenex ®Luna C_{18} (2) 5µm, 150 x4.6mm
Flow rate	1ml/min
Injection volume	20µL
Detection	245nm
Temperature	22°C
Mobile phase	ACN: Water (55:45) %v/v
Operating back pressure	925 psi
Detector sensitivity	0.005 AUFS
Chart speed	2.5mm/min

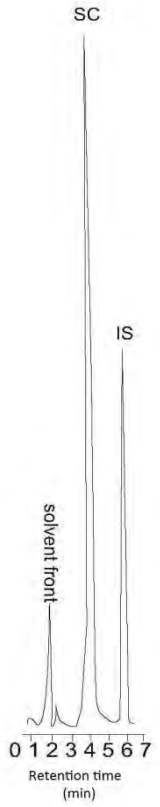


Figure 2.3 Typical chromatogram showing the separation of SC (2 µg/ml) and IS (0.25 µg/ml)

2.5 METHOD VALIDATION

2.5.1 Introduction

The validation on an HPLC method incorporates the following requirements: confirmation of the process by means of experimental studies, and ensuring that the performance of the analytical method meets the accuracy and precision standards required to achieve an acceptable uncertainty limit (80).

The importance of validating the proposed HPLC method is to ensure the reliability and reproducibility of results generated when using that method, even when carried out by different operators using the same analytical equipment.

Although an important aspect of the validation process is to ensure the acceptability of the method that has been developed, it is also to investigate the method thoroughly to determine the limits of allowed variability for the conditions to achieve a desired outcome during analysis (59; 67).

2.5.2 Linearity and Range

The linearity of an analytical method describes the ability of that method to produce results that are directly proportional to the concentration of an API in a series of calibration samples (81). Linearity experiments are typically performed over a wide concentration range, as this approach instils confidence in the accuracy of results (82). The range that is selected should however not be unrealistically broad, as this may result in the rejection of a method that only exhibits linearity over a narrow range of concentrations (81).

The acceptability of linearity data is usually based on an investigation of the correlation coefficient (R^2) for a calibration curve with an R^2 value of > 0.99 generally considered as evidence of an acceptable fit of the regression line to experimental derived data (64).

The linearity of the method was established by plotting the average peak height ratios (PHR) of SC and the IS versus the known concentration of calibration samples. These data are summarized in Table 2.3.

Table 2.3 Linearity and precision data for the analysis of SC

Concentration µg/ml (n=5)	Average Peak Height Ratio	% RSD
0.2	0.22 ± 0.003	1.33
0.3	0.33 ± 0.005	1.54
0.7	0.73 ± 0.018	2.42
1.2	1.20 ± 0.007	0.57
1.5	1.49 ± 0.015	1.01
1.7	1.69 ± 0.018	1.07
2	2.04 ± 0.013	0.63

The resultant calibration curve is depicted in Figure 2.4 and the equation for the line was $y = 1.0161x+0.0298$ with an R^2 value of 0.9992 indicating that the method was linear over the concentration range 0.2 – 2 µg/ml.

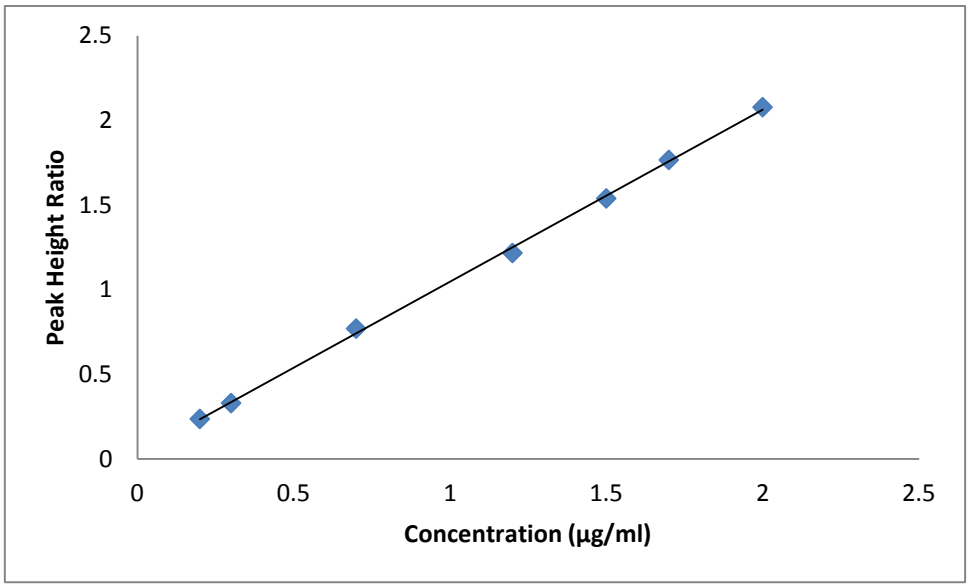


Figure 2.4 Calibration Curve for SC

2.5.3 Precision

Precision is defined as "the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of an homogenous sample" (67). This indicates the degree of random error with an analytical method (83).

The International Conference on Harmonization (ICH) (84) defines three (3) different classes of precision viz., intra-assay precision or repeatability, intermediate precision and reproducibility or robustness (84).

Precision is expressed by the relative standard deviation (RSD) of a set of data. Therefore if a set of *n* measurements is performed on a sample, the average value obtained is defined using Equation 2.5.

$$x = \sum_{i=1}^{n} xi/n$$

Where,

x_i = individual measurements,
n = number of measurements.

The standard deviation can then be calculated using Equation 2.6.

$$SD = \frac{\sqrt{\sum_{i=1}^{n}(xi - x)^2}}{n - 1}$$

The %RSD can then be calculated using Equation 2.7.

$$\%RSD = \frac{SD\ x\ 100}{Mean} \qquad \textbf{Equation 2.7}$$

In these studies the limit for %RSD was set at 5%.

2.5.3.1 Intra-Day Precision or Repeatability

Repeatability is an indication of the precision of a method used under the same operating conditions over a short period of time (85). A minimum of nine determinations covering the specified concentration range are required for repeatability studies (67; 85).

Repeatability was measured by injecting samples (n=5) to obtain an average peak height ratio and calculating the %RSD for each concentration. Samples were selected so as to cover low, medium and high concentrations of the calibration range. The results are summarized in Table 2.4.

Table 2.4 Intra-Day precision data for SC

Concentration (µg/ml)	Peak Height Ratio (n=5)	Standard Deviation	%RSD
0.25	0.270	0.001	0.476
1	1.100	0.007	0.652
1.9	1.933	0.030	1.569

As can be seen the results summarized in Table 2.4, all tests had RSD values of less than 2% indicating that the proposed HPLC method can be accurately repeated.

2.5.3.2 Inter-Day Precision or Intermediate Precision

The intermediate precision of a method expresses its precision under different laboratory conditions. These conditions may include analysis on different days, use of the method by different analysts, or use of different instruments. The intermediate precision of this method was assessed by injecting samples (n=5) to determine the average PHR and %RSD on three consecutive days.

Table 2.5 Inter-day precision data for SC

Day	Concentration (µg/ml)	Mean concentration determined (µg/ml)	%RSD
1	0.25	0.252±0.017	1.569
	1.00	1.08±0.012	0.652
	1.90	1.91±0.010	0.476
2	0.25	0.238±0.031	3.533
	1.00	1.033±0.016	1.556
	1.90	1.93±0.037	0.235
3	0.25	0.241±0.017	3.825
	1.00	1.022±0.023	0.439
	1.90	1.969±0.016	2.154

Table 2.5 summarizes the inter-day precision results generated using the proposed HPLC method. As can be seen from the results, the calculated %RSD never exceeds the set laboratory limit of 5% indicating that the method is able to produce precise results over a long period of time.

2.5.3.3. Reproducibility

The reproducibility of a method relates to the precision of the proposed method when used in different laboratories. This is necessary when standardization of the method is required for collaborative research studies. For the purposes of this research, reproducibility studies were not performed as the proposed method was only intended for use in the same laboratory, performed by the same analyst using the same analytical system.

2.5.4 Accuracy

The ICH guidelines define the accuracy of an analytical method as the closeness between the values accepted as 'true values' and experimentally-derived values for that sample (86). Accuracy may be reported as the percentage recovery from samples analyzed using the proposed analytical method. Percentage Bias can also be used to determine the extent of deviation of a result for a sample from the true value for that particular sample. The closer the percentage recovery from analyzed samples is to 100%, and the lower the value for % Bias, the more accurate the analytical method is considered.

Accuracy was assessed at three levels, *viz.* high, medium and low sample concentrations to ensure accuracy over the range of concentrations under investigation. A tolerance of 5 % was set for the % RSD for accuracy and the limit for % Bias was set at < 5%.

All % RSD values were < 2% which complies with the limits set in many pharmaceutical industries, indicating that the method was accurate (87). Results for accuracy studies are summarized in Table 2.6.

Table 2.6 Accuracy data for SC

Theoretical Concentration µg/ml	Actual Concentration µg/ml	%RSD	%Bias
0.25	0.24	0.48	-4.53
1.0	1.02	0.65	2.43
1.9	1.81	1.57	-4.91

2.5.5 Specificity

The specificity of an analytical method is defined as its capacity to accurately measure the concentration of an analyte in a sample, in the presence of impurities or pharmaceutical excipients (88).

Specificity studies are a crucial aspect of an HPLC method: if a method lacks the capacity to produce sample peaks that are clearly resolved from interference(s) from other materials, the accuracy of the method is likely to compromise the accuracy of the overall results.

Specificity tests were performed by comparison of chromatograms developed from the analysis of a standard solution of SC and the IS and results based on the analysis of commercially available SC tablets.

The peaks of interest were adequately resolved, as shown in Figure 2.5, and it was noted that there were no other peaks present in the chromatogram suggesting that the proposed method can be considered specific for the analysis of SC.

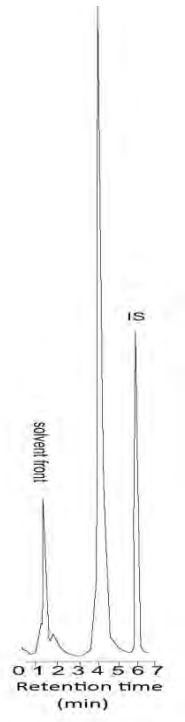


Figure 2.5 Selectivity of the chromatogram peaks

2.5.6 Limits of Quantitation (LOQ) and Detection (LOD)

The LOQ of an analytical method is the lowest concentration of an analyte that can be measured with acceptable accuracy and precision (64). LOD is defined by the ICH guidelines as the lowest amount of analyte that can be detected, which cannot however be quantified to an exact value (89).

Paino and Moore (90) described four approaches for establishing the LOQ and LOD of an analytical method. These include:

i. determining the lowest concentration for which the % RSD ≤ 5%,
ii. plotting the standard deviation versus concentration,
iii. establishing a confidence interval for the best-fit line, or
iv. use of signal to noise ratio approaches.

The United States Pharmacopoeia (USP) recommends the use of signal to noise ratio assessments to establish the LOQ and LOD associated with a particular method. Signal to noise ratio methods compare the signals obtained from samples of low and known concentration to those of blank solutions (80). Signal to noise ratios of 10:1 and 3:1 are considered appropriate for establishing the LOQ and LOD of a method, respectively (80; 81).

This technique is difficult to reproduce as it relies on the response from a specific detector and different detectors produce diverse signal to noise ratios. Thus for the purposes of this research the approach of using the lowest concentration for which the %RSD was < 5% was used. Samples of known and low concentration were injected (n=5) and the lowest concentration that produced precision data in which the %RSD was <5% was selected as the LOQ. The LOD was then calculated as the concentration at one third that that was considered to be the LOQ (90).

Using the above methods, the LOQ and LOD values were established as 0.2µg/ml and 0.06µg/ml, respectively.

2.5.7 Forced Degradation Studies

Forced degradation studies are generally performed to demonstrate the specificity of an analytical method when developing a stability-indicating HPLC assay. These studies provide information on the possible degradation pathways as well as degradation products that could be formed following the storage of dosage forms containing the analyte of interest (91).

Forced degradation studies are performed under different conditions including, hydrolytic, photolytic, acidic, basic and heat environment and at least 10% of the analyte should degrade to ensure method optimization is achieved (92).

2.5.7.1 Methods

Typical chromatograms from freshly prepared SC samples were compared to chromatograms developed following degradation studies. The ICH guidelines state that the analysis of degradation products should be quantitatively and qualitatively analyzed, however for the purposes of this research, the peaks were only assessed qualitatively.

2.5.7.1.1 Sample Preparation

Approximately 10 mg of SC was accurately weighed and transferred into a 10ml A-grade volumetric flask. With the exception of the samples subjected to oxidative degradation studies, SC was dissolved and made up to volume in a medium specific for that degradation study, to yield solutions of approximately 1 mg/ml.

2.5.7.1.1.1 Oxidative Degradation

SC is reportedly susceptible to oxidative degradation (74). Therefore samples were prepared by weighing and transferring approximately 10 mg SC into an A-grade volumetric flask and dissolving the sample in mobile phase. A 2 ml aliquot of 30% v/v H_2O_2 was added to the sample and the mixture was refluxed for 10 hrs at 50 ± 0.5°C. Aliquots (20µL) were harvested hourly and transferred into a volumetric flask followed by the addition of 13 µL of IS solution prepared as described in § 2.3.2.3 and the samples were made up to volume with mobile phase prior to analysis was added.

2.5.7.1.1.2 Alkali Degradation

A 0.1M NaOH solution was prepared by weighing 0.4g NaOH pellets that were transferred to an A-grade 100ml volumetric flask and dissolved in distilled water. The solution was sonicated for 5 minutes to ensure that all pellets had dissolved and then made up to volume with distilled water. Approximately 10mg of SC was accurately weighed and transferred into a 10 ml A-grade volumetric flask and made up to volume with 0.1M NaOH. The sample was refluxed for 10 hrs at 50 ± 0.5°C. Aliquots (20µL) were harvested hourly and transferred to a volumetric flask after which 13µL of the IS solution prepared as described in§ 2.3.2.3 and then made up to volume with mobile phase prior to analysis. .

2.5.7.1.1.3 Acidic Degradation

A 0.1M HCl solution was prepared by transferring 833µl HCl into an A-grade volumetric flask and diluting and making up to volume with distilled water. Approximately 10 mg of SC was weighed transferred to an A-grade 10ml volumetric flask and made up to volume with 0.1M HCl. The sample was refluxed for 10hrs at 50±0.5°C. Sample aliquots were collected both prior to refluxing and every hour during the refluxing process. The aliquots were transferred to a volumetric flask and 13 µL of the IS was added as described in §2.3.2.3. The samples were made up to volume with a solution of acetonitrile and water (55:45).

2.5.7.1.1.4 Photolytic Degradation

Approximately 10 mg of SC was weighed and transferred to an A-grade 10ml volumetric flask. The sample was made up to volume with water and exposed to sunlight for 10 hours. Sample aliquots (20µL) were taken both prior to and hourly during exposure and diluted with 13µL of IS as described in §2.3.2.3 and made up to volume with a solution of acetonitrile and water (55:45).

2.5.7.1.1.5 Heat Degradation

Approximately 10mg of SC was weighed and transferred to an A-grade volumetric flask. The sample was made up to volume with water and heated to 50±0.5°C under reflux conditions for 10hrs. Sample aliquots (20µL) were taken both prior to and during exposure and diluted with 13µL of IS as described in §2.3.2.3 and made up to volume with a solution of acetonitrile and water (55:45). If no degradation was observed the experiment was repeated at an increased temperature of 60°C.

2.5.7.2 Results and Discussion

2.5.7.2.1 Oxidative Degradation

Immediately following the addition of H_2O_2 (hydrogen peroxide) two peaks, (denoted' A' and 'B') were identified at retention times of 1.4 and 2.5 minutes, respectively as shown in the chromatograms in Figure 2.6. Following 10 hrs of refluxing approximately 20 % of the SC remained and a further peak, (denoted 'C') appeared at a retention time of 1.6 minutes. The appreciable oxidative degradation observed in these studies is similar to that observed in other stability studies on SC (74).

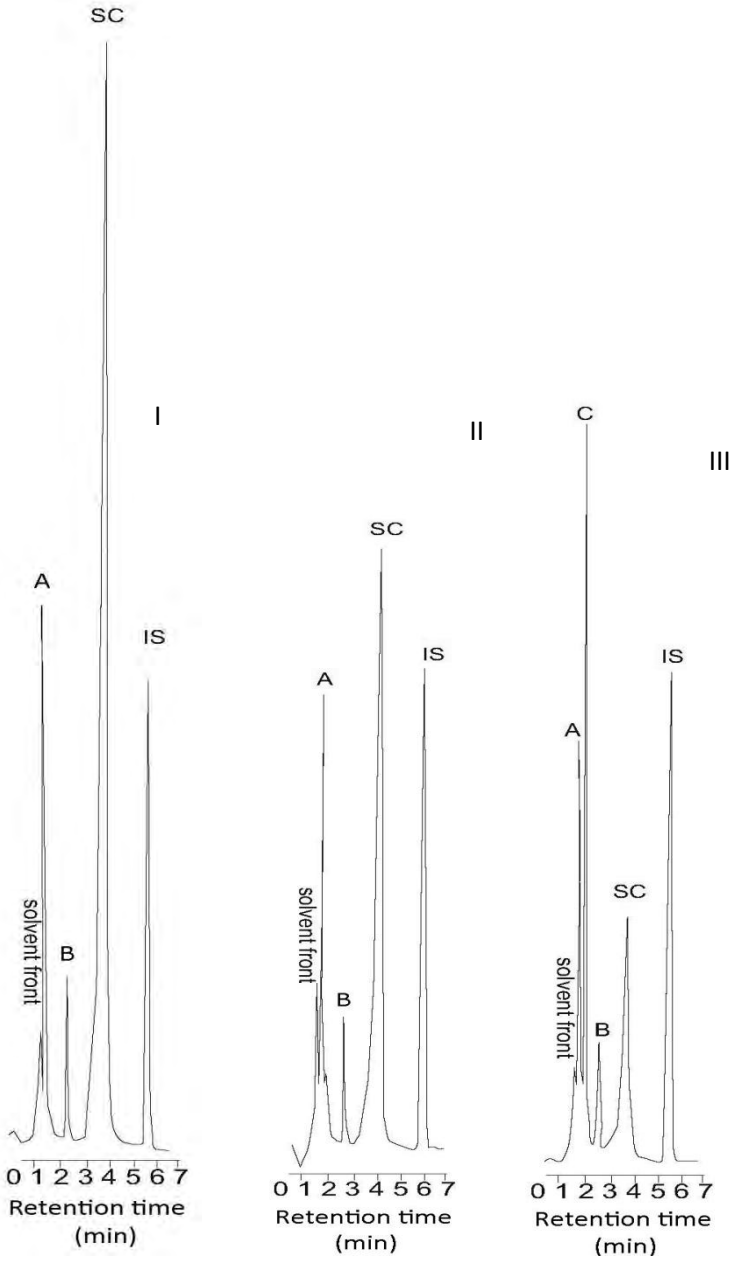


Figure 2.6 Chromatograms following oxidative degradation studies with SC after 1 hr (I) 5 hrs (II) and 10 hrs (III)

The data summarized in Table 2.7 reveals that over the 10 hrs that SC was exposed to H_2O_2, there was a significant decrease in the PHR and so demonstrating the susceptibility of SC to oxidative degradation

Table 2.7 Peak height ratio of SC and IS

1hr	5hr	10hr
2.073	1.777	0.434

2.5.7.2.2 Alkali Degradation

The drug was initially exposed to a 0.1M NaOH solution under reflux conditions but as shown in Figure 2.7, no noticeable degradation was observed following 10 hrs of refluxing This result was similar to that reported following a stability study conducted by Aboul-Enein and Hefnawy (73) who found that SC dissolved in 0.1M NaOH and stored at ambient room temperature for 24 hrs did not degrade appreciably.

In situations where no degradation is observed for a specific sample, the experiments should be repeated under reflux conditions at a higher molar concentrations of the reactant solution (93). Consequently fresh SC samples were prepared as described in §2.5.7.1.1.2 in a 0.2M NaOH solution. As can be seen from Figure 2.8 I, II and III, the only evidence if possible degradation is that peak height of SC decreases and no other peaks appear in the chromatograms. There is a possibility that any possible degradation products do not absorb radiation at the wavelength of analysis or the degradation products formed could be non-chromorphic products or also have decomposed to low-molecular weight fractions suggesting that detection with mass spectrometry may be necessary (93)

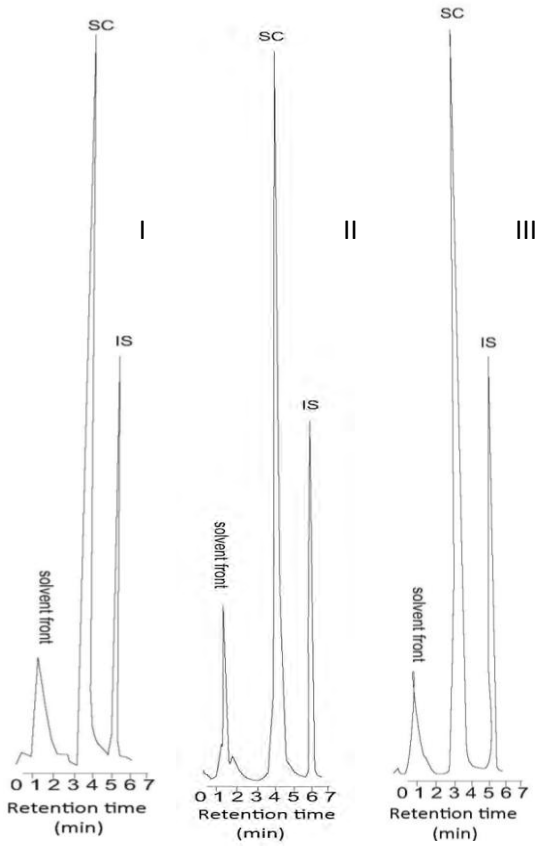


Figure 2.7 Chromatograms developed following refluxing of SC in 0.1N NaOH at 1hr (I) 5 hr (II) and 10 hr (III)

The data summarized in Table 2.8 suggest that SC is stable in 0.1M NaOH as there is no significant change in the PHR of SC and the IS. This indicates that it may be necessary that the drug be degraded in a stronger molar concentration base to achieve significant degradation.

Table 2.8 PHR of SC and IS under basic conditions (0.1M NaOH)

1hr	5hr	10hr
1.967	1.983	1.985

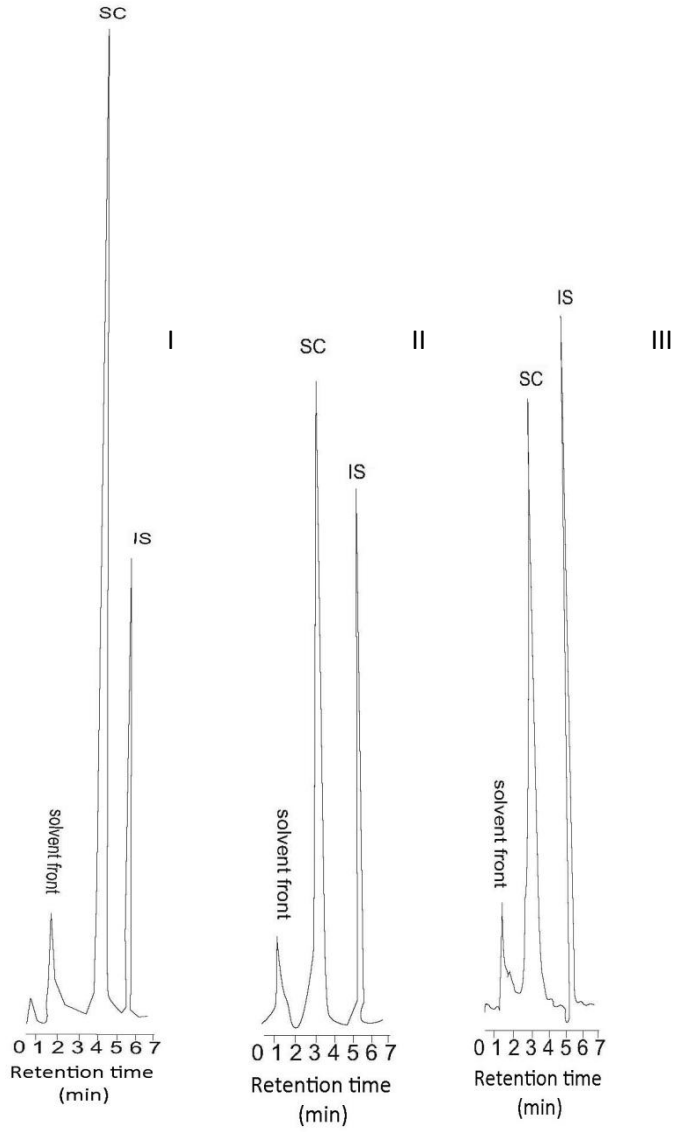


Figure 2.8 Chromatograms depicting the degradation of SC in 0.2M NaOH at 1hr (I) 2 hr (II) and 10 hr (III)

The data listed in Table 2.9 suggest that when SC is exposed to stronger basic conditions degradation of SC may occur as there is a significant decrease in PHR between 1hr and 10 hr indicating a significant decrease of SC present in the sample.

Table 2.9 PHR of SC and IS under basic conditions (0.2M NaOH)

1hr	**5hr**	**10hr**
2.166	1.506	0.917

2.5.7.2.3 Acidic Degradation

In the case of acidic degradation, no degradation was noted after 10 hours as can be seen from Figure 2.9. This result is similar to that observed by Abdoul-Enein (73) that showed that no appreciable degradation of SC occurred after exposing SC to 0.1M HCl at room temperature for 24 hrs. *Bakshi et al* advises that in the case where no degradation is noted, the drug sample should be refluxed under stronger acidic concentrations (93). For this reason fresh samples were prepared as discussed in §2.5.7.1.1.3 and the SC solution were made up to volume in 0.2M HCl. As seen in Figure 2.10 III, after refluxing SC in 0.2M HCl, the drug has completely degraded.

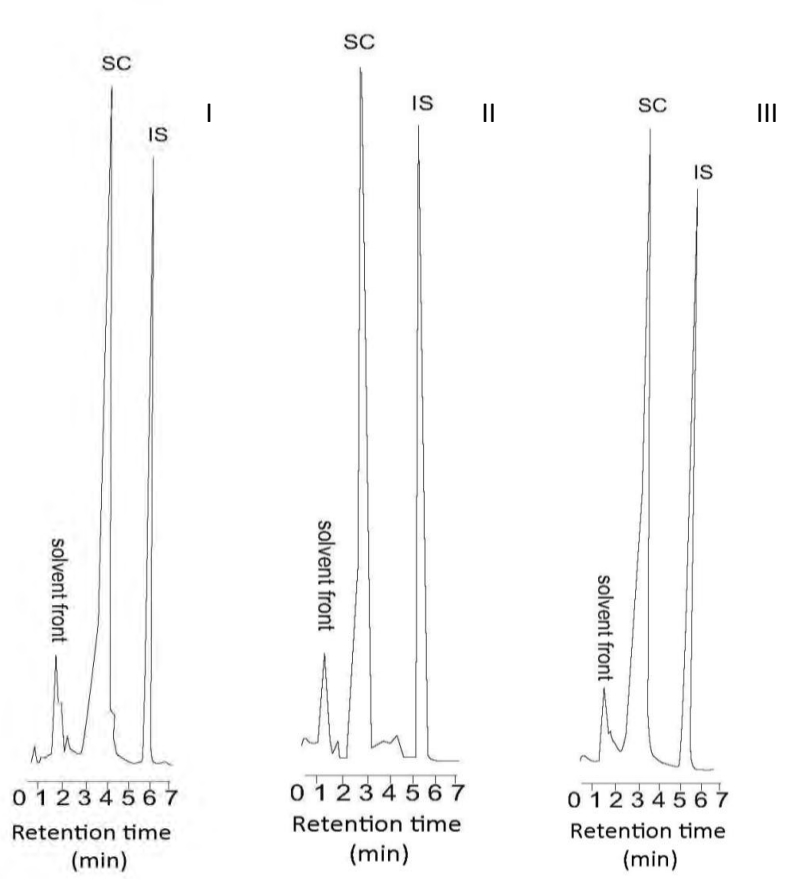


Figure 2.9 Chromatograms depicting the degradation of SC in 0.1M HCl at 1hr (I) 5 hr (II) and 10 hr (III)

The data shown in Table 2.10 indicate the stability of SC in 0.1M HCl as there is no significant change in the PHR of SC and the IS.

Table 2.10 PHR of SC and IS exposed to acidic conditions (0.1M HCl)

1hr	**5hr**	**10hr**
1.054	1.053	1.056

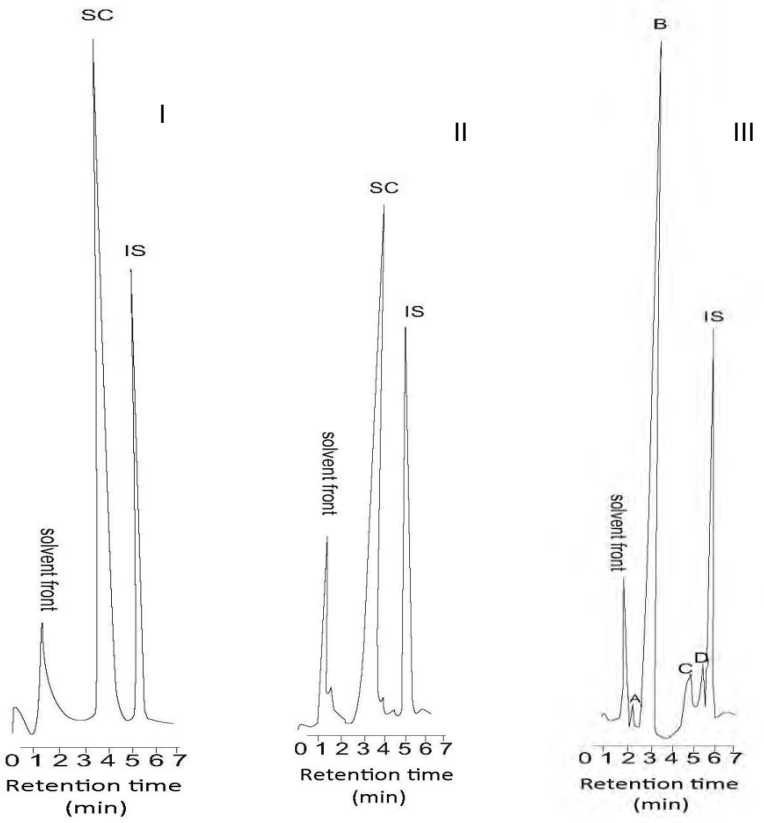


Figure 2.10 Chromatograms depicting the degradation of SC in 0.2M HCl at 1 hr (I) 5 hr (II) and 10 hr (III)

The data summarized in Table 2.11 shows that in the presence of strong acidic conditions, there is significant degradation of SC and after 10 hours there is no SC peak remaining.

Table 2.11 PHR of SC and IS under acidic conditions (0.2M HCl)

1hr	**5hr**	**10hr**
1.496	1.148	No SC remaining

2.5.7.2.4 Photolytic Degradation

The chromatograms generated following injection of samples subjected to light exposure revealed no evidence of degradation and no corresponding decrease in the PHR suggesting that SC is stable in the presence of light.

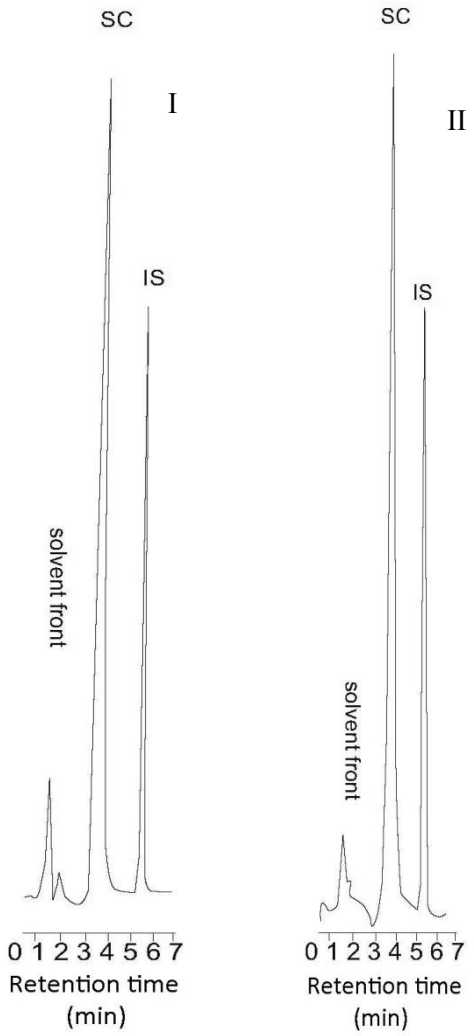


Figure 2.11 Chromatograms of samples following exposure of SC to light for 1 hr (I) and 10hr (II)

The data listed in Table 2.12 displays the fact that no degradation occurs after SC is exposed to light for 24hrs as there is no change in PHR.

Table 2.12 PHR of SC and IS after SC exposure to light

1hr	10hr
1.696	1.694

2.5.7.2.5 Heat Degradation

Sample of SC heated to 50, 60 and 70 °C for 10 hours revealed no obvious evidence of degradation. These results are similar to stress studies that suggested that SC was stable at elevated temperatures (74).

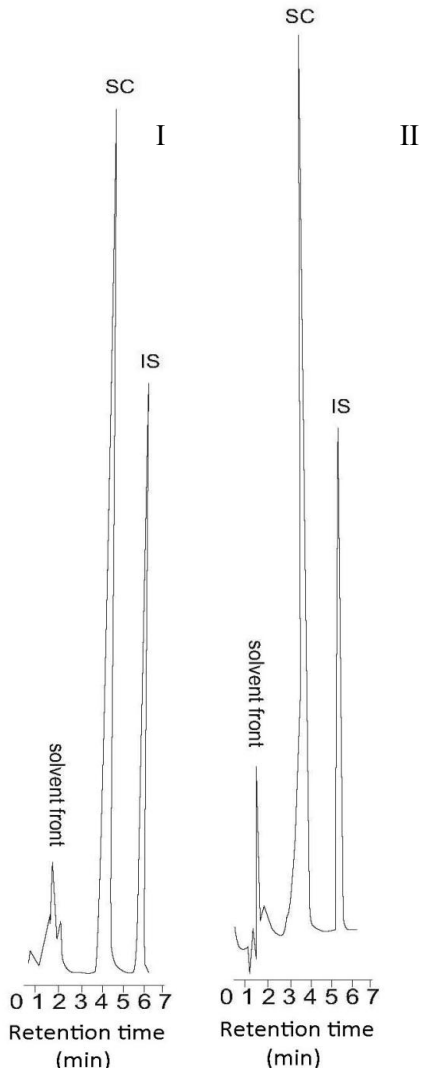


Figure 2.12 Chromatograms depicting no degradation of SC when exposed to heat at 60ºC for 10 hrs (I) and 70ºC for 10 hrs (II)

As can be seen from the data summarized in Table 2.13 there is no change in the peak height ratios when SC was exposed to elevated temperatures showing that SC does not degrade at high temperatures.

Table 2.13 PHR of SC and IS after SC exposed to heat for 10 hrs

50°C	60°C
1.350	1.368

2.6 SYSTEM SUITABILITY

2.6.1 Introduction

System suitability tests were proposed for use by the FDA in 1974 (94). Following validation of a particular method, system suitability tests should be performed to verify the resolution and repeatability of an analytical system is adequate to perform subsequent analyses (85). System suitability tests are based on the concept that the equipment, analytical operations and samples are an integrated organization that can be evaluated as a whole.

Failure of system suitability tests can be indicative of a number of problems with a method including the use of incorrect mobile phase compositions or inadequate preparation of standard solutions. It could also be a sign that leaks may be present in an analytical system or that the column has degraded (88). Therefore it is good practice to perform system suitability tests routinely.

2.6.2 Method

For the purposes of this research visual system suitability tests were performed. Visual comparisons were made between chromatograms developed from test solutions to those following analysis of standard solutions. Chromatograms from standard solutions are example chromatograms derived from the validation process or previous system suitability tests (95).

In additions calculation of the resolution and tailing factor can also be undertaken to ensure system suitability.

2.6.2.1 Resolution

The resolution of a method describes the extent of separation between two peaks and this parameter can be calculated using Equation 2.8.

$$R_s = \frac{(V_{R2} - V_{R1})}{2} X (w_1 + w_2)$$

Where,

Rs = the resolution factor,
V_{R1} = the retention time of peak 1,
V_{R2} = the retention time of peak 2,
w_1 = the width of peak 1,
w_2 = the width of peak 2.

Resolution factors > 1.5 are considered acceptable for analytical purposes (95).

Calculation of the resolution factor from chromatograms resulted in a factor of 1.6 and shows that the peaks are sufficiently resolved to produce accurate results.

2.6.2.2 Tailing Factor

Peak tailing occurs when a peak deviates from a Gaussian or expected symmetrical shape. Peak tailing is commonly observed when separating basic compounds on C_{18} stationary phases due to interaction of the analyte with silanol functional groups located on the stationary phase backbone (67). Excessive peak tailing is an issue as it can decrease peak height and therefore produce inaccurate results.

Peak tailing is also a problem if minor peaks run soon after a larger peak, for example while completing stability-indicating studies. It is possible that a minor peak could be hidden under the tail of a major peak and so reduces the ability of a method to facilitate quantitation of the components of the mixture being analyzed.

The tailing factor can be calculated using Equation 2.9.

$$T_f = \frac{(a+b)}{2a}$$

Where,

T_f = tailing factor,
a = the half width of peak 1 at 5% of the peak height,
b= the half width of peak 2 at 5% of the peak height.

Peaks of interest that have a tailing factor < 1.5 are suitable for quantitation in HPLC. Calculation of the tailing factor revealed that the a value of 1.125 was obtained suggesting that the mobile phase used for this separation is adequate

2.7 CONCLUSIONS

A reversed-phase HPLC method for the quantitation of SC *in vitro* has been developed, successfully validated and subsequently applied to the analysis SC dosage forms.

The use of a validated analytical technique is essential for the collection of reliable data. The main purpose being to ascertain that the peak can be clearly resolved from other peaks in the chromatogram. Successful resolution is reliant on a number of different factors, including the type of column and the composition of the mobile phase.

The internal standard method was made use of whereby the peak height ratio of the peak of interest to the internal standard is determined thereby eliminating injection errors from the results and generating more accurate data. The internal standard chosen for the method was diazepam based on its use in a previous SC HPLC study.

