CHARACTERISATION OF CYSTEAMINE PRODRUGS FOR THE TREATMENT OF CYSTINOSIS AND EVALUATION OF LIQUID FILL TECHNOLOGY

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DECLARATION

This thesis has been composed by myself and has not been submitted in any previous application for a higher degree. The work that is documented was undertaken by myself. All information included from published or unpublished work of persons other than myself has been acknowledged and references provided.

John McCormick

ABSTRACT

Cystinosis is a rare, autosomal, recessive disease characterised by raised levels of the amino acid cystine in the cells of most organs in the body which can cause organ damage. The treatment involves the oral administration of the aminothiol, cysteamine (Cystagon™) but this has an offensive taste and smell, can cause vomiting and its metabolites are excreted from the body via breath (halitosis) and sweat which can cause poor patient compliance.

The main research aim was to characterise two cysteamine prodrugs for the treatment of cystinosis and evaluate the suitability of liquid fill technology using prodrugs, or alternative drug models, to determine excipient usage using solubility, stability and capsule shell compatibility data to potentially develop prototype formulations based on liquid fill technology.

Cysteamine steramide and cysteamine decanamide were characterised using thermoanalytic techniques including, Differential Scanning Calorimetry (DSC), Hot Stage Microscopy (HSM), Thermal Gravimetric Analysis (TGA) and Fourier Transform Infrared Spectroscopy (FT-IR). Hot stage microscopy results appear to correspond with DSC data obtained over the stability program. DSC detected the main melt for cysteamine steramide at 131°C and identified micro-phase separation as a direct result of the heat/cooling cycle. TGA detected no residual solvents or water present in the molecule and indicated that no solvates were present. FT-IR showed no increase, reduction or shift in peaks. DSC and TGA data indicated that both prodrugs were stable at accelerated storage conditions of 25°C/60%RH and 40°C/75%RH. Another insoluble prodrug as an alternative to Cystagon™, cysteamine palmitate, was also sourced and used as a model for a liquid fill capsule stability study to determine the mechanical strength of

capsules containing such a formulation over a 4 week storage period at 40°C/75%RH.

In order to evaluate and develop formulations for oral delivery that are suitable for liquid fill technology, solubility screening using a large variety of commercial solubilisers and physical compatibility studies with cysteamine prodrugs were conducted and all excipient and excipient/prodrug combinations appeared to be compatible, however only low levels of solubility were detected within the solubility study. Propylene glycol solubilised 40mg/ml and Gelucire 44/14 60mg/ml. Prodrugs were not visually soluble in the other excipients examined and therefore prototype formulations were developed as suspensions. Both gelatin and Hypromethylcellulose (HPMC) capsules shells were evaluated at 40°C/75%RH for 6 months, with selected excipients and excipient/prodrug combinations and all were deemed compatible. Texture analyser results indicated a general reduction in compressive peak force required to crush capsules, after 4 week storage at 40°C/75%RH; however, the capsules were still considered satisfactory. Tramadol hydrochloride (water soluble salt) was used as an alternative compound for demonstrating Gelucire's effectiveness in providing liquid fill formulations showing immediate and delayed release dissolution profiles.

All characterisation of cysteamine prodrugs for the treatment of cystinosis was successfully completed using thermal analytical techniques and this data was used to certify the complete stability of these prodrugs in liquid fill technologies.

Keywords: Cystinosis, formulation, Capsules, Encapsulation, Modified release, Gelucire, Liquid fill technology.

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1.0 Introduction

1.1 Cystinosis

Cystinosis is a rare autosomal (non-sex chromosome) genetic recessive disease occurring in about 1 in 200,000 births within developed countries. Cystinosis is a lack of cysteine available for protein synthesis by host tissue. Ingested protein enters the lysosome, where it is hydrolysed, breaking it down to cysteine and its other component amino acids. Cysteine is oxidised to form a disulfide of the amino acid cysteine (cystine) in the lysosome (Figure 1) [1, 2].

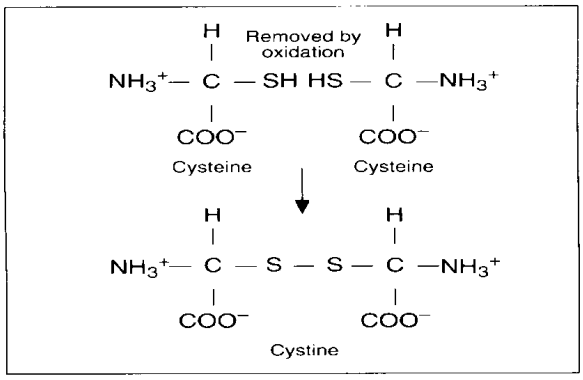
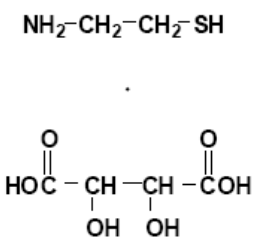


Figure 1: Disulphide of the amino acid cysteine [3].

Cysteamine is an aminothiol compound used to treat cystinosis. Various cysteamine salts are used. The treatment involves the oral administration of the aminothiol, cysteamine bitartrate (Cystagon™) but this has an offensive taste and smell, can cause vomiting and its metabolites are excreted from the body via breath (halitosis) and sweat which can cause poor patient compliance [4], and, therefore, other forms have been developed (Figure 2) [5].



2-aminoethanethiol

L-(+)-Tartaric acid

Figure 2: Cystagon chemical structure.

Side effects of cysteamine treatment include nausea and vomiting, in part because of its repulsive odour and taste [6]. Cysteamine increases gastrin synthesis and gastric acid production. Liquid fill technology can be utilised in multiple formulation strategies to develop an encapsulated product which bypasses the stomach or contains the odour and reduces the tendency to vomit. This can greatly improve patient compliance and allow for targeted delivery.

Cystinosis is inherited in an autosomal recessive manner. Both parents would need to be heterozygous [7] for a child to potentially inherit the condition. When a condition is recessive then one copy of a recessive allele (gene) must be inherited from each parent [7]. This means that each sibling has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. Prenatal testing is also available biochemically, based upon elevated cystine concentration in both chorionic villi and amniocytes [8]. Usually both cystine and cysteine can normally enter the cytoplasm from the lysosomes [9], where cystine is promptly converted to cysteine by glutathione [10]. Cysteine residing in the cytoplasm is incorporated into protein or degraded to inorganic sulfate for secretion from the cell. Cystinosis is caused by mutations in the gene that encodes cystinosin [9]. The CTNS gene is located on chromosome 17p13 and the most common mutation involves a 57kb deletion in approximately 50% of diagnosed patients [1]. Cystinosin is the cystine-lysosomal exporter and due to the defect in cystinosin, cystine accumulates as rectangular, hexagonal, or birefringent crystals in most of the organs within the body [11].

There are three types of cystinosis: nephropathic cystinosis, intermediate cystinosis and ocular cystinosis (non-nephropathic). Infants affected by nephropathic cystinosis initially exhibit poor growth and particular kidney problems (Fanconi syndrome) [11]. Some of the problems that can be exhibited by the kidneys, can be loss of important materials such as; minerals, salts, fluids, and other nutrients which may result in hypophosphatemic rickets which can predominantly affect the legs. This usually occurs between 6 and 12 months after birth [1, 12]. The nutrient imbalances in the body can also lead to increased urination, thirst, dehydration, and acidosis (abnormally acidic blood) in sufferers. Around 16 months after birth, cystine crystals may have developed in the clear covering of the eye (cornea). The accumulation of these crystals in the eye causes photophobia [13] and extreme sensitivity to light. If nephropathic cystinosis is left untreated, children will suffer complete kidney failure by approximately 10 years of age and will require a kidney transplant. Other signs and symptoms of nephropathic cystinosis include muscle deterioration, blindness, swallowing dysfunction, diabetes mellitus, hypothyroidism, hypergonadism, pancreatic exocrine insufficiency, and vascular calcification and nervous system problems [12]. Some patients with severe gastroesophageal reflux may require gastric/jejunal tube replacement or surgery to strengthen the valve muscle between the stomach and the esophagus to prevent stomach acids from being forced back up into the esophagus (Nissen fundoplication)[11] to achieve optimal nutrition.

Intermediate cystinosis begins to affect individuals from the ages of 12 and 15 years of age. Corneal crystals and kidney problems are the main features of this

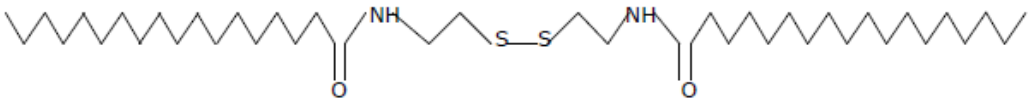
disorder initially observed. Complete kidney failure will occur usually between late teens and mid-twenties [13], if the patient is left untreated.

Non-nephropathic cystinosis is also known as ocular cystinosis; patients only suffer from one condition, which is photophobia. Ocular cystinosis is usually diagnosed by the identification of cystine crystals in the cornea using the slit test [14]. The slit test is a standard examination by an eye doctor using a microscope to assess the structure of the eye. This test can detect the presence of cystine crystals in the cornea [11].

The current treatment for cystinosis is Cystagon™ and is available in 50mg and 150mg concentrations [15]. As discussed earlier, the current treatment involves the oral administration of the aminothiol, cysteamine bitartrate (Cystagon™) but this has an offensive taste and smell and can cause symptoms like vomiting and pungent sweat and bad breath. This is caused by excretion of metabolites) leading to poor patient compliance [4].

A novel way of bypassing the excretion process is to develop prodrugs with low solubility. Cysteamine prodrugs have been developed at RGU (figure 3). These prodrugs are pure API (Please refer to appendix 3 and 4). The original idea was to produce prodrugs with low solubility to counter affect the problems of patient compliance with the noxious soluble cysteamine [16].

Cysteamine Steramide



Cysteamine Decanamide

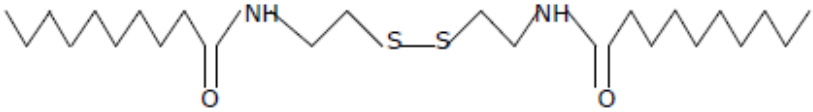


Figure 3: Chemical structure of prodrugs used within the research program, cysteamine steramide and cysteamine decanamide (Supplied by RGU).
Please refer Appendix 3 and 4 (NMR data)).

1.2 Colloidal systems

Colloidal systems are one of the fundamental systems that govern design strategy when considering the solubility of a molecule. Disperse systems contain one component, the disperse phase, dispersed in the bi-continuous phase. Colloids can be lipophilic (fat loving) and lyophobic (solvent repelling). When the solvent is water, the terms hydrophilic and hydrophobic are used [17]. A colloidal system refers to systems where the disperse phase particles are less than 1 μm. Surfactant molecules tend to associate in water into aggregates called micelles and these constitute hydrophilic colloidal dispersions [18]. Colloid-sized clusters of molecules' hydrophobic tails tend to aggregate, and their hydrophilic heads provide protection from external environments [17, 19]. Micelles form only above the critical micelle concentration (CMC) and above the Krafft temperature (minimum temperature at which surfactants form micelles) [11, 20]. Non-ionic surfactant molecules may cluster together in clusters of

1000 or more, but ionic species tend to be disrupted by the Coulombic repulsions (charge) between head groups and are normally limited to groups of between 10 and 100 molecules. They have lower aggregation numbers (n). Shapes of formation vary depending on concentration and can be spherical, flattened spheres close to the CMC, and rod like at higher concentrations [17, 20].

1.3 Microemulsions

Microemulsions are isotropic, thermodynamically stable, transparent (or translucent) systems of oil, water and surfactant, frequently in combination with a cosurfactant (droplet size 20-200 nm) [21]. Classifications can be applied, oil-in-water (o/w) or water-in-oil (w/o) and bicontinuous systems, depending on their structure, and are characterised by ultra-low interfacial tension between the oil and water phases [22]. Hydrophilic and hydrophobic molecules can be successfully formulated due to the presence of both lipophilic and hydrophilic domains. The advantages include spontaneous formation, thermodynamic stability, and improved solubilisation. Absorption of drugs from a microemulsion is influenced by various factors of a formulation [21]. These include particle size, partition coefficient of the drug between the two immiscible phases, the presence of the drug in the interface, site and route for absorption, microemulsion components that can act as absorption enhancers and drug solubility in microemulsion components [23, 24, 25].

An important aspect of microemulsion vehicle design involves assessing the drug delivery potential. The microstructure characteristics should be identified for the vehicles and drug loaded microemulsions. Characteristics determined include rheological and electrical conductivity during the formation process and assessment of the continuing stability of the mixed system [26]. Active

molecules may influence the system due to amphiliphilic (having both hydrophilic and hydrophobic parts) and/or mesogenic properties (tendency to induce the liquid crystalline state) [27]. Alternatively, loaded and unloaded vehicles can be examined using polarised light microscopy. This technique allows the identification of hexagonal mesophases, anisotropic lamellar or isotropic microemulsions by observing whether the sample rotates the plane of polarisation of polarised light [28].

1.4 Liposomes

Liposomes are colloidal particles which commonly consist of phospholipids and cholesterol. Other ingredients such as phytosterols can be incorporated into liposomes [29]. Liposomes surround an aqueous core (figure 4).

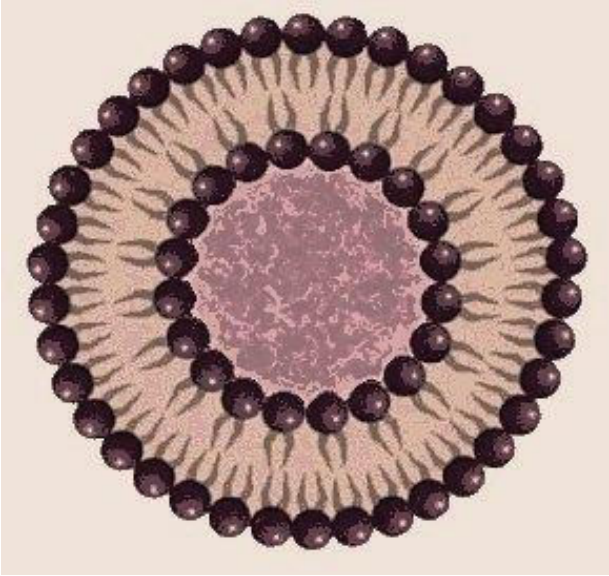


Figure 4: Cross section of a liposome displaying the arrangement of the hydrophilic head and the hydrophobic tail surrounding an aqueous environment [30].

Multilamellar vesicles consist of several lipid layers (in an onion-like arrangement) separated from one another by a layer of aqueous solution [31]. These vesicles are up to several hundred nanometres (nm) in diameter. Small

unilamellar vesicles are also surrounded by a single lipid layer (25–50 nm in diameter). Large unilamellar vesicles are a very heterogeneous group of vesicles that are surrounded by a single lipid layer. The diameter of these liposomes can range from 100 nm up to 1 μm [32, 33].

The most commonly used lipids are the phospholipid phosphatidylcholine (PC) or sphingomyelin. Other phospholipids do not form liposomes in isolation, but they may be combined with other phospholipids to modify bilayer properties. Other phospholipids that can be incorporated into a liposome bilayer are phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidic acid (PA) [34]. These phospholipids are charged and promote electrostatic repulsion which inhibits aggregation or fusion. Incorporating charged phospholipids increases the distance between concentric membranes in multilamellar vesicles [35], increasing entrapment volume [34].

1.5 Solid Solutions/Solid Dispersions

1.5.1 Solid Solutions

A solid solution can be described as a mixture of two or more crystalline solids that coexist as a new crystalline solid, or crystal lattice [36]. The mixing can be achieved by combining at least two solids. The solids are melted and combined during the liquid phase and this mixture is then cooled to form a new crystal lattice. The individual chemical properties and crystalline structure determine how their atoms fit together in the newly formed crystal structure. Atoms from one starting crystal can replace the atoms of another crystal or atoms from one crystal can occupy positions normally vacant in the lattice. The concentrations of each of the mixes can be modified for specific applications [36, 37].

This conversion of the drug into the amorphous state produces material which dissolves more rapidly than the corresponding crystalline drug substance. For example, the incorporation of the drug substance into hydrophilic polymeric materials such as polyvinylpyrollidone (PVP) and polyethylene glycol (e.g. PEG6000) can produce additional solubility enhancing effects. In some cases it is possible to dissolve the drug in the molten polymer and fill directly into hard capsules. On cooling, the drug is entrapped in an amorphous state within the water-soluble matrix [38].