The method was found to be linear over the range 0.2 – 2µg/ml with the calibration curve having an R^2 value of 0.999. The proposed method has acceptable accuracy and precision and can be used for the analysis of SC in raw material and dosage forms. The analyte of interest, SC, and the IS were adequately separated with a retention time of 3.6 and 6 min, respectively. Consequently the method has a relatively short analytical run time of 7 minutes and is appropriate for quality control purposes. Furthermore the method is simple and the mobile phase used for the separation does not require complicated preparation procedures or components.

Forced degradation studies revealed that the method is stability-indicating with no interfering peaks detected and therefore the method can be applied in formulation development and long term stability studies of SC containing dosage forms.

Suitability parameter tests were carried out during the entirety of the method validation process to ensure that the results being produced are accurate.

CHAPTER THREE

PREFORMULATION AND POWDER ASSESSMENT

3.1 INTRODUCTION

The biological and analytical requirements needed for the registration of an active pharmaceutical ingredient (API), whether of natural or synthetic origin are the focus of much attention in the pharmaceutical industry. The ever increasing requirements with regard to ensuring safety, quality and efficacy has meant that the development of new medicines is characterised by higher assay values and lower impurity content (96). However, the quality of a dosage form does not depend only on the characteristics of the active substance but also on the excipients used to manufacture the dosage form and many stability problems seen in development of dosage forms are due to incorrect matching of excipients and an API (97).

Preformulation studies are the first step in the rational formulation of an API into a usable medicine (98). These studies – that investigate the physicochemical properties of the drug substance, alone and in combination with excipients - are designed to identify properties that may influence formulation design and method of manufacture of the resulting dosage form.

There are many options available to a formulator with regards to deciding which preformulation tests should be completed on an API. It is typically important to evaluate the solubility, pKa, particle size and morphology of the API as well as investigating potential interactions that may occur between an API and excipients that may be used to produce the dosage form.

The physiochemical properties of SC were discussed in Chapter One and in this chapter the physicochemical and molecular properties of not only the API but the potential excipients for inclusion in SC orodispersible tablets were investigated to ascertain the influence they could have on the successful development of fast-dissolving tablets.

3.1.1 Physicochemical Properties

3.1.1.1 Particle Size and Shape

The shape of a particle, defined by the exterior morphology of the material, has a significant impact on the particle distribution in a powder (99). A qualitative description of the shape of powder particles can be made by using descriptive terms such as ‘spherical’, ‘granular’, ‘crystalline’, ‘fibrous’ and/or ‘flaky’ (100).

The shape of a particle is an important factor to consider when undertaking preformulation studies as it can influence many critical powder properties, such as flowability, compatibility, content uniformity, and dissolution rates (101). The flow properties of powders form an integral part of preformulation studies, and the design of oral dosage forms as quality control parameters for raw materials and product uniformity must be established. Particle shape plays a crucial role in the success of blending, granulation and other manufacturing processes (102). Irregularly shaped particles generally contribute to poor flow properties of a powder, whereas spherical powder particles tend to exhibit good flowability (103).

The size of the particles in a powder blend is generally the largest dimension of the individual particles in that blend (103). Particle size also plays a major role in the successful blending of powders and therefore the content uniformity of the final dosage form (103). A wide distribution of powder particle sizes results in segregation, and ultimately non-uniformity of dosage forms.

3.1.1.2 Powder Density

3.1.1.2.1 True Density

True density is defined as the density of the material, exclusive of the pores or inter-particle voids that are larger than molecular or atomic dimensions, in a crystal lattice (104).

The true density of organic excipients typically falls in the range 1.0-1.6g/cm^3 (103) whereas the density of inorganic excipients generally exceeds 2.0g/cm^3 (103). A gas pycnometer is commonly used to determine the true density of powders.

3.1.1.2.2 Bulk Density

The bulk density of a powder is calculated using Equation 3.1 and is the mass of the powder divided by the volume that the powder occupies.

$$\text{Bulk Density} = \frac{\text{e ight of the powder (g)}}{\text{olume of the powder (ml)}}$$

The volume of the powder includes air located between the particles of the powder (105; 106). The density of a powder is primarily dependent on the size and shape of the particles in addition to the tendency of particles to adhere to each other (104). The bulk density of a powder is an important parameter as it is used to establish the batch size for manufacture in a specific blender and/or granulator (104).

The bulk density of a material is also used to calculate Carr's Index (CI) which provides an indication of the compressibility of a powder and therefore the potential for use as a suitable tablet formulation component. The CI guidelines are summarized in Table 3.1 and are used by formulators to assess the flowability of powders for tablet manufacture. In general a low index is indicative of excellent flow properties whereas a high index of 40 is indicative of extremely poor flow.

Table 3.1 Carr's Index Guidelines (107)

C rr 's I dex (%)	**Type of Flow**
5-15	Excellent
12-18	Good
18-23	Satisfactory
23-35	Poor
35-38	Very Poor
40	Extremely Poor

3.1.1.2.3 Tapped Density

The tapped density of a powder, calculated using Equation 3.2, is the ratio of the total mass of a powder to the volume occupied by that powder after it has been compacted or tapped for a specific period of time.

$$\text{Tapped Density} = \frac{\text{e ight of the powder (g)}}{\text{olume of powder after being tapped (ml)}}$$

Tapping the powder removes small voids or air entrapped between the particles in a powder blend.

The tapped density is used to calculate the Hausner ratio (HR) that also provides an indication of the flow properties of a powder.

3.1.1.3 Angle of Repose

The determination of the angle of repose (AOR) is also used assess the flowability of a powder.

In order to determine the AOR of a powder the material is poured onto a flat surface where it forms a heap. The particles initially stack, until the angle available for the addition of subsequent particles to the heap is large enough to overcome friction and the particles will slide down the surface of the heap until the gravitational forces balance the inter-particulate forces, and movement ceases (107). The

heap of particles tends to form a conical shape with the sides of the heap producing an angle between the surface on which the powder resides and the free surface of the powder known as the angle of repose, which is indicative of the cohesion between particles and therefore an indirect measure of powder flowability (107). The guidelines for AOR are summarized in Table 3.2 with values of < 25 being the most desirable and indicative of good flow properties. Powders in which constituent particles have rough and irregular surfaces produce high AOR values (108) indicating poor flow characteristics.

Table 3.2 Angle of Repose Guidelines (109)

Angle of Repose (in degrees)	Type of Flow
<25	Excellent
25-30	Good
30-40	Satisfactory
>40	Very Poor

3.1.2 Molecular properties of the drug

3.1.2.1 Polymorphism

An important factor to be established in preformulation studies is the crystal form of the API and whether the API exists in more than one polymorphic form. Polymorphic forms of an API often present with different physicochemical properties including melting point and solubility and other factors that influence dosage form performance (110).

The identification of polymorphs is typically achieved using X-Ray Powder Diffraction that permits the characterisation of the crystal form of an isolated solid (110). It is however important that more than one technique is used to identify the presence of the polymorph and techniques such as Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), Infrared (IR) or Raman Spectroscopy and solid state NMR (Nuclear Magnetic Resonance) are often used to achieve this (110).

Anhydrous SC has no structural isomers or polymorphic forms (111) but a new form of the citrate salt has recently been observed during solubility studies of SC at 4°C (111). This "new" form of SC has been characterized as a hydrate and is known as sildenafil hemi-citrate. It has been shown that material is not produced under the conditions used for the commercial manufacture of SC. It was also

noted that the different stoichiometry of the hemi-citrate hydrate form prevents its formation on storage of either the raw material or solid dosage forms (111).

3.1.3 API-Excipient Interactions

The bulk of a dosage form is generally made up of excipients that are included to facilitate the manufacture of a product. Excipients have historically been considered as inert support for the medicaments, however this view has changed and excipients are now recognised as components that may interact with an API to produce unwanted physical and/or chemical transformation (112).

Drug-excipient interactions may take a long time to manifest during real time stability tests and are not always readily identified or predicted by stress and/or preformulation studies. It is therefore important for a formulator to have knowledge of the susceptibility of an API to undergo degradation reactions by understanding the chemistry of the molecule they are dealing with.

These interactions are not necessarily detrimental; they can also be beneficial. Drug-excipient interactions are usually separated into categories that are designated physical or chemical interactions (113).

3.1.3.1 Physical API-excipient interactions

Physical interactions involve no chemical changes to an API or excipient and can be difficult to detect.

A beneficial physical interaction can occur during mixing when smaller particles, typically the API, interact with the surfaces of larger particles (usually excipients) through physical forces. Consequently, a more homogenous powder blend is produced (113).

In contrast detrimental physical interactions may alter the performance of a dosage form. For example, a primary amine can interact with the microcrystalline cellulose. When dissolution is carried out in water, a small amount of the drug may be bound to the cellulose and will not be released. A dosage form that contains a high API concentration may not be a matter of great concern, however for low dose formulations, this may lead to dissolution changes and batch failure (113).

3.1.3.2 Chemical API-excipient interactions

Chemical interactions between an API and an excipient, impurities or residues of an excipient may occur and are almost always detrimental, as degradation products are likely to result.

One of the few known beneficial chemical API-excipient interactions occurs in association with nystatin, and the excipients, pectin and glycerine. The combination of these materials results in increased therapeutic activity of nystatin and prolonged release due to the formation of intermolecular hydrogen bonds between the hydroxyl functional group of nystatin and the appropriate functional groups located on the excipient molecules (114).

An example of a chemically based API-excipient interaction occurs between diclofenac sodium and the polysaccharide based, polymer, chitosan. The release of diclofenac is inhibited at low pH due to the formation of an ionic complex of the drug and cationic polymer (115).

Many API are susceptible to oxidative degradation. Atorvastatin is one such compound and special consideration needs to be given when this compound is paired with excipients such as fumed silica or fumed titania that have an ability to promote oxidative degradation reactions (98).

3.2 METHOD

3.2.1 SEM

Particle morphology of materials was investigated using a Vega LMU© Scanning Electron Microscope (Tescan, Czechoslovakia Republic). A small amount of SC, microcrystalline cellulose (MCC), mannitol, fructose, crospovidone (CRP), sodium starch glycolate (SSG) and crosmarmellose Sodium (CMS) were dusted onto separate graphite plates and coated, under vacuum, with gold for 20 minutes. The samples were then viewed using SEM at an accelerated voltage of 20kV.

3.2.2 Powder Density

Approximately 10g of SC, MCC, mannitol, CRP, SSG and CMS were separately weighed and passed through a sieve. The powders were sieved in accordance with FDA guidelines that state that the powders must be sieved with apertures to break up agglomerates which may have formed during storage (116). This must be done gently to avoid changing the nature of the powder. Each powder was then transferred into a separate 100ml graduated measuring cylinder where the bulk density was determined by measuring the volume that the powder occupied (V_{bk}). The tapped density of each powder was determined with the aid of a Model SVM 203 tapped density tested (Erweka GmbH,

Heueastamm, Germany) operated at a rate of 220 taps per minute for two (2) minutes. Following the two minutes of agitation the volume of the tapped powder was read (V_{tp}).

The CI, porosity and HR were calculated using Equations 3.3, 3.4, and 3.5, respectively.

$$CI = \frac{(\quad_{tp} \quad _{bk})}{_{tp}} \text{ x } 100$$

$$\varepsilon = \left(1 - \frac{\rho_{tp}}{\rho}\right) \text{ x } 100$$

$$HR = \frac{_{tp}}{_{bk}}$$

Where,

ε = powder porosity
$_{bk}$ =bulk density where v=v_{bk}
$_{tp}$ =tapped density where v=v_{tp}
CI = Carr's Index
HR = Hausner Ratio

The true density of the powder was calculated by compaction of the powders. SC, MCC, mannitol, CMS, CRP and SSG were each filled into a tablet die and manually compressed with a compression force of 30N to form 150mg tablets using an F3 Manesty® single punch press (Manesty Machines Ltd, Liverpool, United Kingdom). This compression force was selected as it is the same force that the tablets are to be compressed at in the manufacturing of the orodispersible tablets (ODT). By using the same compression force to calculate the true density of materials used to formulate the tablets one can ascertain whether the true density will have a detrimental effect on the production of a suitable dosage form. The weight and volume of each tablet (n=5) were measured and the true density was calculated using Equation 3.6.

$$= \frac{m}{v}$$

Where,

= de nsity (g/ml)
m = mass of powder (g)
v= volume occupied by powder (ml)

3.2.3 Angle of Repose

The AOR was measured using a funnel method. Approximately 10g of powder was weighed and placed in a funnel. The height of the funnel was adjusted to a point where the tip of the funnel was just above the apex of the heap of powder. The powder was allowed to flow freely through the funnel onto a glass plate surface. A diagrammatic representation of the angle of repose is seen in image Figure 3.1 and was calculated using Equation 3.7.

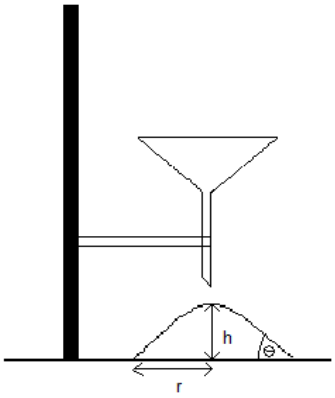


Figure 3.1 Diagrammatic representation of the method of calculating the angle of repose

$$\tan \theta = \frac{h}{r}$$

Where,

h = height of a pile of powder
r = radius of the heap of powder
θ= angle of repose

3.2.4 IR Spectroscopy

The IR absorption spectra of SC and excipients were generated using a Spectrum 100 FT-IR ATR Spectrophotometer (Perkin Elmer®Ltd, Beaconsfield, United Kingdom). The spectra were generated from samples of individual components and 1:1 binary mixtures of SC and excipient. Samples of binary mixtures were prepared by weighing approximately 0.5g of each component and gently blending the mixture using a mortar and pestle. A small amount of the mixture was placed on a diamond crystal and analysed in the wave number range, 4000-650cm^{-1} at a resolution of 4cm^{-1}.

3.2.5 DSC

DSC is the measurement of the energy change that occurs as a sample is heated at a constant rate (117). The principal process involves the heating of two ovens to the same temperature at the same rate. One heater contains the sample in a sealed pan and the other containing an empty pan serving as the reference. If a change in the sample occurs such as melting, energy will be consumed and the process is classified as an endothermic reaction (118) whereas, if the sample were to crystallise, energy is released due to the occurrence of an exothermic reaction. As the reference remains at a constant temperature, a thermogram displaying the thermodynamic events of that sample is produced showing a releases or uptake of energy.

Approximately 2mg samples of SC, potential individual excipients and binary mixtures of SC and individual excipients in a 1:1 ratio of were analysed using DSC. DSC thermograms were generated at temperatures between 30 and 250ºC using a Model DS-60(Shimadzu®, Tokyo, Japan) with equipment and PC control unit TAC 60 (Shimadzu®, Tokyo, Japan) at a heating rate of 10ºC/min and a nitrogen flow rate of 20ml/min. Data analysis was undertaken using Pyris™ Manager Software.

3.3 RESULTS AND DISCUSSION

3.3.1 SEM

SEM imaging is used to obtain information relating to the size and shape of materials and in this case the API and excipients. This provides additional information about the potential flow properties and compressibility of raw materials and the powder blend.

SEM was chosen as a suitable method because of the high resolution of the images obtained and its rapidity and ease of operation, which enables the generation of suitable images within a short period of time.

Furthermore SEM has a high depth of field which allows for analysis of the surface texture of particles, resulting in excellent characterisation of particle morphology. An understanding of the texture of particle surfaces is important, as this property may influence the surface area, settling velocity and adherence of a powder or raw material to the particle (119).

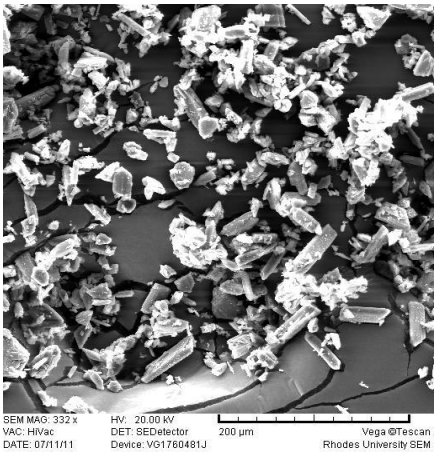
SEM images of SC and raw materials that were considered as components of a tablet formulation are depicted in Figure 3.2.

SEM imaging of SC (Figure 3.2 (I)), reveals the presence of many well-defined rod-shaped crystals. These crystals may at times be brought together forming irregular flat aggregates (120) and therefore suggests that SC may exhibit poor bulk flow properties.

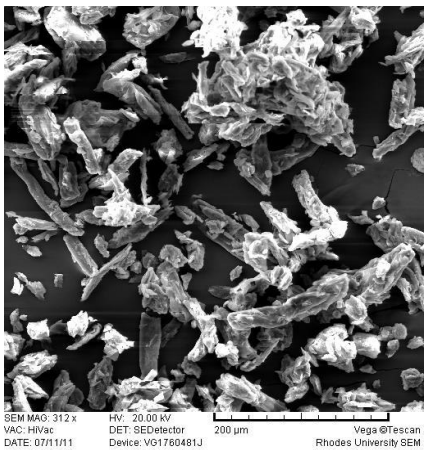
MCC exhibits irregular, elongated crystals (Figure 3.2 (II)). MCC particles vary in size and exhibit rough particle morphology. Based on these properties it is likely that MCC will exhibit poor flow properties. One cannot however make a judgement on the flow properties of a powder simply by looking at an image and further tests such as angle of repose are required before a final assessment can be made. The variable particle size of MCC, revealed by SEM imaging, indicates that it may be necessary to sieve the material when manufacturing a dosage form to ensure that uniformity in the product is achieved.

The particle size and shape of mannitol (Figure 3.2 (III)) and fructose (Figure 3.2 (IV)) were compared to establish which material would be a more appropriate tablet diluent for the purposes of manufacturing a rapidly disintegrating tablet. As can be seen from Figure 3.2 (IV) fructose particles are far larger in size than those of mannitol and all other excipients. Fructose occurs as cubic-shaped crystals with a smooth surface whereas mannitol occurs as smaller cubic and rod-shaped crystals which also have a smooth surface. Therefore, even though both mannitol and fructose exhibit properties that point to good flow properties due to their cubic shape and smooth surface, mannitol would most likely be the most suitable diluent. Mannitol has a similar particle size to the other excipients investigated and therefore there is less chance of segregation of powders occurring.

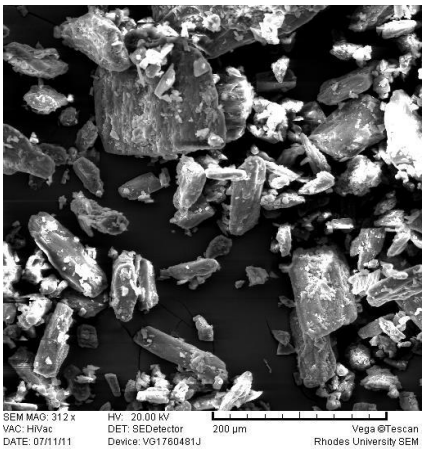
CRP (Figure 3.2 (V)) particles were highly porous and slightly granular in shape. SSG (Figure 3.2 (VI)) particles showed a high degree of sphericity and were nonporous with a very smooth surface which is indicative of SSG having good flow properties. CMS (Figure 3.2 (VII)) particles exhibited a fibrous shape with nonporous characteristics. All three superdisintegrants have similar particle sizes indicating a low probability of segregation occurring.



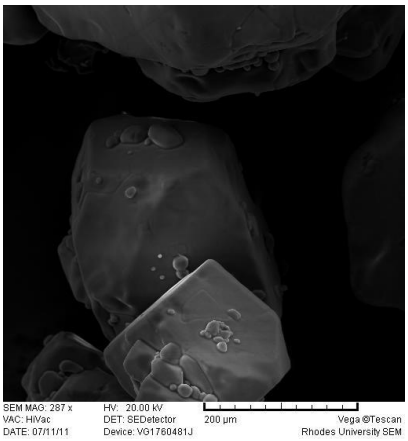
I



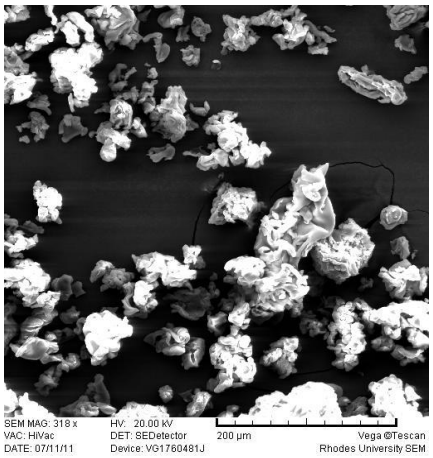
II



III



IV



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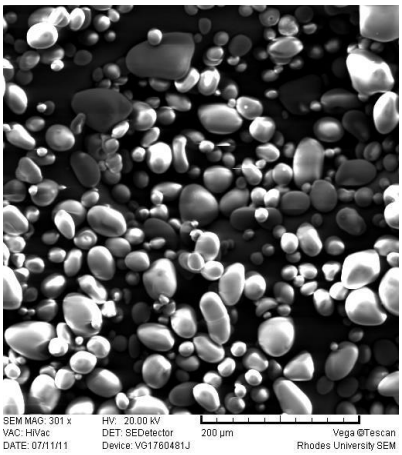

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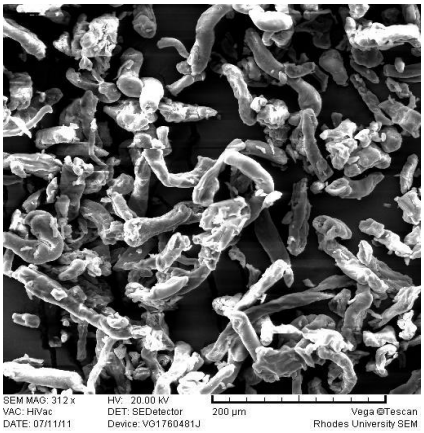


VII

Figure 3.2 Typical SEM images showing the particle morphology of (me) SC, (II) MCC, (III) mannitol, (IV) fructose, (V) CRP, (VI) SSG and (VII) CMS.

3.2.2 Powder Density

3.2.2.1 True Density

The reported and actual true density values determined in this study are summarized in Table 3.3.

Table 3.3 True Density of raw materials

Material	Actual (g/ml)	Literature (g/ml)	Reference
SC	1.62±0.04	No reported values	-
MCC	1.93±0.05	1.51-1.67	(121; 122)
Mannitol	1.27±0.02	1.44	(103)
CMS	1.72±0.08	1.53	(122)
CRP	1.76±0.01	1.3	(123)
SSG	1.69±0.02	1.44	(122)

The true density value of a powder provides useful information that can be applied to the characterization of the mechanical properties of powders on which properties of a tablet such as hardness and tensile strength are reliant (124). Due to the fact that powders flow under the influence of gravity, dense particles are generally less cohesive than low density particles of similar size and shape (125).

Determination of the true density of the API and potential excipients is a vital part of preformulation studies with regard to ODTs as these data are used to determine the porosity of a powder.

The data summarized in Table 3.3 reveal that experimentally determined values for density generally deviate from those reported in the literature. This is most likely due to the fact that true densities are typically calculated using a helium pycnometer (103), an instrument that was not accessible for the purposes of this research. The data was generated empirically by measuring the weight and volume of a tablet compact. The deviations noted may therefore be attributed to the use of this approach and variations in machined tooling.

3.2.2.2 Bulk and Tapped Density

Data on the bulk and tapped density for materials used in these studies are summarized in Table 3.4, with previously reported values for these parameters included for comparative purposes. The Bulk and tapped densities calculated along with the true densities determined in these studies were used to calculate CI, HR and porosity of SC and the potential excipients. These results are summarized in Table 3.5. Comparisons between the actual data and data obtained from literature are in agreement with each other and we can therefore conclude that calculations conducted with the data e.g. CI and HR are accurate.

Table 3.4 Bulk and tapped density values of raw materials

Material	Bulk Density			Tapped density		
	Actual (g/ml)	**Literature (g/ml)**	**Reference**	**Actual (g/ml)**	**Literature (g/ml)**	**Reference**
SC	0.564±0.021	0.595	(126)	0.756±0.023	0.764	(126)
MCC	0.286±0.015	0.26-0.31	(122; 127)	0.385±0.015	0.337	(122)
Mannitol	0.513±0.007	0.430	(122)	0.714±0.115	0.734	(122)
CMS	0.501±0.012	0.46-0.529	(122; 128)	0.769±0.011	0.72-0.819	(122; 128)
CRP	0.313±0.007	0.3-0.4	(122; 128; 129)	0.401±0.011	0.4-0.5	(122; 128)
SSG	0.802±0.021	0.756-0.76	(128; 129)	0.947±0.089	0.98	(122)

Table 3.5 CI, HR and Porosity data of raw materials

Material	CI	HR	Porosity
SC	34.6±1.15	1.51±0.02	53.33±1.42
MCC	25.71±3.59	1.35±0.06	80.05±1.46
Mannitol	26.99±1.91	1.37±0.04	43.78±0.74
CMS	31.85±1.02	1.53±0.02	41.74±1.63
CRP	21.95±3.60	1.25±0.07	77.22±1.60
SSG	19.89±2.47	1.21±0.04	54.50±1.53

The value for porosity is derived from the powder density and is usually determined using mercury porosimetry (104). It is generally thought that granules that exhibit a greater degree of porosity will dissolve faster than denser granules as water is known to pass rapidly through porous substances (104). Porosity is an important consideration in preformulation studies as the intended dosage forms need to disintegrate rapidly and the greater the porosity the more likely the dosage form will disintegrate quickly.

MCC is considered the most compressible of any of the directly compressible excipients and exhibits the greatest porosity (127) and producing a tablet of a specific hardness using MCC requires less compression force than for that using other materials.MCC is therefore usually combined with other fillers to achieve an ideal compactability and flowability for a direct compression formulation. When designing formulations there is a need to use excipients that exhibit a high degree of porosity as the porous surfaces provide adsorption sites to ensure that fine drug particles in low-dose formulations are homogenously distributed (127).

The CI indicate that CRP and SSG are likely to exhibit good flow and compressibility properties whereas the API and other excipients evaluated would exhibit poor flow and compressibility properties. Although the data for SC reveals that it may not exhibit good flow and compressibility properties, this may not be important because only a small dose (3mg) of SC is required in the tablet and therefore such a small amount will not make a great difference when incorporated into a powder blend.

Values for HR are listed in Table 3.5. HR values < 1.25 are generally considered to indicate that material are likely to exhibit good flow properties whereas those > 1.25 tend to exhibit poor flow

(130). The HR data confirm that only CRP and SSG exhibited good flow and therefore reinforce the possible need for a glidant in the formulation.

The data obtained from these studies is valuable as the intended method of manufacture of SC tablets is direct compression. It is important therefore to assess the flowability of the raw materials to ensure that content uniformity will be achieved when the powder flows from the hopper to the die cavity. The data revealed that the direct compression approach may be successful as a method of manufacture of fast dissolving tablets if the formulation includes a glidant.

3.2.3 Angle of Repose

The results of AOR studies are summarized in Table 3.6 and the guidelines of AOR results discussed previously in §3.1.2 were used to assess the flowability of the raw materials.

Table 3.6 Angle of Repose values for raw materials

Material	Actual	Literature	Reference
SC	40.6±0.60	42.3	(126)
MCC	33.6±0.76	34.4	(122)
Mannitol	38.2±1.46	38	(127)
CMS	30.7±1.74	30-36	(130; 131)
CRP	42.2±1.34	43-44	(129; 130)
SSG	31.7±1.70	29-33	(129; 131)

The data summarized in Table 3.6 reveal that the API and all excipients investigated show satisfactory or very poor flow properties, confirming the CI and HR data. These results reinforce the fact that a glidant may be necessary in the formulation in order to ensure the flow properties of the powder blend are adequate to produce high quality, homogenous dosage forms using direct compression.

Glidants improve the flow properties of a powder blend by interposing between particles to reduce surface irregularity and therefore preventing interlocking of particles and decreasing inter-particulate friction that may be present (132).

3.2.4 DSC

DSC is a commonly used thermal analysis method in the pharmaceutical industry(110). Although there is acknowledgement that the presence of a physical or chemical interaction does not necessarily point to an incompatibility, it is generally agreed that any change observed in a DSC thermogram, is explicit proof that an interaction has occurred (133).

There are many advantages of using DSC as a tool that include a rapid evaluation of potential interactions using only small amounts of sample (134). However higher temperatures are used for analysis that may introduce artefacts to the technique and events that are not typically witnessed at room temperature may be produced. This is due to the fact that at increased temperatures the kinetics of reactions, which could possibly alter the physicochemical properties of a sample under investigation, are increased (134).

The DSC thermograms for SC, individual excipients and 1:1 binary mixtures of API and excipient are exhibited in Figures 3.3- 3.5 and 3.7 - 3.20. SC (Figure 3.3) has a melting endotherm peak at 195.4°C which is in agreement with that of a previous DSC study (15). It can be concluded that the bulk SC that is being used in the preformulation studies, and ultimately in the manufacturing process, is pure.

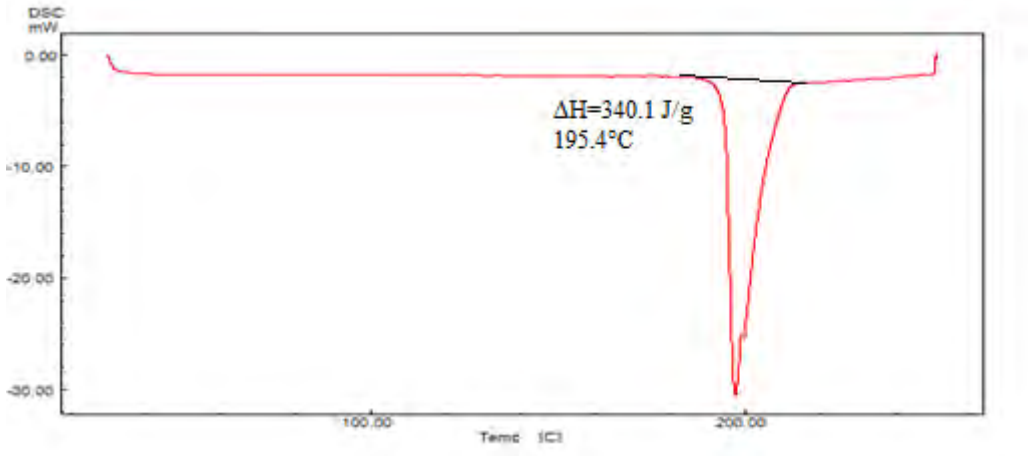


Figure 3.3 DSC thermogram of SC generated at a heating rate of 10°C/min.

As expected the DSC thermogram of MCC (Figure 3.4) reveals no thermal events in the range 30-250°C as MCC does not melt in this temperature range.

The thermogram of the binary mixture of SC and MCC (Figure 3.5) revealed one endothermic peak at 195°C due to the presence of SC, suggesting that no potential interactions between SC and MCC are likely to occur.

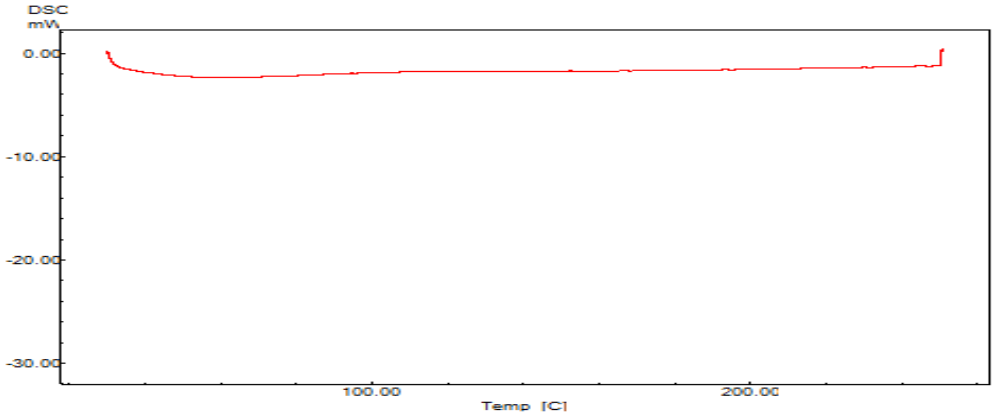


Figure 3.4 DSC thermogram of MCC generated at a heating rate of 10°C/min

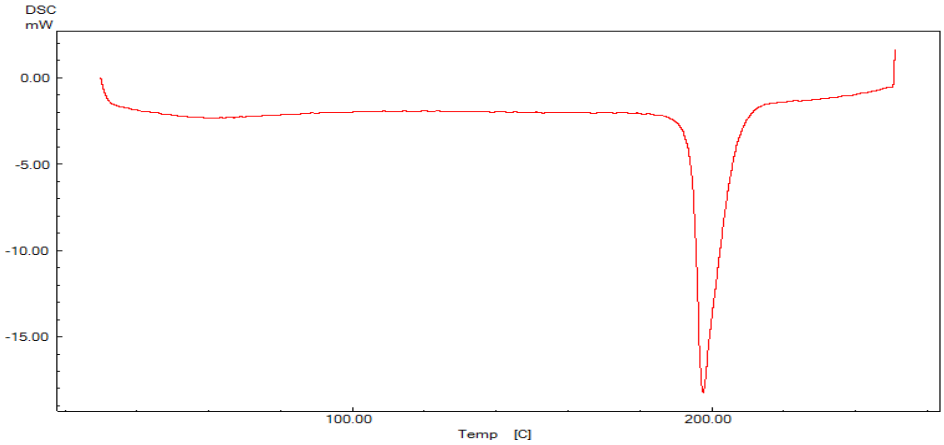


Figure 3.5 DSC thermogram of a binary mixture of SC and MCC generated at a heating rate of 10°C/min.

The thermogram for mannitol reveals one endothermic peak at 168°C (Figure 3.7). This is in agreement with previous thermal analysis studies conducted on mannitol that showed a single endothermic event that occurs at 167°C (135-137).

The thermogram of the binary mixture of SC and mannitol (Figure 3.8) displays a sharp peak at 167°C, indicating the presence of mannitol, in addition to a smaller and broad endothermic peak at 176.7°C. The peak for SC is not present in the thermogram indicating that at high temperatures an interaction between SC and mannitol occurs. This interaction is more than likely due to a small amount of reducing sugar that may be present in mannitol (138; 139), thereby precipitating a Maillard reaction.

The Maillard reaction typically occurs between a reducing sugar and a primary amine however it may also occur in the presence of secondary amines (98). A schematic representation of the Maillard reaction is depicted in Figure 3.6 in which the glycosidic hydroxyl functional group of a reducing sugar interacts with the primary or secondary amine to form an imine structure (103).

An IR scan of a binary mixture SC and mannitol was performed as described in §3.3.4 and all characteristic bands of SC were visible (Figure 3.25). Therefore, due to evidence of a potential interaction when using thermal analysis, long term stability testing may well be necessary for dosage forms in which therapeutic amounts of SC are included with excipients such as mannitol.

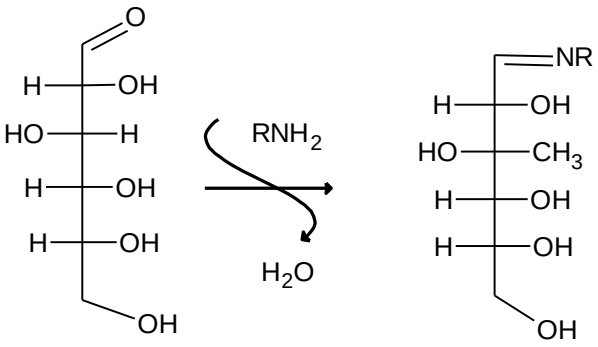


Figure 3.6 Maillard reaction with secondary amine

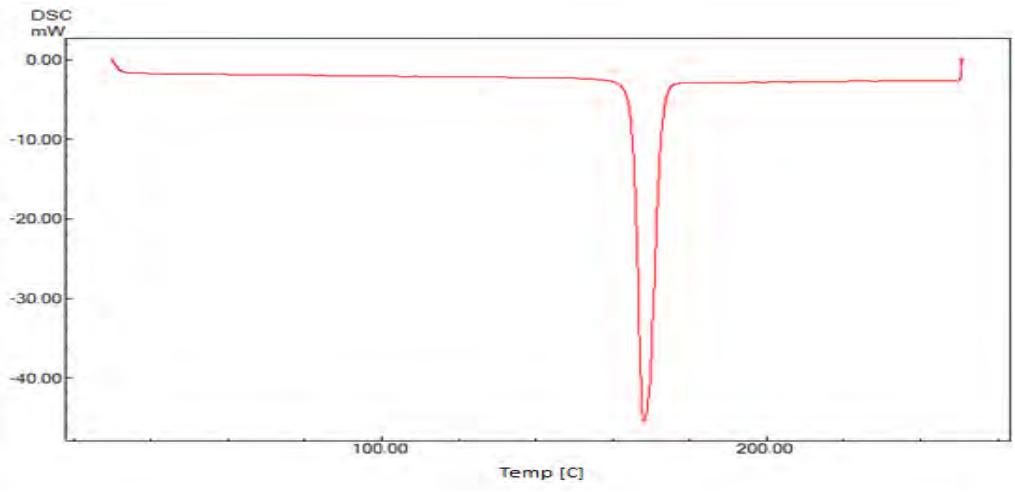


Figure 3.7 DSC thermogram of mannitol generated at a heating rate of 10°C/min.

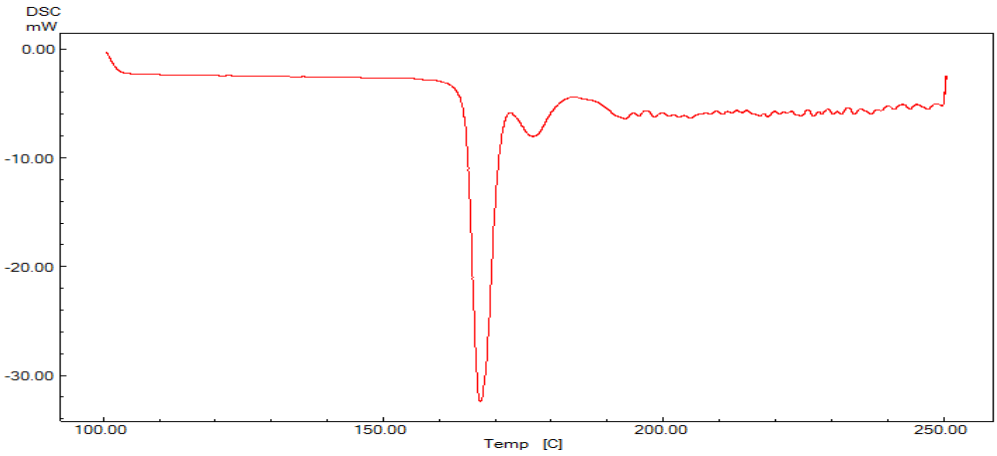


Figure 3.8 DSC thermogram of a binary mixture of SC and mannitol generate at a heating rate of 10°C/min.