1.5.2 Solid Dispersion

A solid dispersion is a two component system containing a solute (API) and solvent (polymer). The two-component systems can form multiple structures depending on their composition and sample processing history [39]. When the drug loading is lower than the equilibrium solubility of drug in polymer, the drug is molecularly dispersed within the polymer matrix and should form a homogenous, thermodynamically stable solution [39]. This is the most desirable structure of a solid dispersion. However, for most drug–polymer pairs, this situation only occurs at low drug loading and can also be impacted by high temperatures. As the temperature is decreased, the API tends to precipitate out due to the solution becoming supersaturated [40].

Solid dispersions can be eutectic mixtures (two components causing a lower melting point than when in a mixed system), crystalline/glass solutions, and amorphous/crystalline suspensions. The amorphous/crystalline combinations on their own or incorporating surfactants can enhance drug release, long term stability and improve bioavailability [39, 41]. Dispersions can be prepared by

melting (fusion); solvent evaporation, freeze drying, hot melt extrusion, melt agglomeration process, spray drying and use of surfactant. [42].

The main advantage of solid dispersions is the reduction of particle size to nearly a molecular level. Therefore as the carrier (containing the insoluble API) dissolves, the insoluble API is delivered as very fine particles to dissolution medium resulting in an increase in both surface area and solubilisation, resulting in faster absorption and dissolution.

In many cases, improvements in drug dissolution and bioavailability can be achieved using dispersions or suspensions of API in appropriate vehicles which are suitable for filling into hard shell capsules. One such material is Gelucire 44/14. Gelucires are polyethylene glycol glycerides composed of mono-, di- and triglycerides and mono- and diesters of polyethylene glycol (PEG) [43]. Each component presents a different affinity for water and acts as surfactant and co surfactant. Di- and triglycerides are lipophilic in nature. Gelucire 44/14 contains surfactant and has self-emulsifying characteristics and can be used as a meltable binder in the melt granulation of poorly water-soluble drugs. In contact with aqueous fluids, Gelucire 44/14 forms a fine emulsion, solubilises the active substance and hence increases its oral bioavailability [44].

The hydrophilic property of Gelucire is a useful characteristic in dissolution enhancement [45]. Gelucire consisting of low hydrophilic-lipophilic balance (HLB) values can be utilised in formulations to control the dissolution rate of an active pharmaceutical ingredients (APIs). Gelucires with high HLB values can be used in formulations for faster release of APIs. In the designation of Gelucire

names, for example, Gelucire 44/14, 44 indicates melting point and 14 indicates the HLB value [44].

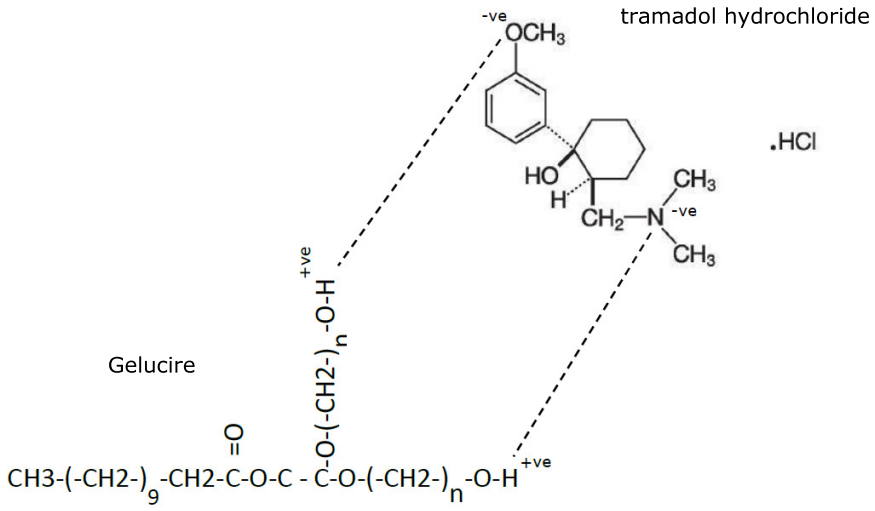


Figure 5: Theoretical interactions of tramadol HCl and Gelucires during dissolution.

1.6 Liquid Fill Technology and processes

Liquid fill encapsulation offers several benefits over solid dosage units. The benefits include processing of low dose products, improved stability, targeted release, delivery of biomolecules, polymorphism solutions, bioavailability enhancement and accelerated clinical development;

Low dose APIs have two main issues, the first is content uniformity and the second is containment of potent APIs (e.g. hormones and cytotoxic) during processing. Content uniformity variation can be minimised by the filling machine pump heads used for dosing liquids into capsules. The pump heads are capable of achieving weight variations of < 1% [46, 47].

Low dose APIs can also be difficult to contain during processing. The incorporation of potent APIs into liquid fill materials has the potential to reduce the risk of exposure of operators to harmful airborne API particles. Swab tests on the surfaces of various parts of the processing equipment can show reduced API contamination compared to powder filling processes [48].

The stability of an API can be adversely affected by environmental conditions such as temperature, humidity and oxidation. Gelatin capsules can provide protection depending on the product [49].

Target release can be achieved by using different excipient combination such as liquids for immediate release, semisolid and waxy excipients for prolonged or delayed release [50]. These materials can also be combined into the same capsule or a capsule within a capsule and utilise the use of enteric or erodible coats [51].

Unlike tablets which require multiple components to aid in compression [52] capsules can be prepared with one lipid or polymer carrier. This lipidic carrier can be used to deliver biomolecules, such as proteins. Polymorphism can be overcome by using excipients to solubilise the API and therefore delivering the formulation as a solution unlike powder fills and tablets.

Lipidic formulations can be combined with bioavailability enhancers (e.g. Labrafil M1944CS [53]) to further enhance release. Multiple surfactants can be added depending on the desired dissolution release profile.

Accelerated clinical development is achievable with liquid fill technology, as the final formulation can be the API and one excipient. Therefore combining multiple excipients may not be necessary based on the stability and dissolution release profile. Hard shells are particularly good at accelerating the development programme as empty hard shells are readily available from multiple suppliers (e.g. Qualicaps or Capsugel) and small batches can be prepared at bench scale with only small amounts of API. This is a significant advantage at the beginning of a development project, as API can be expensive and only a small quantity may be available. Therefore more studies can be performed with this technology to support development programme.

There are several considerations during formulation development for an encapsulated product. Excipient selection is critical when considering an API's solubility, hygroscopicity, known compatibility and desired release characteristics (Table 1).

Table 1: Description of excipients commonly selected for solubility analysis

Excipient	Description of excipient
Miglyol 812	Fractionated coconut oil- commonly used oil compatible with all known shells.
Gelucire 44/14	Gelucire® is a family of naturally derived excipients used in formulations of semisolid oral dosage forms to improve the solubility of active ingredients in a drug. Gelucire®44/14 is composed of surfactants (mono- and diesters of polyethylene glycol, co-surfactants (monoglycerides), and oily phase (di- and triglycerides).
Transcutol HP	It is a high performance solubiliser/solvent for many poorly soluble compounds. It is soluble in both water and oil. Commonly used solvent for poorly soluble drugs.
TPGS (Vitamin E)	Solubility and bioavailability enhancer and water-soluble.
Labrafil M 1944 CS	It is a solubiliser/bioavailability enhancer for oral formulations. It can be used in Self Emulsifying Lipidic Formulations (SELF-type SEDDS/SMEDDS)
Labrasol	It is a solubiliser/bioavailability enhancer or oral formulations
Precirol ATO 5	Can be used in combination with other excipients to modify release profile and taste masking agent
Propylene glycol	It is a solubiliser/bioavailability enhancer or oral formulations but can plasticise gelatin capsules at low concentrations
Miglyol + Aerosil 200	Aerosil is used as a thickening agent. Normally used between 1 and 3%. Miglyol 812 is used as carrier
HPMC + Miglyol 812	HPMC (Methocel K15M) is a water soluble polymer derived from cellulose and offers controlled release in hydrophilic matrix systems. In this context, it's combined with Miglyol 812 (as a carrier for HPMC).
Lauroglycol 90	It is a solubiliser/bioavailability enhancer for oral formulations

Various materials can be processed using liquid fill technology such as lipids, semi-solids and waxes, depending on the desired release profile. Miglyol 812 is a slightly yellowish, clear ester of saturated coconut and palm kernel oil, which

is derived from oil-derived caprylic and capric fatty acids and glycerin triglycerides of the fractionated plant fatty acids C8 and C10 [54]. Miglyol 812 is known to be a pharmaceutically, widely used, inert carrier and can be combined with gelling agents such as fumed silica or HPMC [55] to modify release of APIs and is widely used in liquid fill technology.

If a suspension is selected, the ideal suspension should not settle too rapidly; the particles which do settle to the bottom of the container must not form a hard mass but should be readily dispersed into a uniform mixture when the container is shaken. The suspension must not be too viscous to pour freely from the orifice of the bottle or to flow through a syringe needle. Physical stability of a suspension can be described as particles that do not aggregate and in which they remain uniformly distributed throughout the dispersion [56]. The particles may eventually settle but then they should be easily re-suspended by a moderate amount of agitation. The difference between a pharmaceutical suspension and a colloidal dispersion is one of the sizes of dispersed particles, with the relatively large particles of a suspension liable to sedimentation due to gravitational forces. Suspensions show similar characteristics to colloidal systems and therefore flocculation, both controlled and secondary must be considered [56].

1.6.1 API and excipient compatibility (Emulsion based excipients)

Oral administration of drugs with poor water solubility can be enhanced using self-emulsifying vehicles to develop self-emulsifying drug delivery systems (SEDDS) and self-microemulsifying drug delivery systems (SMEDDS)[57].

These are formulations which form emulsions or micro emulsions spontaneously on contact with aqueous media.

Gelucires are a mixture of glycerides and therefore solidify slowly from the melt, not setting immediately and therefore are useful when formulating APIs. Gelucires contain both hydrophilic and hydrophobic [51] sections and the molten excipient can dissolve the API and on cooling, entrap in an amorphous state within the water-soluble matrix as discussed in section 1.5 of the introduction [38, 44].

Labrafil M 2130 CS is a semi-solid for use in oral and topical formulations; it is a solubiliser/bioavailability enhancer for oral formulations. It can form the basis of Self Emulsifying Lipidic Formulations (SELF-type SEDDS/SMEDD) [57]. It is a co-emulsifier/penetration enhancer for topical formulations and an oily phase for emulsions, micro or nano-emulsions in topical formulations [58].

Nano-emulsions can be categorised as oil/water nano-emulsions that are transparent and easily flowable. Nano-emulsion gels, however can be transparent, or form milky or cloudy emulsions.

Some emulsions can be formed by using semi-solid and liquid solubility enhancers. Semi-solid materials are described in section 1.6.1.1, and liquid solubility enhancers are described in the following sub-sections 1.6.1.2 to 1.6.1.5.

1.6.1.1 Gelucire® 44/14

Gelucire 44/14 is composed of lauroyl macrogol-32 glycerides EP, lauroyl polyoxyl-32 glycerides NF, lauroyl polyoxylglycerides (USA FDA IIG) and is a

non-ionic water dispersible surfactant composed of well-characterized PEG-esters, a small glyceride fraction and free PEG. Gelucire is able to self-emulsify on contact with aqueous media forming a microemulsion [59]. Gelucire improves the solubility and wettability of APIs *in vitro* and *in vivo*. Gelucire has the ability to inhibit the enterocytic efflux transporter (known as P-gP inhibition) which increases bioavailability of APIs and has a Hydrophilic-Lipophilic Balance (HLB) value of 14 [59].

1.6.1.2 Labrafil® M1944CS

Labrafil M1944CS consists of oleoyl macrogol-6 glycerides EP, oleoyl polyoxyl-6 glycerides NF and apricot kernel oil PEG-6 esters (USA FDA IIG). Labrafil M1944CS is a water dispersible surfactant composed of a glyceride fraction and well-characterised PEG-esters. Labrafil has the ability to self-emulsify on contact with aqueous media forming a coarse emulsion (SEDDS). Labrafil M1944CS displays good miscibility with Gelucire 44/14 and Labrasol to form microemulsions (SMEDDS). It can also improve the solubility of APIs *in vitro* and *in vivo* and increases oral bioavailability due to the long chain triglyceride composition and selective absorption of highly lipophilic APIs by the lymphatic transport system. Labrafil M1944CS has an HLB value of 4 [58].

1.6.1.3 Labrasol®

Labrasol is composed of caprylocaproyl macrogol-8 glycerides EP, caprylocaproyl polyoxyl-8 glycerides NF and PEG-8 caprylic/capric glycerides (USA FDA IIG). Labrasol is a non-ionic water dispersible surfactant and can form microemulsion (SMEDDS) as it self-emulsifies on contact with aqueous media.

It can improve the solubility and wettability of APIs *in vitro* and *in vivo* and possesses a HLB of 14 [60].

1.6.1.4 Lauroglycol™ 90

Lauroglycol 90 is composed of propylene glycol monolaurate (type II) EP/NF. It is a water insoluble surfactant for use in self-emulsifying systems forming an emulsion (SEDDS) or microemulsion (SMEDDS). It can also increase oral bioavailability as it is associated with inhibition of the enterocytic drug metabolizing enzyme CYP3A4 and has a HLB of 5 [61]. Enterocytic enzymes (P450) metabolise APIs as well as toxins and therefore by inhibiting this process, bioavailability can increase.

1.6.1.5 Transcutol® HP

Transcutol HP is a purified diethylene glycol monoethyl ether EP/NF that is a powerful solvent for poorly water soluble APIs and therefore ideally suited for the formulation of insoluble prodrugs. Transcutol HP is a hydrophilic co-solvent for use in self-emulsifying lipid formulations forming an emulsion (SEDDS) or fine dispersions known as microemulsions (SMEDDS). Transcutol is also compatible with hard and soft gelatin capsules and can be used at varying levels with other compatible and/or miscible excipients [62].

1.6.1.6 Excipient and API compatibility

Chemical screening of binary mixes which are composed of excipients and API is critical when selecting viable excipients to select candidates to develop prototype formulations. Chemical analysis can be performed by various analytical techniques depending on the API (e.g. High Performance Liquid

Chromatography (HPLC)). This technique involves pumping a sample of the mixture at high pressure through a column with chromatographic packing material (stationary phase). The stationary phase separates and quantifies each component in a mixture against a known concentration of a reference standard at a specific retention time and can show presence of altered /new peaks indicating potential degradation products as a result of chemical incompatibility [63].

For drugs with low solubility or bioavailability, there are a range of formulation options and technologies which will give APIs the best chance of success. These include solid solutions [38] and solid suspensions [44] of drugs in polymeric vehicles, emulsions and self-emulsifying lipidic systems.

1.6.2 Lipidic Formulations

Liquid and semi-solid filled hard capsule formulations are also ideally suited to compounds with low aqueous solubility, poor permeability and consequently low or variable bioavailability. Formulations which increase the solubility of the active or indeed present the drug as a solution can have a significant influence on the bioavailability of such drugs. Lipidic vehicles are generally well absorbed [64] from the GI tract and in many cases this approach can significantly improve the oral bioavailability compared with administration of the solid drug substance. Below is a dissolution comparison of a self-emulsifying formulation versus a lipid formulation using a model compound (Figure 6) [65].

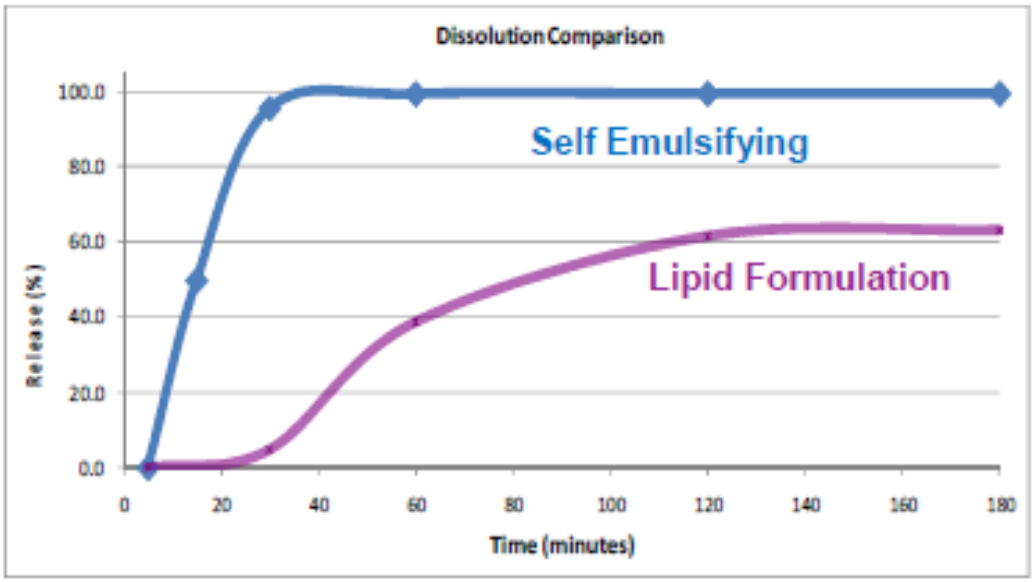


Figure 6: Dissolution comparison of a self-emulsifying formulation vs. lipid formulation using an insoluble model compound. Available from http://www.encapdrugdelivery.com/library.html[66]

1.6.3 Targeted Release

Targeted delivery can be achieved using different routes, such as colonic delivery (enteric/erodible coat), dual release systems (capsule in capsule) [68], multiple release systems (different excipients e.g. waxes and/or solutions). Figure 7 shows the different targeted release strategies.

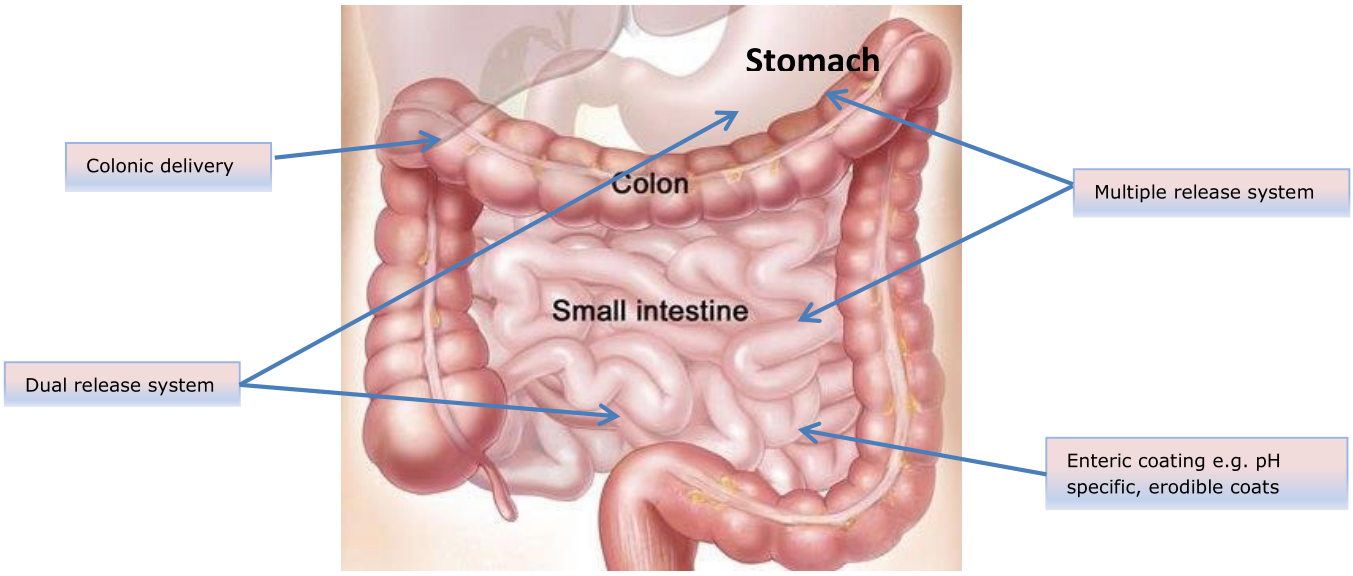


Figure 7: Targeted delivery technology. Image modified from http://www.oregonsurgical.com/surgery_abdomen.htm

Dual release is a single oral dosage unit that comprises of a capsule in a capsule. The two capsule strategy allows for the delivery of incompatible ingredients to two different parts of the gastro-intestinal tract (Figure 8) [68].

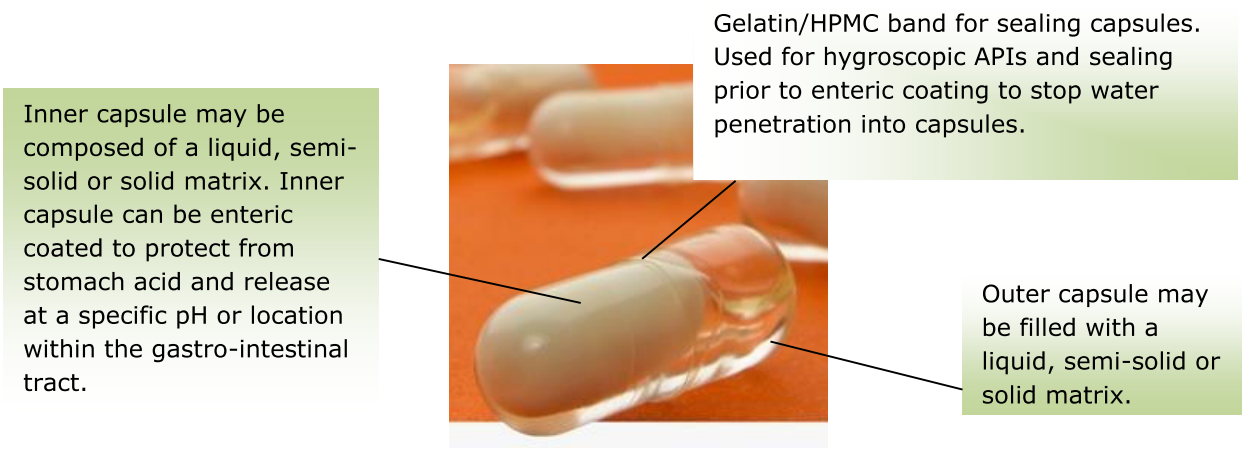


Figure 8: Capsule within a capsule. Image modified from http://www.capsugel.com/ihc/duocap [69].

A Multiphase release profile can be produced using liquid-fill technology. This technology allows for a liquid, immediate release and a semi-solid/solid delayed release profile for the same API in a single capsule unit (Figure 9).

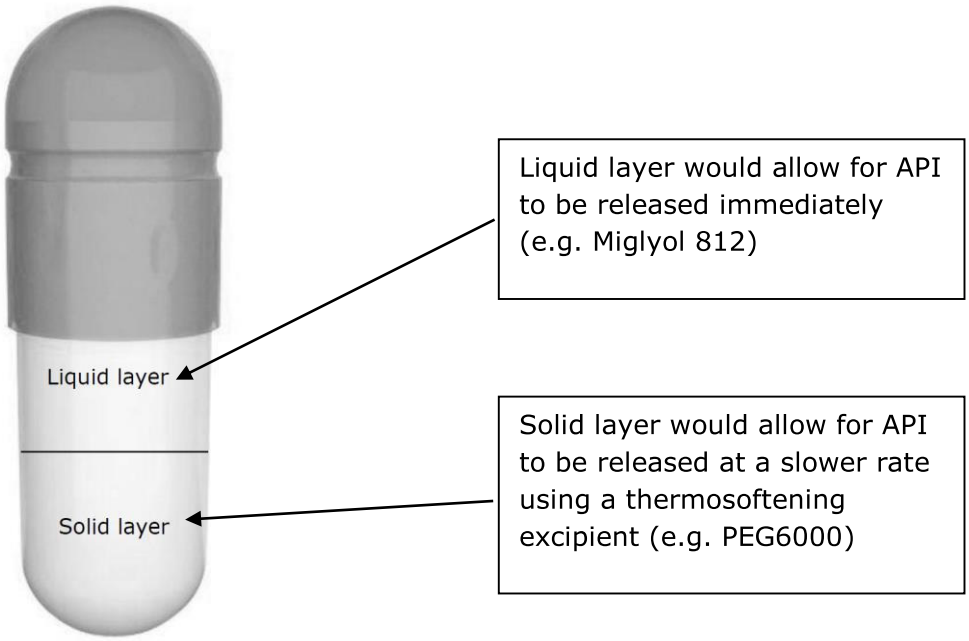


Figure 9: Multiphase release diagram. Image modified from http://www.worldpharmaceuticals.net/contractors/drug-delivery systems/qualicaps1/ [70].

1.6.4 Content Uniformity

An additional advantage of using liquid fill encapsulation can be in relation to improved content uniformity [46]. Low dosage products (under 10mg) can often give rise to low content uniformity. Liquid fill formulations, as solutions or suspensions, in general provide better dose homogeneity and fill accuracy compared to powder fill capsules or tablets [47]. They are often the formulation of choice for low dose products. In addition, liquid fill capsules generally require lower quantities of API and excipients compared to other formulations by comparison, thus minimising API requirements in both the early stages of drug product development and clinical manufacture. These formulations are also easily scaled up from bench to commercial scale when required. Low doses can also be effectively used with highly potent APIs.

1.6.5 Potent Product Manufacturing

Drug products that are intended for use in low doses are particularly difficult to produce in a solid oral dosage form compared to liquid fill encapsulation. The highly potent nature of such drugs (e.g. hormones) means that they can increase the risks of cross contamination and can expose operators to harmful dust during compounding and general manufacturing. Incorporating these APIs into a dust free liquid or semi-solid formulation is an alternative strategy which protects against such risks [48].

1.6.6 Hi-bar bench filling machine

Hi-bar bench filling machine is a semi-automatic liquid filling machine used for encapsulation (e.g. liquid-fill hard capsules (LFHC)) [71]. The instrument runs on compressed air (5 bar) and the temperatures are set for the hopper and the pump on the heater controls depending on excipient properties (e.g. thermo-softening). The pump forward and reverse stroke pressures are adjusted to suit the viscosity characteristics of the material to be filled by adjustment of the needle valves to the rear of the pump. The fill weight is set by releasing the locking grub screw on the micrometer then adjusting the fill volume by altering the micrometer setting. Height adjustment is dependent on a variety of factors such as shot size; dispense speed, product viscosity and capsule size. These parameters need to be determined at set-up for different formulations to best suit the application.

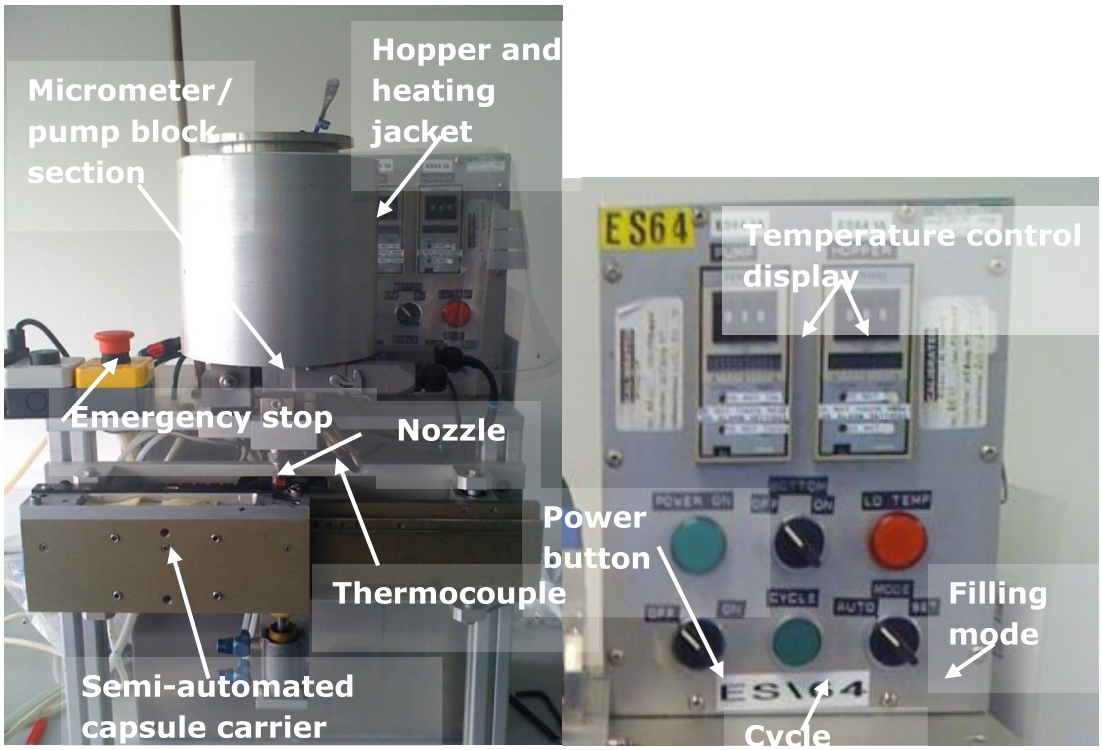


Figure 10: The Hibar filling machine is a semi-automated filling machine for manufacturing liquid-fill hard capsules (LFHC).

1.6.7 Qualiseal benching banding machine

The Qualiseal bench filling machine is a semi-automatic banding instrument that is used to apply a band to the cap and body join to inhibit the leaking of the internal formulation from the capsule. Banding solution can be either gelatin or HPMC [72]. The bath temperature is set to 45-65°C (temperature requirement for various banding solutions). The volume of the wet band will be approximately 3 times the volume of the eventual dry band. The capsules can be banded either by manually or automatically passing the slat over the blade. Capsules should roll continuously (at least 360°) until completely banded by a rotating disc. Normally one pass of banding will achieve a satisfactory band however, if not, a second band may be applied and can be used to dissipate bubbles on the band from residual pressurisation that can be caused during the mechanical forces being applied when the cap is being placed on the body of the capsule. The

capsules are transferred to a drying station or to an oven set to approximately 25-30˚C. This will hold the contents of the capsule at an angle within the body until the band dries. The capsules can be transferred onto stainless steel trays to completely dry for a minimum of 8 hours prior to inspection or leak testing.

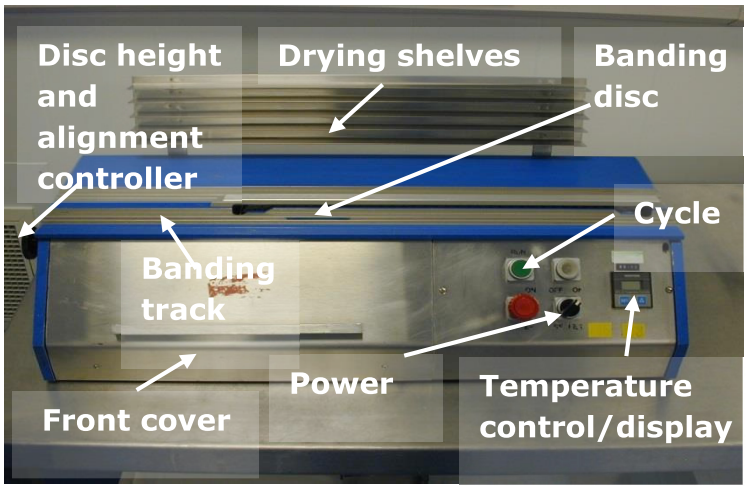


Figure 11: The bench Qualiseal bander is used to apply a band around the cap and body join to produce a sealed product for liquid, semi-solid and solid dosage forms.

1.6.8 Capsule selection

Capsule stability is necessary to evaluate in order to determine physical and chemical changes over the course of a capsule's life span. Soft gel and Hard shells capsules are the two main types of gelatin capsules available. If physical and chemical changes occur then this can be as a result of temperature, moisture, packaging, storage conditions etc [71]. For example, temperature can cause physical weakness in the shell; combined with physical interaction with excipient/s and/or API [72, 73] this can also cause chemical degradation of API and therefore loss in potency and increase in related degradation substances.

1.6.8.1 Soft Gel Capsules

A softgel is an oral dosage form consisting of a gelatin based shell surrounding a liquid fill. Soft gel capsules (SGC) are a combination of gelatin, water, opacifier (opaque and photosensitive barrier) and plasticiser (e.g. glycerin and/or sorbitol(s) [74, 75, 76].