The thermogram generated following DSC analysis of CMS (Figure3.9) reveals no major thermal events in the range 30-250°C as CMS does not melt in this range. However, there is a small but broad peak that occurs over the range 30-70°C which most likely can be attributed to the volatilization of water.

Analysis of a binary mixture of SC and CMS (Figure 3.10) shows that there are no interactions between SC and CMS as only a solitary peak; representative of SC was observed at 195°C.

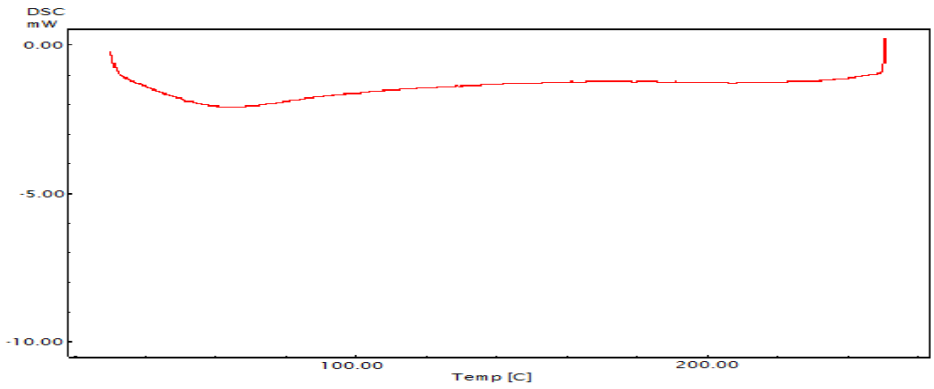


Figure 3.9 DSC thermogram of CMS generated at a heating rate of 10°C/min.

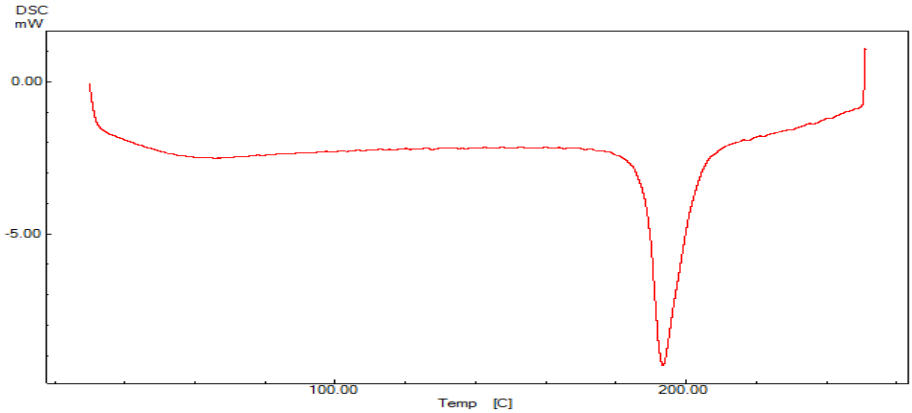


Figure 3.10 DSC thermogram of a binary mixture of SC and CMS generated at a heating rate of 10°C/min.

The DSC thermogram generated following the analysis of CRP (Figure 3.11) reveals the presence of a broad endothermic peak from 30-80°C which is possibly due to the volatilization of adsorbed water (140). No other thermal events were observed in the range investigated as CRP melts at a temperature above those to which the materials were exposed.

Analysis of a binary mixture of CRP and SC revealed no interactions similarly to the DSC analysis of CMS.

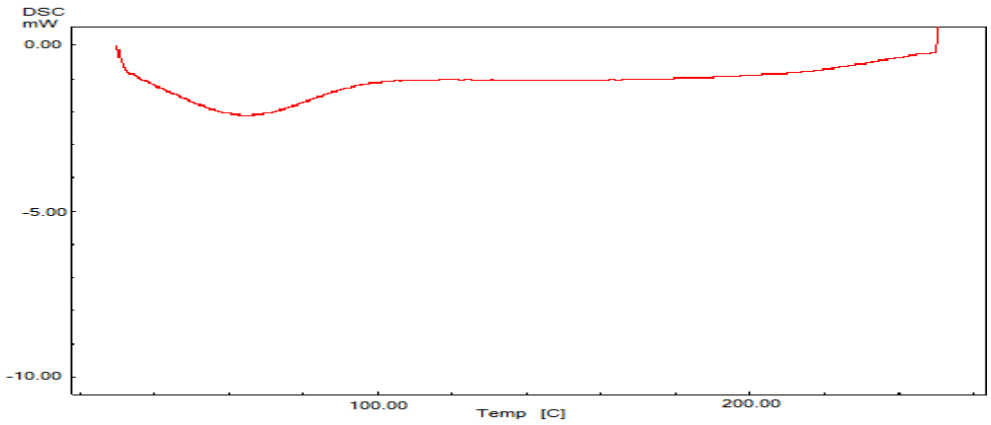


Figure 3.11 DSC thermogram of CRP generated at a heating rate of 10°C/min.

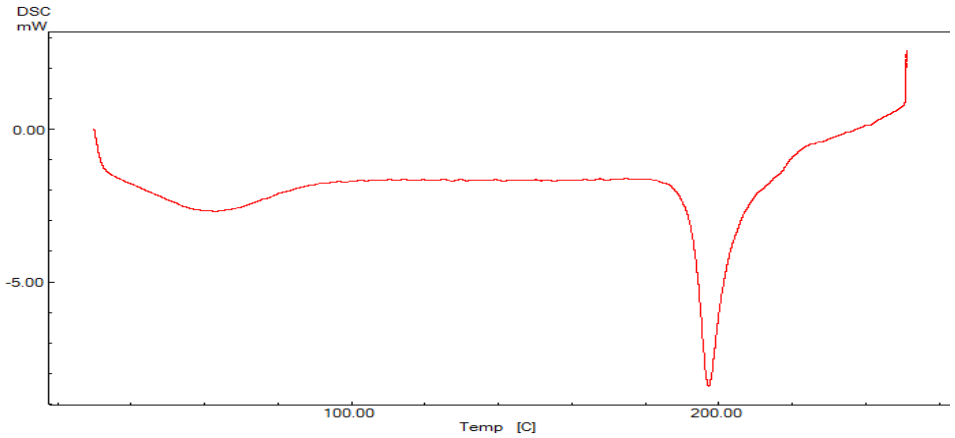


Figure 3.12 DSC thermogram of a binary mixture of SC and CRP generated at a heating rate of 10°C/min.

No endo- or exothermic peak events were observed when SSG was analysed using DSC as the material does not melt, but rather chars or burns at 200°C. There is however a slight endothermic peak at approximately 60°C which is most likely due to volatilization of water. The thermogram generated following thermal analysis of a binary mixture of SC and SSG revealed only one endothermic peak at 195°C that was characteristic of SC, indicating that SC and SSG are more than likely compatible.

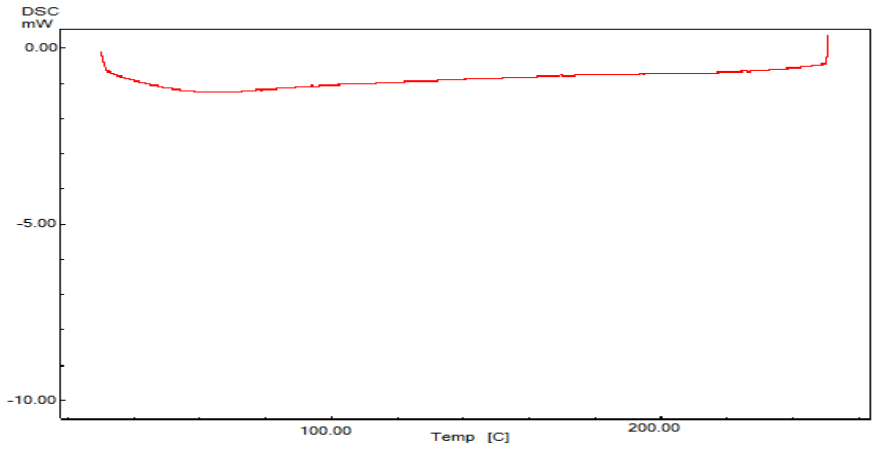


Figure 3.13 DSC thermogram of SSG generated at a heating rate of 10°C/min.

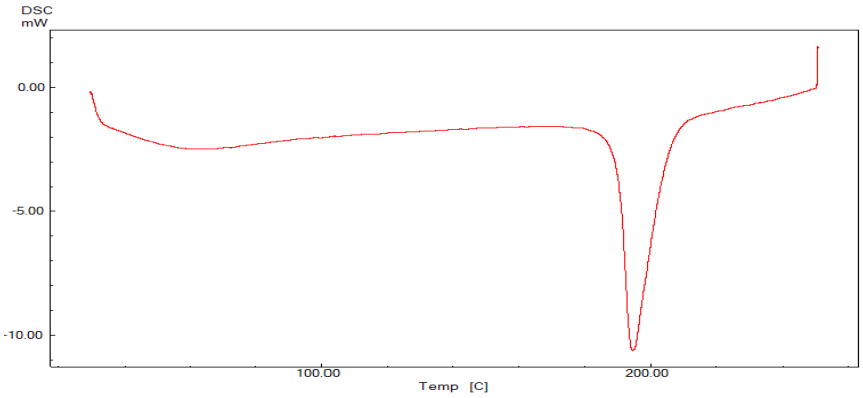


Figure 3.14 DSC thermogram of a binary mixture of SC and SSG generated at a heating rate of 10°C/min

The thermogram generated following DSC analysis of magnesium stearate (Figure 3.15) shows an endothermic peak at a temperature of 110°C that is representative of the melting point of magnesium stearate. There is an additional exothermic peak at a temperature of 182°C that is due to the crystallization of magnesium stearate (141).

Analysis of a binary mixture of SC and magnesium stearate (Figure 3.16) reveals that there is a potential interaction between the API and lubricant magnesium stearate as there appears to be a decrease in the melting point of SC.

It has been suggested that the interaction between API and the lubricant may be due to the presence of impurities, such as magnesium oxide in the magnesium stearate that have the ability to catalyze degradation by altering the pH of the micro-environment to a more alkaline condition. However in

terms of long term compatibility this is unlikely as SC was shown to be stable under alkaline conditions as reported in §2.5.7.2.2.

A more appropriate explanation for the interaction between SC and magnesium stearate has been described by Miller and York (142) who proposed a possible mechanism for the interaction that was observed between acetylsalicylic acid and magnesium stearate. Their position was that the small particle size of magnesium stearate permits the formation of a surface film around the API and the close contact between API and excipient lowered the melting point of acetylsalicylic acid. This scenario may well exist with SC and magnesium stearate, with the fine magnesium particles coating the SC particles and lowering the melting point.

Eutectic mixtures of two or more compounds have a melting point that is lower than each of that of the separate compounds (143) and ibuprofen, for example, in the presence of stearate salts formed a eutectic mixture with a lowered melting that then sublimates (144). This may also have occurred between SC and magnesium stearate but requires further investigation.

Infrared absorption scans show magnesium stearate and SC are compatible at room temperature (§3.2.4).

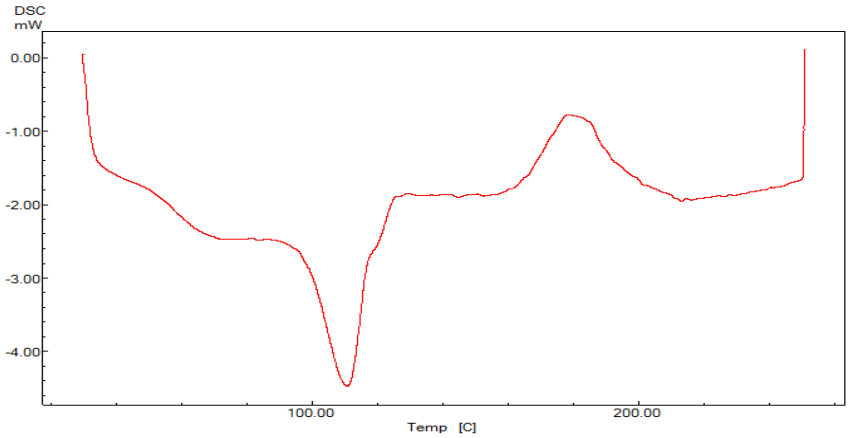


Figure 3.15 DSC thermogram of magnesium stearate generated at a heating rate of 10°C/min.

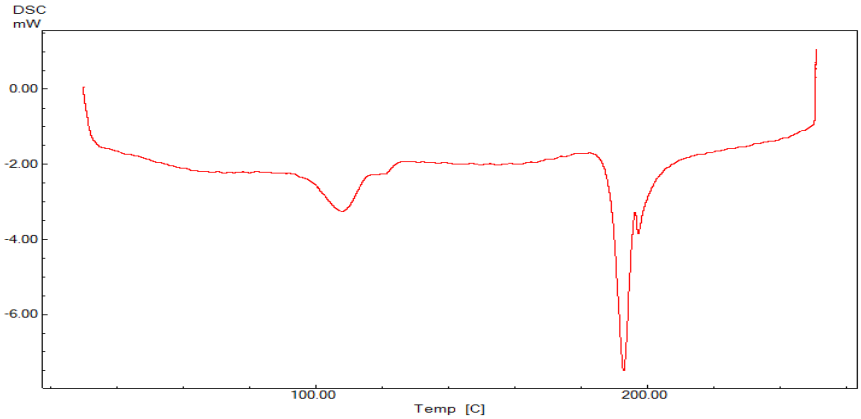


Figure 3.16 DSC thermogram of a binary mixture of SC and magnesium stearate generated at a heating rate of 10°C/min.

No endothermic or exothermic events were observed in the range 30-250°C following DSC analysis of CSD (Figure 3.17). This result is not surprising as CSD melts at a temperature of 1610°C and therefore falls outside the temperature range investigated. The thermogram of a binary mixture (Figure 3.18) of SC and CSD revealed one endothermic peak at 195°C confirming that no interaction between SC and CSD occurs.

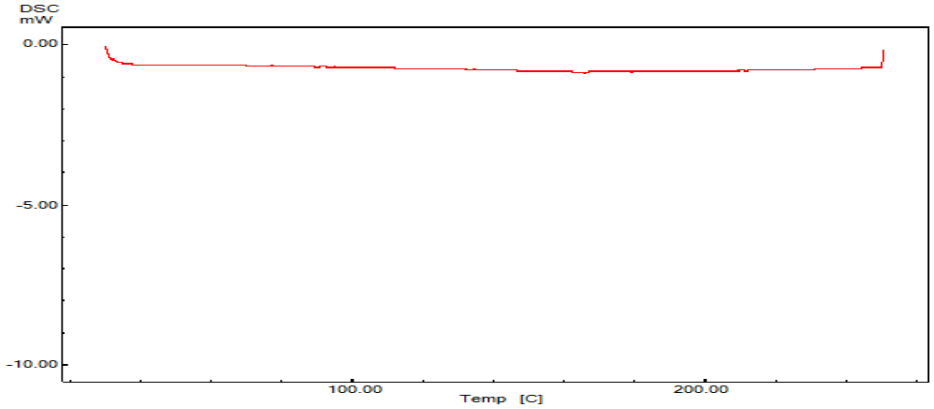


Figure 3.17 DSC thermogram of CSD generated at a heating rate of 10°C/min.

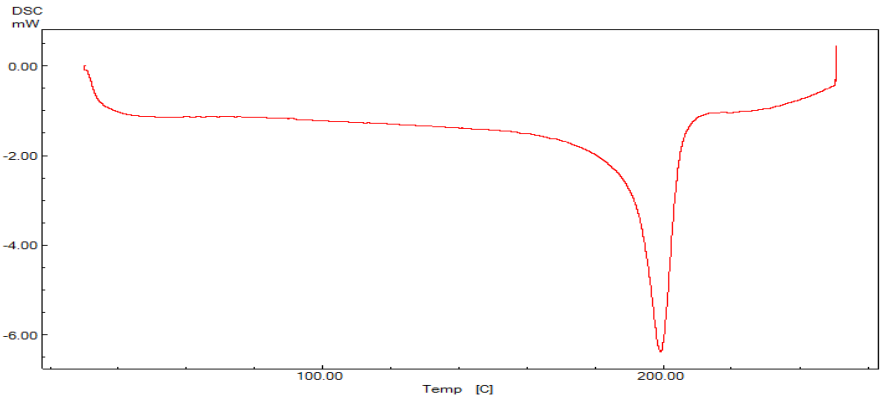


Figure 3.18 DSC thermogram of a binary mixture of SC and CSD generated at a heating rate of 10°C/min.

The DSC thermogram generated following the analysis of talc (Figure 3.19) revealed no peaks as talc has a melting point of approximately 1500°C (145) and therefore does not fall within the temperature range investigated.

Analysis of a binary mixture of SC and talc (Figure 3.20) once again revealed one endothermic peak at 196°C confirming that there are no interactions between the API and talc.

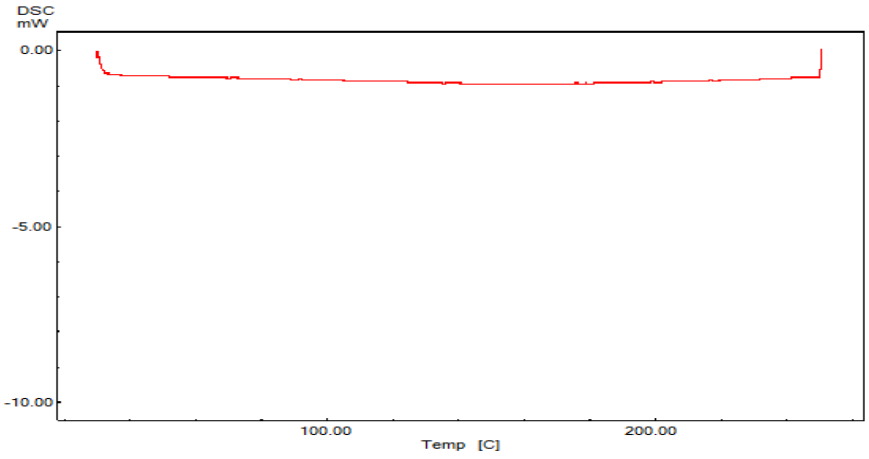


Figure 3.19 DSC thermogram of Talc at a heating rate of 10°C/min

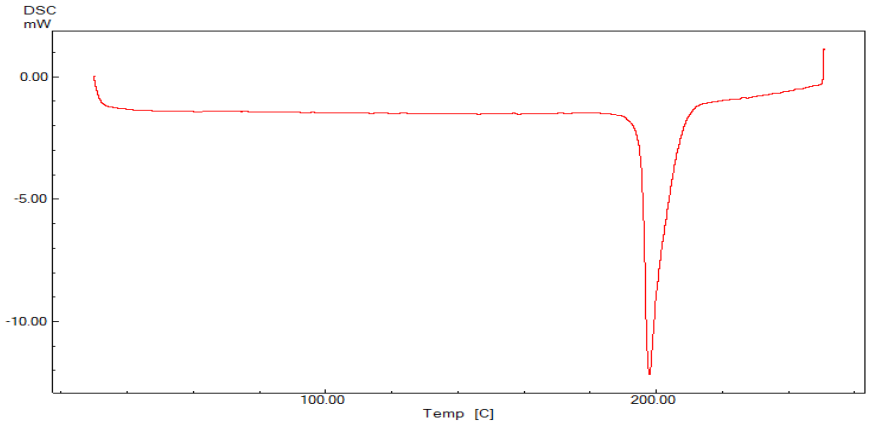


Figure 3.20 DSC thermogram of a binary mixture of SC and talc at a heating rate of 10°C/min

3.2.5 IR Absorption Spectroscopy

IR absorption spectroscopy is used extensively in the pharmaceutical industry for the identification of drug molecules and to confirm molecular structures (146).

The absorption band frequencies used for the interpretation of IR spectra is listed in Table 3.7.

Table 3.7 Infrared absorption band assignment of SC (15)

Wave Number (cm-1)	Group Assignment
3613	OH stretching
3293	NH stretching
3028	CH stretching (aromatic)
3000-2270	CH stretching (aliphatic)
1700	C=O stretching
1600-1500	C=C stretching
1358, 1174	SO_2 stretching
1240	CN stretching

SC has several significant peaks (Figure 3.21). The peak at 1240cm^{-1} is representative of the bridging of the two different ring systems *viz.,* the pyrazolopyrimidine and piperazine ring systems and the presence of this band in an IR absorption spectrum is crucial as it confirms that the two ring systems are attached (126). Another important peak is the broad band found in the spectrum that appears at a wave number of 1700cm^{-1} and is attributed to the C=O functional group present on the pyrazole ring system (126).

The band observed at 3293cm^{-1} represents NH symmetric and asymmetric stretching. The enlargement of peaks in the range 3600-2700cm^{-1} is due to the presence of hydrogen bonds in the citrate complex (120). The three important peaks observed in this range are 3028cm^{-1}, 2942cm^{-1} and 2874cm^{-1} and are attributed to the =C-H aromatic, CH_3 asymmetric and CH_3 symmetric vibrations in the molecule (120).

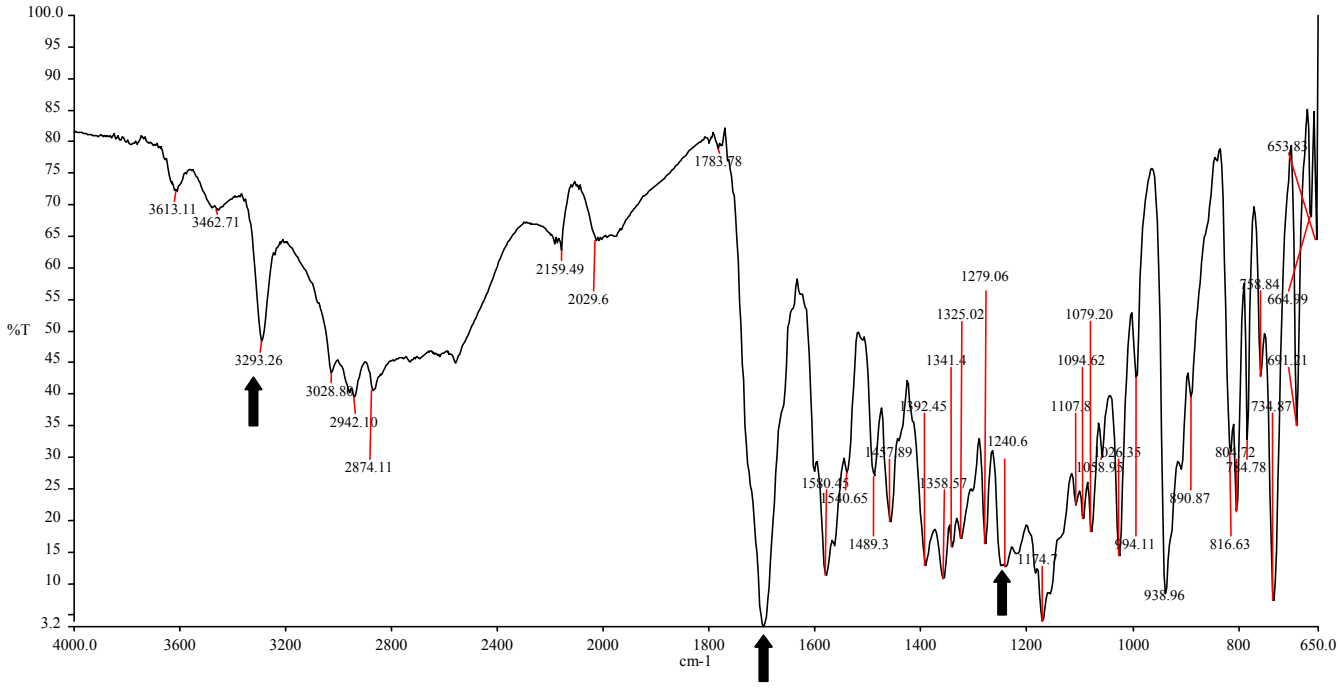


Figure 3.21 Infrared absorption spectrum of SC

Infrared spectroscopic analysis of MCC (Figure 3.22) reveals the presence of several characteristic peaks. The broad peak at 3286cm^{-1} is representative of OH functional group stretching while the peaks at 2899cm^{-1} and 1427cm^{-1} can be attributed to CH and CH_2 vibrations, respectively (147).

The IR spectrum of a 1:1 binary mixture of SC and MCC (Figure3.23) reveals that there are no overt incompatibilities reactions between the API and excipient with all representative bands of SC and MCC present in the IR absorption spectrum of the binary mixture.

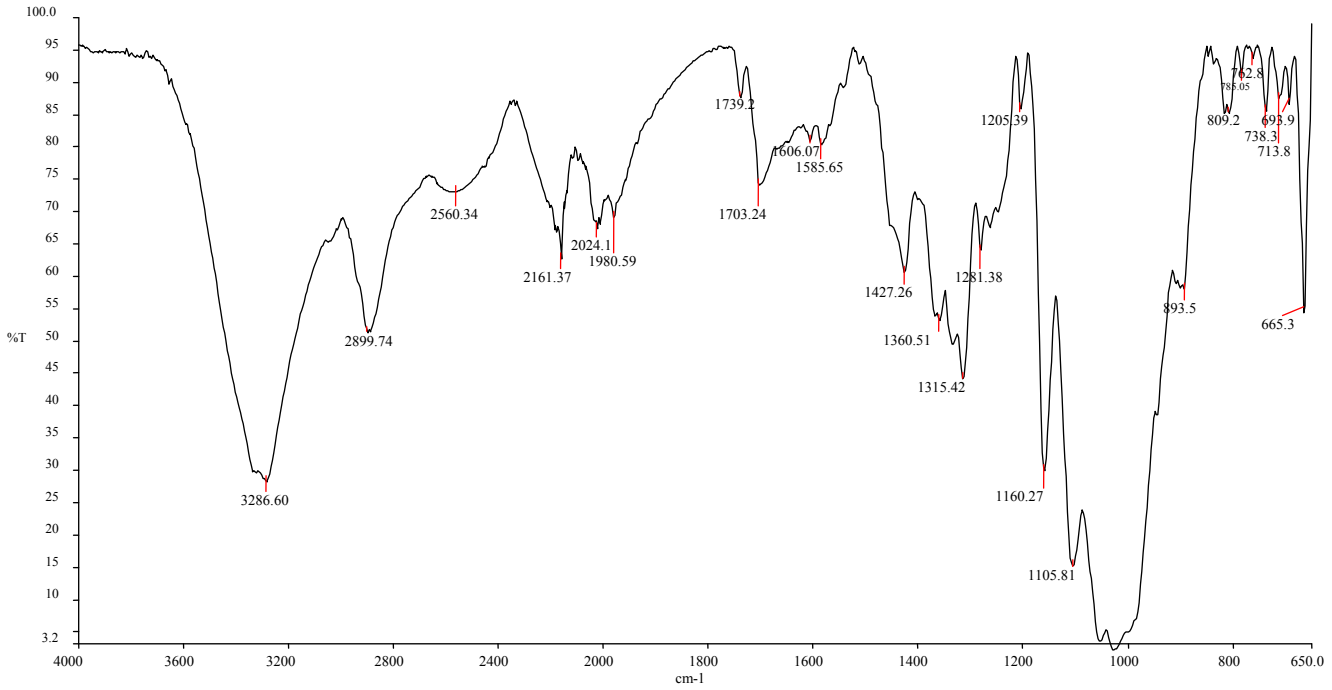


Figure 3.22 Infrared absorption spectrum of MCC.

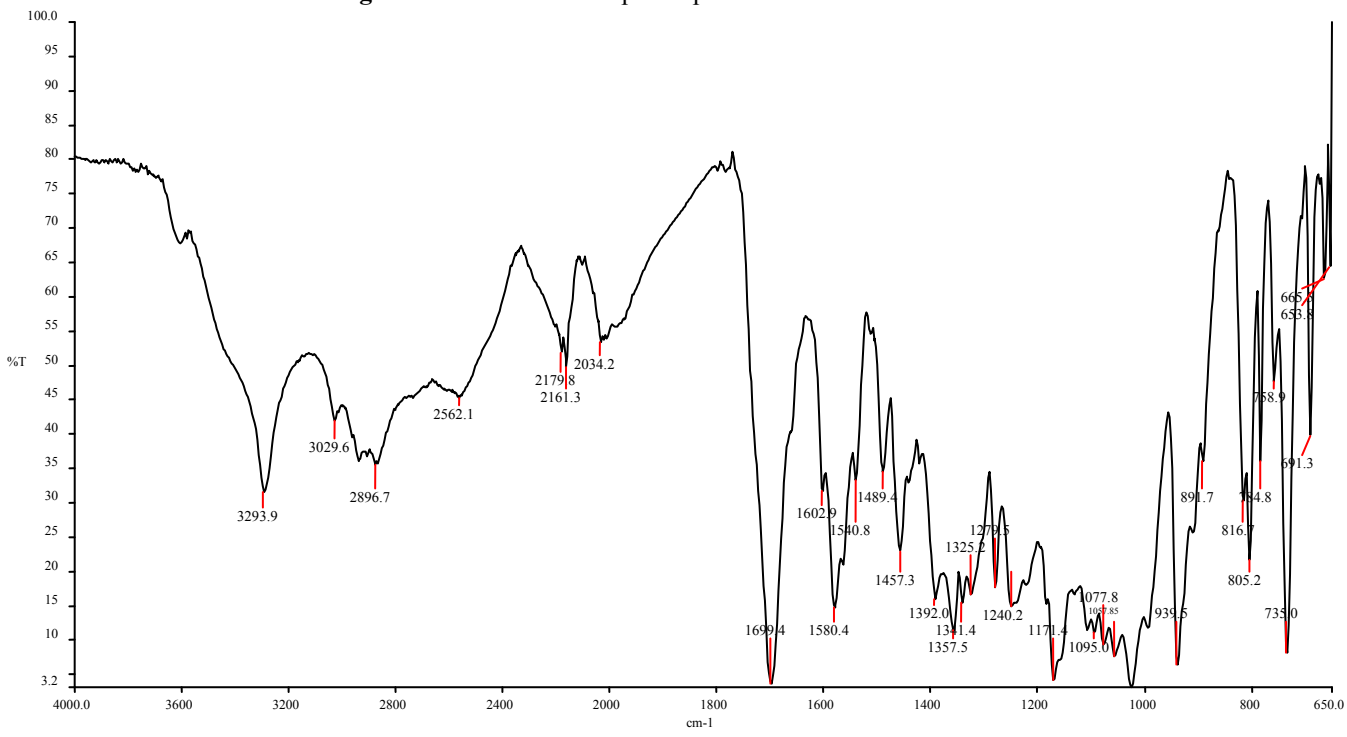


Figure 3.23 Infrared absorption spectrum of a 1:1 binary mixture of SC and MCC

The absorption spectrum for mannitol includes a number of characteristic peaks (Figure3.24). The very broad peak at 3387cm^{-1} represents OH band stretching. The three peaks at 2985cm^{-1}, 2949cm^{-1} and 2903cm^{-1} may be attributed to CH stretching while the peaks at 1280cm^{-1} and 1260cm^{-1} correspond to primary and secondary alcohol OH plane deformation (148). The peaks at 1077cm^{-1} and 1040cm^{-1}represent primary and secondary alcohol CO stretching, respectively (148).

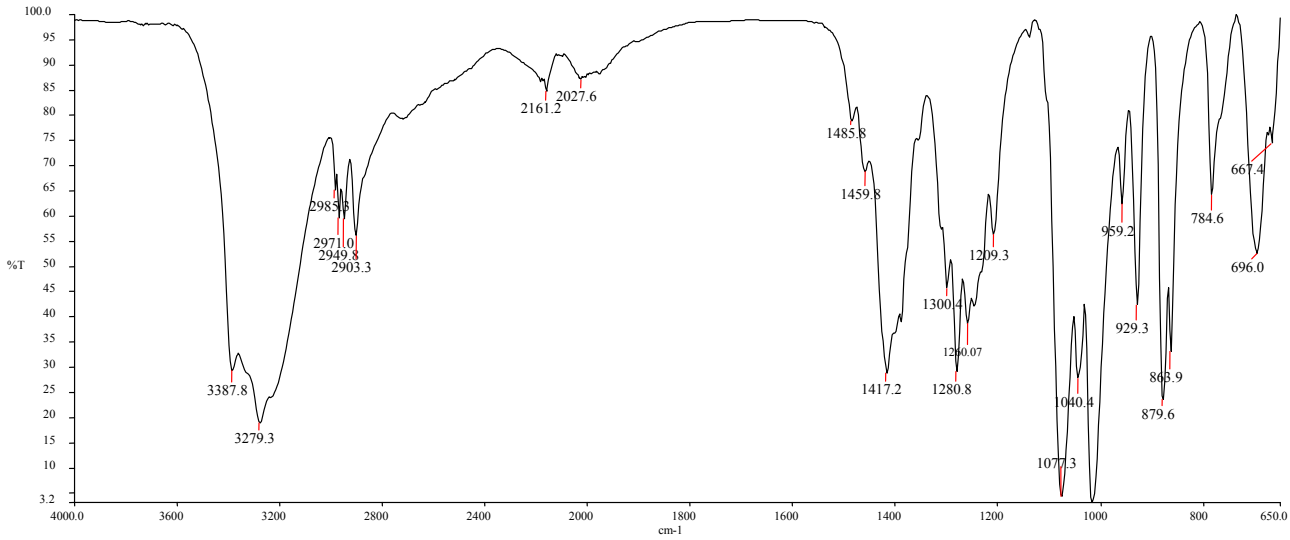


Figure 3.24 Infrared absorption spectrum of mannitol.

To ensure that no interaction between SC and mannitol occurred, an IR spectrum of a 1:1 binary mixture of SC and mannitol was generated (Figure3.25) and revealed the presence of all characteristic peaks for SC and mannitol. DSC results showed that there is an incompatibility between mannitol and SC. However due to the fact that DSC analysis takes place at elevated temperatures, IR was used to determine whether that interaction also occurs at room temperature. It was thought that the Maillard reaction had occurred with reducing sugars found in mannitol, reacting with the secondary amine to form an imine. The spectrum highlights that no imine product was formed as a characteristic peak for this material would appear at a wave number of 1630cm^{-1} (149). The absence of this peak confirms the fact that at room temperatures a Maillard reaction is not likely to have taken place.

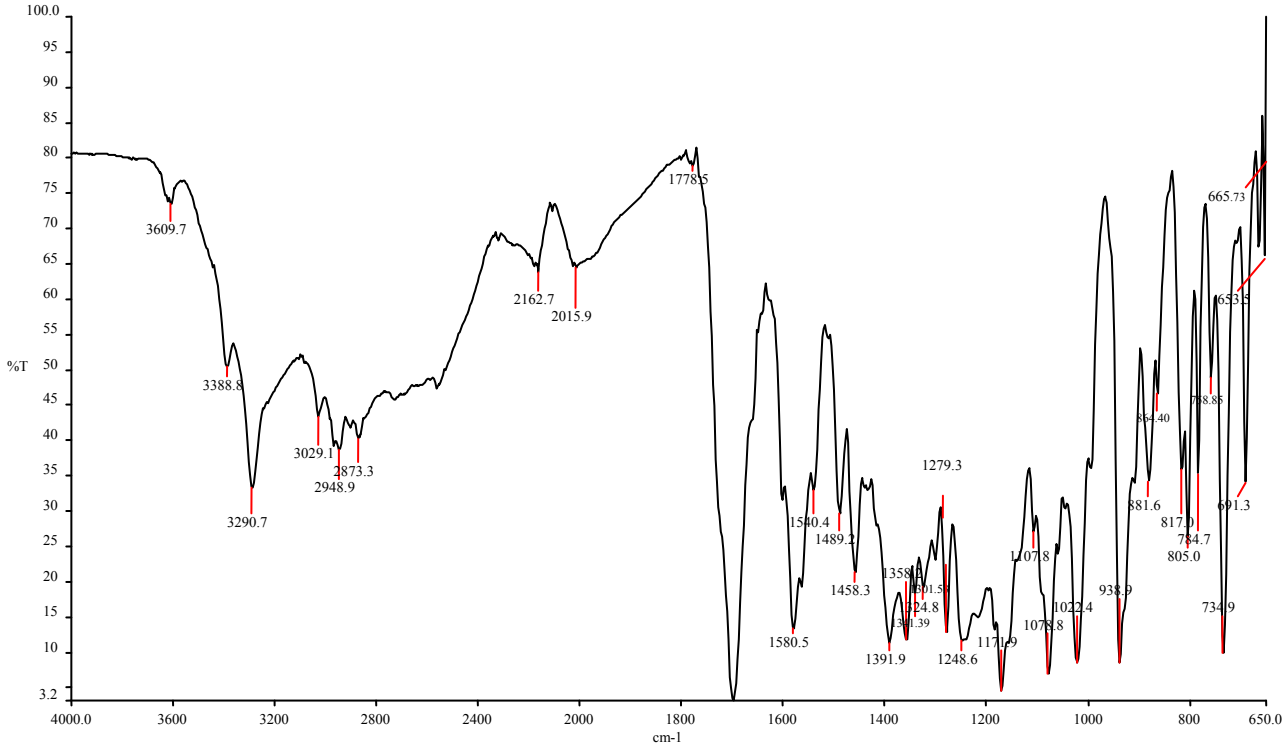


Figure 3.25 Infrared absorption spectrum of a 1:1 binary mixture of SC and mannitol.

IR spectroscopic analysis of CMS reveals the presence of a broad peak at a wavelength of 3275cm^{-1} (Figure 3.26) that is representative of H-bonding while the broad peak at 1015cm^{-1} is characteristic of the C-O bonds.