Softgels are produced in a process known as encapsulation using a Rotary Die Encapsulation (RDE) process [77]. The encapsulation process involves forming of the shells, followed by filling and finally sealed. The forming involves two flat ribbons of shell material being manufactured on the machine and brought together on a twin set of rotating synchronised dies. The dies contain size and shape specific recesses (product specific), which cut out the ribbons into a two-dimensional shape, and form a seal around the outside [77, 78]. At the same time a pump delivers a precise dose of fill material through a nozzle incorporated into a filling wedge whose tip sits between the two ribbons in between two die pockets at the point of cut out. The wedge is heated to facilitate the sealing process. The wedge injection causes the two flat ribbons to expand into the die pockets, giving rise to the three-dimensional finished product. After encapsulation, the softgels are dried for days, depending on the product [78].

A SGC could also include minor additives such as colouring agents, flavours, sweeteners and preservatives [75, 76]. They can also be enterically coated for certain applications. There are non-animal based forms of gelatin called Vegicap® [79] to meet the needs of those wishing alternatives to meat products for vegetarian, ethical or religious reasons. [79].

SGC's are easy to swallow; mask odours and unpleasant tastes, enhance the bioavailability of the active ingredient, can be made into chewable, extended release capsules, ophthalmic preparations and vaginal/rectal suppositories. They can solve many problems associated with tableting, including poor compaction, poor weight or content uniformity [80]. SGC's can reduce oxidation, hydrolysis [81] and increase bioavailability of hydrophobic APIs [82].

Water soluble material is difficult to incorporate into SGC'S due to the forming process [78]. SGC's can be very sensitive to heat and humidity. In hot or humid climates, capsules may stick together or even break open before they can be administered. Efflorescent material cannot be incorporated, they may cause softening/leaching. Deliquescent materials cannot be incorporated. They may become brittle due to dehydration. Larger volumes of bulk formulations are required compared to hard shells and therefore developing a formulation can be expensive. SGC's are manufactured at approximately 40°C [77] and therefore excipient and API are held at elevated temperatures and could degrade temperature sensitive API's. Hard Gelatin Capsules (HGC) can be filled at below room temperature and therefore can be used for temperature sensitive materials.

1.6.8.2 Hard Gelatin Capsules

HGC's are made with Gelatin (animal origin) or HPMC (plant origin). Capsules are manufactured and supplied as empty shells, comprised of a cap and a body (Figure 12). Capsules can be hand filled and therefore is an inexpensive method of developing and evaluating prototype formulations consisting of API and excipient(s) and determining chemical and physical compatibility.

The design of a capsule is important when deciding which capsule to select. For example, capsules may have vents built into the cap or body locking ring or both [71]. Vents, pre-locks and locking rings have different designs of shape, size and position on different manufacturer's capsules but all serve the same function [72].

Pressurisation occurs when the cap is closed (rapidly) and locked onto the body by a filling machine. Capsule vents help release some or all of this excess pressure in the few seconds between closing and ejecting the capsule from the filling machine. Vented capsules are primarily designed for powders, such as Capsugel's Coni-Snap; however they are also suitable for liquid filling due to the pressure in the capsules being allowed to dissipate by the vents. However these vents also need to be sealed with a sealing solution [83] to inhibit leaking due to capillary action and vents allowing the depressurisation. Figure 12 shows a Capsugel Coni-snap gelatin capsule in the pre-lock position and vents for depressurisation.

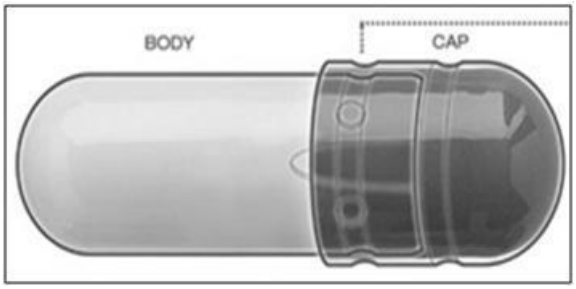


Figure 12: Coni-snap gelatin capsule in the pre-lock position.

'Licaps' are a design of capsules made by Capsugel specifically for liquid filled capsules; they are designed without vents and have a particularly tight locking ring to retain liquids which can cause some pressurisation at high capsule fill

volumes and speed of filling. However, Capsugel have further enhanced their design by incorporating an additional venting system (additional locking ring) to allow adequate de-pressurisation [71, 83].

Some of the advantages of HGCs include that they are easy to swallow; mask odours, unpleasant tastes, enhance the bioavailability of the active ingredient, can be coated or developed with various excipients to allow for immediate or extended release. HGCs can also solve many problems associated with tableting, including poor compaction, poor weight or content uniformity [80]. They can also reduce oxidation, hydrolysis [81] and increase bioavailability of hydrophobic drugs [82].

HGCs have some disadvantages in that they are moisture sensitive but not as sensitive as SGCs as the water content is lower (13-16% w/w). In hot or humid climates, HGCs caps may stick together or break open during storage. They may become brittle due to dehydration or become soft depending on internal formulation plasticisers.

As empty HGCs were available as an off the shelf product and only use small quantities of API compared to softgels it was concluded that HGCs were the ideal choice to evaluate limited quantities of prodrugs.

1.7 Characterisation techniques

1.7.1 Differential Scanning Calorimetry (DSC)

DSC measures temperatures and heat flows associated with thermal transitions in materials [84]. It provides crucial information in regards to the thermal properties of selected molecules, including glass transitions, phase changes, melting, and crystallisation and can be applied to product stability, cure/cure kinetics, and oxidative stability [84, 85]. DSC can be used to identify phase separation or mesophasic structures, in conjunction with other thermoanalytic techniques to determine polymorphic behaviour of long alkyl chains [85] with a similar structure to the two prodrugs of interest within this thesis, cysteamine decanamide and cysteamine steramide. Hot Stage Microscopy (HSM) can be used to visually capture the phase transitions recorded by DSC [86].

1.7.2 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis concerns the measurement of a material's thermal stability [87]. Material is dispensed on to a platinum plan and placed into a sealed furnace. The material is heated at a specific rate within the furnace until fully degraded. TGA measures weight changes in a material as a function of temperature (or time) under a controlled atmosphere [85].

1.7.3 Infrared spectroscopy (IR)

Infrared spectroscopy deals with variations in the vibrational and/or rotational motions of molecules. The information derived from the vibrational and rotational spectra includes: chemical composition of the molecules, the masses of the atoms, molecular structure and binding forces between the atoms [88]. Substances can be characterised, identified and quantified by Fourier

Transform-Infrared spectroscopy (FT-IR) [89]. Attenuated Total Reflectance (ATR) FT-IR operates by measuring the changes that occur in a totally internally reflected beam when the beam comes in contact with a sample. This technique involves an infrared beam being directed on to a crystal with a high refractive index at a specific angle. This internal reflectance extends to the sample that is in contact with the probe. Where the sample absorbs energy (infrared region) the evanescent wave attenuated energy is returned to the IR beam which is read by the detector in the IR spectrometer [90]. Following Fourier transform processing a spectrum is obtained.

1.7.4 Texture analysis (TA)

Texture analysis is a useful technique commonly used in the food industry to determine product quality based on tensile and compressive mechanical measurements (figure 13 to 15) [91]. More recently, it has been used by the pharmaceutical industry in a variety of conformations in order to characterise dosage forms and has been shown to be particularly useful in determining the robustness of capsule shell integrity [92]

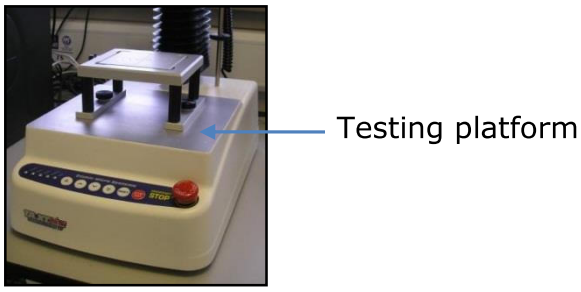


Figure 13: Texture analyser testing platform

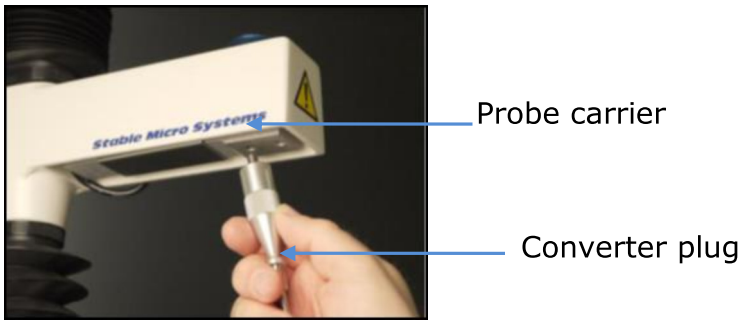


Figure 14: Texture analyser probe and converter

Different probes can be fitted into the texture analyser, depending on the nature of the tests. In order to measure capsule resistance to compression [92], a cylindrical aluminium piston with plane pressure surface was used (figure 15).

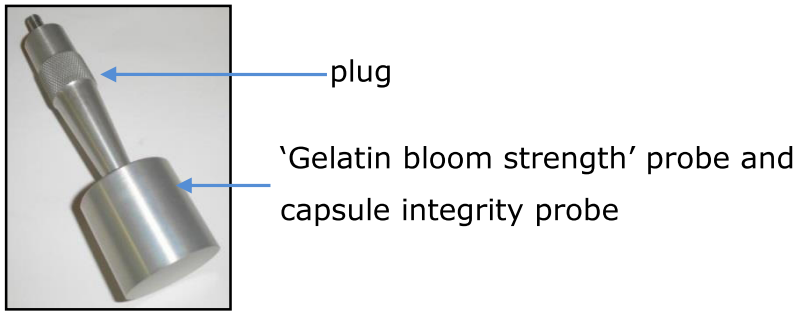


Figure 15: Texture analyser cylindrical aluminium probe

1.8 Research Aim

The main research aim was to characterise two cysteamine prodrugs for the treatment of cystinosis and evaluate the suitability of liquid fill technology. The following objectives were set based on the results of a literature review.

The first objective was to generate chemical/physical characterisation data for cysteamine prodrugs within a stability program.

The second objective was to conduct research activities, such as excipient screening and capsule shell compatibility studies to assess the suitability of prototype formulations using some of the techniques outlined in the introduction.

The third objective was to utilise liquid fill technology and testing to produce a viable dosage unit for further investigation.

2.0 Materials and Methods

2.1 Materials

Table 2: Materials used in these development studies

Name	Description	Grade	Supplier
Cysteamine decanamide	Prodrug of cysteamine	Technical	RGU
Cysteamine steramide	Prodrug of cysteamine	Technical	RGU
Cysteamine palmitate	Prodrug of cysteamine	Technical	RGU
Tramadol HCl	Tramadol Hydrochloride	Technical	Encap
Nitrogen	Inert gas used in DSC study	Technical	RGU
Liquid Nitrogen	Inert gas used for HSM temperature control	Technical	RGU
Miglyol 812	Fractionated coconut oil	EP	Medex
Gelucire 44/14/50/02	Gelucire®44/14 is composed of surfactants (mono- and diesters of polyethylene glycol, co-surfactants (monoglycerides), and oily phase (di- and triglycerides).	EP/USP/NF	Gattefosse
Transcutol HP	Highly purified diethylene glycol monoethyl ether	EP/NF	Gattefosse
TPGS (Vitamin E)	Polyethylene glycol succinate	USP/NF	Antares
Labrasol	Caprylocaproyl macrogol-8 glycerides (EP), Caprylocaproyl polyoxyl-8 glycerides (NF), PEG-8 Caprylic/Capric Glycerides (USA FDA IIG)	EP/NF & IIG	Gattefosse
Precirol ATO 5	Glycerol distearate (type I) (EP) Glyceryl distearate (NF) Glyceryl palmitostearate (USA FDA IIG)	EP/NF & IIG	Gattefosse
Propylene glycol	Plasticising agent		
Aerosil 200	Thickening agent for future use. Increases viscosity of formulation at low concentrations 1%-3%	EP	Azelis UK
HPMC(Methocel K15M)	HPMC (Methocel K15M) is a water soluble polymer derived from cellulose and offers controlled release in hydrophilic matrix systems. In this context, its combined with Miglyol 812 (as a carrier for HPMC) in order to determine compatibility with prodrugs	EP/USP	Colorcon Ltd
Lauroglycol 90	Propylene glycol monolaurate (type II)	EP/NF	Gattefosse
size 0 gelatin capsules	A size 0, two piece, Gelatin capsule, with an opaque white body and an opaque white cap. The body and cap are unprinted.	Technical	Capsugel
Hydroxypropyl Methylcellulose (HPMC)	A size 0, two piece, HPMC (Hydroxypropyl Methylcellulose) capsule, with an opaque white body and an opaque white cap. The body and cap are unprinted.	Technical	Shionogi/Qualicaps
Size 0 Vcaps Plus White/White HPMC (Hypromellose) Capsule Shells	A size 0, two piece, HPMC (Hydroxypropyl Methylcellulose) capsule, with an opaque white body and an opaque white cap. The body and cap are unprinted. Supplied as Vcaps Plus	Technical	Capsugel-Colmar
Sterile water	Sterile water for Irrigation, in two litre, plastic bottles	BP/EP/USP	Fresenius Kabi
Gelatin	Gelatin Bloom, pharmaceutical grade (banding solution preparation)	EP	Gelita AG
HPMC banding solution	Encap specific composition	Technical	Encap
dihydrogen orthophosphate	Phosphate buffer component	Reagent grade	Sigma
Sodium Hydroxide	pH modifier	Reagent grade	Sigma

Table 3: Method and test description for excipient/formulations evaluation

Method Section and Test	Excipient/Formulation
1a. Visual solubility	Miglyol 812 Gelucire 44/14 Transcutol HP TPGS (Vitamin E) Labrafil M 1944 CS Labrasol Precirol ATO 5 Propylene glycol Miglyol 812N + Aerosil 200 Miglyol 812N + HPMC Lauroglycol 90
1b. Gelucire based dissolution release profiles	Gelucire 44/14 (100%) Gelucire 44/14 (50%) + Gelucire 50/02 (50%) Gelucire 50/02 (100%)
1c. Capsule shell compatibility study	Labrafil 1944 CS Labrasol Lauroglycol 90 Miglyol 812N Transcutol HP Labrafil 1944 CS + cysteamine palmitate Labrasol + cysteamine palmitate Lauroglycol 90 + Cysteamine Palmitate Miglyol 812N + Cysteamine Palmitate Transcutol HP + Cysteamine Palmitate

2.2 Characterisation of prodrugs

2.2.1 Differential Scanning Calorimetry (DSC)

Prodrugs cysteamine decanamide and cysteamine steramide were screened using hermetic pans for thermal stability using DSC (Q-100 series, TA Instruments, United States). The DSC was calibrated for T_{zero} and cell constant with the manufacturer supplied standard (e.g. indium) following the instrument calibration guidelines. Separate samples were initially tested at three heating rates i.e. 10, 20 and 30°C/min heating rates under nitrogen (10 ml/min) as the purge gas. Approximately 4.6 mg of each sample of API was subjected to a temperature ramp, starting from room temperature. The sample was heated from approximately 20°C (room temperature) to 200°C to achieve a full melt. A heating, cooling and repeat heating stage was employed to investigate stability and crystal morphology. Three separate cycles were implemented to

fully assess the effect of heating (blue), cooling (red) and secondary heating (green). The data analysis was completed using the TA instrument software.

2.2.2 Hot Stage Microscopy (HSM)

Hot stage microscopy was employed to evaluate prodrug cysteamine steramide, using a polarising microscope (Leica DM2500) with real-time imaging, with a stage compartment fitted with a heated/cooled hot-stage (Linkam Scientific Instruments Ltd, United Kingdom), temperature controlled by computer software. The material for analysis was dispensed on to a microscope slide and placed into the chamber of the hot stage. The temperature program was set using the temperature control software and the melting point, recrystallisation and other significant thermal events for comparison with significant exothermic/endothermic events captured using DSC.

A few crystals of cysteamine steramide were placed on a microscope slide. A cover slip was placed on the crystals to produce a thinly distributed sample for analysis and to achieve optimum resolution. The sample was heated initially at 10°C/min (the heating and cooling rate was modified throughout the program to capture images at various phase transitions) until the sample had reached 200°C, then further cooling to room temperature using liquid nitrogen. The prodrugs were assessed using a magnification of 500x (50x objective lens and 10x optical lens).