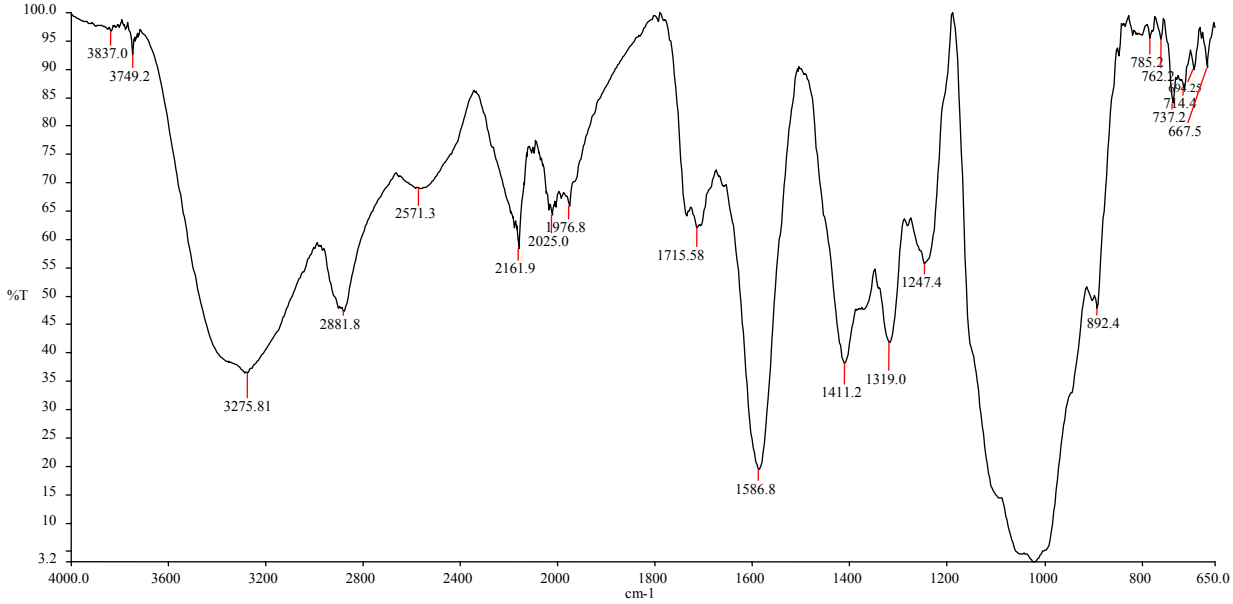


Figure 3.26 Infrared absorption spectrum of CMS.

The infrared absorption spectrum of a 1:1 binary mixture of SC and CMS (Figure 3.27) reveals no interaction between the two powders with the characteristic peaks of SC visible in the spectrum.

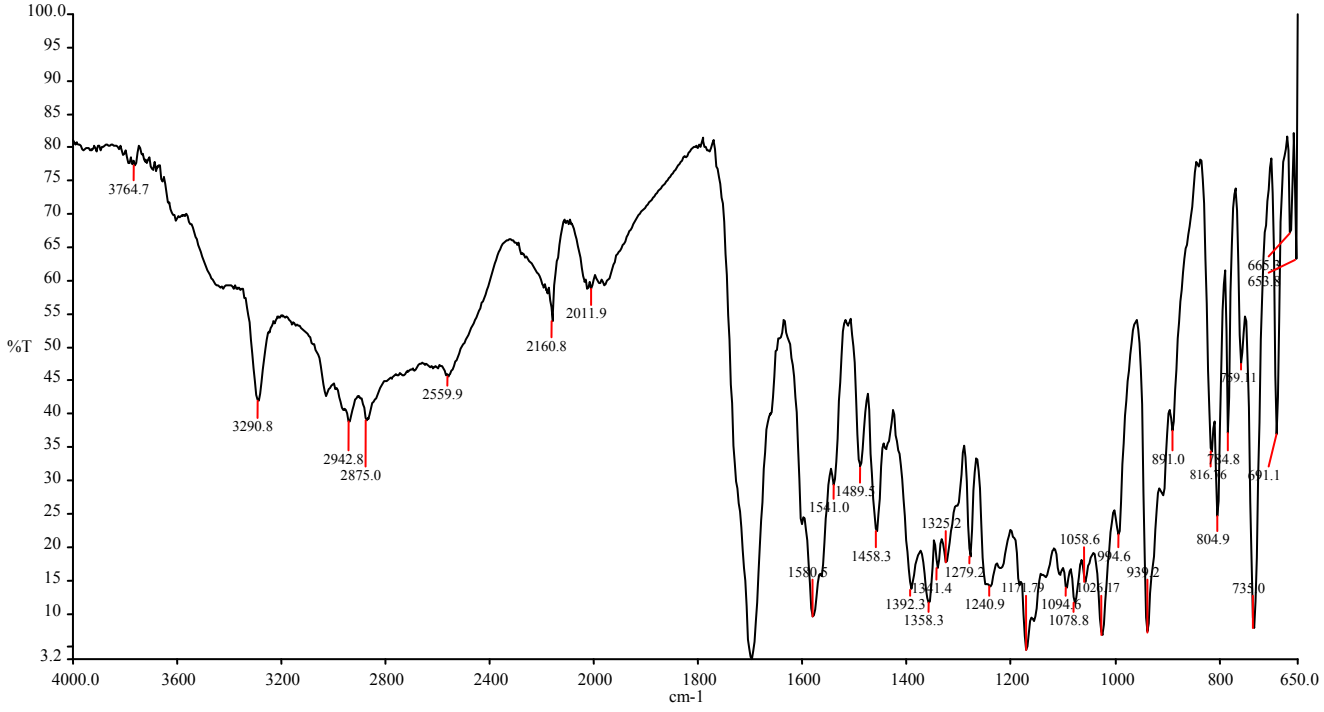


Figure 3.27 Infrared absorption spectrum of a 1:1 binary mixture of SC and CMS

The IR absorption spectrum of CRP (Figure3.28) reveals the presence of a peak at 2944cm^{-1} which is representative of aliphatic CH stretching. The prominent peak at 1678cm^{-1} is attributed to carbonyl stretching while CH_2 band stretching is represented by a peak at a wavelength band of 1420cm^{-1} (123). Analysis of a 1:1 binary mixture of SC and CRP (Figure3.29) revealed the presence of no incompatibilities. The peak at 1678cm^{-1} was not observed, most likely due to peak overlapping as the IR spectrum of SC has a characteristic long and broad peak at 1700cm^{-1}.

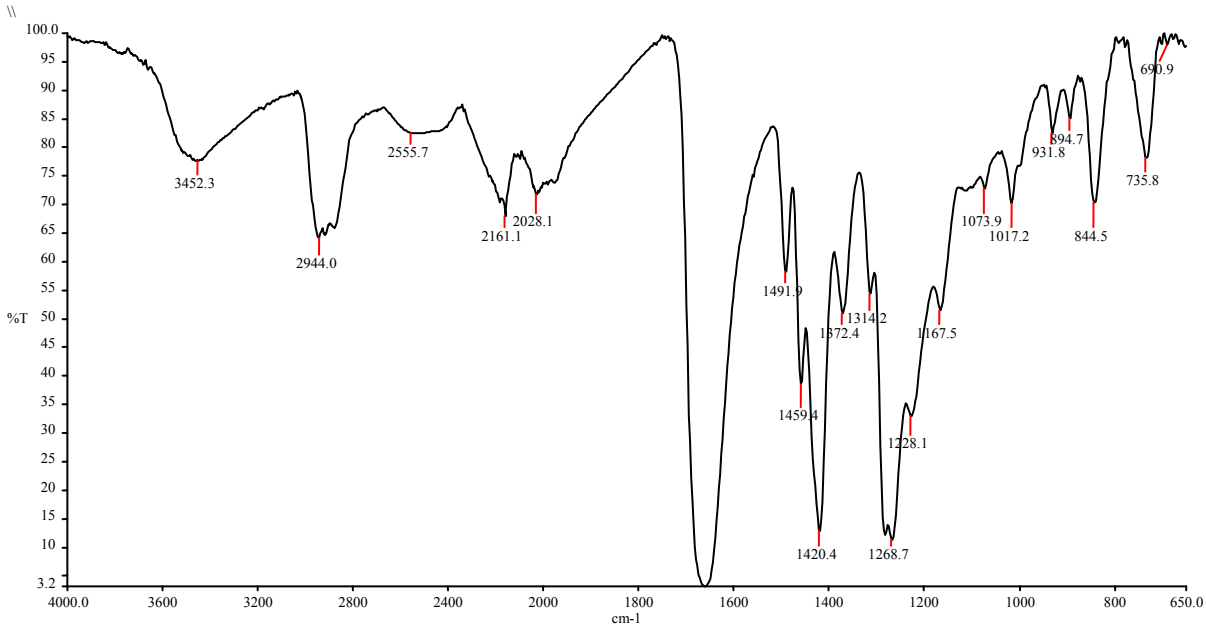


Figure 3.28 Infrared absorption spectrum of CRP.

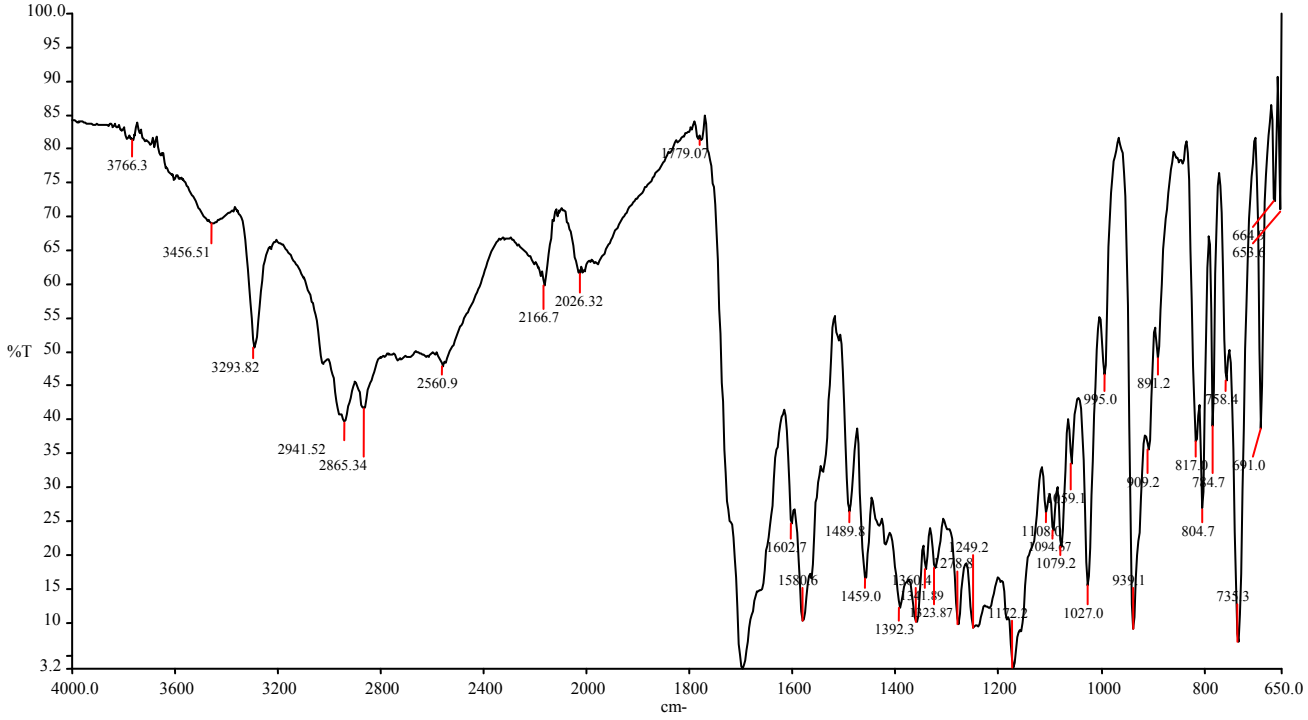


Figure 3.29 Infrared absorption spectrum of a 1:1 binary mixture of SC and CRP.

IR analysis of SSG (Figure 3.30) reveals a broad peak at 3290cm^{-1} that is attributed to H bonding with the broad band at 1000cm^{-1} being representative of the C-O bonds.

The infrared absorption spectrum of a 1:1 binary mixture (Figure 3.31) highlights the fact that no interactions are likely to occur between SC and SSG with all important peaks of not only SC but also SSG visible in the binary mixture spectrum.

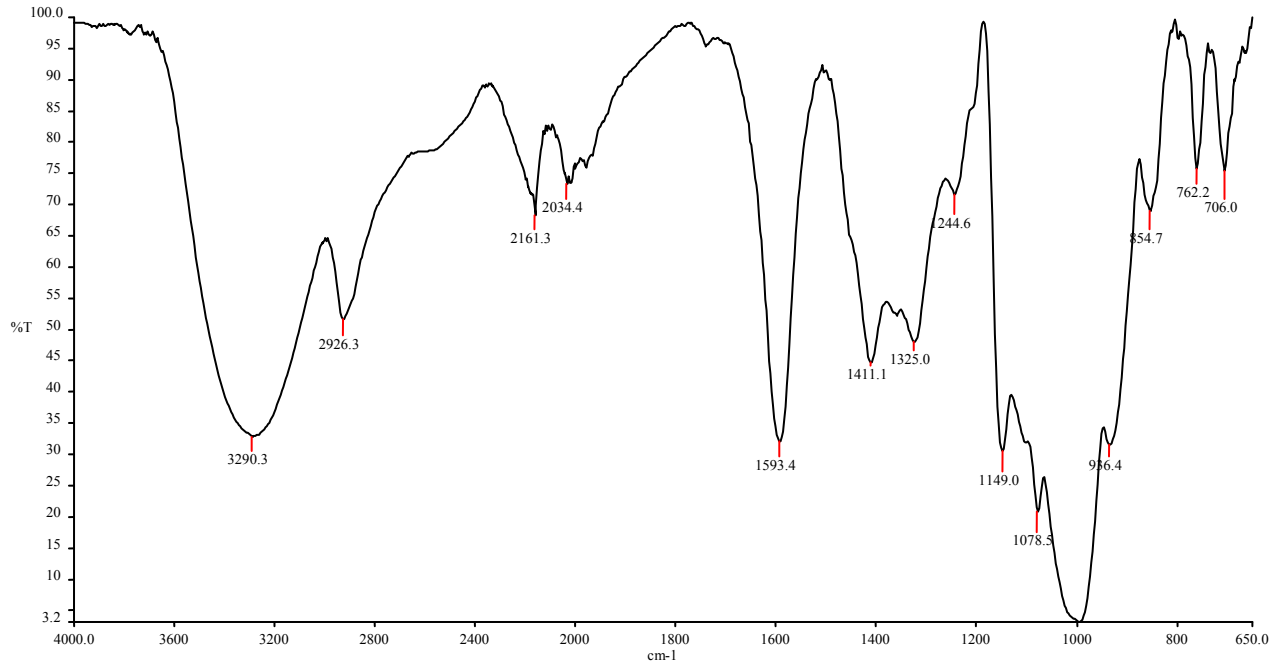


Figure 3.30 Infrared absorption spectrum of SSG.

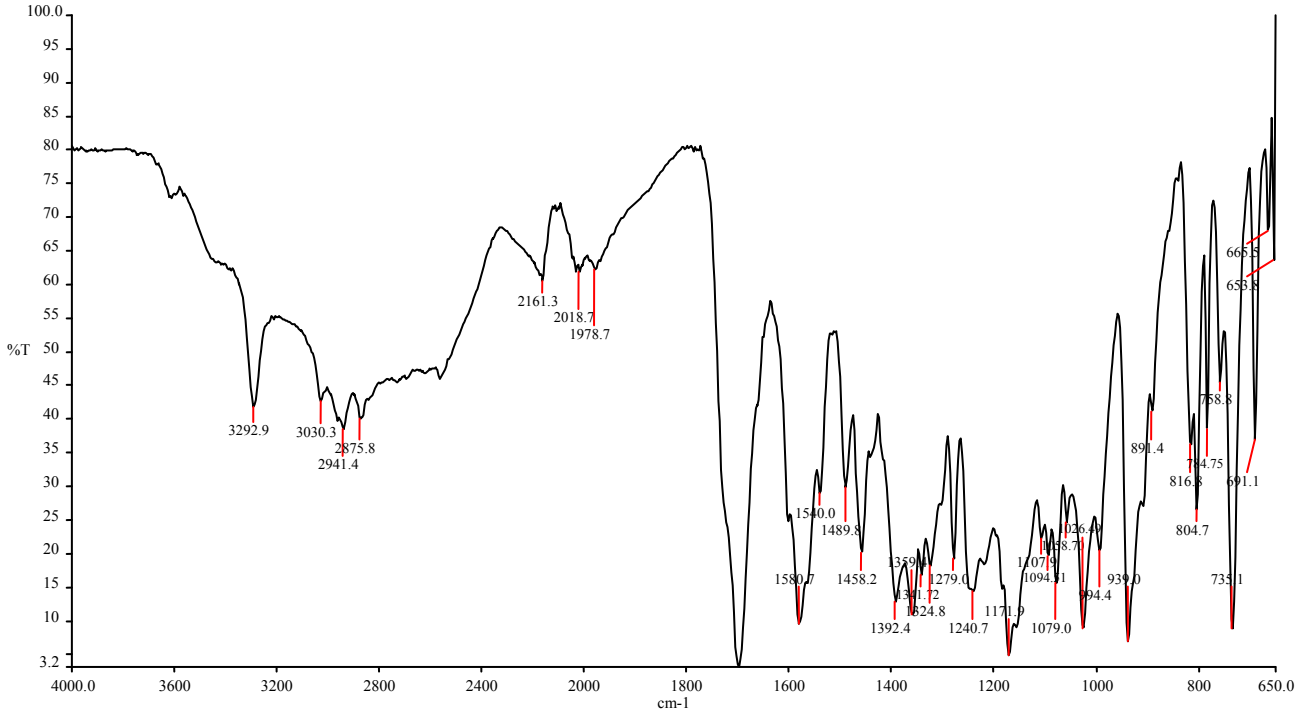


Figure 3.31 Infrared absorption spectrum of a 1:1 binary mixture of SC and SSG.

The OH stretching vibration of the magnesium hydroxide ($MgOH_3$) in talc (Figure 3.32) is represented at a wave number of 3677cm^{-1} (150). The broad peak at approximately 990cm^{-1} is due to Si-O-Si bond vibration while the peak observed at 665cm^{-1} is attributed to Mg-O-Si vibrations (151).

The IR spectrum of a 1:1 binary mixture of SC and talc (Figure 3.33) reveals that there is no interaction between the two powders as all characteristic peaks of both the API and excipient are present. There is however a decrease in the intensity of the Si-O-Si vibration of the talc and the 1695cm^{-1} vibration of SC which is most likely due to the presence of hydrogen bonding. The use of DSC experiments confirm the compatibility of the two powders as discussed in §3.2.4.

There is also a decrease in the intensity of the 1700cm^{-1} vibration of SC that may be attributed to dilution of the drug in the binary mixture.

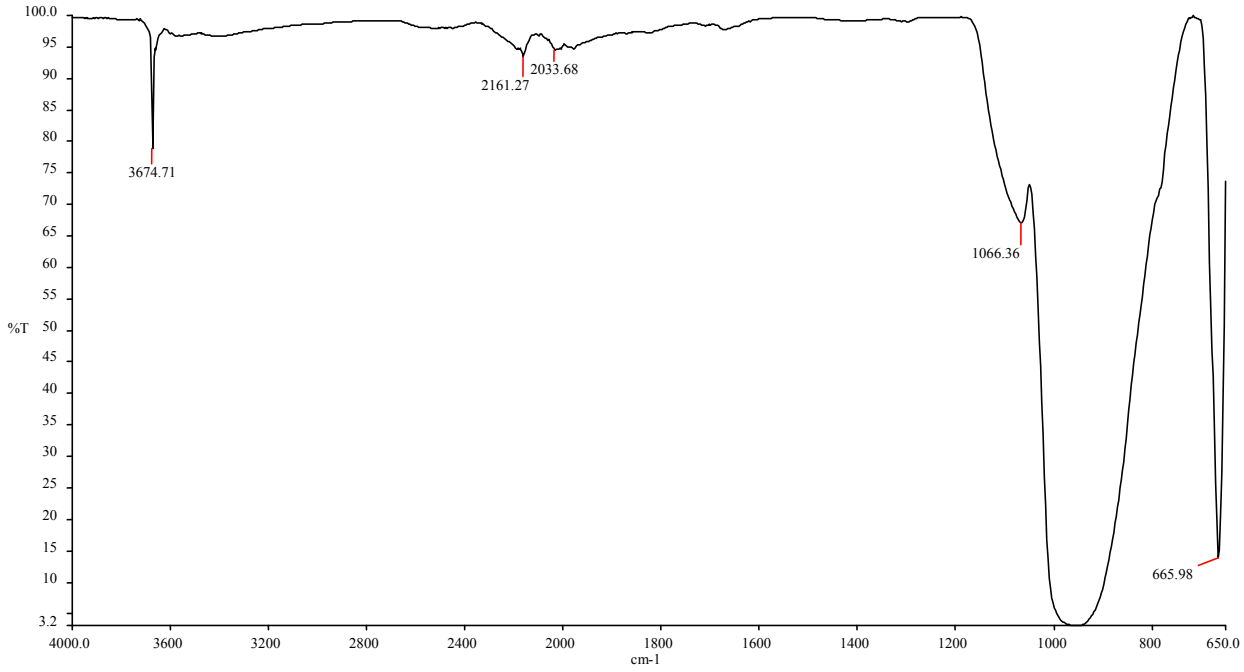


Figure 3.32 Infrared absorption spectrum of talc

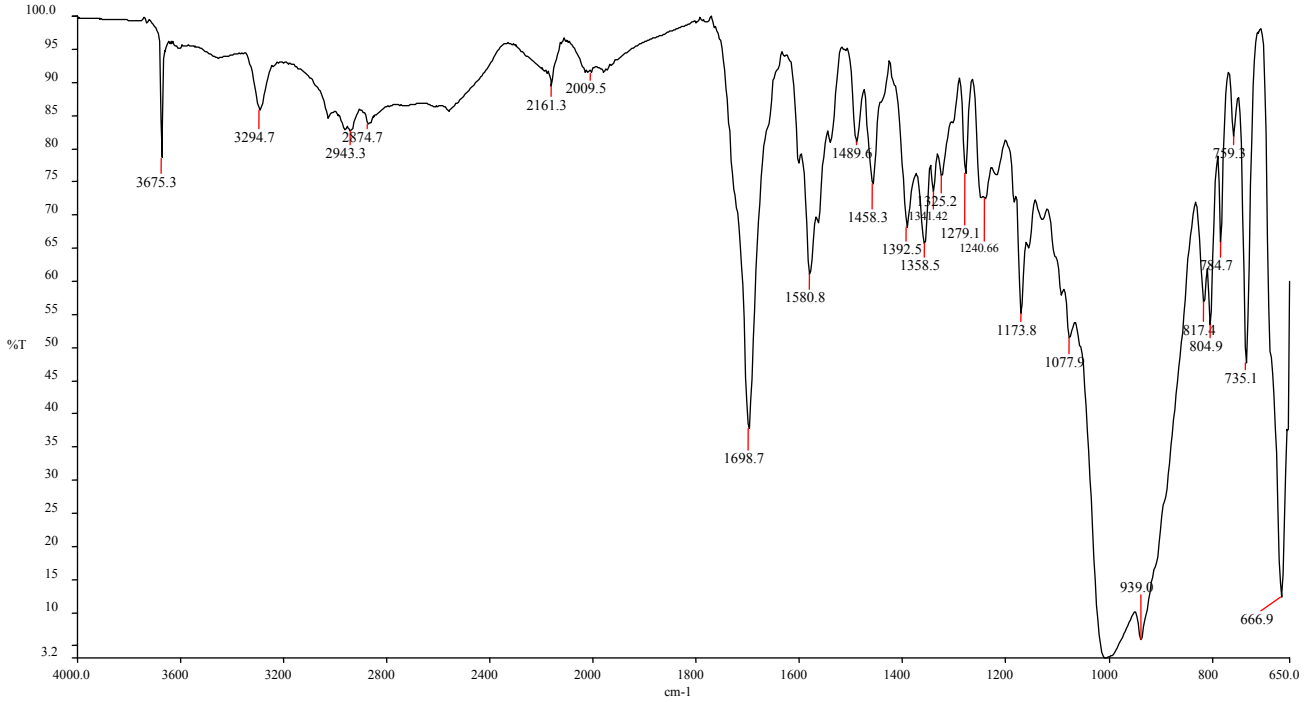


Figure 3.33 Infrared absorption spectrum of a 1:1 binary mixture of SC and talc

The IR spectrum for magnesium stearate (Figure 3.34) reveals twin peaks at 1576 and 1455 cm^{-1} which are attributed to the presence of asymmetric carboxylate (COO^-) stretching and symmetric carboxylate stretching, respectively. The peaks at 2955 and 2849 cm^{-1} represent C-H stretching while the broad peak at approximately 3257 cm^{-1} represents OH stretching of the associated water molecule (152).

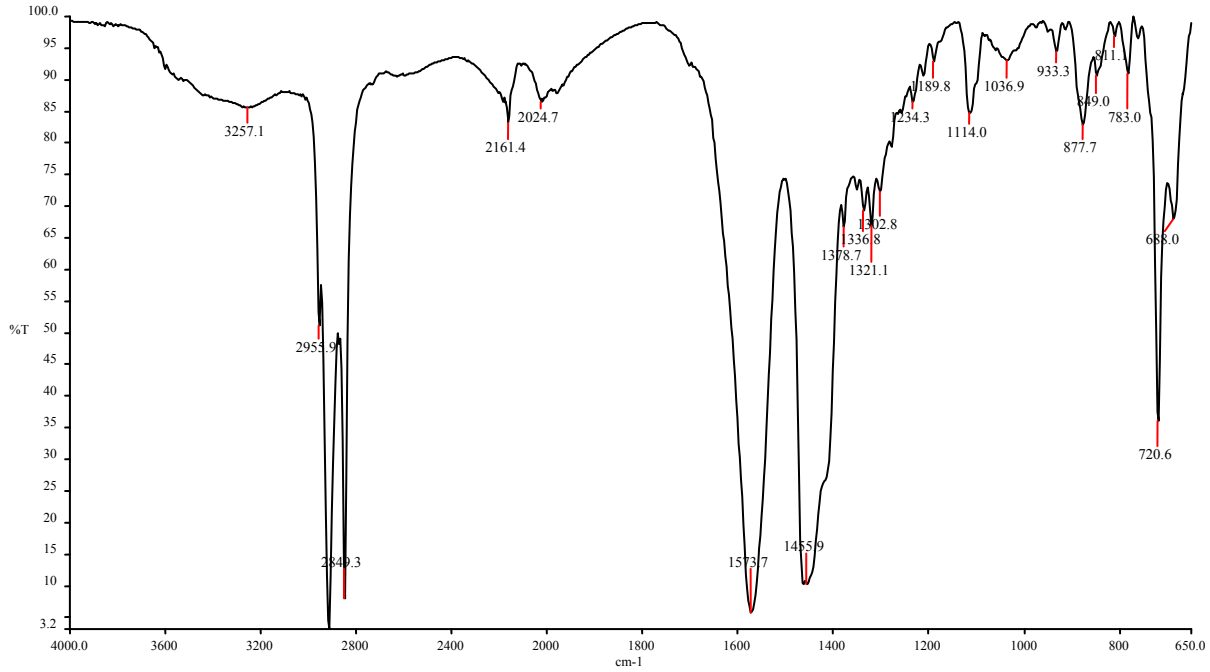


Figure 3.34 Infrared absorption spectrum of magnesium stearate

The IR spectrum of a 1:1 binary mixture of SC and magnesium stearate (Figure3.35) reveals the absence of three peaks at 3042cm^{-1}, 2942cm^{-1} and 2874cm^{-1} of SC which is most likely due to peak overlapping of the strong twin peaks in the IR spectrum of magnesium stearate, which occur at the same wavelength .

The binary mixture of magnesium stearate and SC showed the band at 1700cm^{-1} representing the C=O functional group on the pyrazole ring is present however the intensity is not as great as seen in the SC IR absorption spectrum. This is most likely due to dilution of the drug with the magnesium stearate.

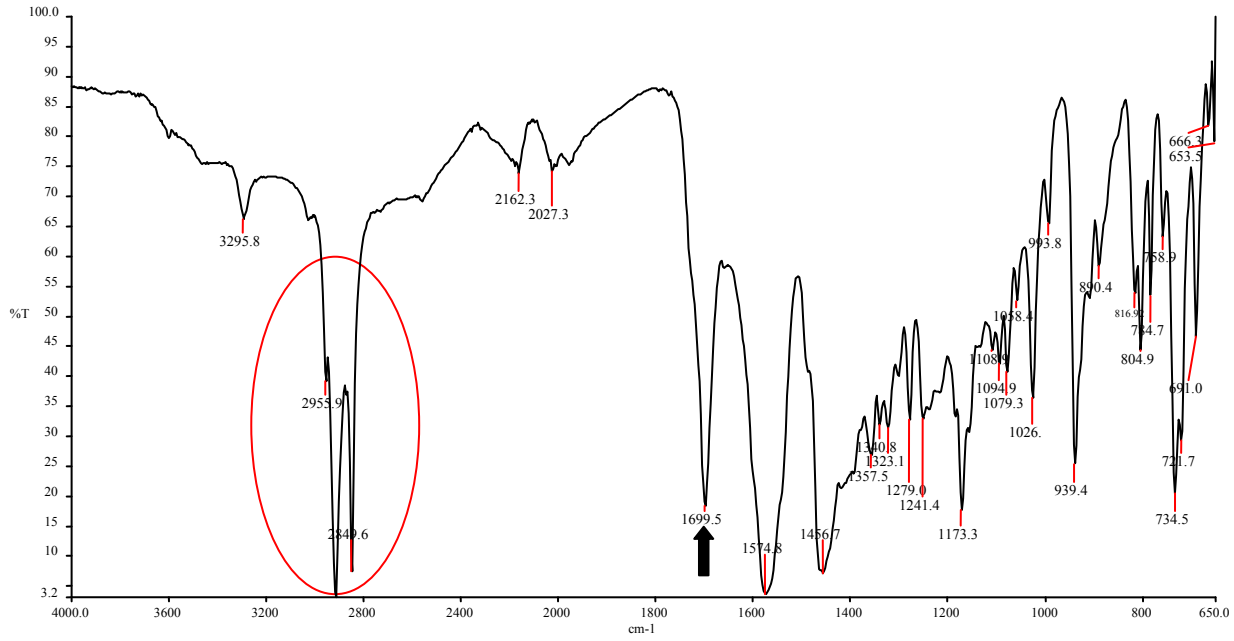


Figure 3.35 Infrared absorption spectrum of a 1:1 binary mixture of SC and magnesium stearate.

3.4 Conclusion

Preformulation studies form a strategic and critical step in the development of a dosage form. The results of these experiments provide the formulator with important information with regard to the potential behaviour of an API and excipients during the manufacturing process as well as elucidating information pertaining to the stability of an API alone or in combination with prospective excipients.

During preformulation studies it is important to establish the range of excipients that can be used in a formulation in order to ensure that an efficient drug product development process is undertaken. However it is imperative that the chemical and physical properties of an API are clearly understood, prior to commencing excipient selection studies (153).

SEM was successfully used to assess particle size and shape. SEM was chosen as the preferred method of microscopy as it is a high resolution technique that is easy to use and does not require large amounts of time to generate images. SEM results revealed that particle morphology may play an important role in the selection of excipients, and that certain excipients may need to be milled to facilitate the production of homogenous blends.

The true density, bulk and tapped densities of SC and potential excipients were determined. These results were then used to calculate the CI, HR and porosity of the raw materials. The importance of

these physical characteristics is that it allows the formulator to identify potential problems with regard to content uniformity of the dosage form. If the powder blend does not exhibit good flow properties the tablet dies will not be filled correctly, and hence the production of non-uniform tablets would occur.

Of all excipients evaluated, MCC exhibited good compressibility and porosity. Powder property assessments however revealed that the some excipients, for example CMS, displayed poor flow properties. The inclusion of a glidant would therefore be a most likely requirement for the successful manufacture of a quality and homogenous dosage form if those excipients were to be included in the formulation.

Thermal analysis is a popular analytical technique that provides crucial information with respect to the existence of polymorphic forms of an API as well as the compatibility of an API with excipients. However, as far higher temperatures than those to which a product would usually be exposed are used, preformulation studies should also include IR absorption spectroscopy to confirm or negate the results generated using DSC.

DSC analysis highlighted the fact that there are interactions between mannitol, magnesium stearate and SC, albeit at high temperatures. These interactions were not confirmed using IR analysis, which are performed at room temperature and therefore it can be inferred that these interactions are most likely due to the high temperatures that the binary mixtures were exposed to.

IR analysis revealed no incompatibilities between the API and the potential raw materials and showed that IR analysis used in combination with thermal analysis are important methods to ascertain interactions between API and drug excipients.

It can be concluded that preformulation studies conducted in this chapter revealed that none of the potential excipients showed any incompatibility with the intended API. However, long term real time stability studies may be necessary if these materials are to be used in the production of SC containing dosage forms. The data also provided an indication that with regard to the flowability characteristics of the raw materials, direct compression may be a suitable method of manufacture during the development of orodispersible SC tablets. This approach is further investigated and reported in Chapter Four.

CHAPTER 4

FORMULATION DEVELOPMENT AND MANUFACTURE OF FAST ORODISPERSIBLE SILDENAFIL CITRATE TABLETS

4.1 INTRODUCTION

Unless a disease affects the paediatric population to a large extent, most medicines are not tested or labelled for use in infants and children. The main reason for the lack of appropriate dosage forms for paediatric patients is the fact that neonates and infants are seen as being a relatively small population group and therefore provide a limited return on the substantial investment pharmaceutical companies make in drug product development studies (154). There is also the perception of a need for more stringent ethical considerations, legal liability and regulatory requirements when conducting clinical studies in children (154).

The lack of paediatric formulations is a worldwide challenge, with approximately 70% of drugs approved for adult use in the United States of America not labelled for use in infants and children (155). In Europe a similar situation is observed, where only 33% of all medicines licensed by the European Agency for Evaluation of Medicinal Products (EMEA) in the period from October 1995 to September 2005 were labelled for paediatric use, 23% for use in infants and 9% in newborn babies (156).

The lack of availability of suitable dosage forms for use in this population group often results in the use of extemporaneously manufactured medicines. Consequently it is common practice to crush SC tablets, dissolve the residue in water and to administer this mixture to the patient via a naso-gastric tube. Alternatively SC suspensions are manufactured using methylcellulose or a combination of Ora-Sweet and Ora-plus as the suspending agent (157). These suspensions have been shown to be stable over a period of 91 days with no change in pH, odour or physical appearance noted (157). Stability issues however are not the only concern with regard to extemporaneous preparations.

Extemporaneous preparations are not ideal dosage forms for use in paediatric patients as there are many issues in respect of the stability, safety and dosing of these preparations.

For example, potent drugs such as morphine are too concentrated for accurate measurement of the small doses required to treat neonates and infants (154). Therefore it may be necessary to dilute a commercial preparation, which may then result in the production of a dosage form in which the preservative efficacy is questionable. There is also the difficulty that in seriously ill neonates, fluid intake restrictions exist and there is a limit to the volume of medication that can be administered at any one time.

Due to the dangers of administering extemporaneously manufactured medications, different dosage forms that will allow for the safe and effective delivery of a useful API to paediatric patients need to be further investigated.

Fast dissolving and orodispersible tablets are currently an important research niche and are an alternate means of delivering an API, to extemporaneous preparations, since they can be easily administered to a paediatric patient and are formulated to dissolve and release the API for absorption, rapidly.

4.1.1 Tablet Compression

Solid oral dosage forms are the most popular method of formulating an API for administration as they are safe and convenient to administer and are economical to manufacture on a large scale, by the pharmaceutical industry (158).

The basic principles of tablet compression in which granular or powdered materials are compressed in a die between two punches using a tablet press have remained the same since the tablet, as a dosage form was initially conceptualized (159).

A typical manufacturing process for the production of tablets includes milling, granulation, drying, blending, compression and tablet coating (160), with each unit operation possibly further disaggregated into several additional processing steps.

The process of converting a powder into a tablet is fundamentally dependent on an interparticulate bonding process. The classification of these bonds is broadly divided into five types according to the Rumpf classification (161):

i. Formation of Solid bridges
ii. Bonding by liquids (capillary and surface tension forces)
iii. Binder bridges (viscous binders and adsorption layers)
iv. Intermolecular and electrostatic forces
v. Mechanical interlocking

In the case of direct compression the two predominant bonds thought to be important in ensuring dosage form integrity are those that occur due to intermolecular forces and the formation of solid bridges (161). Bonding by intermolecular forces is also known as adsorption bonding and occurs when two solid surfaces are brought into close contact so that adhesion or adsorption to one another is achieved. Common intermolecular forces include an der a al's forces and hydrogen bonding (162) in addition to dispersion forces(161).

The formation of solid bridges is also referred to as the diffusion theory of bonding (161). The formation of these bonds occurs when solids are mixed and become slightly fused at their interface, thereby forming a continuous phase between the two particles (161). It is the presence of the continuous face between particles that makes solid bridges the strongest of all bonds between particles in a tableting process (162).

Mechanical interlocking describes the hooking of irregular shaped particles (161) and it is thought to also play a role in the bonding of powders in directly compressed tablets, however they are considered to be less significant in comparison to the other two types of bonds, previously discussed.

4.1.2 Orodispersible Tablets (ODT)

Solid oral dosage forms are one of the more popularly used dosage forms, however they do have some shortcomings. The common drawback of these dosage forms is the difficulty that some patients have in swallowing and therefore their use may lead to non-adherence in patients, especially in the paediatric and geriatric populations (158; 163).

ODTs provide an alternate approach to delivering an API to these populations and can be referred to by variety of different names including orodispersible tablets, orally disintegrating tablets, quick disintegrating tablets, fast dissolving tablets, rapid dissolving tablets, porous tablets, quick melt tablets rapid melt tablets and fast disintegrating tablets. The European Pharmacopoeia has adopted the term orodispersible tablet "for a tablet that disperses or disintegrates in less than three minutes in the mouth before swallowing" (164).

In general, an ODT is a tablet that dissolves or disintegrates in the oral cavity without the need for administration with water or chewing to facilitate tablet break up.

An ideal ODT should (165):

i. require no water for oral administration, yet dissolve/disperse/disintegrate in the mouth in a matter of seconds.
ii. have a pleasing feel in the mouth.
iii. have an acceptable taste or be appropriately taste-masked.
iv. demonstrate acceptable hardness and friability properties.
v. leave minimal or no residue in the mouth after administration.
vi. exhibit low sensitivity to environmental conditions such as humidity.
vii. allow for the manufacture of the tablet using conventional processing and packaging equipment.

4.1.2.1 Advantages of ODT

ODT exhibit all the advantages of solid dosage forms including enhanced stability, accurate dosing, ease of handling by patients and a small packing size (164). In addition they are easily administered to persons that are unable to swallow, such as paediatric and elderly patients. Furthermore some API are absorbed from the mouth, pharynx and oesophagus as the saliva passes down alimentary canal into the stomach, and therefore formulating these compounds as an ODT can increase the bioavailability of that drug (166). This increased bioavailability is due to reduced pregastric metabolism and may also result in the need for administration of a lower dose with an associated decrease in unwanted side effects (166).

ODT can easily be administered to patients who do not have immediate access to potable water and therefore can be a valuable dosage form for delivering essential medicines in developing world countries.

4.1.2.2 Limitations of ODT

ODT tablets typically have low mechanical strength and are usually more friable than other solid dosage forms and therefore require special handling and packaging techniques (167).The tablets may also have an unpleasant feel in the mouth due to the presence of a high percentage of disintegrant(s) in the tablet formulations.

Tablets with large doses of API are, in general, difficult to formulate in comparison to low dose formulations due to the fact that if a formulation only requires a small amount of API, there is more freedom to incorporate excipients that facilitate disintegration to the formulation.

ODT may be difficult to administer to patients who take anti-cholinergic medication or who suffer from conditions that result in a dry mouth and decrease in salivary grand secretions, such as Sjogren's Syndrome (168).

A further disadvantage of ODT is that they are usually hygroscopic and therefore careful consideration with regards to manufacturing parameters, environment and packaging need to be taken into account (163).

4.1.3 Methods of manufacture of ODT

4.1.3.1 Direct compression

The direct compression method of manufacture is the simplest and most cost-effective approach to produce solid dosage forms. This manufacturing approach involves the use of conventional manufacturing equipment in addition to commonly available tableting excipients. Heat and water are not used in the manufacturing process and therefore the stability of the product may also be improved (161).

The manufacturing process involves only a limited number of individual steps and can be summarized as a method in which powders are blended and then compressed between two punches in a die to form a tablet (169).

The disintegration and dissolution of a ODT manufactured using the direct compression method is based on the activity of disintegrants and the presence of water soluble excipients. Disintegration efficacy is strongly affected by tablet size and hardness and large, hard tablets can have a disintegration time greater than those usually desired for application as ODT (170).

4.1.3.2 Moulding

Moulded ODT are manufactured using water soluble excipients. A powder mixture is moistened with a solvent, typically ethanol or water and then compressed into tablets under a pressure lower than that used for conventional tablet compression (170). More recently moulded dosage forms have been manufactured by preparing a molten matrix in which the drug is dissolved or dispersed (heat moulding) or by evaporating the solvent from the drug solution/suspension at standard pressure (no-vacuum lyophilisation) (170). Moulded tablets are less compact than compressed tablets and exhibit a porous structure that increases disintegration and subsequent dissolution of the API (168).

4.1.3.3 Freeze drying (Lyophilization)

Freeze drying was one of the first techniques applied to the manufacture of ODT (166) and is a process in which water is removed from a frozen product by sublimation. The API is dissolved or dispersed in an aqueous solution of a carrier/polymer and the resultant mixture is then poured into the wells of blister packs, after which the trays holding the blister packs are passed through a frozen liquid nitrogen tunnel to freeze the API solution (171). The freeze drying process continues after which the blister packs are moved to refrigerated cabinets.

The ideal characteristics of an API for use in a freeze drying process is a degree of aqueous insolubility, fine particle size in addition to exhibiting good stability in disperse systems (166). Difficulties that could possibly be experienced when having to manufacture a dosage form with a water soluble API using the freeze drying approach is the formation of eutectic mixtures, as the freezing point depression and formation of glassy solids that occurs on freezing may collapse the structure following sublimation of the vehicle (166).

The advantage of using a freeze drying method of manufacture of dosage forms at low or non-elevated temperatures is the elimination of the potential deleterious effects on stability of the API when using high temperatures.

4.1.3.4 Melt Granulation

Melt granulation is a process whereby pharmaceutical powders are agglomerated by use of a binder that melts at a relatively low temperature. The advantage of this technique over conventional granulation approaches is that no water or organic solvents are used in the process and therefore no drying of the granules is required, resulting in shorter production times.

The binder is typically a hydrophilic wax like material such as Superpolystate©, PEG-6-stearate (165) which has a melting point of between 33 and 37°C. The material not only acts as a binder in the formulation but aids in the disintegration of the tablet as it will melt in the mouth at body temperature (172).

4.1.3.5 Choice of Method of Manufacture

The method of manufacture selected for the production of ODT was direct compression. Direct compression was selected since it is an economical approach to use with regards to excipients, time and equipment. The method makes use of commonly available excipients that are not excessively expensive, and does not require a great number of steps in the production of the tablets resulting in short manufacturing times. There is the added advantage that tablets manufactured using direct compression disintegrate into primary particles and not granules which occurs when manufactured using wet or dry granulation (173). This eliminates the need for granule disintegration and ensures rapid dissolution of API occurs following administration.

4.1.4 Excipients

The development and manufacture of a successful solid oral dosage form is dependent on the choice and amount of excipients that are incorporated into the formulation of that dosage form.

Excipients are ideally inactive, non-toxic and should not interact with the API or other excipients to be included in the dosage form (174). Excipients are included in the pharmaceutical dosage form in order to aid the manufacturing process, support or enhance stability and/or enhance patient acceptability of the medicine (174). However, the safe and successful use of excipients in the manufacture of products for use in adult patients does not necessarily imply they will be safe for use in children. Excipients should be used with special care, particularly in neonate and infant patients due to the fact that their physiology and organ development are very different to those of adult patients (175).

Excipients are categorized by the primary role they play in the manufacture of a pharmaceutical dosage form and include dilution, disintegration, binding, lubrication, flavouring, sweetening amongst others.

4.1.4.1 Binders

Binding agents are incorporated in a solid oral formulation to promote the formation of agglomerates during granulation or cohesive compacts during the compression process (176). The binder is often dissolved in a vehicle or granulating fluid that is added to a powder blend when tablets are manufactured using the wet granulation method. In the case of direct compression tableting, the binder is added to the powder mixture directly and blended prior to compression.

Most binders used in the wet granulation process are polymeric in nature and starch, gelatine and polyvinylpyrrolidone (PVP) are popular options (176). The most effective binder used in direct compression manufacture is MCC (176).

4.1.4.2 Diluents

In general the dose of an API is low in relation to the size of a tablet and therefore formulation must include excipients that are used to increase the size of the tablet so as to ensure it is of suitable dimensions for compression and ease of handling by patients or care-givers. The excipients that fulfil this role are known as diluents and should be inert materials as they are generally used in large quantities in solid dosage forms (177).

The common assumption relating to diluents is that they are inert however they have the potential to influence the stability and performance of a pharmaceutical product. For example, dibasic calcium

phosphate is an inorganic salt that is commonly incorporated in formulations as a filler/binder in direct compression formulations. The surface of this material is alkaline and therefore incompatible with drugs that may be sensitive to alkaline environments (160).

There are a number of materials that are suitable for use as diluents with many formulation scientists in the pharmaceutical arena favouring the use of lactose and MCC for many applications (122).

4.1.4.3 Disintegrants

Disintegrants are included into a tablet formulation to facilitate the breakdown of a tablet into granules (178) thereby increasing the surface area of API exposed to the gastrointestinal fluids and facilitating dissolution of the compound. A schematic representation of this process is shown in Figure 4.1.

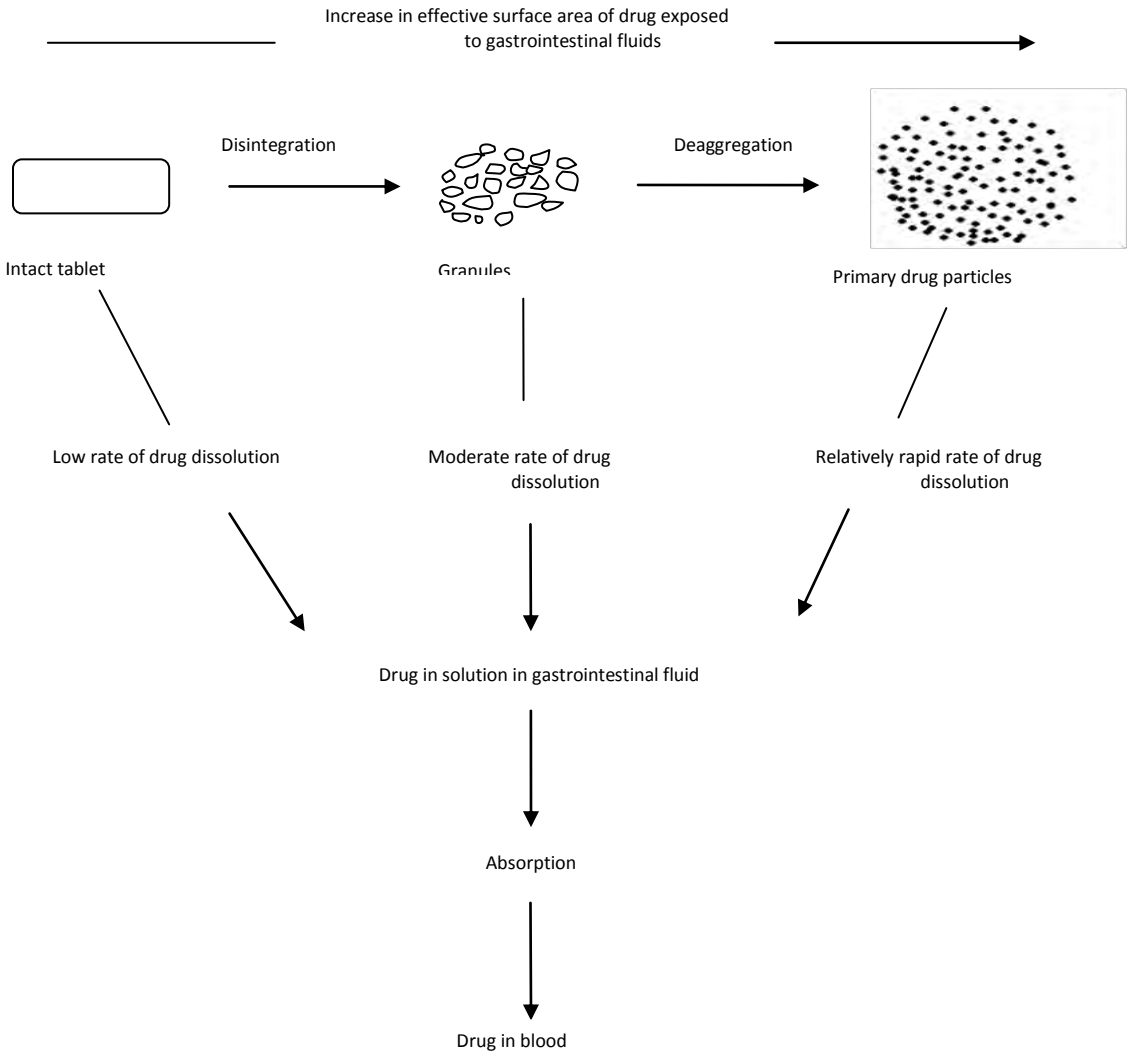


Figure 4.1 Schematic representation of the action of the disintegrant. Adapted from Wells, J.I. and Rubinstein, M.W. (1976) (179)

Tablet disintegrants are categorized into two classes *viz*., traditional or superdisintegrants. The term superdisintegrants was first used when referring to SSG, CMS and CRP and relate to their increased effectiveness in comparison to traditional disintegrants such as starch or alginic acid that were used previously (180).

4.1.4.3.1 Mechanism of Action of Disintegrants

Disintegrants may function by a number of mechanisms that may operate in isolation on some occasions or where multiple mechanisms might be in evidence. These mechanisms include swelling, wicking, repulsion and elastic deformation of raw materials.

Swelling occurs due to the affinity of a disintegrant for water. The disintegrant absorbs water and particles subsequently swell to a point when the adhesiveness of the other excipients in the formulation are surpassed, with the result that the tablet falls apart (181).

Wicking is also referred to as capillary action that occurs due to the porosity of a tablet and provides a pathway for the penetration of liquids into the core of the tablet. The liquid is drawn or "wicked" into these pathways through capillary action and destroys the inter-particulate bonds that hold the tablet together, thereby facilitating tablet disintegration (181).

Materials that recover the energy of elastic deformation following powder compaction may also fulfil the role of a disintegrant in a formulation. Starch is thought to be "elastic" in nature suggesting that the particles deform when placed under pressure yet return to their original shape when the applied stress is removed (181). Following application of tableting compression forces to a powder blend containing starch, it is thought that the starch particles are permanently deformed and are "energy rich". hen the tablet is subsequently exposed to water the energy potential of the deformed starch is triggered leading to disintegration of the tablet (182).

Repulsion mechanisms are an attempt to explain the swelling of tablets that are formulated with 'non swellable' disintegrants and it has been proposed that non swelling particles facilitate disintegration by the introduction of electric repulsive forces, following the addition of water, thereby promoting tablet disintegration (181).

4.1.4.4 Anti-frictional agents

4.1.4.4.1 Lubricants

The purpose of a lubricant in a formulation is to overcome or reduce friction. In the tableting context the friction that occurs between the die wall and the sides of the tablets are the frictional forces that should be overcome (183). Inadequate lubrication can result in the production of tablets with a pitted or scratched surface and is the consequence of the failure of a tablet to be detached from the surfaces of the die cavities. Lubricants are classified according to their aqueous solubility v*iz*. insoluble and soluble lubricants (178).

Insoluble lubricants are generally added to a powder blend during the final mixing stage prior to compression. The concentration of the lubricant used is an important consideration in formulation as lubricants can have a deleterious effect on tablet disintegration and subsequent dissolution of an API. Commonly used insoluble lubricants include magnesium stearate, stearic acid and glyceryl behenate (178).

Soluble lubricants are included in a formulation when it is necessary to overcome the potential detrimental effect of using insoluble lubricants and examples include polyethylene glycol and lauryl sulphate salts (178).

Lubricants tend to play a secondary role when included in ODT formulations. Lubricants may make a tablet more palatable as it disintegrates as the lubricant has the ability to remove grittiness and facilitate the transport of an API from the mouth to the stomach (184).

4.1.4.4.2 Glidants

Glidants are included in pharmaceutical formulations to reduce friction that may be generated between particles (185) and therefore serve to improve the flow properties of the components of a powder blend.

Glidants are thought to enhance the flow properties of a blend by one or more of several possible mechanisms (186) such as reducing roughness by filling surface irregularities, reducing attractive forces by physically separating bulk powder particles, modifying electrostatic charges, acting as moisture scavengers or acting as ball bearings between bulk powder particles.

Commonly used glidants for tablet formulations include talc and colloidal silicon dioxide (159).

4.1.4.4.3 Anti-adherents

The function of an anti-adherent is to reduce the adhesion between powders and punch surfaces thereby prevent particles sticking to punch surfaces resulting in a reduction of tablet imperfections.

Many powders can adhere to the punch surfaces and this phenomenon is known in the pharmaceutical industry as ‘picking’ or sticking’ that can be a result of a high moisture content of a powder blend or humidity of the manufacturing environment (161). The adherence of particles to punches can lead to the build-up of a thin layer of material on the surface which can cause the production of uneven tablets (161).

Commonly used anti-adherents include talc and starch however many lubricants such as magnesium stearate have some anti-adherent properties and are included in a blend to fulfil both functions.

4.1.4.4 Adsorbents

Adsorbents are included into tablet formulations when liquid or semi-solid excipients or API are to be included in a dosage form. The successful production of a tablet requires that only solid components be compressed and the use of an adsorbent excipient to which the liquid/semisolid component is bonded during a mixing process ensures that compaction is possible (178). The use of adsorbents is not without its challenges and may lead to the production of abrasive tablets. It is therefore important that fine and grit-free grade adsorbents are used when manufacturing such tablets (176) and commonly used adsorbents include kaolin, bentonite or magnesium carbonate.

4.1.4.5 Colourants and Flavourants

Colourants are included into a tablet formulation to increase the aesthetic appeal of a dosage form and to ensure ease of identification (178). Colourants are usually added during the coating stages of production but can also be included in a blend prior to compression where the dye is either added as an insoluble powder or dissolved in the granulation liquid (161).

Concerns over the safety of colouring agents used in formulations have been raised and relate to the unwanted side effects that were noted when food colourants are used [6). These colourants are therefore subject to regulations that are not usually associated with other pharmaceutical excipients that are otherwise required to be safe and non-toxic (160). Country specific legislation to control the use of colorants in pharmaceutical formulations also provide for purity specifications [6) and have led to a decrease in the number of permitted colours for pharmaceutical applications (160).

Colourants are classified as water-soluble dyes or water-insoluble pigments. Water-soluble dyes are usually applied in the granulation process to ensure the dye is evenly distributed throughout a formulation. Uneven distribution of colours can occur since the migration of the dye during drying may occur (160). To overcome the problem of uneven colour distribution water-insoluble pigment can be dry blended prior to the final compression.

Flavourants and other taste-masking excipients are incorporated into a formulation to produce a more palatable dosage form. Flavouring agents are often thermolabile and cannot be added prior to a unit operation involving heat (161). Flavourants are included in formulations to manufacture more pleasing dosage forms that will hopefully improve patient adherence to therapy.

4.2 METHODS

4.2.1Materials

SC was purchased from MTT Pharma and Bio-Technology Co., Ltd (Shanghai, China) and MCC (Emcocel 90M) was purchased from Penwest Pharmaceuticals Co. (Nastola, Finland). CRP was purchased from BASF® (Ludwigshafen, Germany) and CMS, SSG, CSD, mannitol (SD200), talc and magnesium stearate were donated by Aspen Pharmacare (Eastern Cape, South Africa)

4.2.1.1. Microcrystalline cellulose

MCC may be included in a formulation as a filler, although it can also be used as a binder and also facilitates tablet disintegration (122; 160). MCC is both crystalline and amorphous depending on the orientation of the cellulose chains in the material (161). MCC is available in a variety of different grades that are reflective of alternate particle size and moisture content (122) with the larger particles sizes exhibiting better flow ability as discussed in Chapter Three, *vide infra*.

MCC is compatible with many drug molecules, however it is hygroscopic and must be used with caution in formulations that contain molecules that are susceptible to hydrolysis in the solid state (161).

4.2.1.2 Mannitol

Mannitol is used as filler in many chewable formulations. It is a white crystalline non-hygroscopic powder with a faint odour (122) which makes it an ideal excipient for use in ODT formulations.

Although the primary role of mannitol in formulations is a filler it fulfils multiple roles and since it is soluble in water (128) it enhances the disintegration of tablets. Furthermore mannitol, a sugar, has a negative heat of solution and produces a cooling sensation in the mouth with a pleasant taste when chewed or dissolved (128).

Mannitol can be used in varying amounts in a formulation based on the dose of API to be delivered from the formulation.

4.2.1.3 Crospovidone (CRP)

CRP is included in tablet formulations as a disintegrant. It is a white to creamy-white powder that is hygroscopic, has a faint odour and is insoluble in water (122). As CRP is non-ionic, the disintegration properties that it imparts to a tablet are not influenced by pH (128).

The optimum concentration of CRP for use in formulations is 2-5% w/w, however tablets containing up to 13% w/w CRP have been successfully produced (187). The mechanism by which CRP promotes disintegration of tablets is predominantly though capillary action (181).

4.2.1.4 Croscarmellose sodium (CMS)

CMS is a white, free flowing powder that is used in tablet formulations as a disintegrant (122).

CMS is a cross linked polymer of carboxymethyl cellulose sodium (188) that is insoluble in water but is a highly absorptive material with exceptional cold water swelling properties (188). Due to the high density of cross linking, CRP has the ability to swell rapidly in water without forming a gel (181) and disintegration occurs by a process of wicking and swelling (181).

4.2.1.5 Sodium starch glycolate (SSG)

SSG is the sodium salt of the carboxymethyl ether of starch and is a very fine white to off white powder that is free flowing, odourless and practically insoluble in water (122).

The effect of the presence of a large hydrophilic carboxymethyl group disrupts the hydrogen bonding within the polymer structure and allows water to penetrate the molecule with the polymer becoming water soluble (181). SSG is widely used as a disintegrant in both tablets and capsules with a recommended concentration for use in a formulation of between 2-8% w/w (122).

4.2.1.6 Colloidal silicon dioxide (CSD)

CSD is a non gritty amorphous powder that is bluish white in colour. It is odourless and consists of fine spherical particles of small size and excellent water-adsorbing properties (160).

CDS is included in formulations as a glidant and to impart smoothness to the surfaces of punches, however it is often required to be used in combination with additional lubricants to facilitate tablet ejection from die cavities (160).

4.2.1.7 Talc

Talc is a compound that is primarily comprised of magnesium silicate and on occasion, small quantities of aluminium silicate (177). Talc is a very fine white to off white crystalline powder and is used in formulations as a glidant. It has been shown that there is no significant difference between using 0.25% w/w and 1% w/w talc in tablet formulations (189).

The effectiveness of talc is limited by its hydrophobic nature and the use of increasing levels of the material result in a significant decrease in tablet wetting and consequently, reduced dissolution rates.

4.2.1.8 Magnesium stearate

Magnesium stearate is a commonly used lubricant in tablet formulations. It is a fine, white bulky powder with a characteristic odour and is insoluble in water (177) that when used in concentrations greater than 5% w/w retards drug release from solid dosage forms (122). The impact of blending time on dosage form performance must be evaluated when formulating dosage forms with magnesium stearate, as increased blending times may reduce dissolution rates and the crushing strength of tablets (122).

4.2.2 Manufacturing equipment

All raw materials were weighed using a Mettler Model PM4600 top loading analytical balance (Mettler Instruments, Zurich, Switzerland) with a sensitivity of 0.01g. Blending was performed using a cube mixer (Erweka®, Munich, Germany) attached to a drive unit (Kraemer Electronik GmbH®, Damstadt, Germany). Tablet compression was performed using a Manesty®F3 single punch press (Manesty®., Liverpool, England) tooled with 7mm flat-faced punches at a speed of 20 r.p.m. All materials were sieved prior to processing using wire cloth sieves conforming to a DIN 4188 standard.

4.2.3 Method of manufacture

Tablets were manufactured using a direct compression method to produce dosage forms that each contained 3 mg SC. The dose of SC was based on the data following a literature review and evaluation of the typical doses used to treat neonate patients (Chapter One).

Mannitol or fructose was used as the primary diluents in combination with microcrystalline cellulose (MCC) as an additional diluent. CRP, CMS and SSG were included in the formulation as disintegrants. Magnesium stearate, talc and CSD were included as anti-frictional agents.

A diagrammatic representation of the method of manufacture is located in Figure 4.2

SC, diluents and disintegrants were screened through a #20 sieve and magnesium stearate, talc and CSD which were screened through a #44 mesh sieve. The powders were placed into the cube blender and mixed for 10 minutes without magnesium stearate. The powders were blended for a further three minutes following addition of the magnesium stearate.

The blend was compressed to form 7mm diameter flat-faced tablets using a Manesty® F3 single punch press set to a compression force of 30N.

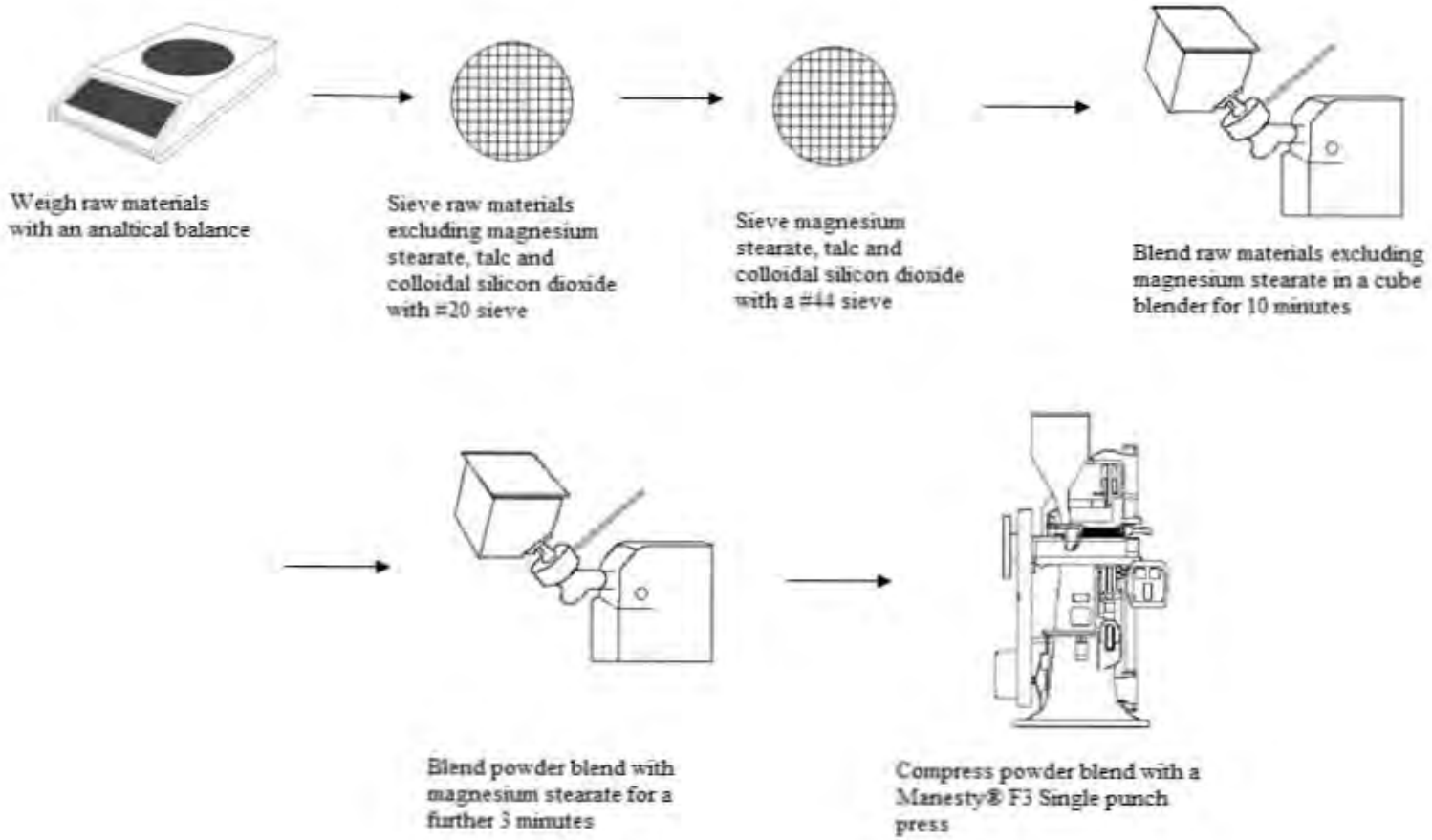


Figure 4.2 Method of manufacture of SC ODT

ODT have a high sensitivity to humidity, as many of the excipients used to produce the tablets are hygroscopic. It was noted during the initial stages of developing a suitable formulation that if the tableting was performed in an environment in which the relative humidity was > 50% picking occurred, resulting in batch failure. Picking is an action in which solid particles of a tablet blend adhere to the surfaces of a punch resulting in small pits becoming apparent on the surface(s) of the tablet.

Consequently the RH of the tableting room was constantly monitored during formulation and manufacture of SC tablets. Furthermore, to ensure continuity when Central Composite Design experiments were conducted, and in the absence of an environmentally controlled tableting facility, production was performed between 00H00 - 06H00 am when the RH of the facility was found to be at the lowest level.

The initial formula was developed following a literature review of formulae used for the manufacture of orodispersible tablets. However the literature review revealed that the dose of SC used was far higher than the dose that was required in this formulation. Previous formulae also did not appear to disintegrate rapidly enough as was required for this formulation. Modification with regard to the concentration of diluents and disintegrants were therefore required to distil a suitable formula for use.

The batch formulae used for the development of a suitable ODT are summarized in Table 4.1.

Table 4.1 Formulae used for the manufacture of SC ODT by direct compression

	SC 001 % w/w	**SC 002 % w/w**	**SC 003 % w/w**	**SC 004 % w/w**	**SC 005 % w/w**	**SC 006 % w/w**	**SC 007 % w/w**
SC	2	2	2	2	2	2	2
CMS	8.5	8.5	8.5	8.5	8.5	8.5	8.5
CRP		3	3	3	3	8.5	13.5
SSG	-	-	-	4	8	8	8
MCC	15	15	15	15	15	15	15
Talc	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mg stearate	1	1	1	1	1	1	1
CSD	-	-	1	1	1	1	1
Fructose	70.4	-	-	-	-	-	-
Mannitol	-	70.4	69.4	65.4	61.4	55.9	50.9

Following the initial development of these formulations, Batch SC003 was further optimized in respect of disintegration by increasing disintegrant concentration and ensuring that the tablet retained suitable mechanical strength characteristics. The success of the optimization procedure was evaluated by assessing content uniformity of the tablets using a validated HPLC method (Chapter Two) in addition to assessing the disintegration time of these tablets.

The content limits of the API was set at 100±10% with an RSD of <5%. The friability limit was set at < 1% and other physical characteristics such as disintegration and wetting time in addition to *in vitro* dispersion time were required to be as low as possible.

4.2.4 Physical characteristics of ODT

4.2.4.1 Physical properties of powder blends

All powder blends were assessed by evaluating bulk and tapped density, flowability and compression characteristics.

Approximately 10g of each powder blend was assessed as discussed in §3.2.2 and § 3.2.3 and the bulk and tapped density, angle of repose, CI and HR established**.**

4.2.4.2 Physical characteristics of tablets

4.2.4.2.1 Mechanical strength of tablets

The mechanical strength of a tablet is an important characteristic that must be measured as it provides a formulation scientist with an indication of the extent to which a tablet can withstand the mechanical shock that it will be exposed to during manufacture, packaging and transportation (167).

The most commonly used methods for testing mechanical strength are attrition-resistance or fracture-resistance methods.

4.2.4.2.1.1 Attrition-resistance tests

Attrition-resistance test methods, mimic forces to which a tablet is subjected during handling through production to administration of a tablet to a patient (161) and are also referred to as friability tests.

During friability testing, tablets are subject to repeated abrasion through rotation for a specified number of cycles in a friability tester. The tablets are held in a transparent drum containing a blade that carries tablets to a central height that permits them to fall as the drum rotates (190). The movement of the drum and tablets results not only in continuously falling from a set, small height but that they also rub against each other. The tablets are dusted and weighed prior to and following testing and the percent lost from the original weight is calculated. A friability of less than 1% is considered acceptable, whereas friability values > 1% are cause for batch rejection.

The friability of the tablets was determined using a Model TA3R friabilator (Erweka GmbH, Hausenstamm, Germany). Twenty (20) tablets were randomly selected, de-dusted and weighed using a Mettler Model PM 4600 top-loading balance (Mettler Instruments, Zurich, Switzerland). The tablets were tumbled at a rate of 25 rpm for 4 minutes or 100 drop cycles and then removed from the friabilator, de-dusted and reweighed. The friability of the tablet was calculated using Equation 4.1.

Equation 4.1

Where,

w_1 = weight prior to testing
w_2 = weight after testing
Fr = friability

4.2.4.2.1.2 Fracture-resistance tests

Analysis of the fracture resistance of tablets involves the application of a load to the tablet and the determination of the force needed to fracture or break the tablet along the diameter of the compact. A schematic representation of the tensile strength test is shown in Figure 4.3.

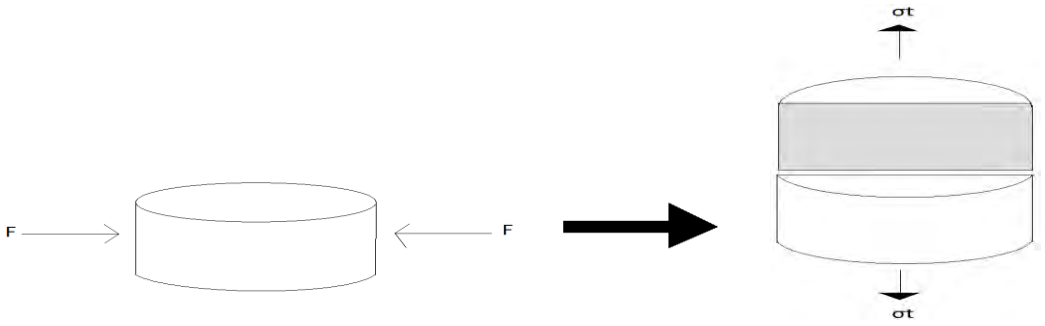


Figure 4.3 Diagram of the tensile strength of a tablet

The tensile strength of the manufactured tablets was calculated using Equation 4.2.

$$\sigma_0 = \frac{2F}{\pi dT} \qquad \textbf{Equation 4.2}$$

Where,

σ_o = tensile strength (MPa)
F = crushing strength (N)
d = tablet diameter (mm)
T = tablet thickness (mm)

4.2.4.2.2 Weight Uniformity

Weight uniformity is an important criterion for evaluation of tablets within a batch as it provides an indication of the content uniformity of that batch. Therefore it can be seen as an indication of the efficiency of mixing of an API and the excipients that make up a formulation. If the tablets tested exceed the limits of weight variation as set out by the United States Pharmacopoeia (USP) [9] as shown in Table 4.2 the batch must be rejected [9].

Table 4.2 Weight variation limits as specifies in the USP (163)

Average weight of tablet	% Deviation
80 mg or less	± 10
More than 80 mg but less than 250 mg	± 7.5
250 mg or more	± 5

The individual weights of 10 randomly selected tablets were measured using a Mettler Model AG 135 top-loading electronic balance (Mettler Instruments, Zurich, Switzerland) that had a sensitivity of 0.1 mg. The average weight and %RSD for the batch was then established.

4.2.4.2.3 Crushing strength and diameter

The crushing strength of the tablet is defined as the force that is applied across the diameter of a tablet in order to break the tablet (168). The harder the tablet the greater resistance the tablet exhibits to chipping, abrasion or breakage when stored or handled prior to use.

The crushing strength and diameter of the tablets were measured using a Model PTB 311 E Hardness Tester (PharmaTest AG®, Hainburg, Germany). Each tablet was placed in the tester and a crushing force applied to the tablet and the crushing strength and diameter were measured simultaneously.

4.2.4.2.4 Disintegration test

The disintegration time is a crucial characteristic that must be monitored during the development of a ODT, due to the fact that the formulation needs to disintegrate rapidly to exert a therapeutic effect.

The disintegration time of tablets was measured using a Model ZT 61 tablet disintegration apparatus (Erweka GmbH®, Heusenstamm, Germany). Six tablets were randomly selected from each batch and a single tablet was placed into separate cylinders of the basket-rack and covered with a disc. The basket was set to oscillate vertically at a speed of 30 oscillations per minute in a beaker containing 800ml of distilled water that was maintained at 37 ± 0.2°C. The time for disintegration of each tablet was recorded and noted at the completion of disintegration testing

4.2.4.2.5 Tablet assay

Twenty tablets were randomly selected and ground into a fine powder using a mortar and pestle. An aliquot equivalent to the weight of one tablet (150mg) was weighed using a Model AG 135 top loading analytical balance (Mettler Instruments®, Zurich, Switzerland). The powder blend was transferred into a 25ml A-grade volumetric flask and dissolved in ACN: water in a ratio of 55:45 and sonicated for 5 minutes using a Model B-12 Ultrasonic bath (Branson Cleaning Equipment Co., Shelton, Connecticut, United States of America). The solution was then made up to volume with an ACN: water mixture in a ratio of 55:45. Approximately 2ml of the solution was filtered through a 0.22µm Acrodisc® PSF syringe filter (Pall Corporation, Port Washington, New York, United States of America) and an aliquot of 167µl of the filtered solution was transferred into a 10ml A-grade volumetric flask. A 13µl aliquot of IS solution was added to the volumetric flask and the resultant solution was made up to volume with an ACN: water mixture in a ratio of 55:45.

The solution was analysed using a validated HPLC method and analysis was performed in triplicate.

4.2.4.2.6 Water Absorption Ratio Studies

The water absorption ratio is usually investigated in the development of ODT to provide an understanding of the capacity of the disintegrants included in the formulation to swell and/or wick in the presence of a small amount of water (191).

The most popular method used to determine the water absorption ratio is undertaken by using a piece of tissue paper folded twice and placed on a petri dish containing 6ml buffer solution. The tablet is placed on the tissue paper and allowed to completely wet. The tablet is then removed from the petri dish and weighed.

As the tablets disintegrated rapidly after wetting, it was not possible to be move and reweigh the individual units. Therefore a simple approach was to place the tissue paper and petri dish on a tarred scale and then to place the tablet on the petri dish to ascertain the initial weight of the system. Buffer was then added drop wise with a plastic pipette until the tablet was completely wetted. The final weight of the tablet was then established and the water absorption ratio calculated. All studies were performed in triplicate. The water absorption ratio was calculated using Equation 4.3.

$$\text{a ter Absorption Ratio} = \frac{a - b}{b} \times 100$$

Where,

Wa= weight of the tablet after wetting
Wb= weight of the tablet before wetting

4.2.4.2.7 Wetting Time

The wetting time of ODT is an important physical characteristic as it provides an indication of the disintegration efficiency of the tablet (192) with faster wetting times implying faster tablet disintegration and more rapid drug release.

The wetting time of the tablets (n=6) were determined by folding a piece of tissue paper twice (12cm x 10.75cm) and placing the tissue paper onto a petri dish containing 6ml buffer solution (pH6.8). The

tablet was placed on the tissue paper and the time taken to completely wet the tablet was recorded. All determinations were performed in triplicate.

4.2.4.2.8 *In vitro* Dispersion Test

The use of *in vitro* dispersion tests as additional indicator of the disintegration time of the tablets is common and the dispersion test defines the time taken for tablets to undergo uniform dispersion.

The *in vitro* dispersion time of tablets (n=3) from each batch that was manufactured was determined by placing the tablet in a beaker containing 10ml buffer solution (pH 6.8). No degree of agitation was used to enhance dispersion and the end point of the test was set at the time taken for the tablet to lose its original shape. Upon visual inspection the separation of the tablet into clearly defined particles was considered the end-point and this time was noted as the *in vitro* dispersion time.

4.2.4.2.8.1 Preparation of buffer

The buffer used in these studies was prepared by weighing 0.68g of dihydrogen orthophosphate on Mettler AG 135 top loading balance (Mettler Instruments, Zurich, Switzerland) and then transferring into a 1000ml A-grade volumetric flask. The powder was dissolved in distilled water and sonicated for 5 minutes using a Model B-12 Ultrasonic bath (Branson Cleaning Equipment Co.,Connecticut, United States of America) and then made up to volume with distilled water. The pH of the buffer was adjusted to pH 6.8 with sodium hydroxide.

4.2.5 Blend homogeneity studies

Blending can be thought of as a "reshuffling" process involving the movement of individual and/or groups of particles to produce a homogenized blend of solids in bulk (193).

Blending times that are typically used for the manufacture of tablets range between 2 and 10 minutes (194), however with low dose formulations longer blending times may be necessary. The USP recommends that blend uniformity testing be performed on dosage forms that contain < 50mg API per dosage unit or if the API content of the dosage form is <50 % w/w of the each unit (195). The acceptance limits set by the USP for blend uniformity testing is 100±10% of the expected content with an associated % RSD of < 5% (196).