2.2.3 Thermal Gravimetric Analysis (TGA)

TGA measurements were obtained using a TGA500 (TA Instruments, United States). An empty platinum pan was tared prior to dispensing prodrug powder. A small sample of each of the prodrugs, cysteamine decanamide and cysteamine steramide (1.647-7.799mg) was dispensed into the platinum pans for testing and subjected to a temperature ramp. The samples were tested at a heating rate of 10°C/min under nitrogen (10 ml/min) as the purge gas. The sample was heated to 500°C to achieve full degradation of the sample in the furnace. The data analysis was completed using the TA instrument software. The temperature range of interest, for direct comparison with DSC data was room temperature to 200°C. The presence of residual solvents from synthesis of the prodrugs and the possibility of solvate formation was of primary interest.

2.2.4 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

A small quantity of between 1-2 g of cysteamine decanamide and cysteamine steramide powder was placed on the platform of the FTIR in direct contact with the ATR crystal (zinc selenide) and compressed using the FTIR probe to ensure direct contact and reduced interference from external sources. The FTIR is controlled using the computer software and the instrument scans from 400cm^{-1} to 4000cm^{-1}. A spectrum is generated and functional groups identified by the peaks present at characteristic wavenumbers.

2.3 Excipient solubility study

The prodrug was added in increments of 10-20mg to the potential solubilising agent contained in a 50ml beaker to determine the visual solubility of both prodrugs in each of the selected excipients. A theoretical target of 50mg per 0.5ml was set in order to replicate one of the current available doses of Cystagon™. Each of the individual excipients were mixed with the prodrugs using high shear mixing (Silverson small scale tubular high shear head with square perforations) at intervals of 2 minutes maximum or until visually homogeneous/soluble. The procedure was conducted in this way to ensure minimal use of the limited availability of the two prodrugs being assessed. Section 1a of Table 3 in materials section 2.1 details the excipients used in the visual solubility study.

During preparation, the perceived mixability (visual homogeneity (e.g. suspension) and flowability (Visual viscosity and physical characteristics (e.g. Thixotropic)) was assessed using a syringe to examine each mix in relation to the potential transfer to bench scale filling equipment (Hi-Bar semi-automated filling machine).

2.4 Tramadol HCl/Gelucire®

For development work undertaken with Gelucires, limited availability of the Cysteamine prodrugs necessitated the use of another compound, namely Tramadol HCl. Tramadol HCl was available onsite at Encap and was tested using UV analysis (refer to section 2.4.1-2.4.3). This section uses Gelucire 44/14 (soft) and Gelucire 50/02 (hard) to show the impact on dissolution if the ratio between a 'soft' Gelucire versus a 'hard' Gelucire self-emulsifies on contact with

aqueous media forming a fine microemulsion (SMEDDS). Gelucire 44/14 also improves the solubility and wettability of APIs *in vitro* and *in vivo*. Gelucire 50/02 is considered a 'hard Gelucire' and erodes over time and therefore combining both Gelucires can potentially modify API release without the use of additional surfactants and thus, demonstrates the effectiveness of this excipient. The Tramadol HCl was used at 20% (w/v) and the percentages displayed in Table 3 only references the excipient percentage for clarity. A 100 g bulk of each of the formulations listed below was prepared. This consisted of 80g of excipient or excipient mixtures and 20 g of tramadol HCl. These were combined using a Silverson high shear mixer for 2 minute intervals or until visually homogenous. Size 0 gelatin capsules were filled using the Hibar semi-automated filling machine with a target weight of 500mg ± 7.5% and banded using the Qualiseal semi-automated bander. Bands were allowed to dry overnight prior to initial testing. Section 1b of Table 3 in materials section 2.1 specifies the Gelucire based formulations.

2.4.1 Calibration curve and wavelength selection

The calibration curve and the wavelength were determined by UV/vis, with a Unicam UV2-400 spectrophotometer and a 1cm Quartz cell. λmax was determined by dissolving 75mg of tramadol HCl in 600ml of water (equivalent to 0.125mg/ml). The 0.125mg/ml tramadol HCl solution was poured in to a cuvette and analysed. The sample was run from 200-400nm in order to determine the wavelength which produced the highest absorbance AU value. Once determined the tramadol HCl samples would be tested at the same wavelength to produce release profiles of various Gelucire mixes.

2.4.2 Dissolution testing of Tramadol HCl/Gelucires

A standard dissolution apparatus, USP II (711), paddle and sinker method was used to determine the dissolution profile of capsules containing 100mg of tramadol hydrochloride and Gelucires 44/14 and 50/02 (50:50).

2.4.3 Preparation of dissolution medium

For each litre of dissolution medium, dihydrogen orthophosphate (6.8 g) and sodium hydroxide (0.9 g) were dissolved in deionised water (1000 ml). The final pH was measured using a pH meter (Serial 1227207031, Mettler Toledo Seven-multi pH meter) and adjusted to 6.80 ± 0.02 using 1.0 M sodium hydroxide. Solutions were degassed (using sonication bath) prior to use.

2.4.4 Dissolution procedure

Dissolution medium (600 ml) was placed in 6 dissolution vessels and allowed to equilibrate to 37°C ± 0.5°C. Filters (40 μm) were attached to the sample probes and individual capsules (with sinker attached) were placed in each vessel. Paddle stirrers were set at 75 r.p.m. and sampling was at intervals of 15 mins until the total API release reached a maximum concentration (total maximum release). API concentrations were determined by UV/vis, Caleva 9ST dissolution apparatus with a Unicam UV2-400 spectrophotometer, using a Watson Marlow 205U peristaltic pump and 1cm Quartz cell, λmax = 271 nm, a cuvette containing a reference sample of dissolution medium was used and the sample cuvettes change in absorbance (AU) was measured against the reference sample (placebo blank). Once the absorbance values plateaued this was considered to represent 100% release of tramadol hydrochloride from the Gelucire matrix. The spectrophotometer was fitted with a six cell auto-changer

permitting continuous automatic recording of cell solution absorbances. The capsules were weighed down with 316 stainless steel sinking wire, wrapped round each capsule.

2.5 Capsule shell compatibility study

To evaluate shell compatibility, each formulation was filled into white/white size 0 gelatin capsules (Capsugel), Hydroxypropyl Methylcellulose (HPMC) (Shionogi) and Hydroxypropyl Methylcellulose (HPMC) V cap plus capsules respectively. Miglyol 812 is a widely used inert excipient and has been tested in all types of capsules without any incompatibility issues at Encap. Each shell type has capsule fill weight of 500 ± 7.5%. A 100 g bulk was prepared for each of the formulations specified in Table 3, section 2.1 materials section. This consisted of 95 g of Miglyol 812N and 5 g (5% equivalent to 25 mg of prodrug per 500mg capsule unit) of cysteamine decanamide or cysteamine steramide. Each capsule was banded using either a gelatin or HPMC band corresponding to the same shell type used, on the semi-automatic bench Qualiseal. After overnight drying, the capsules were further subjected to a leak test at approximately -26 in Hg and were pressure tested after storage by physically squeezing the capsule between the forefinger and index finger and assessing mechanical resistance, signs of cracking, gross deformation or embrittlement. After initial assessment, capsules were packed into 60ml sealed amber jars and placed on stability storage at 25°C/60%RH and 40°C/75%RH. Approximately 20 capsules were placed in each container. Samples were taken at one, two, three and six month intervals. The capsules were examined for visual changes, brittleness or physical defects. Section 1c of Table 3 specifies the excipient or

excipient and cysteamine palmitate based formulations assessed in the capsule shell compatibility study, using the texture analyser (section 2.5.1).

2.5.1 Mechanical testing of capsule strength (texture analyser)

After the initial studies were completed using the manual physical integrity check, a Texture Analyser (XT Plus, Stable Micro Systems) fitted with a 15 mm Cylindrical Probe and 30 kg load cell was used to test the compressive strength of 'size 0' capsules filled with 500 mg of formulation containing cysteamine palmitate (50 mg). Individual capsules were placed in the centre of an adjustable platform (100 x 90 mm) and a compressive test (detailed below) undertaken on five individual capsules (n = 5).

Table 4: Test parameters used for capsule strength measurements

Parameter	Setting
Test mode	Compression
Pre-test speed	1.00 mm/sec
Test speed	0.50 mm/sec
Post-test speed	5.00 mm/sec
Target mode	Distance
Distance	5.3 mm
Trigger force	0.49N

All capsules were deemed physically compatible at this stage, gelatin capsules were selected for texture analyser studies using cysteamine palmitate. Section 1c of Table 3 in the material section 2.1 specifies the excipient or excipient and cysteamine palmitate based formulations assessed in the capsule shell compatibility study, using the texture analyser.

2.6 Semi-automated production of liquid fill capsules

2.6.1 Hi-Bar™ bench filling machine

2.6.1.1 Operating the Hi-bar bench filling machine

The Hibar uses compressed air for mechanical movements and requires 5 bar of pressure. If required, temperatures can be set using the heater controls for the hopper and the pump separately (e.g. thermosoftening excipients). If the heating is not required the thermocouples can be disengaged by removing the electrical pins. Size 0 capsule carriers were used for all filling processes. Degassed formulation was transferred to the hopper and material allowed to equilibrate (if applicable) to temperature.

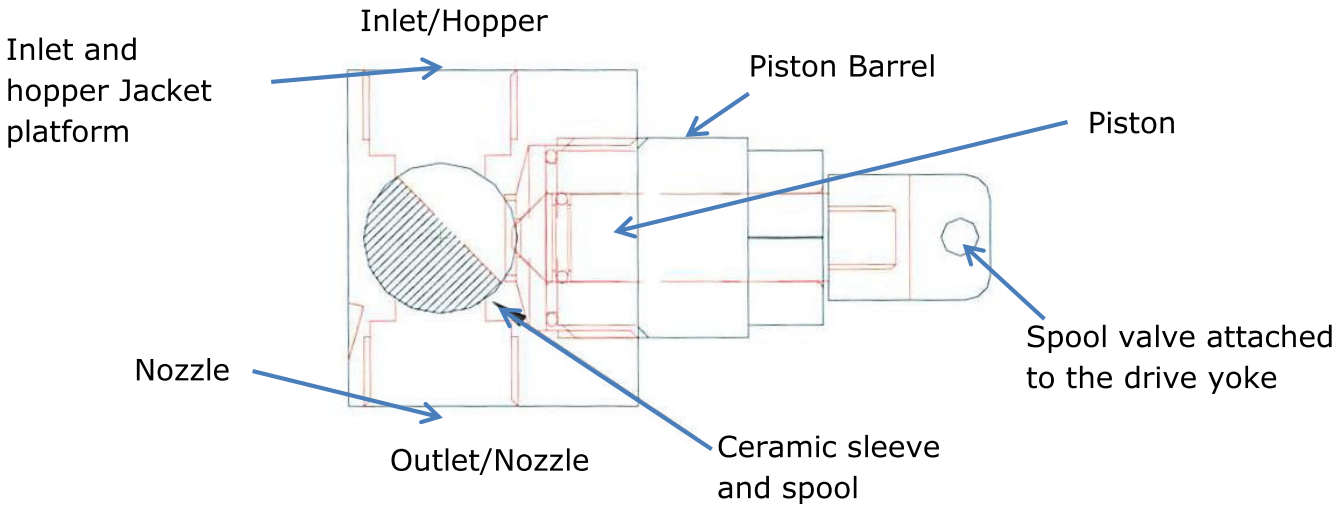


Figure 16: Pump assembly diagram

The pump forward and reverse stroke pressures were set to suit the viscosity characteristics of each formulation being filled and is a visual assessment based on flow characteristics. Adjustments were made via the marked needle valves to the rear of pump. Turning the valve clockwise reduces the pressure and vice versa. To set the fill weights an empty capsule shell was placed under the nozzle and filling control was switched to the 'SET' position. This relieves the

compressed air pressure on the piston to allow for volume adjustment. The locking grub screw on the micrometer was released allowing the fill volume to be adjusted by altering the micrometer setting. This was repeated until the target fill weight of 500mg was achieved. The micrometer grub screw was then tightened to secure the fill volume. The Hibar was set to 'MAN' i.e. manual and a carrier consisting of 3/4 capsules was positioned underneath the filling nozzle to confirm the delivered fill weight and ensure repeatability. The next filling control selected was 'AUTO' for automatic filling. A full carrier of 24 empty capsule bodies were placed on the filling table and filling initiated by pressing 'START'. At end of the first semi-cycle (12 capsules), the carrier is manually rotated through 180 degrees and the remaining 12 capsules were filled. The capsule fill weights were checked and any required changes to the fill volume were made. The caps are manually placed on the filled capsule bodies and stored upright or transferred directly to the Qualiseal bander.

2.6.2 Bench Qualiseal™

The discs, spacers and doctor blades were selected for size 0 capsules. (Each piece is marked with its application size). The discs were mounted on the spacer, (V-groove), noting the correct orientation (i.e. size identification and serial number to front). The assembly was mounted above the bath using the knurled drive pin. The doctor blades were fitted so that they just clear the rotating discs, (approximately 0.1mm).

The bander lid is then lowered and placed on the slat on the track so that the slat end with the indentation is to the right hand side. All capsules were placed in the pockets in the same orientation. The pockets within the slat are slightly

angled and this directs all capsules to the top of the holder ensuring all capsules are in the same position and therefore banding solution is applied at the same location for all capsules. In addition this keeps the capsules slightly raised to minimise leaks. The disc position is set, by adjusting the lid position, so that the discs are centred on the cap edge. The slats are also designed to allow sufficient air flow around the band to allow the band to dry. The slat is designed to hold the top of the cap and bottom of the body. The band therefore does not come into contact with the slat.

2.6.2.1 Capsule banding

On the control panel the bath was set to the required temperature (set to approximately 25°C for gelatin banding solution or 35°C for HPMC banding solution). Once the bath had reached temperature, the banding solution was transferred to the bath. The banding solution bath was then elevated using the lever to its operating position. The front panel was secured to maintain the temperature (when the bath is in the upper position, the discs will rotate and the bath heater will operate) (Figure 17).

The capsules were orientated with the cap facing away and placed in the banding slat. The amount of banding deposited was adjusted by two methods; the first method involved adjustment of the knurled screw on the machine left side and the second was by changing the clearance of the doctor blades.

The volume of the wet band was approximately 3 times the volume of the eventual dry band. The slat was placed on the forward drive and capsules banded automatically, by operating the drive control to produce the complete band and functional seal (Figure 17). A functional seal inhibits the formulation

from leaking out. The band also offers structural support for the capsule by fusing the cap and body together in a 'locked' position and protection from environmental conditions (e.g. moisture).

The capsules were monitored to ensure they roll continuously and were completely banded. Normally one pass of banding will achieve a satisfactory band however, if not (e.g. bubbles due to pressurisation), a second band may be applied. During a second band, the slat is transferred to the rear drive within 20 seconds of the first pass. The bath was constantly monitored to maintain a sufficient level of banding solution throughout the banding process. Additional stock was prepared to top up bath. The viscosity of the banding solution is visually monitored throughout the banding process and when the banding solution thickens, the reservoir contents were discarded and topped up with fresh solution.

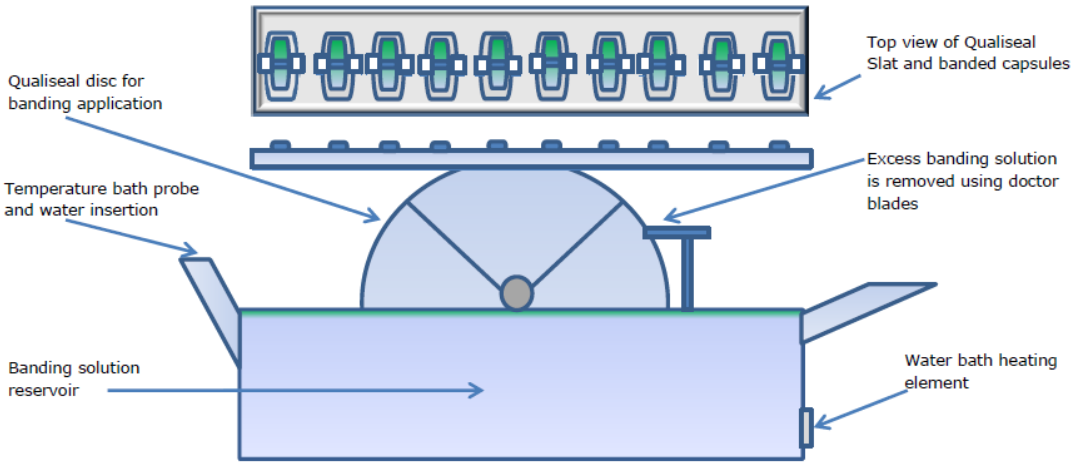


Figure 17: Qualiseal bander diagram

The capsules were transferred to the drying station on the Qualiseal (racks). Slats were placed on the drying station with the slat indentation to the left hand

side. This holds the contents of the capsule within the body until the band dries. Capsules were then transferred (after a few minutes) onto stainless steel trays lined with 60gsm PE bleached kraft uncoated tray lining paper, and allowed to completely dry at room temperature, for at least 8 hours, prior to inspection or leak testing. The capsules were then progressed for dissolution and stability testing.