During initial formulation studies, a blending time of 10 minutes without the addition of magnesium stearate and a further 3 minutes blending of the powder mixture with the magnesium stearate was selected for use. A blend homogeneity study was performed on Batch SC 003 only. Blend

homogeneity studies were only performed on this batch to evaluate the mixing process and the optimum blending time was further evaluated using an experimental design approach and is reported in Chapter Five, *vide infra*.

Blend homogeneity studies were performed by measuring the API content of the powder samples of blend at after being mixed for13 minutes. Approximately 100mg (n=6) of the powder blend was collected from six separate areas of the cube blender using a stainless steel laboratory spatula and weighed using a Mettler AG 135 top loading balance (Mettler Instruments, Zurich, Switzerland) and transferred into a 50ml A-grade volumetric flask. The powder was dissolved in an ACN: water mixture (55:45) and sonicated for 5 minutes using a Model B-12 Ultrasonic bath (Branson Cleaning Equipment Co., Shelton, Connecticut, United States of America) and made up to volume with the ACN: water mixture(55:45). Approximately 2ml of each solution was filter through a 0.22µm Acrodisc® PSF syringe filter (Pall Corporation, Port Washington, New York, United States of America) and 500µl of the filtered solution was transferred into a10ml A-grade volumetric flask with 13µl of IS solution. The sample was made up to volume with an ACN: water mixture (55:45) and analysed using a validated HPLC (Chapter 2).

4.2.6 Content uniformity of tablets

Following the establishment of an appropriate blending time the content uniformity of the tablets of a batch was investigated. Content uniformity is the measurement of variation in API from one dose unit to the next.

Content uniformity testing should be conducted on all coated tablets other than film coated tablets, transdermal systems, suspensions in single-unit containers or in soft capsules, pressurized metered-dose inhalers and suppositories (196).

During manufacture of any dosage form the excipients are added at different stages and factors such as powder density, particle sizes and shapes may contribute to differences in the resultant uniformity of content of the tablets in a batch (190). It is therefore necessary to test for uniformity of content of each batch that is manufactured to ensure that each tablet conforms to the acceptance criteria and that each batch can be considered of suitable quality, for use.

Ten tablets from the batches under investigation were randomly selected and crushed separately using a mortar and pestle. Approximately 100mg of each powder blend, equivalent to 2mg of SC was weighed and transferred into 50ml A-grade volumetric flask. The powders were dissolved in an ACN: water mixture (55:45) and sonicated for 5 minutes using a Model B-12 Ultrasonic bath (Branson

Cleaning Equipment Co., Shelton, Connecticut, United States of America) and then made up to volume with ACN: water (55:45). Approximately 2ml of each of the solutions was filtered through a 0.22µm Acrodisc® PSF syringe filter (Pall Corporation, Port Washington, New York, United States of America) and 500µl of each filtered solution transferred into a 10ml A-grade volumetric flask containing 13µl of IS solution. Each sample was made up to volume and analysed using a previously validated HPLC method.

4.3 RESULTS AND DISCUSSION

4.3.1 Physical properties of the powder blend

A summary of the properties of the different powder blends that were tested is summarised in Table 4.3.

Table 4.3 Assessment of Powder blends

Batch No.	Bulk Density	Tapped Density	Angle of Repose	CI	HR
SC 001	0.510	0.680	41.63	25	1.33
SC 002	0.504	0.642	33.69	21.46	1.27
SC 003	0.525	0.671	30.96	21.63	1.28
SC 004	0.510	0.674	28.61	24.39	1.32
SC 005	0.520	0.679	28.61	23.41	1.31
SC 006	0.500	0.644	26.57	22.39	1.29
SC 007	0.491	0.636	28.30	22.86	1.30

Batch SC 001, which made use of fructose as the primary diluent exhibited slightly poorer flow properties than other blends, as can be seen from the relatively high angle of repose for Batch SC 001 in comparison to the other batches. This is an indicator to potential content uniformity problems.

When mannitol was used as the primary diluent, the powder blends exhibited improved flow properties when compared to fructose.

As can be seen from the data for Batch SC 003, the incorporation of a glidant improved the flow properties of the blend further and inclusion of a glidant is most likely necessary to ensure uniformity with regards to the content of API present in the tablets.

The addition of increased amounts of disintegrant in Batches SC004 -SC 007 did not reduce the flow properties of the blend, suggesting that an increase in the amount of disintegrant to facilitate faster

disintegration times should not have a negative impact on the flow properties of powder blends and should not result in large variations in content uniformity

4.3.2 Physico-mechanical properties of the tablets

A summary of the physico-mechanical properties of following testing of all batches manufactured are summarised in Table 4.4.

All tablets that were produced passed the uniformity of weight test as all batches had weight data with relative standard deviations of < 7.5%. Weight variations tests are very important consideration when formulating dosage forms as large variations in weight may be an indication of poor flow properties of powders (103) and will most likely result in the production of batches of tablets that are not uniform with regards to API content. The results of weight variation analyses indicate that the batches of SC tablets produced will most likely exhibit a suitable content uniformity. However since the formulation is a low-dose product, weight variation cannot be the only method used to assess content uniformity and other methods such as quantitative determination of the content of SC in each tablet must be established using a validated analytical method.

The tablets manufactured with fructose showed a large variation in disintegration times and had assay values that exceeded the USP limits of 100±10%. This is likely due to the large particle size of fructose that was confirmed using SEM imaging (Chapter Three). Larger particles tend to have better flow properties than smaller particles but when formulated with smaller particle sizes, the different particles may segregate(160). Segregation of the powder blend may then result in inaccurate filling of dies and lead to the production of tablets that have a large variation in dose within a single batch (161). Mannitol was therefore thought to be a more appropriate diluent as the particle size of mannitol was similar to the other excipients used in the formulation.

All tablet batches conformed to the friability limit of 1% despite exhibiting relatively low hardness, that is a required feature of ODT. These results indicated that the tablets were mechanically strong enough to withstand shock but not excessively hard so as to result in increased disintegration times.

The water absorption ratio is an important characteristic that needs to be investigated in the development of an ODT formulation as it provides a relatively good indication of the speed of disintegration. The data produced in these studies suggest that there is an inverse relationship between the water absorption ratio and the disintegration time for these tablets, as an increase in the disintegrant concentration led to an increase in the water absorption ratio. Since the water absorption ratio is indicative of the time for disintegration it can be inferred that an increase in disintegrant

concentration will result in an increase in the water absorption ratio and ultimately a decrease in disintegration times. The standard deviation observed for Batch SC 001 was high due to the fact that segregation of the blend may have occurred and therefore inaccurate filling of the die results in varying amounts of disintegrant in each tablet and consequently variable disintegration times.

An inverse relationship was noted for wetting and *in vitro* dispersion times and the concentration of the disintegrant and it was revealed that increased disintegrant concentrations shortened the wetting and dispersion times for these products. The short disintegration time is a neceesary and desirable property of orodispersible tablets and therefore an important factor that needs to be monitored in the development of an appropriate formulation for SC tablets.

Table 4.4 Physico-mechanical properties of SC tablets

Batch No.	Weight mg	Crushing Strength N	Diameter mm	Tensile Strength	Friability %	Disintegration time s	Water Absorption Ratio	Wetting time s	*In vitro* dispersion s	Assay %
SC 001	188±6.96	19.17±4.29	7.23±0.03	0.712	0.98	51±23	55.47±4.18	18.13±0.15	45.53±2.60	164.19
SC 002	153.5±5.87	21.33±2.07	7.23±0.03	0.803	0.41	80±3	61.83±0.68	20.33±1.40	45.89±1.43	95.64
SC 003	151±7.18	20.58±2.97	7.23±0.03	0.872	0.85	81±2	61.22±0.74	15.93±0.25	43.89±0.33	102.49
SC 004	145±6.07	23.44±2.30	7.24±0.01	0.873	0.78	23±2	64.99±1.42	14.43±0.50	38.46±0.49	98.02
SC 005	153.5±6.71	26.01±2.65	7.26±0.08	0.958	0.62	10±2	68.70±0.54	12.23±0.32	36.16±0.35	98.50
SC 006	150.5±5.10	22.01±2.54	7.24±0.01	0.813	0.66	9±1	68.94±0.65	8.57±0.70	35.28±0.52	98.34
SC 007	149.5±5.99	19.65±3.98	7.25±0.03	0.728	0.69	8±1	71.05±1.49	4.2±0.17	35.28±0.49	101.45

4.3.3 Blend homogeneity

The difficulty with blending excipients used when formulating and manufacturing ODT is that the majority of the materials are hygroscopic and high humidity environments can have a significant impact on the cohesiveness of a blend. Consequently an increase in the moisture content can lead to the formation of unwanted agglomerates. Therefore optimal blending times must be established so as to achieve uniformity of a blend without compromising the quality of the materials required for compression, through moisture uptake (193).

When blending under conditions in which agglomerates may form, the uniformity of a blend improves to a point where agglomerates are formed after which increasing agglomerate sizes result in a subsequent decreases in uniformity (193).

The results of blend homogeneity tests conducted on Batch SC 003 are summarized in Table 4.5.

Table 4.5 Blend Homogeneity study on Batch SC 003

Batch	Sample	Expected Content (mg)	Actual Content (mg)	Content %	%RSD
SC 003	1	2	2.10	104.80	3.87
	2	2	1.95	97.30	
	3	2	2.10	105.22	
	4	2	2.09	104.42	
	5	2	2.15	107.32	
	6	2	2.10	104.90	

The data summarized in Table 4.5 reveal that the limits set by the USP regarding blend uniformity analysis for this batch were in the range 100±10% with a RSD of <5%, had been achieved. Therefore it can be concluded that a blend time of 10 minutes without magnesium stearate followed by blending for a further 3 minutes with magnesium stearate are appropriate blending times to produce uniform ODT of SC.

4.3.4 Content Uniformity

The use of weight as an indicator of the uniformity of dose is not appropriate for tablets, unless the expected dose to be delivered is high and the API forms a large proportion of the tablets. For tablets in which low doses of drug are included, low variations in weight do not guarantee uniformity of content, whereas large weight variations are likely to preclude content uniformity being achieved

(160). It is therefore necessary to assay a small number of dose units from each batch to ensure that the correct dose is present in each of those units.

The content uniformity limit set by the USP are of 85-115% (197) and the results of content uniformity testing are summarized in Table 4.6.

Tale 4.6 Content uniformity results

Batch	Expected Content (mg)	Actual Content (mg)	Content %	%RSD
SC 004	3	2.75±0.48	91.78	5.09
SC 005	3	2.91±0.50	96.91	5.84
SC 006	3	2.92±0.50	97.39	5.74
SC 007	3	2.76±0.50	92.06	4.70

The data reveal that despite changes in the amount of mannitol and disintegrants used in the formulation a blend time of 13 minutes was sufficient to produce tablets that were uniform in content and that falls within the USP limits set for that parameter. It can therefore be concluded that the method of manufacture is suitable to produce SC tablets formulated and manufactured using the proposed method.

4.4 CONCLUSIONS

An ODT containing SC has been successfully developed and manufactured using the direct compression approach. The tablets have acceptable mechanical strength attributes while at the same time disintegrate rapidly which is acceptable for these types of dosage form. The use of three superdisintegrants in combination in the formulation resulted in tablet disintegration occurring in approximately 8 seconds.

Direct compression was successfully used to manufacture an ODT formulation of SC. The use of direct compression is a convenient method of manufacture as the approach facilitates shortened manufacturing times whilst making use of commonly available equipment and excipients.

Mannitol was selected as an appropriate filler as it has a similar particle size to SC and the other excipients that were used to manufacture the SC ODT. The similar particle size decreases the chance of particle segregation during blending and manufacture as witnessed when fructose was used as a diluent. The minimization of segregation resulted in the manufacture of tablets of suitable content

uniformity and the batch fell within the specification for the dose limits that had been set for this parameter

A blending time of 13 minutes was established as suitable to produce a blend and tablets that met the USP acceptance criteria with each blend sample containing 100±10% with an associated % RSD < 5%. All tablets that were manufactured met the specification limits for friability and mechanical strength and would be able to withstand transport and handling and yet exhibited desirable and fast disintegration times.

Extemporaneous preparations, although not always the safest way to administer medications to paediatric patients are often the only option available to a pharmacist who has to initiate therapy in marginalized patient populations. Research efforts have now turned to producing orodispersible tablets to offer pharmacists a safe and effective alternative to using extemporaneous preparations. Extensive research is however required with regard to developing and manufacturing these formulations as they are not without their own challenges in considering excipient selection and stability attributes.

The tablets that were produced in these studies form the basis of a new SC containing ODT and the current formulation must therefore be further optimized. The tablets produced in this work formed the basis of a new SC containing ODT and the formula was further optimized as described in Chapter 5.

CHAPTER FIVE
THE APPLICATION OF RESPONSE SURFACE METHODOLOGY IN THE OPTIMIZATION OF A SILDENAFIL CITRATE ORODISPERSIBLE FORMULATION AND QUALITY CONTROL TESTING OF THE TABLETS

5.1 INTRODUCTION

For many years formulations have been developed by scientists using the knowledge gained from previous production experience. This approach is generally appropriate for the development of formulations that do not have critical challenges such as inclusion of poor solubility compounds, high or low doses into a dosage form or that require a product to have exact physical-chemical properties for effective performance using a specific route of administration. Consequently at times, adequate formulations only, are developed as the selection of appropriate controllable variables can be complicated by the presence of competing objectives. It is therefore important to have a clear understanding of controllable (independent) variables and performance (dependent) variables when attempting to develop a successful dosage form (198).

An efficient and effective method for obtaining the required information that is needed to clearly understand the relationship between input variables and outcomes is the use and application of statistical principles. The use of a statistical design approach allows for the efficient application of resources and also provides a means of generating a mathematical model that can subsequently be used to optimize a formulation or manufacturing process (198; 199).

The Experimental Design approach is one that affords a researcher the opportunity of setting up a series of experiments to simultaneously evaluate a number of factors, at a specific number of levels in a predetermined number of assessments performed randomly (200).

Design of Experiments (DOE) is an extremely efficient tool for this purpose, as the outcome provides a fixed amount of information that has been generated with considerably less effort than if one were to use the traditional " modification of one variable at a time" approach (199). An important feature of the DOE approach is the random order in which experiments are performed, thereby ensuring that premature decisions relating to an outcome are not made prior to considering all the evidence provided from all the data generated (199). The use of this approach also ensures that a random distribution of errors is likely to occur during experimentation.

Amongst the many advantages of using an experimental design approach are savings in time, money, API and the ability to evaluate interactions between input variables when optimizing a formulation or manufacturing process (201).

5.1.1 Response Surface Methodology

Response surface methodology (RSM) is an experimental strategy that was developed in the 1950's (202). RSM is comprised of a group of mathematical and statistical techniques that are based on fitting experimental data generated from studies established using an experimental design, to empirical models and that are subsequently used to define a relationship between the responses observed and the independent input variables (203; 204). RSM is able to define the effect of independent variables alone and in combination with the manufacturing processes under investigation.

A typical RSM study begins initially with the definition of a problem to be investigated and involves establishing which variables and associated responses are to be studied, monitored, measured and how these will be measured. A summary of the subsequent RSM approach includes (202):

i. Performance of the relevant DOE.
ii. Estimation of the coefficient in the relevant response surface equation.
iii. Checking of the adequacy of the equation to describe the fit.
iv. Studying the response surface to identify and evaluate the region(s) of interest.

The relationship between a response and an input variable can be described by Equation 5.1 (204).

$$y = f(x_1, x_2, \ldots\ldots, x_n) + \varepsilon$$

Where,

y = relevant response
f = unknown function of a response
$x_1, x_2, \ldots\ldots x_n$ = independent variables
n= number of independent variables
ε = statistical error that represents other sources of variability not accounted for by f

The term RSM originates from the graphical perspective generated after fitness of the mathematical model has been established (203; 204) with a graphical representation of the data presented primarily as a three-dimensional (3D) image and/or as contour plots.

Contour plot can be described as:

i. Mound-shaped that has elliptical contours with a stationary point at the position of a maximum response.
ii. Saddle-shaped that has a hyperbolic system of contours with a stationary point that is neither a maximum nor minimum point.
iii. Constant (stationary) ridge response surface in which the contours are presented as concentric elongated ellipses with a stationary point in the region of the design region.
iv. A rising (or falling) ridge response surface with a stationary point that is outside the design region (205).

The stationary point is a combination of design variables where the surface presents as either a maximum and/or a minimum in all directions. If the stationary point is a maximum in one direction and minimum in another direction, the stationary point is termed a saddle point. When the surface is curved in one direction but is fairly constant in another this is considered a ridge response (206).

By plotting a response, y, against one or two input variables a surface, known as the response surface can be generated in two or three dimensions. In general the form of the function, f, is unknown and may be very complicated depending on the effect of the input variables on the response. Therefore RSM aims at approximating f by use of a suitable, ordered polynomial equation in some region(s) of the values for the independent process variables (207). The mathematical or polynomial equations that describe the relationship(s) between the independent and dependent variables may be first, second or third order, depending on how the output variables or responses react to changes in the input variables.

If the response is a linear function of the independent variables, then the function can be written as a first order model (Equation 5.2). In this model the response variables that fit a linear model are generally variables that are significantly affected by a small change in the value of the input factors and that exhibit little or no interaction(s) between the input variable terms.

Second order equations are used to generate linear and quadratic response equations that exhibit interactions between the input factors and can be represented by Equation 5.3.

It has been reported that second order models are also applicable to input factors that exhibit extensive variability over an experimental domain and these relationships are best described using Equation 5.4

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \varepsilon$$ **Equation 5.2**

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_{12} + \ldots + \varepsilon$$ **Equation 5.3**

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} {x_1}^2 + \beta_{22} {x_2}^2 + \ldots . + \varepsilon$$

Where,

y= response
$x_1, x_2, \ldots\ldots x_n$ = input factors
β_0 = constant that represents the intercept
β_i = coefficient of first order term
β_{ii} = coefficient of second order term
β_{ij} = coefficient of second order interaction

The values of the coefficients in the model are generated through multiple linear regression analysis of the data that has been collected. A coefficient with a positive value points to an agonistic effect of the input factor on the response, whereas coefficients with negative values indicate an antagonistic effect.

5.1.1.1 Choice of Response Surface Design

5.1.1.1.1Central Composite Design (CCD)

A CCD was originally presented by Box and Wilson and is based on a factorial design with additional points to estimate the curvature of that design. CCD encompasses a full factorial or fractional factorial approach which can be represented, as shown in Figure 5.1, as the eight corners of a cube.

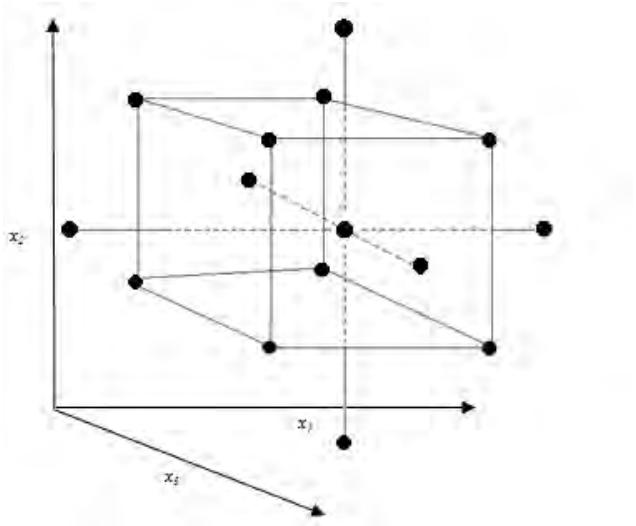


Figure 5.1 Schematic diagram representing the levels studied in a Central Composite Design (203)

There are the six points, known as the axial or star points, located in the centre of each face of the cube with a final point located in the middle of the cube that is known as the centre point (203). The axial points are experimental runs where all but one of the factors to be investigated are set at the intermediate level under consideration. The axial points are all equidistant from the centre point and are denoted using the symbol, alpha (α). The factors under consideration are usually investigated at five different levels and are always represented by coded values *viz.,* -α, -1, 0, +1 and +α. The distance of the axial points from the centre point is dependent on the number of factors investigated in the design and is established using Equation 5.5.

$$\alpha = 2^{\frac{k}{4}}$$

Where,

k= the factor number
α = axial point

The number of experiments required for a CCD approach is calculated using Equation 5.6

$$N = k^2 + 2k + C_0$$

Where,

N= the experiment number
k= the factor number
C_0= the replicate number of the central point

The number of experiments required in an experimental study is important as it determines how much data will be generated, in addition to being an indicator of the amount of time that will be required to conduct the study.

5.1.1.1.2 Box-Behnken Design (BBD)

The BBD describes a class of second-order designs based on a three-level incomplete factorial approach which are also represented as coded values *viz.,* -1, 0 and +1 (208). In this design approach, the treatment combinations are located at the midpoint(s) of the edge of the process space and at the centre, as represented in Figure 5.2.

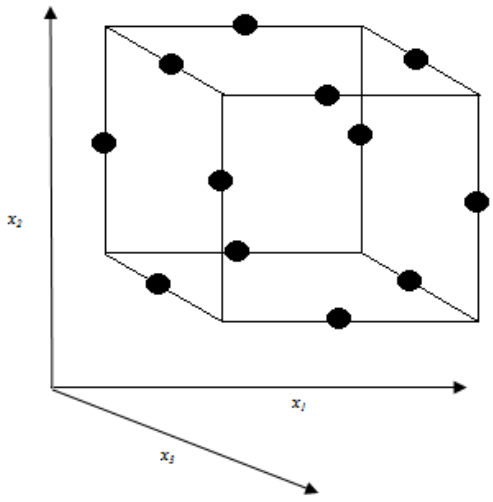


Figure 5.2 Schematic diagram representing the levels studied in a Box-Behnken Design (203).

The number of experiments for Box-Behnken Designs can be calculated using Equation 5.7.

$$N = 2k\ (k-1) + C_0$$

Where,

N= the number of experiments
k= the factor number
C_0 = the replicate number of the central point

For experiments in which there are three or less input variables the BBD design offers some advantage over the CCD approach, in that a fewer number of experimental runs are required. However this advantage does not exist when four or more parameters are to be investigated. A further advantage of BBD is that it does not include the need to evaluate situations in which all factors are simultaneously held at their highest and lowest levels. The use of a BBD therefore allows a formulation scientist to avoid undertaking experiments that are to performed under extreme conditions and that may produce substandard results due to the inclusion of data generated from these extreme high and low levels (203).

5.1.1.1.3 Doehlert Design

The Doehlert design is an experimental design approach in which different factors can be studied at different levels simultaneously (209). This aspect of the Doehlert design is an important characteristic when using some input variables that may be subject to restrictions such as for example cost or experimental constraints (limited amounts of raw material or limited amount of time available) thereby making it a practical and economic alternative to other, second-order experimental design approaches (203). This design describes a circular domain of two input variables, a spherical domain for three input variables and a hyper-spherical space for situations in which more than three input variables are to be investigated and which highlights the uniformity of the input variables to be studied in the experimental domain (203).

The schematic design space of a Doehlert design for two variables is shown in Figure 5.3 , and is represented by a central point and six points of a regular hexagon.

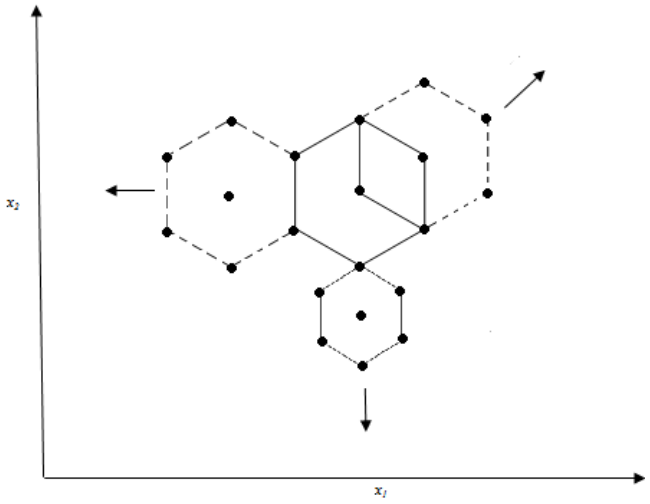


Figure 5.3 Doehlert design for the optimization of two variables with some possibilities for the displacement of the initial design, using previous points (203)

An interesting feature of the Doehlert design is that new factors may be introduced during the course of a study without losing relevant and/or valuable information from the data already generated from the experimental runs that have already been completed.

The number of experiments required for a Doehlert design is determined using Equation 5.8 (203).

$$N = k^2 + k + C_0$$

Where,

N= the number of experiments
k= the factor number
Co= the replicate number of the central point

5.1.1.1.4 Choice of Experimental Design

An evaluation of the main effects and interactions of the input and response (output) variables was performed using CCD statistical screening design and is summarized in Tables 5.1 and 5.2, respectively.

Table 5.1 Summary of the factors evaluated, the actual amounts used and corresponding coded values

Independent Variables	Levels Used, Actual (Coded)				
	Very Low (-2)	Low (-1)	Medium (0)	High (+1)	Very High (+2)
x_1=CMS (%, w/w)	6.80	7.65	8.5	9.35	10.2
x_2=CRP (%,w/w)	10.80	12.15	13.5	14.85	16.2
x_3=SSG (%, w/w)	6.40	7.20	8.00	8.80	9.60
x_4 = Blending Time (min)	7	10	13	16	19

Table 5.2 Summary of the Dependent variables monitored and the constraints set for these variables

Dependent Variables	Constraints
y_1= Disintegration time (D_t)	Minimal
y_2= Wetting Time	Minimal
y_3= Assay of content	90<y3<110

The tablet formulation developed in Chapter 4, Batch SC 007, was used as a basis for optimization. As discussed in §4.3.2 it was shown that as the concentration of the disintegrant increases, there was a decrease in both disintegration and wetting times. As ODT need to disintegrate as rapidly as possible the concentration of tablet disintegrant(s) in a formulation is an important independent factor and each of the three concentrations of superdisintegrant were selected as independent factors to be evaluated for their ability to shorten the D_t. The fourth independent variable to be evaluated was the blending time of the powder mix in the manufacturing process. This variable is important, as the blending time is crucial for the successful manufacture of high quality, low dose solid oral products.

The three dependent variables monitored were Dt, wetting time and API content. Wetting time and content assay were selected as they are indicative of the rate of disintegration and the suitability of the blending time, respectively.

A CCD design was selected as it has the advantage that it requires five levels for each factor to be evaluated, resulting in a better estimation of the responses that are monitored and achieved. The use of the Box-Behnken design for example, results in experiments being conducted at three levels only and therefore a greater level of uncertainty exists in the data that are generated.

5.1.1.2 Mathematical Optimization

Optimization is a mathematical method used to determine an optimum response and is defined as the most advantageous state of existence of the system under investigation (199). Multiple linear regression equations generated from statistically designed experiments provide a description of the change of a response with a change in input factors and further, allows for the determination of input variables that will produce an optimized response.

A difficulty that occurs in optimization procedures, is the need to establish a compromise between the anticipated response variables. This challenge is often encountered in the process of optimization of tablets where the optimum tablet may be one that has superior strength and little or no friability, yet must also have a short disintegration time. Often an increase in tablet hardness results in an increase in the disintegration time of a tablet and therefore a compromise between these contradictory response variables is necessary to achieve an optimized formulation.

5.1.1.3 Advantages of RSM

The primary advantage of RSM in relation to classical experimental methods and approaches of data evaluation in which only one variable is investigated at a time, is that a large amount of information can be generated from a relatively small number of experiments (204). RSM is therefore less time and cost consuming than the classical approach that requires a large number of experiments to be conducted to be able to explain the behaviour of a system (204; 205).

A further advantage, with the use of RSM is that it is possible to observe interaction effects of the independent input parameters on the response(s) being monitored (204). The model equation that is generated from the data is able to be used to explain the effect of combinations of independent input variables on the outcome of a process or product.

5.1.1.4 Disadvantages of RSM

A primary disadvantage of RSM is that fitting data to a second order polynomial for systems that contain some curvature is often not well accommodated by the second order polynomials that are produced. If the system cannot be explained by a first or second order polynomial, it may be necessary to reduce the range of independent input variables under consideration as this may then increase the accuracy of the model being considered (204).

Another disadvantage is that although RSM has the potential to evaluate interaction effects of the independent input parameters, it is unable to be used to explain why an interaction(s) has occurred

(210). A further disadvantage is that RSM is poor at predicting the potential outcomes for a system operated outside the range of study under consideration (210).

5.1.2 Quality Control Testing

5.1.2.1 In vitro drug release testing

Drug absorption from a solid dosage form following oral administration is dependent on the release of the API from that dosage form, subsequent dissolution and/or solubilisation of the API under physiological conditions and also the permeability of the API through the gastrointestinal tract membranes (GIT) (211). Since these steps are of significant importance for effective drug delivery, *in vitro* dissolution testing is highly relevant to the prediction of the potential *in vivo* performance of dosage forms.

Dissolution testing is a quality control technique that is used to assess drug release profiles from pharmaceutical dosage forms. Dissolution testing is considered important as drug release from a dosage form is essential in order to produce a therapeutic effect. An API must be released from the dosage form and dissolve in the gastrointestinal tract (GIT) fluids prior to absorption (212). Consequently dissolution testing may be considered an indicator of the potential for drug release from a solid dosage form.

Drug dissolution is crucial to ensure that there is batch to batch consistency within a manufacturing process.

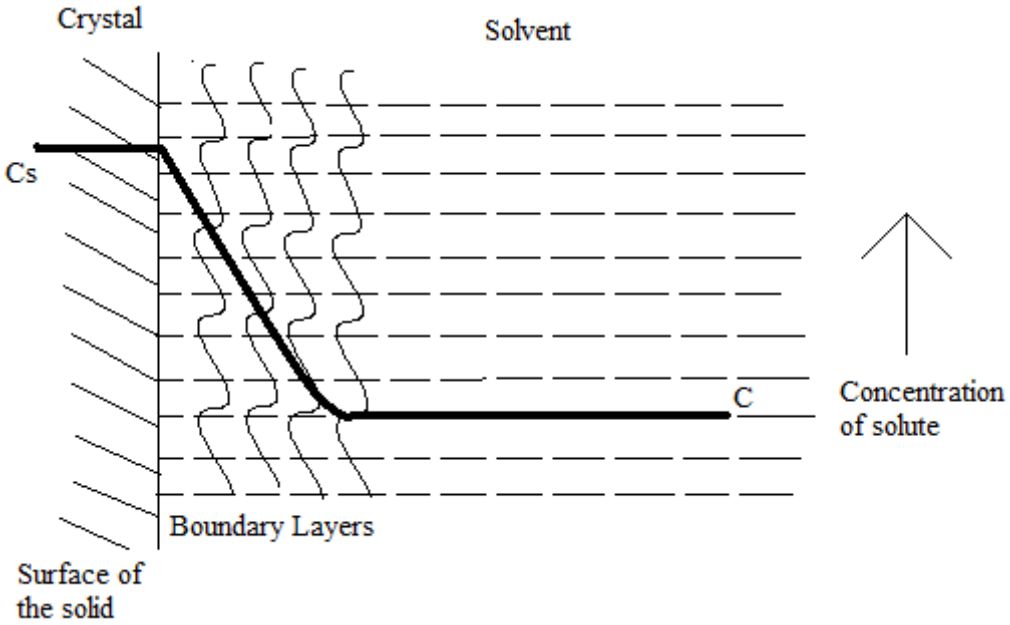
5.1.2.2 Theory of Dissolution

The dissolution of a solid into a liquid can be separated into two distinct stages (213). Initially an interfacial reaction results in the release of solute molecules from a solid phase and involves a phase change that permits molecules of the solid to become molecules of a solute in that solvent. The solution in contact with the solid is saturated since it is in direct contact with the solid in that liquid. Subsequently the molecules of the solute migrate through the boundary layers surrounding that solute, via a passive diffusion process, to the bulk of the solution. The transport of the molecules away from the solid-liquid interface into the bulk of the liquid follows a process that is described by the Noyes-Whitney relationship. The Noyes-Whitney equation is discussed in §5.1.2.2, *vide infra*. .

As is the case with any reaction that involves more than one step, the overall rate of reaction is determined by the rate of the slowest step of that process. In the case of dissolution testing the

interfacial step is instantaneous and therefore the diffusion of a dissolved solute across the static boundary is the rate determining step in dissolution of solid into a liquid.

A diagram depicting the boundary layers and change in concentration surrounding a dissolving particle is shown in Figure 5.4



Where,

C= the concentration of the solute in the bulk liquid

Cs= the concentration of solute in the saturated solution

Figure 5.4 The stages of dissolution (213)

5.1.2.3 Factors affecting API dissolution

Sold drugs need to dissolve prior to absorption. The dissolution of a solid or API can be described by the Noyes-Whitney equation (Equation 5.9) (214), which describes the dissolution of spherical particles, when the dissolution process is diffusion controlled and involves no chemical reaction to facilitate the process of dissolution.

$$\frac{dC}{dt} = \frac{DA(C_s - C)}{h}$$

Where,

$\frac{dC}{dt}$= rate of dissolution of drug particles
D= diffusion coefficient of the drug in solution in the gastrointestinal fluids
A = the effective surface area of the drug particles in contact with the gastrointestinal fluids
h= the thickness of the diffusion layer around each particle
C_s= the saturation solubility of the drug in solution in the stagnant or diffusion layer
C= the concentration of the drug in the gastrointestinal fluids

Consequently the physicochemical properties of an API are an important factor when considering the dissolution of that material. The dissolution medium is usually an aqueous based fluid that may include a buffer or in rare cases, purified water. Therefore the aqueous solubility of an API is a critical determinant in the rate of solution of the raw material. Additional factors that can impact the rate of solution of a molecule include the particle size and crystalline state of the API (215).

Furthermore several key properties of a pharmaceutical product, including dosage form type, expected potency, desired mechanism of release and the excipients used in the dosage form are also important factors to consider when selecting a dissolution test method. For example, USP Apparatus I or the basket method is known to be prone to gelatin build-up if the baskets are not cleaned properly following each test (216).

When developing an appropriate dissolution test procedure the impact of test parameters that may affect the dissolution rate must also be evaluated. The degree of agitation of the dissolution method is one of the important factors that affect the dissolution of an API (217). To ensure the elimination of variability in the rate of drug release from a delivery technology, it is important that factors such as temperature, pH, in addition to the nature, composition and volume of dissolution medium to be used are adequately controlled (215). By way of example, a change is in test method parameters that may impact dissolution rates include changes in temperature that may result in release of dissolved gas, and the resultant bubbles may adversely impact dissolution rate measurements (215).

In addition the dissolution test apparatus may also have an impact on the release of an API from a dosage form. To ensure that the release of a drug is accurately assessed, the dissolution apparatus needs to be appropriately calibrated and used in accordance with the relevant specifications. Specifically, vibration and wobble of the Apparatus must be eliminated so that the flow patterns of the dissolution medium remain consistent from test procedure to test procedure (215). A summary of the most significant factors that can affect drug dissolution rates are summarized in Table 5.3.

Table 5.3 Summary of the factors affecting API dissolution

Factor	Physicochemical parameter	Physiological parameter
Surface area of drug	Particle size, wettabilty	Surfactants in GIT fluids
Solubility in diffusion layer	Hydrophilicity, crystal structure, solubilization	pH, buffer capacity, bile, food composition
Amount of drug already dissolved		Permeability, transit
Diffusivity of drug	Molecular size	Viscosity of luminal contents
Boundary layer thickness		Motility patterns and flow rate
Volume of solvent available		GIT secretions, co-administered fluids

5.1.2.4 Dissolution Test Apparatus

The major components of dissolution testing equipment include a heated water bath, paddles or baskets and shafts, vessels, sampling and analytical test procedures and equipment (216).

The official compendia *viz.,* BP and USP list several official apparatus for dissolution testing in addition to providing other relevant information relating to the conduct of these tests. The official methods listed in both compendia include Apparatus 1 or rotating basket, Apparatus 2 or paddle apparatus, Apparatus 3 or reciprocating cylinder, Apparatus 4 or flow-through cell, Apparatus 5 or Paddle over disk, Apparatus 6 or rotating cylinder and Apparatus 7 or reciprocating holder methods (216).

USP Apparatus 1 and USP Apparatus 2 are the most commonly used dissolution apparatus despite the fact that sources of error can be introduced, if procedures for testing are not closely inspected prior to and monitored during use. The paddles for USP Apparatus 2 may be coated with Teflon in certain methods and if the material peels from the surface of the paddle and is shed, it could result in the disturbance of the fluid dynamics in the dissolution vessel. The hydrodynamics of a test system can also be disturbed when using paddles that have rusted or that have dents present on their surface and can lead to contamination of the test medium if they are not cleaned between test procedures (216). It is therefore important to ensure that the paddles are cleaned correctly after each experimental run. The basket or USP Apparatus 1 method is also not without some challenges as the baskets can become frayed and misshaped following excessive use leading to a change in the mesh size which ultimately may affect the dynamics of the test system(216).

5.2 EXPERIMENTAL

5.2.1 Materials and Equipment

All materials and equipment that were used for the studies conducted and reported in this chapter are listed in §4.41 and 4.4.2, respectively, *vide infra*.

5.2.2 Statistical analysis of data

The significance of the model(s) that were elucidated in these studies was analysed using Analysis of Variance (ANOVA) type three (partial sum of squares) studies at a 5% level of significance. Design Expert® (Stat-Ease Inc., Minneapolis, Minnesota, United States of America) statistical design computer software was used to analyse the data that was generated. The predicted residual error sum of squares (PRESS) was used to assess which of the input factors had a significant impact on the response(s) under investigation.

5.2.3 Physical properties of tablets

The tablets were subject to dissolution, assay, disintegration, friability, crushing strength, tensile strength, wetting*, in vitro* dispersion, thickness, diameter and weight testing. These procedures were performed as described in §4.2.4.

5.2.4 *In vitro* release testing

In vitro release studies of SC ODT tablets was performed using USP Apparatus 2 (Hanson Research SR 8 PLUS , California, United States of America) fitted with an Autoplus™ Multifill™ and Maximizer Syringe Fraction Collector. Six tablets were dropped into the dissolution vessels, each containing 900ml of vacuum degassed 0.05M Phosphate buffer (pH 6.8). The buffer was prepared as described in § 4.2.4.2.8.1.

The paddles were set to rotate at 75rpm, despite the fact that a speed of 50rpm is recommended for dissolution testing of ODT, and slower paddle speeds may be used to generate dissolution profiles that may be compared due to appropriate discrimination of the resultant dissolution profiles generated using the method. However a speed of 50rpm resulted in the formation of a mound of particles at the base of the dissolution vessel and therefore accurate sampling may not have been possible. USP Apparatus 1 may have certain application for ODT testing for *in vitro* studies but is not commonly used due to the physical properties of the tablets under investigation. Tablet fragments from the

disintegrated tablet, for example, may become trapped on the inside and at the top of the basket near the spindle where little effective stirring occurs, thereby reducing the reliability of the test method, with the result that variable and poorly reproducible results may be generated (218).

The temperature of the dissolution medium was maintained at 37±0.5°C for all studies and 5ml aliquots of the dissolution fluid was collected for analysis at 5, 10 and 15 minutes following the commencement of the test. An equal volume of medium was replaced after each sample had been collected. A 3ml aliquot of each sample was harvested using an electronic pipette (Boeckel & Co. GmbH, Hamburg, Germany) and transferred into a 5ml A-grade volumetric flask followed by the addition of 6µl of IS solution that had been prepared as previously described in §2.3.2.3, for HPLC analysis. The resultant solution was made up to volume using an ACN: water solution in a ratio of 55:45 that had been filtered through a 0.22µm Acrodisc® PSF syringe filter (Pall Corporation, Port Washington, New York, United States of America) prior to analysis. Sample content was quantitatively determined using the validated HPLC method that has been described in Chapter Two, *vide infra*.

5.2.5 Experimental Design

The mathematical relationship between the input and response variables was generated using Design Expert® 8.0.4 software (Stat-Ease Inc, Minneapolis, Minnesota, United States of America). The independent variables were studied at five levels *viz.* very high, high, medium, low and very low. In these studies, in respect of the amount of superdisintegrant used in the formulation, the medium point was considered the amount of the excipient(s) used to manufacture Batch SC 007, an ODT technology manufactured as described in Chapter Four.

The coded and actual values generated using CCD design of experiments for the experimental formulations is summarized in Table 5.4

Table 5.4 Coded and actual values of independent variables generated using a CCD design

Batch No.	Independent Variables							
	x_1		x_2		x_3		x_4	
	Coded	Actual	Coded	Actual	Coded	Actual	Coded	Actual*
SC 008	0	8.5	0	13.5	0	8	2	16+3
SC 009	1	9.35	-1	12.15	-1	7.2	1	13+3
SC 010	1	9.35	1	14.85	1	8.8	-1	7+3
SC 011	-1	7.65	1	14.85	1	8.8	-1	7+3
SC 012	-1	7.65	-1	12.15	1	8.8	-1	7+3
SC 013	-1	7.65	1	14.85	-1	7.2	-1	7+3
SC 014	0	8.5	-2	10.8	0	8	0	10+3
SC 015	0	8.5	0	13.5	0	8	0	10+3
SC 016	0	8.5	0	13.5	0	8	0	10+3
SC 017	-1	7.65	-1	12.15	1	8.8	1	13+3
SC 018	-2	6.8	0	13.5	0	8	0	10+3
SC 019	2	10.2	0	13.5	0	8	0	10+3
SC 020	0	8.5	0	13.5	0	8	-2	4+3
SC 021	0	8.5	0	13.5	0	8	0	10+3
SC 022	-1	7.65	1	14.85	1	8.8	1	13+3
SC 023	1	9.35	-1	12.15	1	8.8	-1	7+3
SC 024	0	8.5	0	13.5	0	8	0	10+3
SC 025	0	8.5	2	16.2	0	8	0	10+3
SC 026	0	8.5	0	13.5	0	8	0	10+3
SC 027	1	9.35	-1	12.15	1	8.8	1	13+3
SC 028	1	9.35	-1	12.15	-1	7.2	-1	7+3
SC 029	-1	7.65	-1	12.15	-1	7.2	1	13+3
SC 030	0	8.5	0	13.5	-2	6.4	0	10+3
SC 031	1	9.35	1	14.85	-1	7.2	1	13+3
SC 032	0	8.5	0	13.5	0	8	0	10+3
SC 033	-1	7.65	1	14.85	-1	7.2	1	13+3
SC 034	0	8.5	0	13.5	2	9.6	0	10+3
SC035	-1	7.65	-1	12.15	-1	7.2	-1	7+3
SC 036	1	9.35	1	14.85	-1	7.2	-1	7+3
SC 037	1	9.35	1	14.85	1	8.8	1	13+3

*As discussed in Chapter 4, the blending of powders was separated into two different segments. The first segment involved blending all excipients excluding magnesium stearate and the second segment where the powders were blended for a further 3 minutes followed the addition of magnesium stearate.

5.3 RESULTS AND DISCUSSION

5.3.1 Physical characteristics of powder blend

A summary of the physical characteristics of the powder blends that were manufactured using a CCD are summarized in Table5.5. The results indicate that all powder blends had a CI < 24 and a HR < 1.3 indicating that the flow properties of all powder blends, was satisfactory. These results were confirmed by angle of repose measurements with all values for this parameter being established as < 30°. From these results it can be concluded that the flow properties of the blends are adequate to assume that flow of powder blends from the feed hopper on the press, into the die cavity should result in the production of tablets of uniform weight.

Table 5.5 Physical properties of the powder blends manufactured according to a CCD approach

Formulation	Bulk Density(g/ml)	Tapped Density(g/ml)	CI	HR	Angle of Repose
SC 008	0.50	0.65	21.95	1.28	29.74
SC 009	0.54	0.70	22.50	1.29	29.74
SC 010	0.52	0.67	21.43	1.27	26.57
SC 011	0.50	0.63	20.00	1.25	23.20
SC 012	0.51	0.64	21.43	1.27	21.04
SC 013	0.51	0.65	22.50	1.29	23.20
SC 014	0.54	0.68	20.51	1.26	28.30
SC 015	0.50	0.63	21.43	1.27	21.80
SC 016	0.52	0.65	18.92	1.23	23.20
SC 017	0.48	0.59	19.05	1.24	24.78
SC 018	0.50	0.64	21.95	1.28	26.57
SC 019	0.50	0.64	21.95	1.28	26.57
SC 020	0.53	0.67	21.05	1.27	26.57
SC 021	0.52	0.67	21.43	1.27	26.57
SC 022	0.48	0.62	22.50	1.29	30.65
SC 023	0.50	0.64	21.95	1.28	26.57
SC 024	0.51	0.65	21.05	1.27	24.78
SC 025	0.50	0.65	22.50	1.29	26.57
SC 026	0.52	0.65	19.51	1.24	29.74
SC 027	0.52	0.65	19.51	1.24	27.41
SC 028	0.49	0.65	23.81	1.31	23.20
SC 029	0.53	0.69	22.50	1.29	24.78
SC 030	0.51	0.63	19.05	1.24	29.74
SC 031	0.49	0.63	21.43	1.27	28.30
SC 032	0.51	0.65	21.95	1.28	26.57
SC 033	0.53	0.67	21.05	1.27	28.30
SC 034	0.55	0.69	20.51	1.26	28.30
SC 035	0.49	0.62	20.00	1.25	28.30
SC 036	0.53	0.67	21.05	1.27	29.74
SC 037	0.54	0.69	21.43	1.27	24.78

5.3.2 Physical characteristics of tablets

A summary of the results following an evaluation of the physical characteristics of the tablets produced is listed in Table 5.6. All tablets that were produced were white in colour and had a smooth feel on the surface indicating that no picking or sticking had occurred.

The crushing strength of the tablets was similar for all batches and the variability of the disintegration times was minimal. All batches passed the weight variation test with % RSD values < 7.5% for all batches, further indicating that the powder blends exhibited good flow properties.

All tablets met the compendial standards for friability with values of < 1% reported (219). The successful development of a solid oral dosage form requires that the resultant tablets have a low crushing strength to facilitate rapid disintegration, whilst still demonstrating a friability of < 1%.

The *in vitro* dispersion and wetting times of the tablets infer that a rapid disintegration time for these tablets is possible and this was confirmed when evaluating the results of disintegration testing with a maximum time of 11s recorded for all batches.

The high water absorption ratios that are reported are a consequence of the large amount of disintegrants that were included in the formulation to facilitate the rapid disintegration of the tablets that had been manufactured.

Content uniformity testing of each of the batches of tablets were carried out to establish content uniformity of the manufactured batches. The analysis was performed as described in §4.2.6. It was noted that formulation SC 020 exhibited poor content uniformity that was evident by the low assay value and high standard deviation of the result. It was thought that the result may be due to a short blending time of that particular formulation however this relationship could only be confirmed with the use of CCD.

Table 5.6 Physical Characteristics of Tablets

Batch No.	Weight (n=10) mg	Thickness (n=10) mm	Diameter (n=10) mm	Crushing Strength (n=10) N	Tensile Strength MPa	Friability (%)	Dt (s)	Wetting Time (s)	Water Absorption Ratio	In Vitro Dispersion Time (s)	Assay (n=3) %
SC 008	159.54±1.60	2.89±0.07	7.33±0.02	18.37±1.32	0.55	0.65	8	4.29±0.02	151.62±9.24	35.92±0.17	98.65±4.40
SC 009	156.67±7.07	2.85±0.14	7.32±0.03	18.17±2.49	0.55	0.61	8	4.29±0.06	121.98±10.42	36.68±0.69	92.81±4.44
SC 010	154.44±8.81	2.90±0.10	7.33±0.02	20.41±3.40	0.61	0.59	8	3.89±0.19	141.17±6.83	26.76±0.32	86.67±4.41
SC 011	153.75±7.44	3.00±0.00	7.33±0.02	19.77±2.13	0.57	0.64	11	4.46±0.23	145.67±10.98	38.60±0.60	101.47±1.22
SC 012	154.07±10.73	2.85±0.15	7.30±0.01	19.98±3.66	0.61	0.62	8	4.79±0.18	140.98±8.79	35.79±0.46	92.09±3.70
SC 013	145.83±6.69	2.85±0.04	7.32±0.01	17.69±1.94	0.54	0.69	5	4.44±0.23	120.45±14.51	39.54±0.56	82.05±4.97
SC 014	151.43±9.00	2.86±0.04	7.33±0.00	18.55±2.61	0.56	0.69	8	4.38±0.15	115.09±14.32	36.64±0.55	95.63±3.87
SC 015	158.77±3.50	2.79±0.10	7.32±0.01	18.51±1.76	0.58	0.98	8	4.26±0.24	145.84±19.52	35.41±1.20	96.76±2.89
SC 016	149.03±7.38	2.64±0.17	7.31±0.02	18.98±1.90	0.63	0.64	7	4.32±0.16	146.61±6.65	35.89±0.17	96.16±3.04
SC 017	154.74±5.13	2.86±0.02	7.25±0.02	18.81±3.25	0.58	0.65	9	4.64±0.17	128.78±6.70	36.67±0.14	103.89±1.78
SC 018	151.25±8.34	2.83±0.10	7.32±0.02	19.88±1.98	0.61	0.64	8	4.58±0.29	117.19±9.19	37.68±0.45	96.41±3.25
SC 019	154.30±4.89	2.72±0.16	7.33±0.02	17.41±2.79	0.56	0.65	6	4.55±0.26	129.31±10.84	36.45±0.17	97.88±2.87
SC 020	150.13±7.39	2.67±0.19	7.31±0.01	20.18±3.70	0.66	0.66	7	4.25±0.26	139.93±5.51	36.23±0.20	75.11±8.66
SC 021	158.28±5.03	2.75±0.11	7.33±0.01	17.13±1.37	0.54	0.67	6	4.31±0.10	146.63±7.88	35.98±.0.67	98.42±2.63
SC 022	153.65±7.12	2.79±0.16	7.32±0.01	19.46±2.11	0.61	0.67	7	4.15±0.08	149.89±5.67	36.12±0.18	97.54±2.69
SC 023	156.60±6.75	2.79±0.13	7.29±0.03	17.90±4.54	0.56	0.54	8	4.37±0.23	146.22±13.42	35.42±0.21	114.98±6.03
SC 024	149.72±7.46	2.79±0.17	7.32±0.02	19.33±2.29	0.60	0.74	7	4.32±0.20	143.48±16.44	35.78±0.17	98.63±3.87
SC 025	155.76±4.23	2.74±0.07	7.32±0.02	18.09±2.74	0.57	0.61	7	4.26±0.14	161.61±9.17	35.69±0.23	96.51±4.03
SC 026	154.92±8.45	2.80±0.16	7.32±0.01	19.34±2.56	0.60	0.63	10	4.29±0.12	143.77±10.90	35.90±0.15	98.91±4.59
SC 027	152.06±7.04	2.60±0.15	7.27±0.01	24.71±1.93	0.83	0.74	8	4.42±0.09	139.91±16.71	35.79±0.19	99.03±3.56
SC 028	156.69±6.99	2.80±0.22	7.31±0.02	17.46±2.48	0.54	0.82	9	4.30±0.24	142.45±6.55	35.92±0.18	88.37±5.01
SC 029	147.92±5.49	2.78±0.18	7.31±0.03	16.88±4.17	0.53	0.65	9	4.44±0.22	112.54±13.48	36.20±0.23	96.23±4.12
SC 030	148.05±7.23	2.72±0.17	7.30±0.04	19.96±2.51	0.64	0.65	8	4.42±0.15	132.47±8.99	35.32±0.22	96.21±3.89
SC 031	152.41±8.23	2.79±0.07	7.31±0.02	19.64±3.05	0.61	0.74	8	4.32±0.16	154.61±13.45	35.34±0.40	98.45±2.98
SC 032	152.30±5.66	2.82±0.15	7.31±0.01	18.76±2.34	0.58	0.65	8	4.30±0.17	141.54±7.84	35.81±0.12	99.13±2.46
SC 033	150.26±7.19	2.64±0.17	7.31±0.02	18.98±1.90	0.63	0.63	10	4.49±0.16	161.71±11.23	35.40±0.96	97.44±1.99
SC 034	152.12±10.91	2.79±0.15	7.30±0.01	18.45±3.74	0.58	0.63	8	4.55±0.22	114.12±12.85	35.76±0.32	96.72±3.70
SC 035	149.83±7.33	2.68±0.28	7.30±0.01	18.86±3.26	0.61	0.65	9	4.42±0.23	121.68±17.46	36.62±0.13	89.90±4.81
SC 036	155.71±6.73	2.77±0.22	7.31±0.01	19.48±3.34	0.61	0.62	8	4.49±0.19	152.90±14.08	34.85±1.6	90.01±2.56
SC 037	156.20±7.19	2.78±0.23	7.31±0.02	20.75±3.79	0.65	0.66	8	4.05±0.09	160.01±15.34	36.68±0.69	99.82±4.01

5.3.3 Response Surface Modelling

5.3.3.1 Disintegration time (D_t)

As the purpose of these studies was to develop an ODT, the impact of the D_t of the tablets was investigated using RSM to establish which of the input variables may have had a significant impact on the performance of the dosage forms.

The data was analysed to determine the significance of the models that were produced. However it was discovered that the resultant model was not significant when fitted to a quadratic model and therefore this approach was not suitable to navigate the intended design space. Therefore the model was reduced to a linear form and the linear model was also not significant indicating that the data did not fit the model.

A possible reason for the lack of fit to both models is that the critical concentration of the disintegrants had been reached in the formulation and no further increase in the amount of any of the superdisintegrants used would have had a noticeable effect on the D_t of the tablets.

The critical concentration of disintegrants plays an important role in the disintegration time of an ODT and use of amounts of material below this concentration usually results in disintegration times that are inversely proportional to the disintegrant concentration, whereas higher concentrations result in approximately constant or increased disintegration times (158; 170).

5.3.3.2 Wetting Time

The wetting time for SC ODT tablets was best described by the use of a quadratic model. The polynomial model used to investigate the relationship between the input factors and the wetting time is summarized in Equation 5.10.

$$y_2 = 4.30 - 0.073x_1 - 0.068x_2 - 0.023x_3 - 0.012x_4 + 7.50E - 0.003x_1x_2 - 0.057x_1x_3 + 0.026x_1x_4 - 0.12x_2x_3 - 0.011x_2x_4 - 8.75^{-0.003}x_3x_4 + 0.064{x_1}^2 + 2.91^{-0.003}{x_2}^2 + 0.019{x_3}^2 - 9.583^{-0.003}{x_4}^2$$

Where,

y= Response
x_1 = amount of CMS
x_2 = amount of CRP
x_3 = amount of SSG
x_4 = Blending time

RSM analysis reveals that the concentration of CMS and CRP in the formulation had a significant effect on the wetting time of the tablets and the amount of SSG and the blending time did not play a significant role in the performance of the dosage form. These results indicated that the addition of CMS and CRP at higher levels significantly decreases the wetting time of the formulation and may perhaps decrease the D_t slightly.

As can be seen in the Figure 5.5 (I) the contour graph displays a 'rising ridge' shape and both Figure 5.5 (I) and (II) reveal that an increase in the amount of CMS and CRP results in a decrease in the wetting time of the tablets as an increase in the disintegrant concentration in the formulation leads to an increase in the porosity of the dosage form, thereby facilitating the uptake of water.

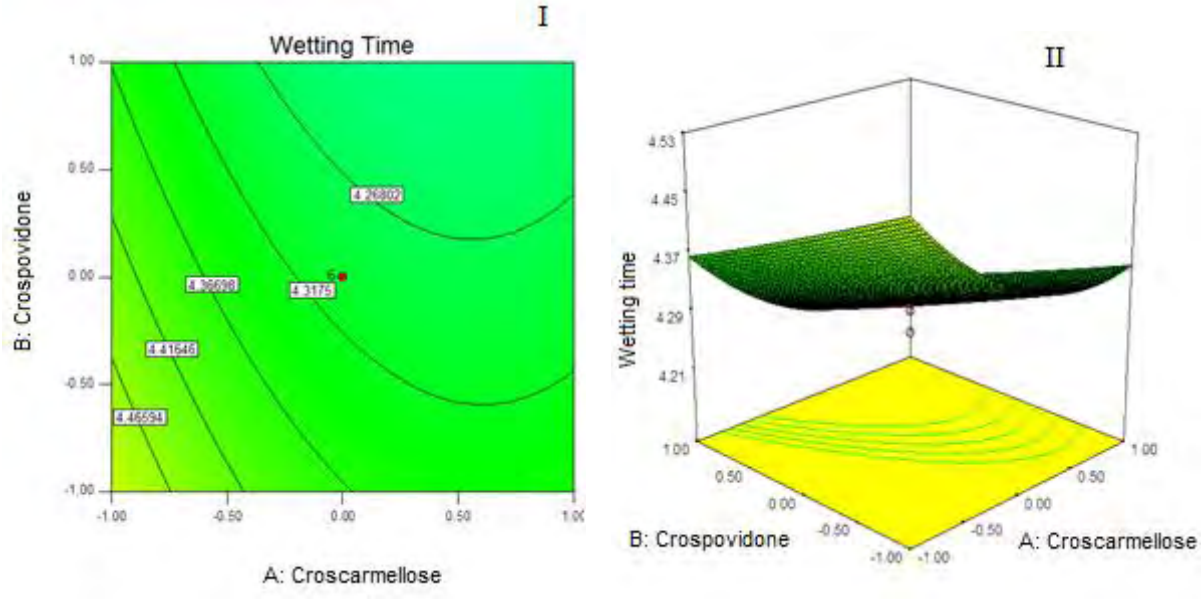


Figure 5.5 Contour plot (I) and 3D representation (II) of the interaction of CMS and CRP on the wetting time of SC ODT

As discussed in Chapter Four, CRP and CMS facilitate tablet disintegration via different mechanisms. CMS disintegrates via a swelling process whereas CRP causes tablets to disintegrate by a process of wicking as liquid is drawn through porous pathways in the core resulting in disruption of inter-particulate bonds and tablet break up. These differences in mechanism of disintegration may result in a synergistic relationship in the ODT where the combination of the disintegrants results in a more significant decrease in the wetting time of the tablets, than if only one disintegrant were used.

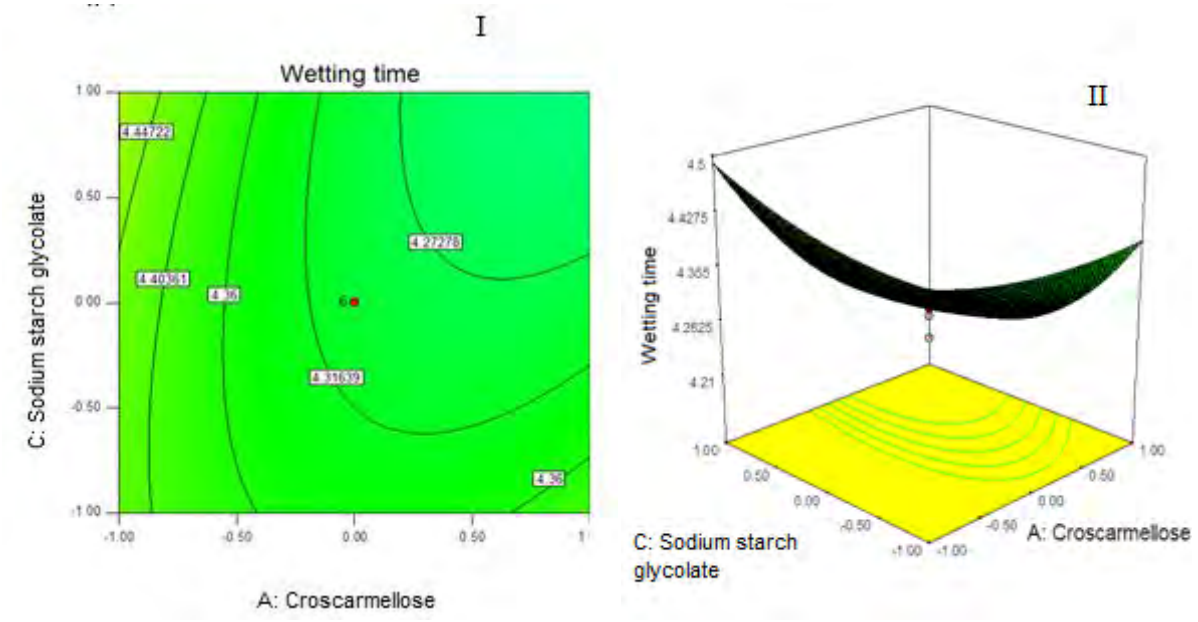


Figure 5.6 Contour plot (I) and 3D representation (II) of the effect of CMS and SSG on the wetting time of SC ODT

SSG did not play a significant role in the wetting time of the SC ODT. As shown in Figure 5.6 low levels of SSG result in a wetting time that is maximized and as higher levels of SSG are reached the wetting time increases which is most likely due to the swelling effect of SSG when used in concentrations over 8% w/w in formulations. It has been observed that SSG can have a detrimental effect on the wetting and disintegration times of tablets since it is able to form a viscous gel layer that is a barrier to the penetration of a dissolution medium (220).

As depicted in the contour (Figure 5.7 (I)) and 3D representation plots (Figure 5.7 (II)) blending time does not appear to play a significant role in the wetting time of the dosage forms. As blending times increase the wetting time of the tablet does not appear to change significantly. However the use of CRP does affect the wetting time of the tablets and as the amount of CRP in the formulation increases there is a decrease in the wetting time of the tablets. This can be clearly observed in the 3D plot where there is a steep decline in the surface plot as the amount of CRP increases in the formulation . In contrast the plot remains straight when the blending times are change indicating that this parameter has little effect on wetting times.

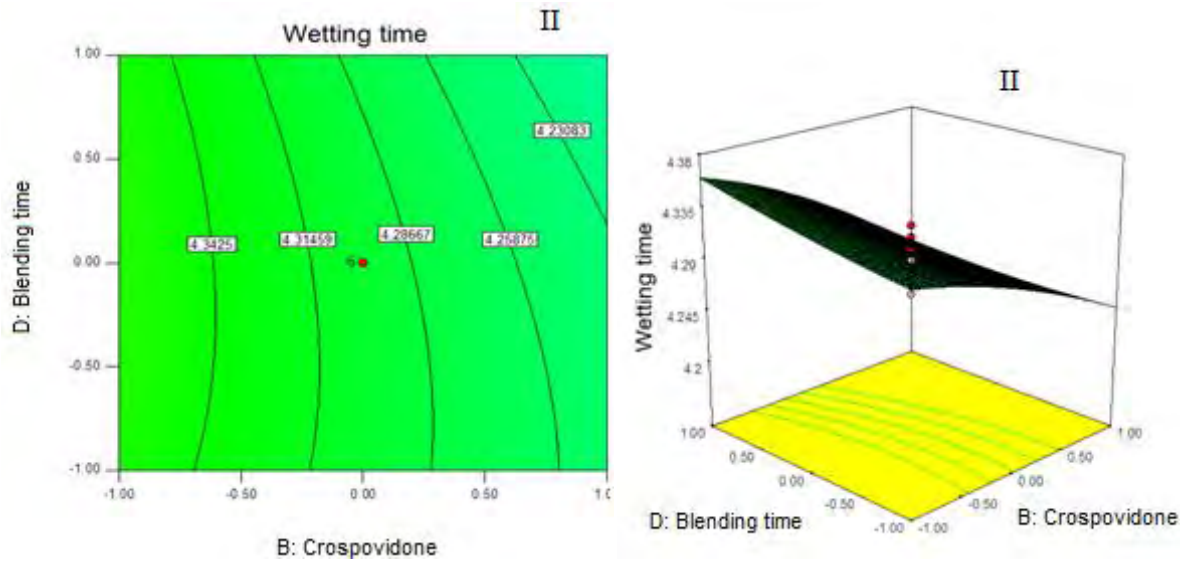


Figure 5.7 Contour plot (I) and 3D representation (II) of the effect of CRP and blending time on the wetting time of SC ODT

Similarly increasing the amount of CMS results in a decrease in the wetting time of the SC ODT and blending time has little impact on this performance characteristic (Figure 5.8).

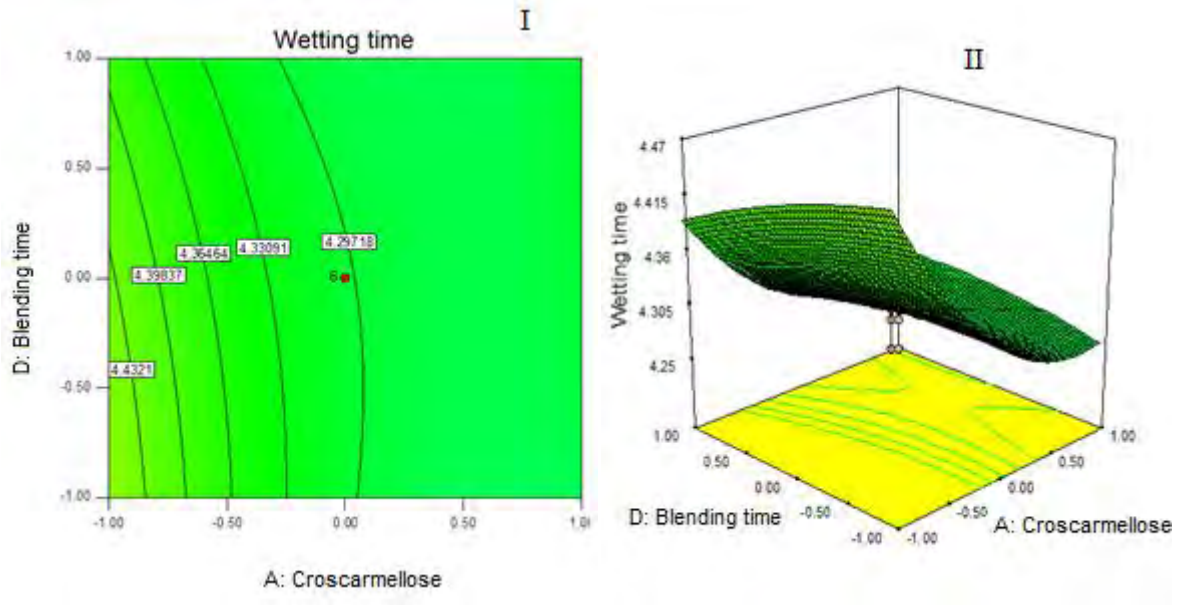


Figure 5.8 Contour plot (I) and 3D representation (II) of the effect of CMS and blending time on the wetting time of SC ODT

5.3.3.3 Content Uniformity

Following RSM analysis it was found that there was a linear relationship between the API content and the blending time and the concentration of any of the disintegrants had an effect on the uniformity of content. This relationship between blending time and content uniformity is summarized as Equation 5.11.

$$y=95.7 +0.52A\ 0.92B+2.55C+ .61D$$

where,

A= Amount of CMS
B = Amount of CRP
C = Amount of SSG
D = Blending time

The uniform distribution of each of the components of a powder mixture is essential to ensure that there is a uniform subdivision of the individual components of that mixture (173). The uniform distribution of the individual components is also critical for the excipients used to produce or manufacture a dosage form with the appropriate performance characteristics. For example the non-uniform distribution of magnesium stearate can lead to over lubrication and potential dissolution rate and processability issues (176).

The data depicted in Figure 5.9 indicate that there is a direct correlation between the blending time and the content uniformity of the tablets that were manufactured in these studies. As the blending time increases there is an increase in the content uniformity of the tablets manufactured in these studies. Efficient mixing of low dose drugs can be challenging due to issues related to segregation, content uniformity and physical stability of a low dose solid oral dosage form (221).

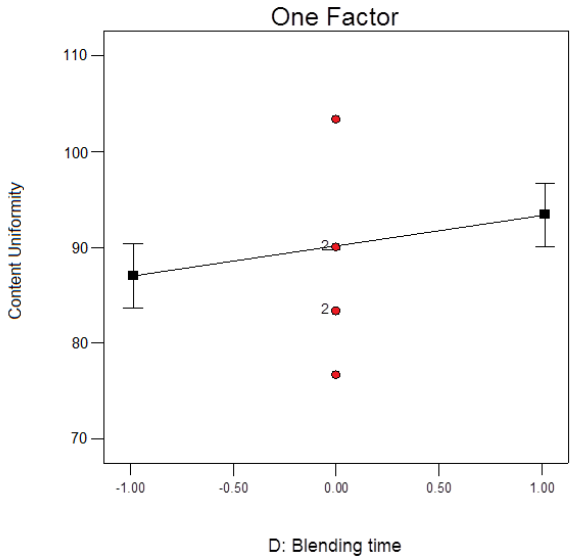


Figure 5.9 Graphical representation of the linear relationship between blending time and content uniformity of SC ODT

Therefore it is important to establish an appropriate blending time for a manufacturing process, as inefficient mixing of a powder blend will result in an uneven distribution of the API and the tablet batch will not be uniform with respect to API content. If however the powder blend is mixed for a period longer than what is optimal the adhesive tendency of drug particles can increase (222) which can lead to segregation and once again the tablet batch will not be uniform with respect to API content.

5.3.4 In vitro dissolution

Unless an API falls into the Biopharmaceutics Classification System (BCS) Class I quadrant which is indicative that 80% of the API dissolves in 15 minutes across the pH range or using one of three preferred media *viz.,* 0.1M HCl, an acetate buffer of pH 4.5,or a phosphate buffer of 6.8, it is necessary to develop a dissolution method that is able to generate a dissolution curve with a discernible profile shape (216). Essentially the dissolution of an API should be gradual so that the results generated from dissolution testing can be compared at several time points. SC is a BCS Class I compound (52) and dissolution testing was performed using a phosphate buffer of pH 6.8 to mimic the pH of the oral cavity. Furthermore since the tablets disintegrate rapidly, plotting dissolution profiles for comparison is difficult as the individual profiles tend to be superimposable.

Consequently the % drug released at 5 minutes (Q_5), 10 minutes (Q_{10}) and 15 minutes (Q_{15}) was monitored from each batch of tablets manufactured and these data are summarized in Table 5.7.

Table 5.7 *In vitro* dissolution data for CCD experimental batches

Batch No.	Q_5	Q_{10}	Q_{15}
SC 008	83.45±2.32	92.97±3.02	104.56±2.77
SC 009	80.34±1.78	95.67±1.78	106.79±2.31
SC 010	85.05±1.48	91.52±1.93	100.77±1.90
SC 011	79.45±3.45	90.20±2.91	103.77±2.64
SC 012	78.99±2.81	92.36±2.78	103.56±4.42
SC 013	84.50±2.75	95.61±2.40	100.68±3.09
SC 014	82.67±2.39	97.44±1.91	100.92±2.10
SC 015	86.78±2.11	95.73±30	101.83±1.86
SC 016	88.03±2.76	90.94±2.62	100.26±2.04
SC 017	88.92±2.60	96.30±3.40	100.54±2.61
SC 018	87.26±1.16	95.77±4.89	100.01±4.39
SC 019	86.51±1.17	99.14±1.07	102.09±3.59
SC 020	76.56±8.23	97.34±10.21	116.72±8.90
SC 021	89.06±3.79	97.79±1.49	101.53±2.90
SC 022	85.23±4.90	91.65±1.07	102.66±0.50
SC 023	71.52±3.51	88.63±2.86	98.19±0.19
SC 024	79.99±4.46	84.52±3.46	98.99±0.54
SC 025	80.11±2.33	95.43±1.91	103.65±2.37
SC 026	74.10±1.92	84.60±2.13	97.00±6.21
SC 027	79.78±2.66	93.48±2.89	104.50±2.16
SC 028	75.99±4.70	84.10±2.13	107.34±6.82
SC 029	76.62±3.63	100.21±3.35	107.44±6.98
SC 030	72.62±0.52	99.46±2.51	99.89±5.04
SC 031	79.62±3.80	99.15±3.66	109.63±2.62
SC 032	86.45±3.45	97.84±3.45	99.45±2.12
SC 033	91.43±10.11	99.54±3.48	99.57±7.43
SC 034	82.58±2.62	95.27±2.77	108.90±3.81
SC 035	87.31±3.18	94.96±6.64	96.29±4.17
SC 036	79.91±3.78	89.36±6.26	98.45±6.19
SC 037	88.43±0.64	93.63±2.00	107.58±2.93

A suitable drug release for tablets that disintegrate rapidly is a quantum of 85% API released in 15 minutes. It can be concluded therefore that all matches meet the specification (223).

Since the disintegration time of the tablets is short, model drug release from the respective tablets relates exclusively to dissolution rate of the API and not the disintegration of the tablet (224).

The incorporation of magnesium stearate into a formulation may impart some useful properties for ODTs including a resultant decrease in disintegration times (225) or crushing strength. A decrease in crushing strength can be attributed to the reduction in interparticulate bonding caused by the formation of the lubricant film around the particles of a formulation (226). The lubricant may however have a negative impact on drug dissolution and retard the rate at which the drug dissolves. Lubricants are, in general, hydrophobic in nature and magnesium stearate, which is an extremely fine material, can coat other particles that are present in a powder blend. This renders the particle surface

hydrophobic and may result in an increased disintegration time with a resultant decrease in the dissolution rate (227). In the context of these studies and evaluation of *in vitro* release data it can be concluded that the lubricant, does not delay the dissolution of SC from these ODT.

All manufactured ODT meet the specification for rapidly disintegrating tablets and at least 85% of the SC is released in 15 minutes (Q_{15}) and changes in the amount of disintegrant did not appear to affect the rate of drug release, to any great extent. However blending time appears to have an effect on the quality of the tablets, for example Batch SC020 was blended for only 4 minutes and had non- uniform content and erratic drug release as can be seen by the relatively high standard deviation for these samples.

5.3.5 Formulation optimization

Following an evaluation of the RSM data generated after the analysis of tablets manufactured using a CCD approach, a numerical optimization technique was used for the development of an optimized formulation that would ensure that responses achieved would meet the relevant specifications of a tablet that has a minimal disintegration and wetting time, while at the same time exhibiting content uniformity. The constraints were retained and are listed in Table 5.3 together with the designated responses. The predicted response values and respective factor compositions are summarized in Table 5.8.

Table 5.8 Values for input variables and respective response variables observed

Factor Composition				**Response**		
x_1	x_2	x_3	x_4	y_1	y_2	y_3
0.87	1	1	0.48	8	4.14	99.45%

Table 5.9 Experimental and predicted response values with percent error for prediction of the optimized formula

Response	**Experimental value**	**Predicted value**	**Percent prediction error**
y_1	8.00	7.97	0.38
y_2	4.15±0.10	4.04	2.68
y_3	100	99.45	0.55

The actual responses observed for the optimized formulation are in close agreement with those of the predicted responses with the error of prediction of as shown in Table 5.9. The percent prediction error is very low for all three responses indicating the accuracy of the model.

The physical characteristics of the tablets manufactured using the optimized formulation and manufacturing process are summarized in Table 5.10.

Table 5.10 Physical characteristics of the optimized formulation and *in vitro* dissolution testing of the optimized formulation

Parameter		Results
Weight (mg)		155.46±5.67
Thickness (mm)		7.31±0.10
Diameter (mm)		2.80±0.01
Crushing strength (N)		18.74±1.81
Tensile strength (MPa)		0.58±0.05
Friability (%)		0.63
Disintegration time (s)		8±0.00
Wetting time (s)		4.15±0.10
***In vitro* dispersion time (s)**		35.65±0.15
Water absorption ratio		147.45±12.40
Q_5	**Q_{10}**	**Q_{15}**
88.74±1.92	96.53±2.30	104.71±1.89

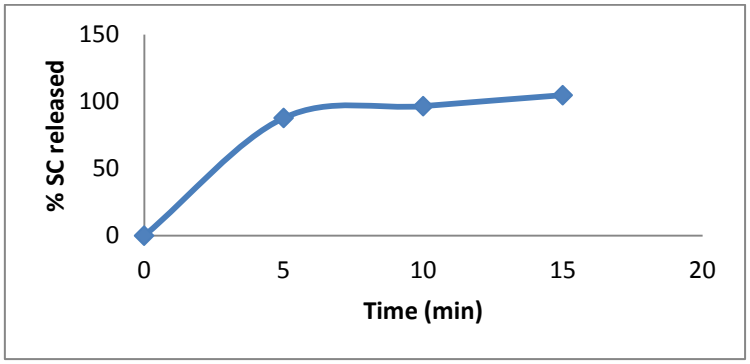


Fig 5.10 Dissolution profile of optimized formulation

The tablets that were produced were white in colour and had a smooth texture with no evidence of picking or any cracking. The physicochemical and mechanical parameters that were measured comply with the specifications that had been set for these tablets i.e. suitable friability, minimal crushing and tensile strength and minimal D_t.

The *in vitro* dissolution results for the optimized formulation are also summarized in Table 5.10 and reveal that the optimized formulation is an immediate release dosage form and meets the compendial limits of at least 85% API release within 15 minutes of commencing testing.

5.4 CONCLUSIONS

The use of Response Surface Methodology can play an important role in the pharmaceutical industry for the purposes of formulation, manufacturing process and analytical method optimization. In order to generate accurate data using a RSM approach it is important that the experimental procedures are performed in a manner such that reproducible and precise results can be generated for each individual experimental run.

The benefits of making use of RSM in formulation optimization is that more than one factor can be investigated at a time and therefore is able to generate a large amount of information at the same time thereby ensuring a more efficient process with respect to time and cost. There is the added advantage that the equations generated for the models that are produced provide a formulation scientist with an indication of whether the effect(s) between factors are synergistic or antagonistic in nature.