3.0 Results

3.1 Characterisation of prodrugs

3.1.1 Differential scanning calorimetry (DSC)

The following DSC thermograms (below) were obtained using approximately 4.6mg of prodrug in hermetic pans. Following initial testing of three distinct heating rates, 10, 20 and 30°C/min, no extra thermal events were detected and subsequent measurements were undertaken at a heating rate of 10°C/min.

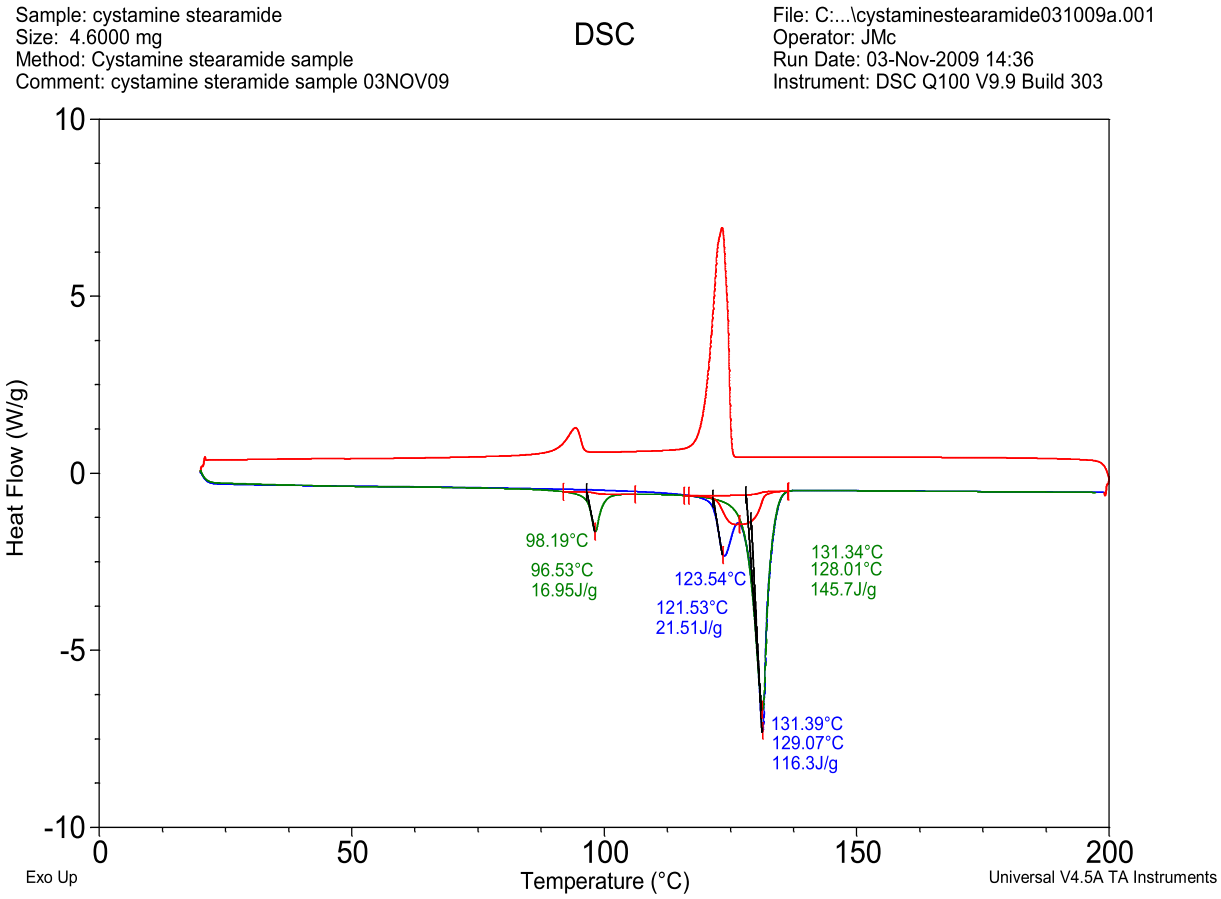


Figure 18: DSC thermogram of cysteamine steramide at week 0 (T_0). A heat/cool/heat cycle (10°C/min) indicates the crystalline melting endotherms and recrystallization exotherms associated with the prodrug. 1st heat cycle (blue), cool cycle (red) and 2nd heat cycle (green).

On the first heating cycle (blue) two distinct endothermic events for cysteamine steramide (CS) were apparent with onsets of 121.5 and 129.0°C respectively (Figure 18). It appeared that CS was mesophasic *i.e.* two distinct crystalline

phases. On subsequent cooling (red), two crystallisation events were observed around 120 and 90°C (Figure 18) respectively. Upon reheating (green), it was clear that a new event with a significantly lower onset of 96.5°C (Figure 18) was now present. The main melting event reappeared with onset 128.0°C (Figure 18).

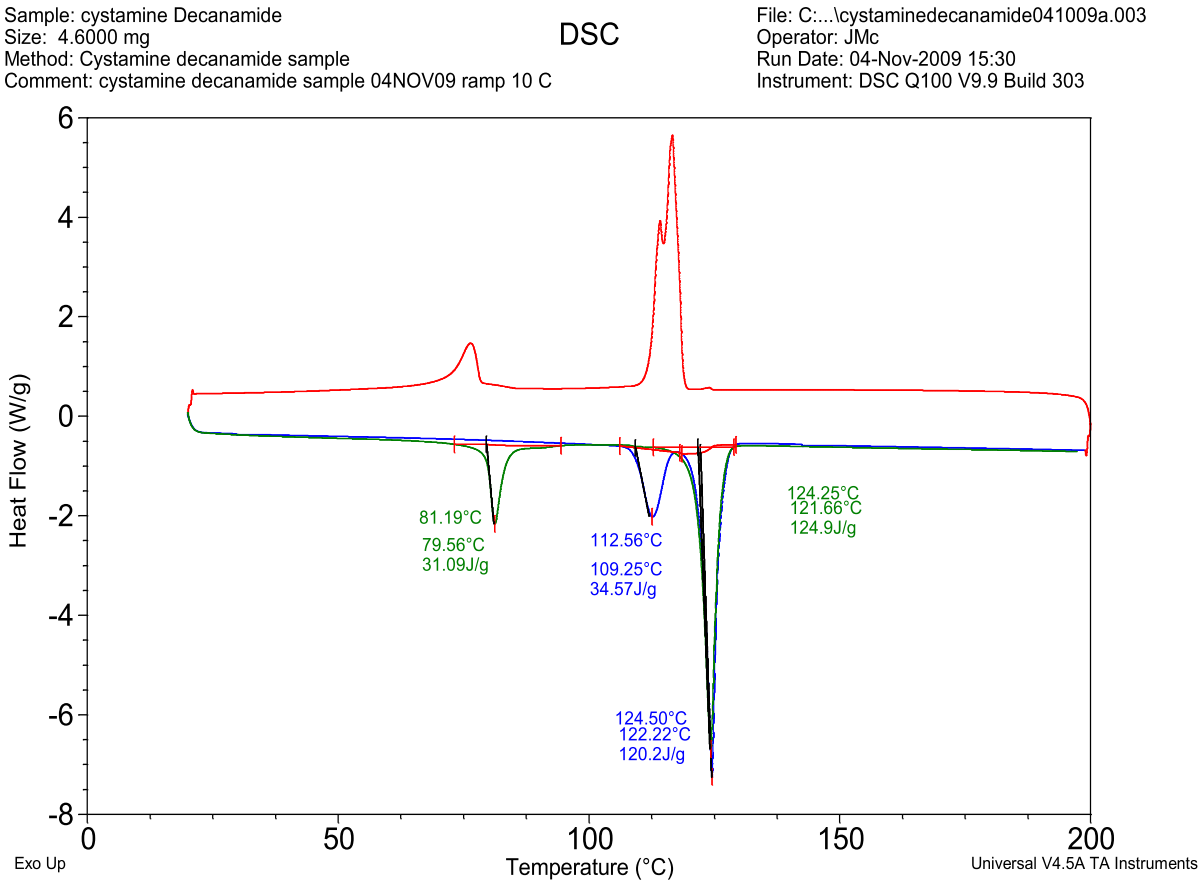


Figure 19: DSC thermogram of cysteamine decanamide at week 0 (T_0). A heat/cool/heat cycle (10°C/min) indicates the crystalline melting endotherms and recrystallization exotherms associated with the prodrug. 1st heat cycle (blue), cool cycle (red) and 2nd heat cycle (green).

For cysteamine decanamide (CD), a similar result was obtained. On the first heating cycle (blue) two distinct endothermic events were apparent with onsets of 109.2 and 122.2°C respectively (Figure 19). On subsequent cooling (red), two crystallisation events were observed around 120 and 80°C (Figure 19)

respectively. Upon reheating (green), it was clear that a new event with a significantly lower onset of 79.6°C (Figure 19) was now present. The main melting event reappeared with onset 121.6°C (Figure 19).

3.1.2 Hot Stage Microscopy

The images captured in figure 20 represent the DSC thermal events captured in the heating/cooling cycles. The images displayed below are for cysteamine steramide at 500x magnification at a scale of 50 micron.

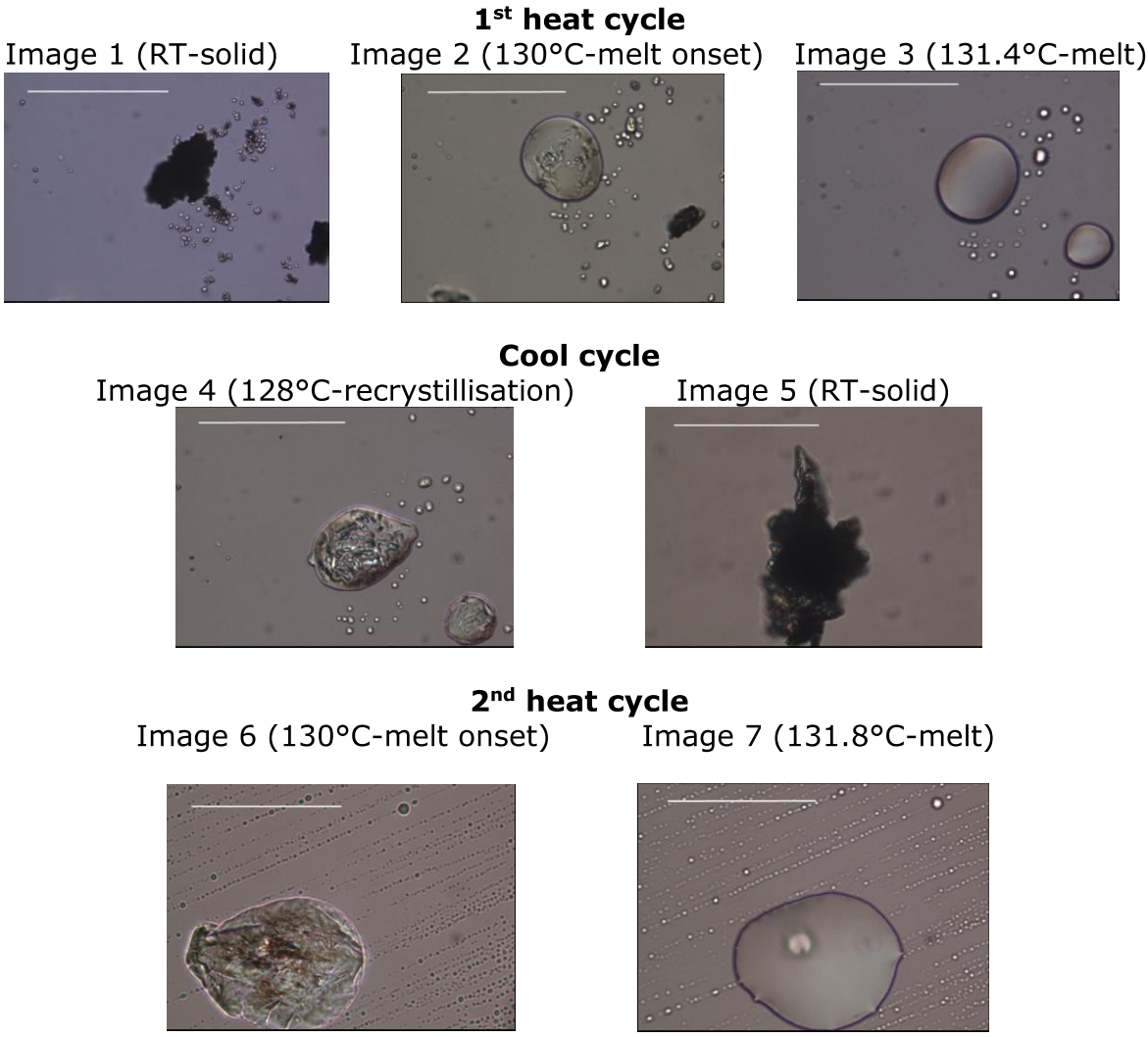


Figure 20: Microscopy images for cysteamine steramide, recorded during the 1st heat cycle, cool down and 2nd heat cycle. Heating rate is 10°C/min

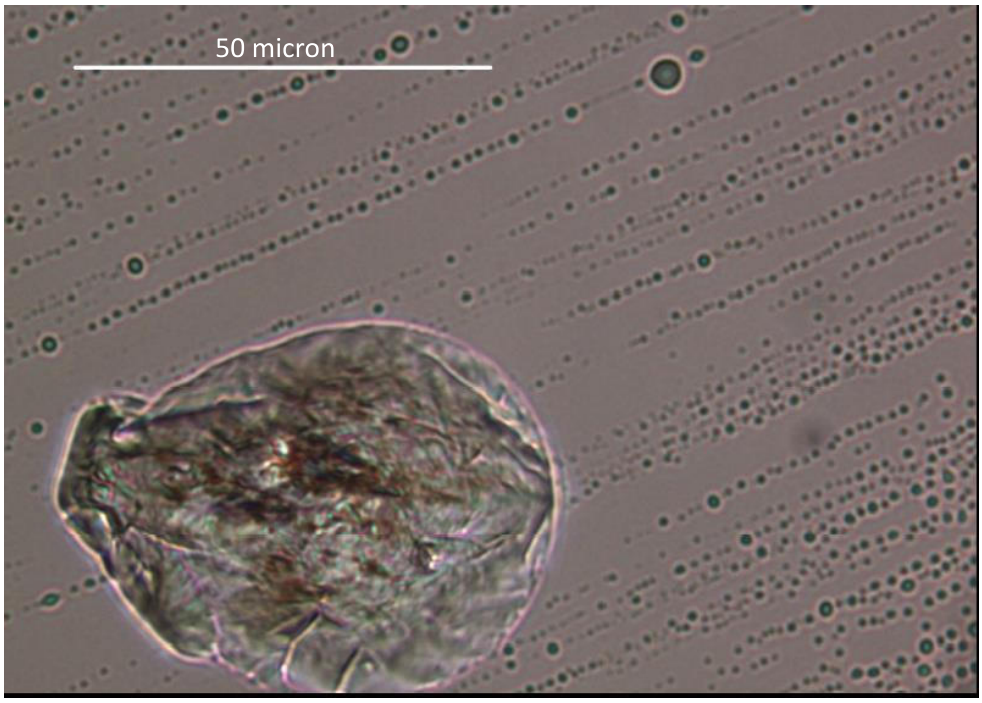


Figure 21: Enhanced image of the crystal structure-From Image 6 in Figure 20

The first heat cycle is depicted in images 1 and 2. Image 1 shows the appearance of cysteamine steramide at room temperature and Image 2 shows the onset of melt at approximately 130°C and Image 3 shows the prodrug completely melted (liquid phase) at 131.4°C. The cool cycle is depicted in images 4 and 5. Image 4 shows large crystals beginning to form on cooling at approximately 128°C and Image 5 shows the prodrug completely crystallised (solid phase) at room temperature (15-25°C). The second heat cycle (reheat) is depicted in images 6 and 7. Image 6 shows the onset of melt at approximately 130°C and Image 7 shows the prodrug completely melted (liquid phase) at 131.8°C.

During the heating stage in the 2nd heat cycle, internal movement was observed in the crystals from around 85°C, which indicated a partial melt, but the majority of the melt occurred at 131°C. The same phenomenon was observed for both prodrug according to DSC data (Figure 18 and 19), however only cysteamine

steramide was evaluated using hot stage microscopy due to limited cysteamine decanamide.

3.1.3 Thermogravimetric Analysis (TGA)

TGA was used in this case to assess the prodrugs thermal stability. The 40°C/75%RH samples for both prodrugs were tested using TGA. The samples were exposed to a 10°C/min heat ramp until full samples degradation was achieved (500°C). The TGA scans confirmed that no additional solvents or water were present as a straight line (zero weight loss) was recorded in the region of interest (0-200°C) (Figure 22 and 23) and no phase transitions other than the eventual thermal degradation was detected. This data corresponded with the NMR scans (Appendix 3 and 4) and confirmed that the samples were extremely pure.

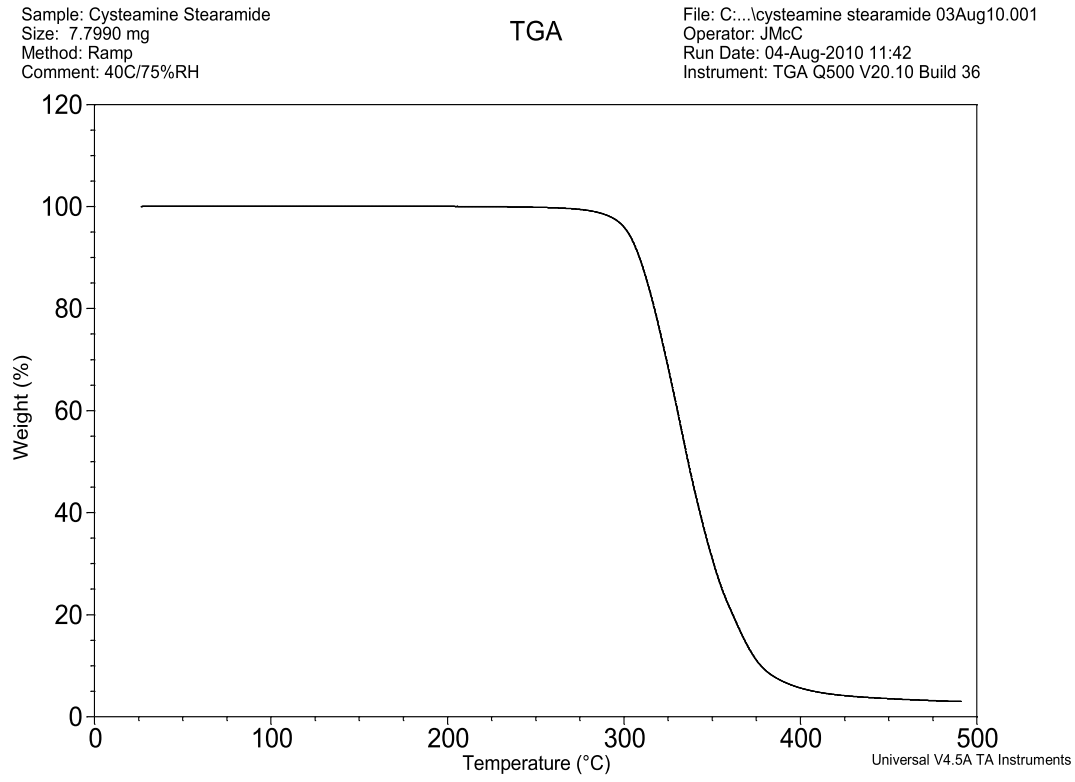


Figure 22: TGA plot of cysteamine steramide after 9 months storage at 40°C/75%RH. Heating rate is 10°C/min.

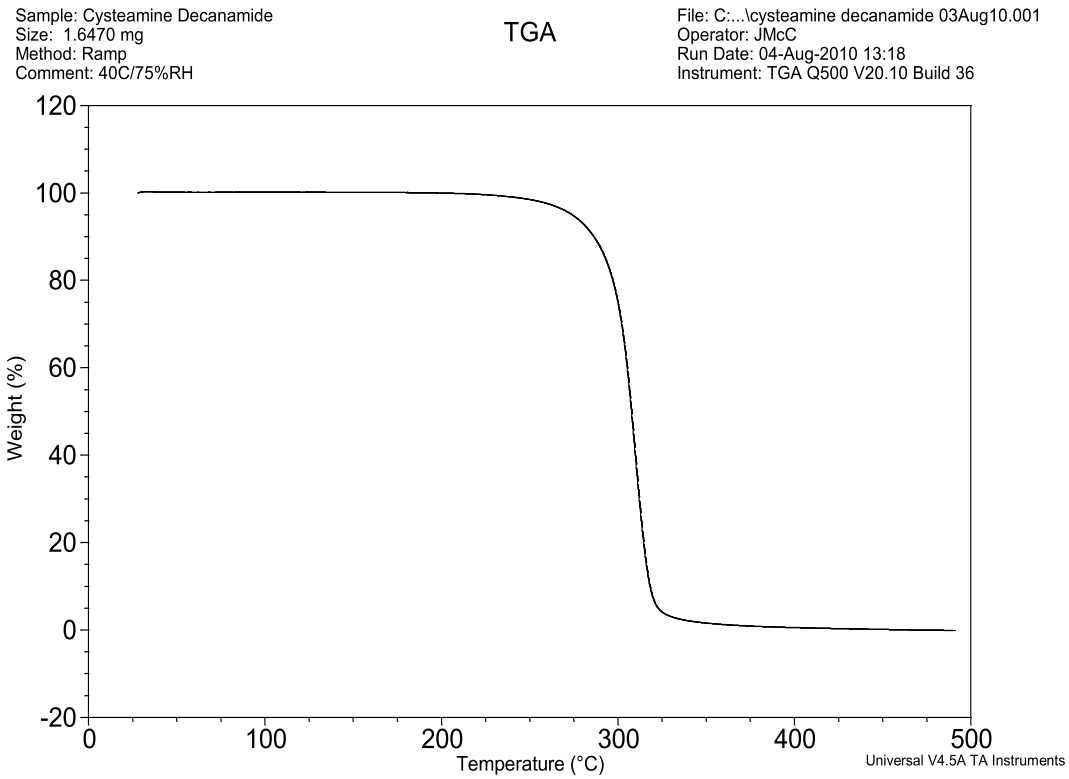


Figure 23: TGA plot of cysteamine decanamide after 9 months storage at 40°C/75%RH. Heating rate is 10°C/min.

3.1.4 Fourier Transform-Infrared Spectroscopy (FT-IR): Attenuated Total reflectance (ATR)

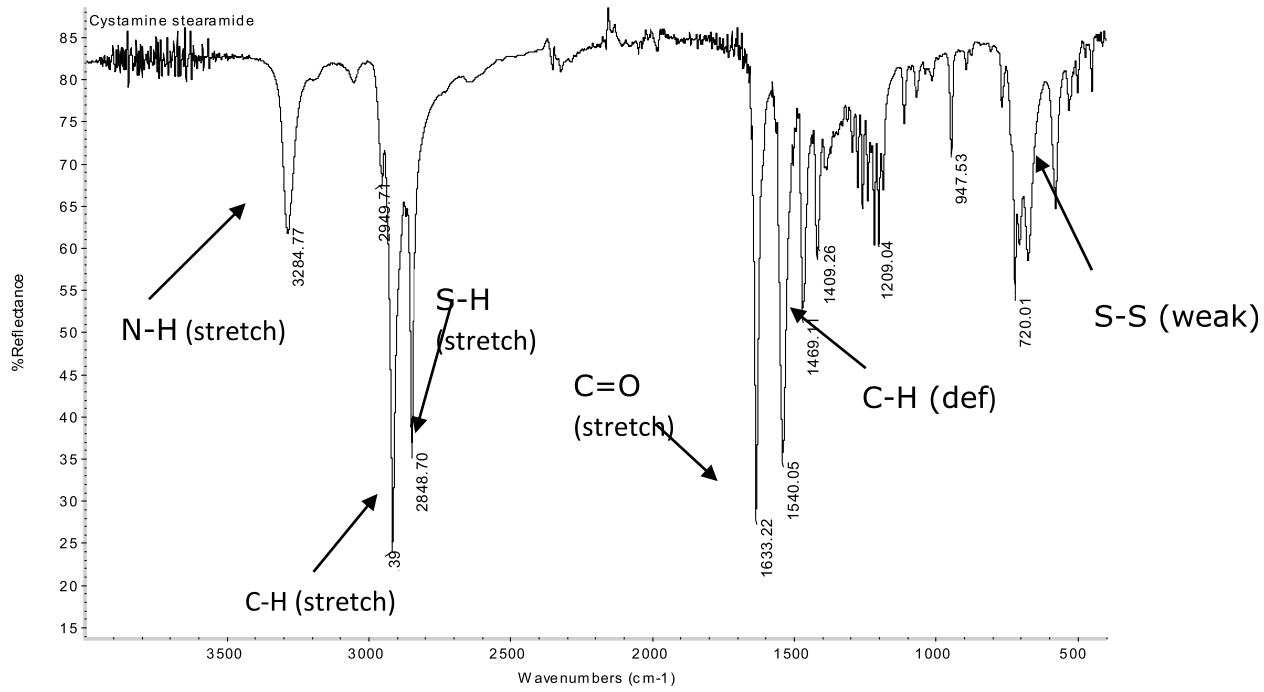


Figure 24: FT-IR spectra of cysteamine steramide at 0 weeks

Table 5: FT-IR spectra of cysteamine steramide and corresponding wavenumbers (cm^{-1}) at 0 weeks of the functional groups identified

Functional group	Wavenumbers (cm^{-1})
N-H	3285
C-H	2916
S-H	2849
C=O	1633
C-H	1469
S-S	~500

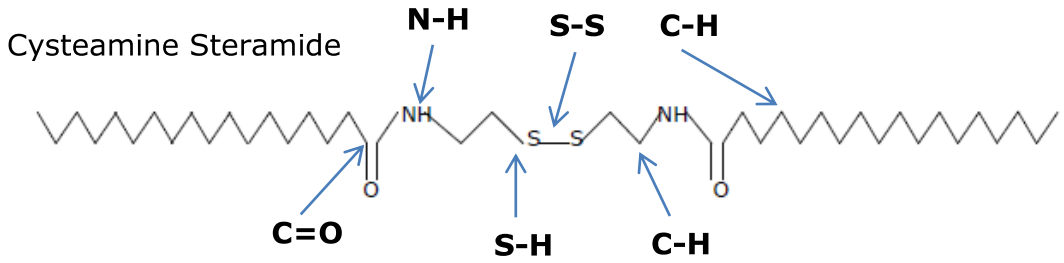


Figure 25: Cysteamine Steramide functional groups identified using FTIR method.

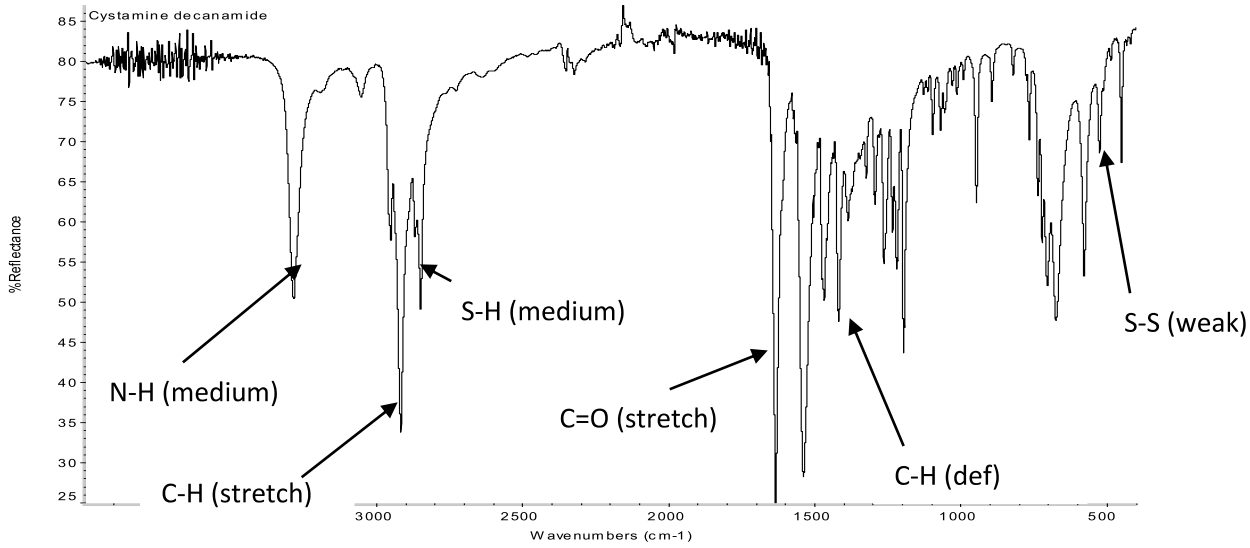


Figure 26: FT-IR spectra of cysteamine decanamide at 0 weeks

Table 6: FT-IR scan of cysteamine decanamide and corresponding wavenumbers (cm^{-1}) at 0 weeks of the functional groups identified

Functional group	Wave numbers (cm^{-1})
N-H	3285.91
C-H	2918.21
S-H	2849.92
C=O	1633.20
C-H	1467.11
S-S	~500

Cysteamine Steramide

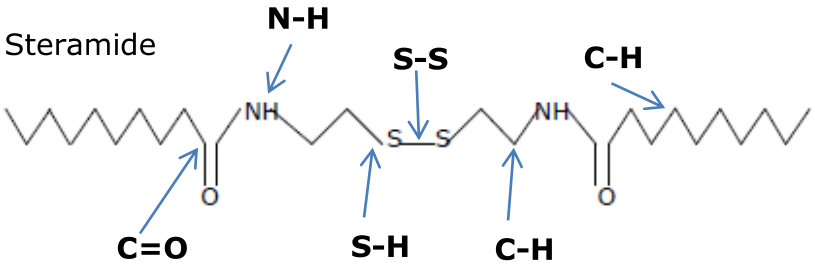


Figure 27: Cysteamine Decanamide functional groups identified using FTIR method.

The IR spectra contain common features such as disulphide bonds (S-S). The disulphide bonds were both identified at 550-400cm^{-1}. The disulphide is used to mask the unpleasant smell associated with thiols (S-H). Each scan showed a strong C-H absorption between 2918 and 2916cm^{-1} due to stretching mode of aliphatic hydrogens. The C=O groups absorption was around 1633cm^{-1} and the N-H medium absorption was around 3285cm^{-1} and represents the amine group. These IR spectra were compared against all spectra acquired over a nine month storage period (40°C/75%RH) and showed no visible growth, reduction or disappearance of any peaks. Please refer to appendix 1 FT-IR scan of cysteamine decanamide and corresponding wavenumbers (cm^{-1}) at 9 months

3.2 Qualitative study of prodrug solubility in a wide range of known solubilisers

Table 7: Excipient solubility table (up to 10% drug loading)

Excipient	Visual Solubility (mg/ml)	
	CS	CD
Miglyol 812	<10	<10
Gelucire 44/14	~60	~60
Transcutol HP	<10	<10
TPGS (Vitamin E)	<10	<10
Labrafil M 1944 CS	<10	<10
Labrasol	<10	<10
Precirol ATO 5	<10	<10
Propylene glycol	~40	~40
Miglyol + Aerosil 200	<10	<10
HPMC + Miglyol 812	<10	<10
Lauroglycol 90	<10	<10

<10 = visually poorly soluble (after the 1st addition 10-20mg of prodrug)

Gelucire 44/14 (API-60mg/ml) and propylene glycol (API-40mg/ml) appear to solubilise the CS and CD prodrugs but at prodrug concentrations significantly below the levels that are required to match the clinical dose (50mg).