The formulation that was developed and reported in Chapter 4 was used for CCD studies and the amount of the three disintegrants used in the formula in addition to the blending time were identified as the four crucial factors to be investigated to ensure that a tablet with a rapid disintegration time, uniformity of content and dosage form integrity was produced.

All tablets that were produced were assessed in terms of their friability, weight, tensile and crushing strength, D_t, wetting time, *in vitro* dispersion time and water absorption ratio. All tablets that were manufactured had no cracks and exhibited satisfactory friability and crushing strength results.

RSM revealed that neither the blending time nor disintegrant content had a significant effect on the D_t. A possible reason for this result is that the critical disintegrant concentration had been reached and any further increase in the amount of did not decrease the D_t further.

The results of RSM analysis reveal that CMS and CRP had a significant effect on the wetting time of the tablets whereas SSG and blending time were shown to have little or no significant effect. As the concentration of CMS or CRP increased, there was a marked decrease in the wetting time of the tablets that is most likely due to the different disintegration mechanisms by which CRP and CMS promote disintegration and therefore, the effects are potentiated. A surprising result was that SSG did not play a significant role in the wetting time and formulations in which low levels of SSG were used, wetting times were optimal and as the levels increased, there was a corresponding increase in the wetting time of the tablets. This result can most likely be attributed in part to the mechanism by which SSG facilitates disintegration. SSG swells and at concentrations > 8% w/w the material forms a gel layer that delays wetting and also disintegration.

CCD revealed a linear relationship, shown in Figure 5.9 and Equation 5.11 between blending time and content uniformity of the tablets that were manufactured and as the blending time was increased there was an improvement in the uniformity of content of the API in the tablets.

Dissolution studies were performed on the experimental batches manufactured using the CCD approach and it was shown that all batches met the specifications for an ODT and 85% of the API was release at Q_{15} for all batches investigated.

With the aid of mathematical modelling it was established that an optimized SC ODT formulation requires 8.13% CMS, 14.85% CRP and 8.8% SSG to ensure rapid disintegration. A blending time of 7.68 minutes was necessary to ensure that tablets of a suitable quality were produced. The optimized tablet was white with a diameter of 7.31 mm and thickness of 2.80 mm with no visible evidence of cracking, lamination, picking and/or sticking. The tablets exhibited suitable friability and tensile strength characteristics while a disintegration time of only 8s was achieved.

RSM was successfully used for the development of an optimized ODT containing SC and these have potential application for use in paediatric patients.

CHAPTER SIX

CONCLUSIONS

SC is a compound that is commonly used for the treatment of erectile dysfunction in adults and has become popular for the treatment of Pulmonary Hypertension (PH) in adult and paediatric patients. Although the use of SC in paediatric patients has not been approved, controlled clinical studies to investigate the use and effect of SC in paediatric patients suffering from PH have been undertaken. The results of these studies reveal that there is a significant decrease in vascular resistance when SC is administered and the API is generally well tolerated by patients. Dose ranging studies conducted in these patients reveal that a dose of 0.5 µg/kg is suitable for the treatment of PH in neonatal and paediatric patients.

Many of the available treatments for PH are not suitable for use in children and despite the fact that SC is well tolerated and has reasonable efficacy in this patient group, a major problem is the fact that SC is only available in tablet form, for adult use. These dosage forms are not suitable for use in paediatric patients who are not able to swallow large dosage units. Therefore the pharmacist is left with no other option other than to formulate an extemporaneous product that contains SC derived from a raw material source or commercially available dosage forms. These extemporaneous suspensions can at times, be harmful as the stability of the formulation in the vehicle that is use is often unknown and there is also always the chance of human error in respect of calculating the amount of API required for the manufacture of such products. Therefore the formulation and manufacture of an ODT may be a viable alternative to the use of extemporaneous preparations SC administration. Since these tablets offer an advantage in terms of oral delivery due to rapid disintegration they can be administered to paediatric patients with minimal risk of choking.

An HPLC method for the analysis of SC in dosage forms was developed and validated in terms of the ICH guidelines (228). The HPLC separation was developed by investigating the impact of different mobile phase compositions and flow rate on retention time, peak sharpness and resolution between the analytical response for SC and the IS, DZ. DZ was selected for use as the IS as it had successfully been used in a previous study (25). The separation was achieved using a Phenomenex® Luna C_{18} (2) 5µm, 150 x 4.6mm i.d. maintained at 22 ± 0.5 °C and a mobile phase composition of ACN: water in a ratio of 55:45 % v/v. The method was found to be linear and precise over the concentration range of 0.2-2µg/ml and was shown to be clearly selective for SC when analysing raw material and pharmaceutical dosage forms with a clear resolution between SC and the IS peak.

Stability indicating studies of SC were undertaken in different environments *viz.* elevated temperature, acidic, basic, oxidative and light exposure conditions. SC was shown to be stabile in the presence of

elevated temperature, mildly basic and acidic conditions in addition to exposure to light. However, considerable degradation of SC was observed when exposed to strongly basic, acidic and oxidative conditions. All degradation peaks were well resolved from those of SC and the IS and therefore the method can be considered stability indicating.

System suitability tests *viz.* resolution and peak tailing factor determination was also conducted throughout the development and validation of an HPLC method to ensure that the results generate would be appropriate for these studies.

Preformulation studies form an initial step in the determination of rational formulation development process. The primary goal of preformulation studies is the investigation of important physicochemical factors that may affect API purity and the stability in a dosage form. Prospective excipients and API were assessed with respect to particle size, shape and morphology using SEM. The particle size and shape of a material are important considerations when attempting to produce a successful tablet formulating as these parameters can affect the flow properties of a powder and ultimately the content of the resultant tablets. Powders that have particles with a relatively large size generally exhibit better flow properties than powders with particles of a smaller diameter. However, combining powders of varying particle size may result in particle segregation that may lead to a lack of blend and tablet homogeneity.

SEM imaging revealed that SC was mainly comprised of rod-shaped needle like particles suggesting that there may be a possibility of poor flow properties being exhibited during processing. The determination of CI, HR and angle of repose confirmed this assumption with values of 34.6, 1.5.1 and 40.6 for these indices, respectively. However, this was not of great concern in respect of manufacturing a tablet formulation of this low-dose API as the poor flow properties of the raw material can be masked by use of excipients that exhibit superior flow characteristics.

Particle flow properties of all raw materials were assessed by establishing the bulk and tapped densities and these data were used to calculate CI and HR for these compounds. The angle of repose was also measured as an additional approach to ascertain the flow properties of the powders to be considered for use and this data was used to confirm the result of studies to establish the HR and CI.

Potential chemical interactions between the API and excipient were investigated using DSC and IR analysis. The API and excipients were analysed alone and as a binary mixture of API and excipient in a 1:1 ratio. DSC analysis revealed the possibility of an interaction between SC and magnesium stearate or mannitol. However, it was thought that these interactions were primarily a consequence of

evaluation at the elevated temperatures at which DSC analysis was conducted as these interactions were not evident when the samples were analysed using IR at an ambient room temperature of 22°C.

The direct compression method of tablet manufacture was selected as the most appropriate approach to produce the ODT. The direct compression method was selected for use since many commonly used excipients have been successfully compressed into tablets using this approach and there is a further advantage that fewer manufacturing steps are required, thereby minimizing the potential for introducing errors into dosage forms. In addition, as fewer unit operations are required for the manufacture of dosage forms, the process times are reduced thereby potentially reducing the cost of production. The direct compression method is not without its challenges and care needs to be taken when producing a formulation with these excipients as many of these materials are hygroscopic and can agglomerate, possibly leading to blend homogeneity issues. To ensure batch to batch uniformity, production continuity, and in the absence of an environmentally controlled manufacturing suite, the tablets were manufactured between 00h00 am and 06h00 am to minimize the impact of humidity on the production process.

The formulation to be manufactured included three different superdisintegrants, viz., SSG, CMS and CRP to maximise the rate of disintegration. Mannitol and MCC were included as diluent and binder respectively and MCC also has disintegration properties. Two glidants, viz., talc and CSD were added to the formulation to improve flow properties and magnesium stearate was used as a lubricant.

The initial blending time for manufacture was 10 minutes without the addition of lubricant and then for a further three minutes with lubricant. The primary reason for not blending for > 3 minutes with the magnesium stearate is due to the fact that excessive blending with this material can reduce the crushing strength of the tablets and delay the release of the API in *in vitro* and *in vivo*. Blend homogeneity studies were undertaken to ensure that the blending time was suitable for the manufacture of this formulation and to establish the optimum blend time to ensure that blend homogeneity is achieved and that particle segregation did not occur. It was established that a blend time of 10+3 minutes was suitable and no further changes were made to manufacturing process that would be optimised at a later stage.

Flat faced tablets of 7 mm diameter were compressed using a Manesty®F3 single punch press at a compression force of 30N. Increasing the amount of disintegrant in the formulation resulted in an increase in the water absorption ratio of the blends and resulted in a decrease in the disintegration, wetting and *in vitro* dispersion times. The tablets produced at this stage of the research process met all requirements for an orodispersible tablets and included a low crushing strength, short disintegration time whilst maintaining the mechanical strength of the unit to facilitate handling with a friability of <

1%. The initial formulations were evaluated and single formulation, Batch SC 007 was selected for further optimization using DOE.

DOE is an efficient statistical approach that has become a popular tool used in the pharmaceutical industry to optimize formulation compositions, analytical methods and manufacturing processes as this approach allows for the investigation of several input factors at the same time whilst not using the tedious and traditional " modification of one variable at a time" approach.

A CCD approach was selected for optimization of the amount of disintegrant to be included and the blending time for production. The formulation responses that were monitored were disintegration and wetting times in addition to content uniformity. CCD was selected for use as it permits the evaluation of input variables at five levels and therefore provides a comprehensive set of data within the design space under investigation. Contour and three-dimensional response surface plots together with mathematical relationship (equations) were generated and examined to assess the nature of the relationship(s) between the levels of input factors used on the measured responses that were monitored.

Following evaluation of the plots and equations and with the aid of mathematical modelling it was established that an optimized SC formulation required 8.13% w/w CMS, 14.85% w/w CRP and 8.8% w/w SSG to facilitate disintegration and a blending time of 7.68 minutes was necessary to ensure high quality and robust tablets were produced. The use of this approach to evaluate the data revealed that no change in disintegrant concentration or blending time had an effect on the disintegration times of the formulations investigated. This is most likely due to the fact that the critical disintegrant concentration had been reached and increasing the amount of disintegrant further had no significant effect on the disintegration time. It was also established that a change in the amount of CMS and CRP altered the wetting time of the tablets significantly, whereas SSG did not affect the wetting time of the tablet greatly. This is most likely due to the fact that at high concentrations, SSG swells greatly forming a gel-like layer and delaying D_t and wetting time. Finally it was noted that there is a linear relationship between blending time and uniformity of content of the dosage forms and therefore an optimal blending time was identified.

In vitro dissolution studies were performed to assess the rate at which the API was released from these dosage forms. All batches that were manufactured met the compendial standards that pertain to orodispersible and 85% of the API was released within 15 minutes of commencing the studies.

All experimental batches and the optimized batch were subject to quality control testing for friability, crushing and tensile strength, disintegration, wetting and *in vitro* dispersion time in addition to water absorption ratio. The optimized batch of tablets that were produced passed the uniformity of weight and friability tests had a crushing strength of 18.74N and a tensile strength of 0.58 MPa. The tablets had a disintegration time of 8s and exhibited a high degree of content uniformity. The DOE approach was therefore successfully used to optimize a SC formulation for the manufacture of an ODT tablet with the potential for paediatric use.

Although a successful ODT containing SC has been formulated, the focus of the research was ensuring that the tablet disintegrated rapidly while still retaining suitable mechanical strength. A further focus of the project was to ensure that content uniformity of all batches was achieved and maintained. However, additional research is required to ensure the palatability of the tablet. SC has a bitter taste even despite the inclusion of mannitol and therefore the excipients that could be included into the formulation that would improve palatability and patient adherence must be considered.

Furthermore stability studies will also need to be completed. Stability studies are used to assess potential changes in the quality of an API and dosage form under the influence of different temperature and humidity conditions. Accelerated studies in addition to long term studies should both be undertaken to assess the chemical stability and physical performance of dosage forms over an extended period of time.

Appendix 1 Batch Record Summaries

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	15 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 008
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	20.25	13.5	X051707
SSG	12	8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.35	50.9	RM000200

Tablet Parameters

Weight (mg)	159.54±1.60
Diameter (mm)	7.33±0.02
Thickness (mm)	2.89±0.07
Crushing Strength (N)	18.37±1.32
Tensile Strength (MPa)	0.55
Disintegration time (s)	8±1
Wetting time (s)	4.29±0.02
In vitro dispersion time (s)	35.92±0.17

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
83.45±2.32	92.97±3.02	104.56±2.77

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	15 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 009
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	14.03	9.35	RM000098
CRP	18.23	12.15	X051707
SSG	10.8	7.2	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	78.3	52.2	RM000200

Tablet Parameters

Weight (mg)	156.67±7.07
Diameter (mm)	7.32±0.03
Thickness (mm)	2.85±0.14
Crushing Strength (N)	18.17±2.49
Tensile Strength (MPa)	0.55
Disintegration time (s)	8±1
Wetting time (s)	4.29±0.06
In vitro dispersion time (s)	36.68±0.69

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
80.34±1.78	95.67±1.78	106.79±2.31

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	15 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 010
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	14.03	9.35	RM000098
CRP	22.28	14.85	X051707
SSG	13.2	8.8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	71.85	47.9	RM000200

Tablet Parameters

Weight (mg)	154.44±8.81
Diameter (mm)	7.33±0.02
Thickness (mm)	2.90±0.10
Crushing Strength (N)	20.41±3.40
Tensile Strength (MPa)	0.61
Disintegration time (s)	8
Wetting time (s)	3.89±0.19
In vitro dispersion time (s)	26.76±0.32

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
85.05±1.48	91.52±1.93	100.77±1.90

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	16 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 011
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	15°C
Relative Humidity:	40%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	11.46	7.65	RM000098
CRP	22.28	14.85	X051707
SSG	13.2	8.8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	74.4	49.6	RM000200

Tablet Parameters

Weight (mg)	153.75±7.44
Diameter (mm)	7.33±0.02
Thickness (mm)	3.00±0.00
Crushing Strength (N)	19.77±2.13
Tensile Strength (MPa)	0.57
Disintegration time (s)	8
Wetting time (s)	4.46±0.23
In vitro dispersion time (s)	38.60±0.60

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
79.45±3.45	90.20±2.91	103.77±2.64

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	16 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 012
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	15°C
Relative Humidity:	40%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	11.46	7.65	RM000098
CRP	18.23	12.15	X051707
SSG	13.2	8.8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	78.45	52.3	RM000200

Tablet Parameters

Weight (mg)	154.07±10.73
Diameter (mm)	7.30±0.01
Thickness (mm)	2.85±0.15
Crushing Strength (N)	19.98±3.66
Tensile Strength (MPa)	0.61
Disintegration time (s)	8
Wetting time (s)	4.79±0.18
In vitro dispersion time (s)	35.79±0.46

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
78.99±2.81	92.36±2.78	103.56±4.42

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	16 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 013
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	15°C
Relative Humidity:	40%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	11.46	7.65	RM000098
CRP	22.28	14.85	X051707
SSG	10.80	7.2	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.8	51.2	RM000200

Tablet Parameters

Weight (mg)	145.83±6.69
Diameter (mm)	7.32±0.01
Thickness (mm)	2.85±0.04
Crushing Strength (N)	17.69±1.94
Tensile Strength (MPa)	0.54
Disintegration time (s)	5
Wetting time (s)	4.44±0.23
In vitro dispersion time (s)	39.54±0.56

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
84.50±2.75	95.61±2.40	100.68±3.09

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	17 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 014
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	44%

FORMULA

Material	Unit Dose Mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	16.2	10.8	X051707
SSG	12	8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	80.40	53.6	RM000200

Tablet Parameters

Weight (mg)	151.43±9.00
Diameter (mm)	7.33±0.00
Thickness (mm)	2.86±0.04
Crushing Strength (N)	18.55±2.61
Tensile Strength (MPa)	0.56
Disintegration time (s)	8
Wetting time (s)	4.38±0.15
In vitro dispersion time (s)	36.64±0.55

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
82.67±2.39	97.44±1.91	100.92±2.10

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	17 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 015
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	44%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	20.25	13.5	X051707
SSG	12	8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.35	50.9	RM000200

Tablet Parameters

Weight (mg)	158.77±3.50
Diameter (mm)	7.32±0.01
Thickness (mm)	2.79±0.10
Crushing Strength (N)	18.51±1.76
Tensile Strength (MPa)	0.58
Disintegration time (s)	5
Wetting time (s)	4.26±0.24
In vitro dispersion time (s)	35.41±1.20

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
86.78±2.11	95.73±30	101.83±1.86

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	17 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 016
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	44%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	20.25	13.5	X051707
SSG	12	8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.35	50.9	RM000200

Tablet Parameters

Weight (mg)	149.03±7.38
Diameter (mm)	7.31±0.02
Thickness (mm)	2.64±0.17
Crushing Strength (N)	18.98±1.90
Tensile Strength (MPa)	0.63
Disintegration time (s)	7
Wetting time (s)	4.32±0.16
In vitro dispersion time (s)	35.89±0.17

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
88.03±2.76	90.94±2.62	100.26±2.04

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	18 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 017
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	16.7°C
Relative Humidity:	45%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	11.48	7.65	RM000098
CRP	18.23	12.15	X051707
SSG	12.2	8.8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	78.45	52.3	RM000200

Tablet Parameters

Weight (mg)	154.74±5.13
Diameter (mm)	7.25±0.02
Thickness (mm)	2.86±0.02
Crushing Strength (N)	18.81±3.25
Tensile Strength (MPa)	0.58
Disintegration time (s)	9
Wetting time (s)	4.64±0.17
In vitro dispersion time (s)	36.67±0.14

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
88.92±2.60	96.30±3.40	100.54±2.61

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	18 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 018
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	16.7°C
Relative Humidity:	45%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	10.2	6.8	RM000098
CRP	20.25	13.5	X051707
SSG	12	8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	78.9	52.6	RM000200

Tablet Parameters

Weight (mg)	151.25±8.34
Diameter (mm)	7.32±0.02
Thickness (mm)	2.83±0.10
Crushing Strength (N)	19.88±1.98
Tensile Strength (MPa)	0.61
Disintegration time (s)	8
Wetting time (s)	4.58±0.29
In vitro dispersion time (s)	37.68±0.45

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
87.26±1.16	95.77±4.89	100.01±4.39

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	18 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 019
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	16.7°C
Relative Humidity:	45%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	15.3	10.2	RM000098
CRP	18.23	13.5	X051707
SSG	12.2	8.0	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	73.8	49.2	RM000200

Tablet Parameters

Weight (mg)	154.30±4.89
Diameter (mm)	7.33±0.02
Thickness (mm)	2.72±0.16
Crushing Strength (N)	17.41±2.79
Tensile Strength (MPa)	0.56
Disintegration time (s)	6
Wetting time (s)	4.55±0.26
In vitro dispersion time (s)	36.45±0.17

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
86.51±1.17	99.14±1.07	102.09±3.59

Batch Production Record

Product:	SC ODT
Date of Manufacture:	19 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 020
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	18.23	13.5	X051707
SSG	12.2	8.0	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.35	50.9	RM000200

Tablet Parameters

Weight (mg)	150.13±7.39
Diameter (mm)	7.31±0.01
Thickness (mm)	2.67±0.19
Crushing Strength (N)	20.18±3.70
Tensile Strength (MPa)	0.66
Disintegration time (s)	7
Wetting time (s)	4.25±0.26
In vitro dispersion time (s)	36.23±0.20

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
76.56±8.23	97.34±10.21	116.72±8.90

Batch Production Record

Product:	SC ODT
Date of Manufacture:	19 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 021
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	18.23	13.5	X051707
SSG	12.2	8.0	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.35	50.9	RM000200

Tablet Parameters

Weight (mg)	158.28±5.03
Diameter (mm)	7.33±0.01
Thickness (mm)	2.75±0.11
Crushing Strength (N)	17.13±1.37
Tensile Strength (MPa)	0.54
Disintegration time (s)	6
Wetting time (s)	4.31±0.10
In vitro dispersion time (s)	35.98±.0.67

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
89.06±3.79	97.79±1.49	101.53±2.90

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	19 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 022
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	11.48	7.65	RM000098
CRP	22.28	14.85	X051707
SSG	13.2	8.8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	74.4	49.6	RM000200

Tablet Parameters

Weight (mg)	153.65±7.12
Diameter (mm)	7.32±0.01
Thickness (mm)	2.79±0.16
Crushing Strength (N)	19.46±2.11
Tensile Strength (MPa)	0.61
Disintegration time (s)	7
Wetting time (s)	4.15±0.08
In vitro dispersion time (s)	36.12±0.18

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
85.23±4.90	91.65±1.07	102.66±0.50

Batch Production Record

Product:	SC ODT
Date of Manufacture:	20 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 023
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	15.8°C
Relative Humidity:	37%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	14.03	9.35	RM000098
CRP	18.23	12.15	X051707
SSG	13.2	8.8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	75.9	50.6	RM000200

Tablet Parameters

Weight (mg)	156.60±6.75
Diameter (mm)	7.29±0.03
Thickness (mm)	2.79±0.13
Crushing Strength (N)	17.90±4.54
Tensile Strength (MPa)	0.56
Disintegration time (s)	8
Wetting time (s)	4.37±0.23
In vitro dispersion time (s)	35.42±0.21

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
71.52±3.51	88.63±2.86	98.19±0.19

Batch Production Record

Product:	SC ODT
Date of Manufacture:	20 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 024
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	15.8°C
Relative Humidity:	37%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	20.25	13.5	X051707
SSG	12.2	8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.35	50.9	RM000200

Tablet Parameters

Weight (mg)	149.72±7.46
Diameter (mm)	7.32±0.02
Thickness (mm)	2.79±0.17
Crushing Strength (N)	19.33±2.29
Tensile Strength (MPa)	0.60
Disintegration time (s)	7
Wetting time (s)	4.32±0.20
In vitro dispersion time (s)	35.78±0.17

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
79.99±4.46	84.52±3.46	98.99±0.54

Batch Production Record

Product:	SC ODT
Date of Manufacture:	20 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 025
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	15.8°C
Relative Humidity:	37%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	24.3	16.2	X051707
SSG	12.2	8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	72.3	48.2	RM000200

Tablet Parameters

Weight (mg)	155.76±4.23
Diameter (mm)	7.32±0.02
Thickness (mm)	2.74±0.07
Crushing Strength (N)	18.09±2.74
Tensile Strength (MPa)	0.57
Disintegration time (s)	7
Wetting time (s)	4.26±0.14
In vitro dispersion time (s)	35.69±0.23

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
80.11±2.33	95.43±1.91	103.65±2.37

Batch Production Record

Product:	SC ODT
Date of Manufacture:	21 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 026
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	19°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	20.25	13.5	X051707
SSG	12.2	8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.35	50.9	RM000200

Tablet Parameters

Weight (mg)	154.92±8.45
Diameter (mm)	7.32±0.01
Thickness (mm)	2.80±0.16
Crushing Strength (N)	19.34±2.56
Tensile Strength (MPa)	0.60
Disintegration time (s)	10
Wetting time (s)	4.29±0.12
In vitro dispersion time (s)	35.90±0.15

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
74.10±1.92	84.60±2.13	97.00±6.21

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	21 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 027
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	19°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	14.03	9.35	RM000098
CRP	18.23	12.15	X051707
SSG	13.2	8.8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	75.9	50.6	RM000200

Tablet Parameters

Weight (mg)	152.06±7.04
Diameter (mm)	7.27±0.01
Thickness (mm)	2.60±0.15
Crushing Strength (N)	24.71±1.93
Tensile Strength (MPa)	0.83
Disintegration time (s)	8
Wetting time (s)	4.42±0.09
In vitro dispersion time (s)	35.79±0.19

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
79.78±2.66	93.48±2.89	104.50±2.16

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	21 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 028
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	19°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	14.03	9.35	RM000098
CRP	18.23	12.15	X051707
SSG	10.8	7.2	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	78.3	52.5	RM000200

Tablet Parameters

Weight (mg)	156.69±6.99
Diameter (mm)	7.31±0.02
Thickness (mm)	2.80±0.22
Crushing Strength (N)	17.46±2.48
Tensile Strength (MPa)	0.54
Disintegration time (s)	9
Wetting time (s)	4.30±0.24
In vitro dispersion time (s)	35.92±0.18

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
75.99±4.70	84.10±2.13	107.34±6.82

Batch Production Record

Product:	SC ODT
Date of Manufacture:	22 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 029
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	20°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	11.48	7.65	RM000098
CRP	18.23	12.15	X051707
SSG	13.2	8.8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	78.45	52.3	RM000200

Tablet Parameters

Weight (mg)	147.92±5.49
Diameter (mm)	7.31±0.03
Thickness (mm)	2.78±0.18
Crushing Strength (N)	16.88±4.17
Tensile Strength (MPa)	0.53
Disintegration time (s)	8
Wetting time (s)	4.44±0.22
In vitro dispersion time (s)	36.20±0.23

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
76.62±3.63	100.21±3.35	107.44±6.98

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	22 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 030
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	20°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	20.25	13.5	X051707
SSG	9.6	6.4	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.35	50.9	RM000200

Tablet Parameters

Weight (mg)	148.05±7.23
Diameter (mm)	7.30±0.04
Thickness (mm)	2.72±0.17
Crushing Strength (N)	19.96±2.51
Tensile Strength (MPa)	0.64
Disintegration time (s)	988
Wetting time (s)	4.42±0.15
In vitro dispersion time (s)	35.32±0.22

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
72.62±0.52	99.46±2.51	99.89±5.04

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	22 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 031
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	20°C
Relative Humidity:	41%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	14.03	9.35	RM000098
CRP	22.28	14.85	X051707
SSG	10.8	7.2	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	74.25	49.5	RM000200

Tablet Parameters

Weight (mg)	152.41±8.23
Diameter (mm)	7.31±0.02
Thickness (mm)	2.79±0.07
Crushing Strength (N)	19.64±3.05
Tensile Strength (MPa)	0.61
Disintegration time (s)	8
Wetting time (s)	4.32±0.16
In vitro dispersion time (s)	35.34±0.40

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
79.62±3.80	99.15±3.66	109.63±2.62

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	23 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 032
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	18.2°C
Relative Humidity:	44%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	20.25	13.5	X051707
SSG	12.2	8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.35	50.9	RM000200

Tablet Parameters

Weight (mg)	152.30±5.66
Diameter (mm)	7.31±0.01
Thickness (mm)	2.82±0.15
Crushing Strength (N)	18.76±2.34
Tensile Strength (MPa)	0.58
Disintegration time (s)	8
Wetting time (s)	4.30±0.17
In vitro dispersion time (s)	35.81±0.12

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
86.45±3.45	97.84±3.45	99.45±2.12

Batch Production Record

Product:	SC ODT
Date of Manufacture:	23October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 033
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	18.2°C
Relative Humidity:	44%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	14.48	7.65	RM000098
CRP	22.28	14.85	X051707
SSG	10.8	7.2	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	76.80	51.2	RM000200

Tablet Parameters

Weight (mg)	150.26±7.19
Diameter (mm)	7.31±0.02
Thickness (mm)	2.64±0.17
Crushing Strength (N)	18.98±1.90
Tensile Strength (MPa)	0.63
Disintegration time (s)	10
Wetting time (s)	4.49±0.16
In vitro dispersion time (s)	35.40±0.96

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
91.43±10.11	99.54±3.48	99.57±7.43

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	23 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 034
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	18.2°C
Relative Humidity:	44%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	12.75	8.5	RM000098
CRP	20.25	13.5	X051707
SSG	14.4	9.6	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	73.95	49.3	RM000200

Tablet Parameters

Weight (mg)	152.12±10.91
Diameter (mm)	7.30±0.01
Thickness (mm)	2.79±0.15
Crushing Strength (N)	18.45±3.74
Tensile Strength (MPa)	0.58
Disintegration time (s)	8
Wetting time (s)	4.55±0.22
In vitro dispersion time (s)	35.76±0.32

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
82.58±2.62	95.27±2.77	108.90±3.81

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Batch Production Record

Product:	SC ODT
Date of Manufacture:	24 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 035
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	42%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	14.48	7.65	RM000098
CRP	18.23	12.15	X051707
SSG	10.8	7.2	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	80.85	53.9	RM000200

Tablet Parameters

Weight (mg)	149.83±7.33
Diameter (mm)	7.30±0.01
Thickness (mm)	2.68±0.28
Crushing Strength (N)	18.86±3.26
Tensile Strength (MPa)	0.61
Disintegration time (s)	9
Wetting time (s)	4.42±0.23
In vitro dispersion time (s)	36.62±0.13

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
87.31±3.18	94.96±6.64	96.29±4.17

Batch Production Record

Product:	SC ODT
Date of Manufacture:	24 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 036
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	42%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	14.03	9.35	RM000098
CRP	22.28	14.85	X051707
SSG	10.8	7.2	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	74.25	49.5	RM000200

Tablet Parameters

Weight (mg)	155.71±6.73
Diameter (mm)	7.31±0.01
Thickness (mm)	2.77±0.22
Crushing Strength (N)	19.48±3.34
Tensile Strength (MPa)	0.61
Disintegration time (s)	8
Wetting time (s)	4.49±0.19
In vitro dispersion time (s)	34.85±1.6

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
79.91±3.78	89.36±6.26	98.45±6.19

Batch Production Record

Product:	SC ODT
Date of Manufacture:	24 October 2011
Formulator:	Bianca Dagnolo
Batch Number:	SC 037
Batch Size:	600 tablets
Target Weight:	150 mg
Temperature:	17.2°C
Relative Humidity:	42%

FORMULA

Material	Unit Dose mg	Batch G	Raw Material Number
SC	3	2	RM000109
MCC	22.5	15	RM 000100
CMS	14.03	9.35	RM000098
CRP	22.28	14.85	X051707
SSG	13.8	8.8	X00074
Talc	0.15	0.1	5726/04
Magnesium Stearate	1.5	1	X052015
Colloidal Silicon Dioxide	1.5	1	X069339
Mannitol	71.85	47.9	RM000200

Tablet Parameters

Weight (mg)	156.20±7.19
Diameter (mm)	7.31±0.02
Thickness (mm)	2.78±0.23
Crushing Strength (N)	20.75±3.79
Tensile Strength (MPa)	0.65
Disintegration time (s)	8
Wetting time (s)	4.05±0.09
In vitro dispersion time (s)	36.68±0.69

In vitro dissolution

Q_5 %	Q_{10} %	Q_{15} %
88.43±0.64	93.63±2.00	107.58±2.93

Appendix 2

Response Surface Methodology Statistics

DISINTEGRATION

Table 7.1 ANOVA for Response Surface Quadratic Model for Disintegration time

Source	Sum of Squares	df	Mean Square	F Value	P-value
Model	11.05	14	0.79	0.37	0.9644
A-Croscarmellose	2.04	1	2.04	0.96	0.3428
B-Crospovidone	1.04	1	1.04	0.49	0.4948
C-Sodium starch glycolate	0.042	1	0.042	0.020	0.8906
D-Blending time	0.37	1	0.37	0.18	0.6806
AB	0.063	1	0.063	0.029	0.8662
AC	0.56	1	0.56	0.26	0.6146
AD	0.56	1	0.56	0.26	0.6146
BC	1.56	1	1.56	0.73	0.4050
BD	0.062	1	0.062	0.029	0.8662
CD	3.06	1	3.06	1.44	0.2489
A^2	0.027	1	0.027	0.013	0.9122
B^2	0.24	1	0.24	0.11	0.7411
C^2	1.31	1	1.31	0.62	0.4445
D^2	0.24	1	0.24	0.11	0.7411

Table 7.2 ANOVA for Response Surface Linear Model for Disintegration time

Source	Sum of Squares	df	Mean Square	F Value	P-value
Model	3.50	4	0.87	0.55	0.6978
A-Croscarmellose	2.04	1	2.04	1.29	0.2662
B-Crospovidone	1.04	1	1.04	0.66	0.4243
C-Sodium starch glycolate	0.042	1	0.042	0.026	0.8722
D-Blending time	0.38	1	0.38	0.24	0.6719

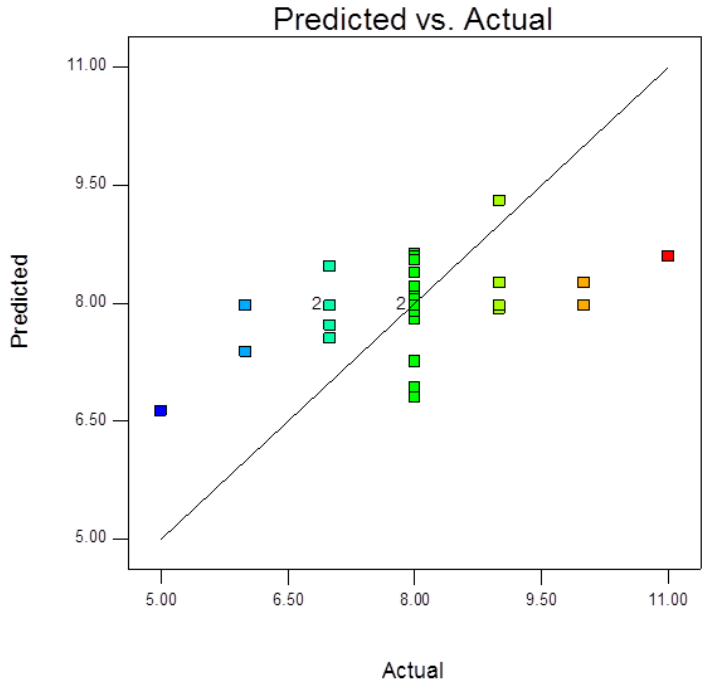

Figure 7.1 Predicted vs. Actual plot for Blending time

WETTING TIME

Table 7.3ANOVA for Response Surface Quadratic Model for Wetting time

Source	Sum of Squares	df	Mean Square	F Value	P-value	
Model	0.69	14	0.049	5.02	0.0018	Significant
A-Croscarmellose	0.13	1	0.13	13.14	0.005	Significant
B-Crospovidone	0.11	1	0.11	11.13	0.0045	Significant
C-Sodium starch glycolate	0.013	1	0.013	1.33	0.2668	
D-Blending time	3.267E-003	1	3.267E-003	0.33	0.5727	

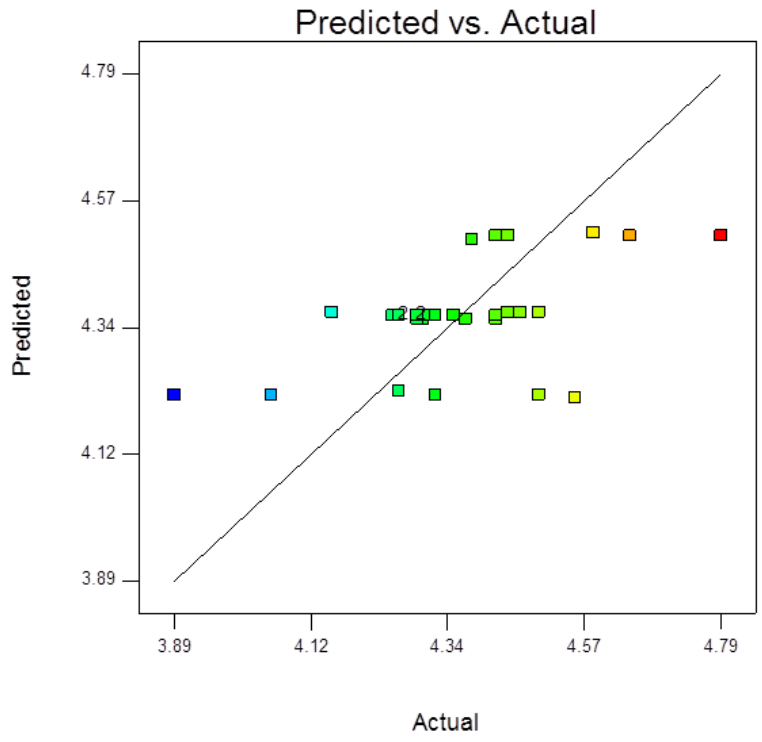

Figure 7.2 Predicted vs. Actual plot for Wetting time

CONTENT UNIFORMITY

Table 7.3 ANOVA for Response Surface Linear Model for content uniformity

Source	Sum of Squares	df	Mean Square	F Value	P-value	
Model	496.69	14	4	3.43	0.0230	Significant
A-Croscarmellose	6.48	1	1	0.18	0.6760	
B-Crospovidone	20.33	1	1	0.56	0.4608	
C-Sodium starch glycolate	156.32	1	1	4.31	0.0582	
D-Blending time	313.57	1	1	8.65	0.0069	Significant

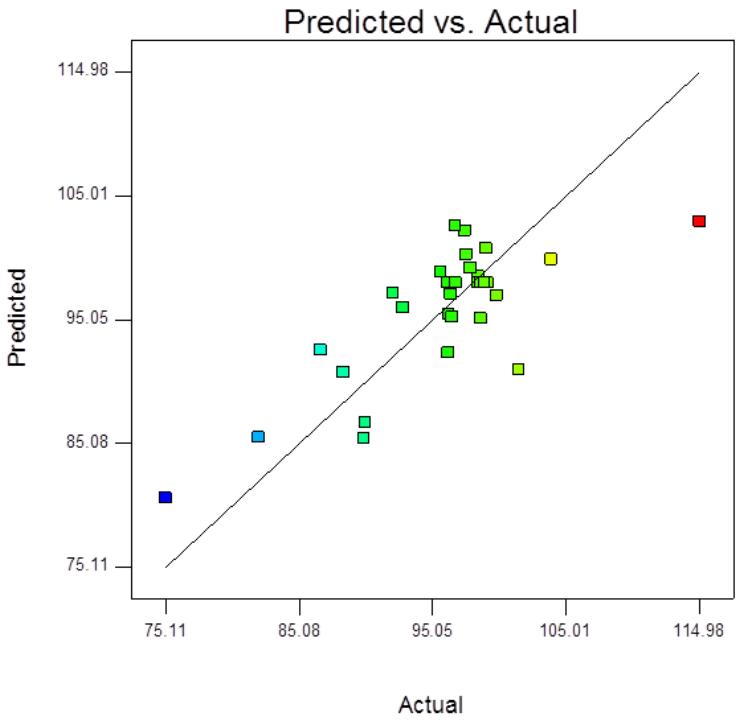

Figure 7.3 Predicted vs. Actual plot for Blending time

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