3.3 Release studies on API/excipient combinations

3.3.1 Calibration curve and wavelength selection

Tramadol HCl in aqueous solution shows an absorbance maximum between 240nm and 290nm with a maximum at 271nm (Figure 28). It starts to show increasingly strong absorbance below the minimum at 240nm to 200nm however absorbance in this area is shown by many compounds so observations in the more definitive region of 240 to 290nm was selected with 271nm chosen as the preferential wavelength of observation. A plot of the UV spectrum of tramadol HCl in water is shown in Figure 28.

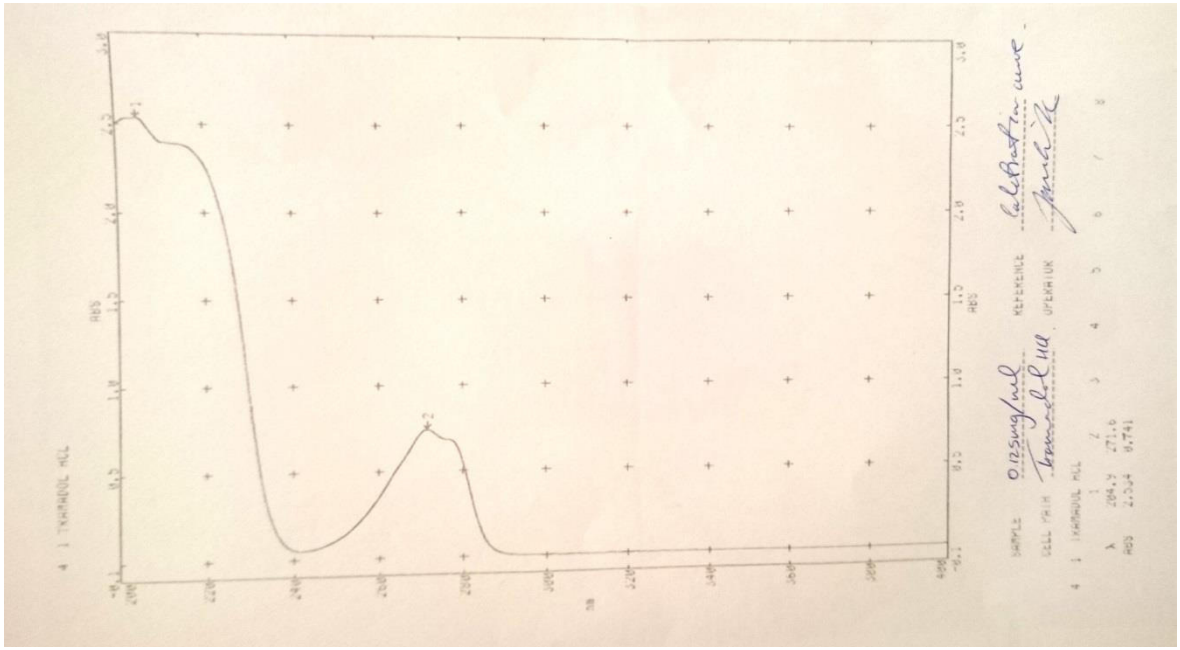


Figure 28: Image of UV calibration spectrum of 75mg of tramadol HCl dissolved in water to select the nominal wavelength (nm) with the strongest absorbance.

The calibration curve shows a λ_{max} at 271nm with an absorbance of 0.741 au for a concentration of 0.125mg/ml of tramadol HCl. This is equivalent to an absorbance of 0.741 for 75mg dissolved in 600ml.

3.3.2 Dissolution results

Figure 29 indicates Gelucire 44/14 (soft) and Gelucire 50/02 (hard) combinations at specific percentages to show the impact of two different types of Gelucires on dissolution release profiles of a model compound, in this instance tramadol hydrochloride. CS or CD was not available in sufficient quantities to evaluate at this time. It can be concluded from the data that release can be modified without the use of additional surfactants and thus, demonstrates the effectiveness of this excipient. If a surfactant was required then this would help reduce the interfacial tension between API and formulation enhancing wettability and therefore accelerate dispersal during dissolution. Section 1b of

table 3 specifies the Gelucire based formulations that were analysed using the dissolution procedure provided in section 2.4 of the methods section.

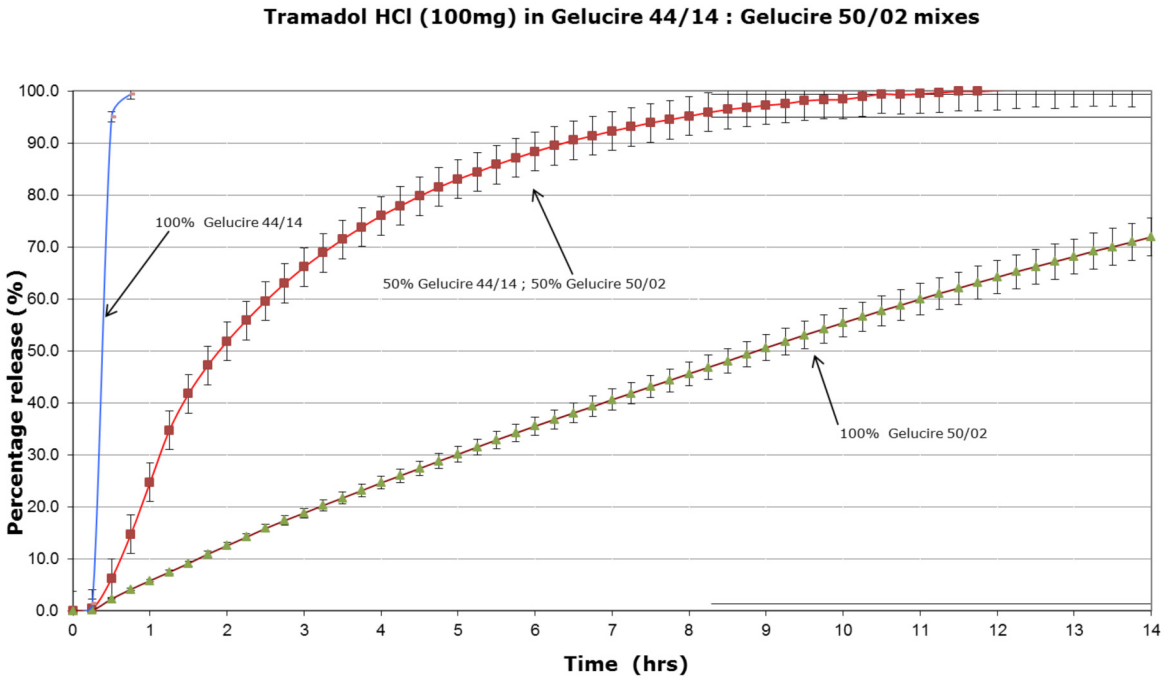


Figure 29: Percentage release (%) of 100mg of tramadol hydrochloride (water soluble) in Gelucire mixes (n=2) using UV analysis (λ max 271nm)

The results indicate that addition of Gelucire 44/14 significantly accelerates dissolution, obtaining a 100% release in less than 1 hour due to its self-emulsifying and surfactive properties. A ratio of 50:50 of Gelucire 44/14 and Gelucire 50/02 modifies full dissolution release to approximately 12 hours. Alternatively, using 100% Gelucire 50/02 the plot shows a steady release of Tramadol hydrochloride in the dissolution medium with just over 70% released after 14 hours.

A large range of different dissolution profiles, from fast to very slow, can be achieved by simply varying the Gelucire 44/14 to Gelucire 50/02 ratios without the need for additional excipients due to its non-ionic water dispersable

surfactant properties and its ability to self-emulsify on contact with aqueous media forming a microemulsion.

3.4 Capsule shell compatibility study

The physical test by hand was performed on 5 capsules at 0, 1, 3, 6 and 9 months. Gelatin capsules, HPMC and V-caps plus capsules were deemed viable after 9 months storage at 40ºC/75%RH. The shell compatibility study may be repeated with a higher dose as the dose selected (25mg) was due to the limited amount of prodrugs available.

3.4.1 Texture Analysis

The physical capsule compression test was performed on five capsules and each capsule was placed directly underneath the compression tool (Figure 30 and 31) (15 mm cylindrical probe) and centralised on the 100mm x 90mm adjustable platform using a 30 kg load cell using the testing parameters as specified on Table 4, section 2.5.1. The adjustable platform was then used to position the next capsule under the probe and the test initiated. This process was repeated for each of the sample groups listed in the methods section. Basic statistical analysis (St Dev & Mean) was performed using Minitab version 16 (please refer to appendix 5); in order to correlate the compressive strength of capsules during accelerated stability (40°C/75%RH).

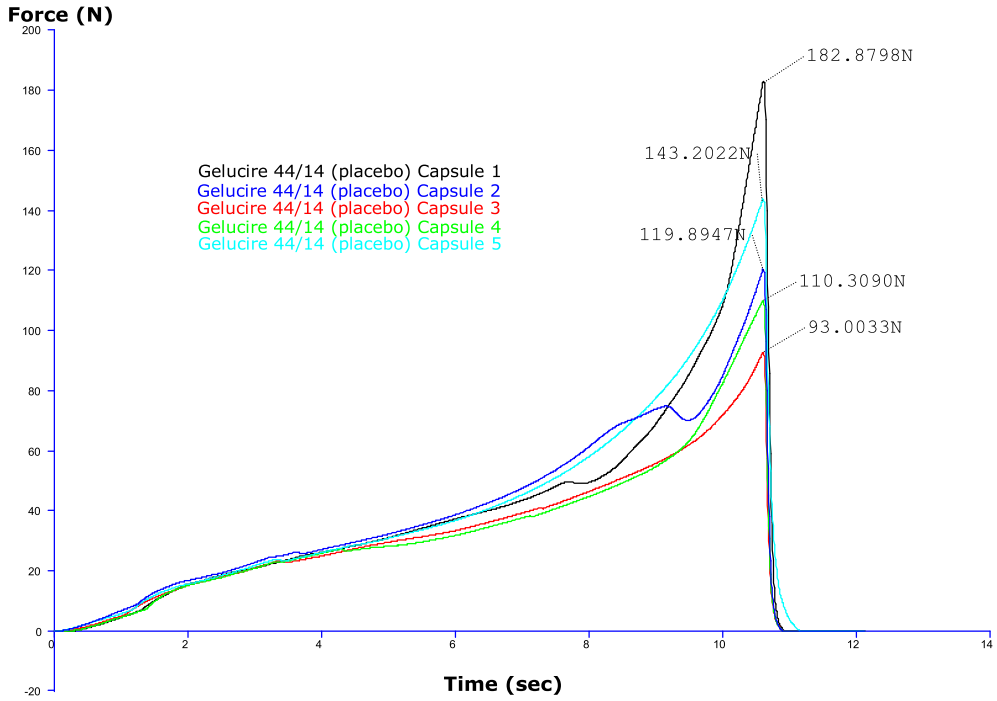


Figure 30: Determination of compressive strength (N) for a size 0 gelatin capsule containing Gelucire 44/14 at T_0

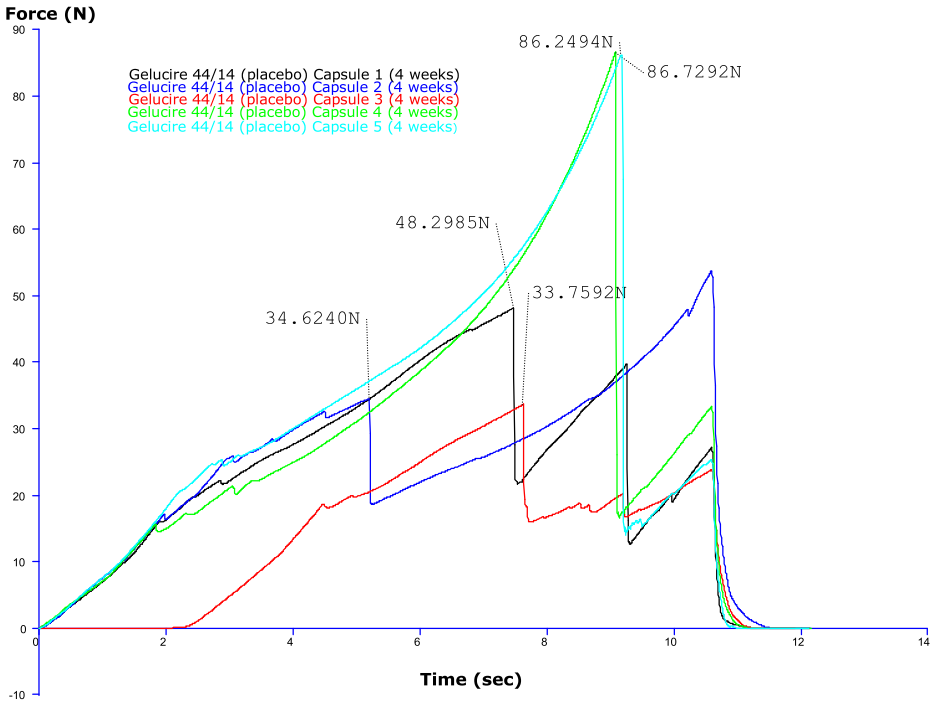


Figure 31: Determination of compressive strength (N) for a size 0 gelatin capsule containing Gelucire 44/14, after 4 weeks' storage at 40°C/75%RH.

Table 8: Determination of compressive strength (Peak Force (N)) at T_0 (initial) and after 4 weeks storage at 40°C/75%RH, using the TA compression test for a size 0 gelatin capsule containing Gelucire 44/14.

Capsule	Peak Force (N)	
	Initial	4 weeks
1	182.88	48.30
2	119.89	34.62
3	93.00	33.76
4	110.31	86.73
5	143.20	86.25
SD	34.75	26.83
Mean	129.86	58.03

The compressive strength peak force (N) recorded for a selected excipient, namely Gelucire 44/14 in figure 30 and 31 is summarised in Table 8. The Force (N) required to break a filled capsule is represented by the first peak to be recorded for each capsule. The mean peak force for a sample group of 5 capsules at T0 is 129.86 N. There is a high variability (SD 34.75) in the individual results potentially due to capsule movement on the testing platform. After 4 weeks of accelerated stability conditions at 40°C/75%RH, the average peak force recorded for another set of 5 capsules from the same sample group of capsules reduced to 58.03 N and the SD lowered to 26.83. The high degree of variability may have been caused by capsule movement during the compression test. The difference in force required indicates that 4 week storage at accelerated conditions has affected the capsules' physical strength.

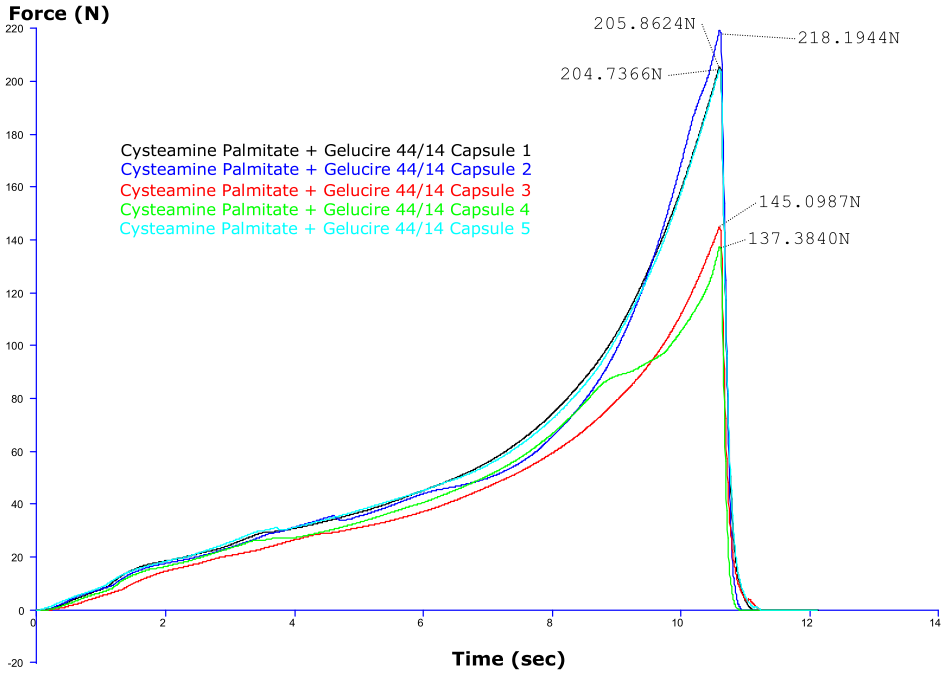


Figure 32: Determination of compressive strength (N) for a size 0 gelatin capsule containing Gelucire 44/14 + cysteamine palmitate at T_0

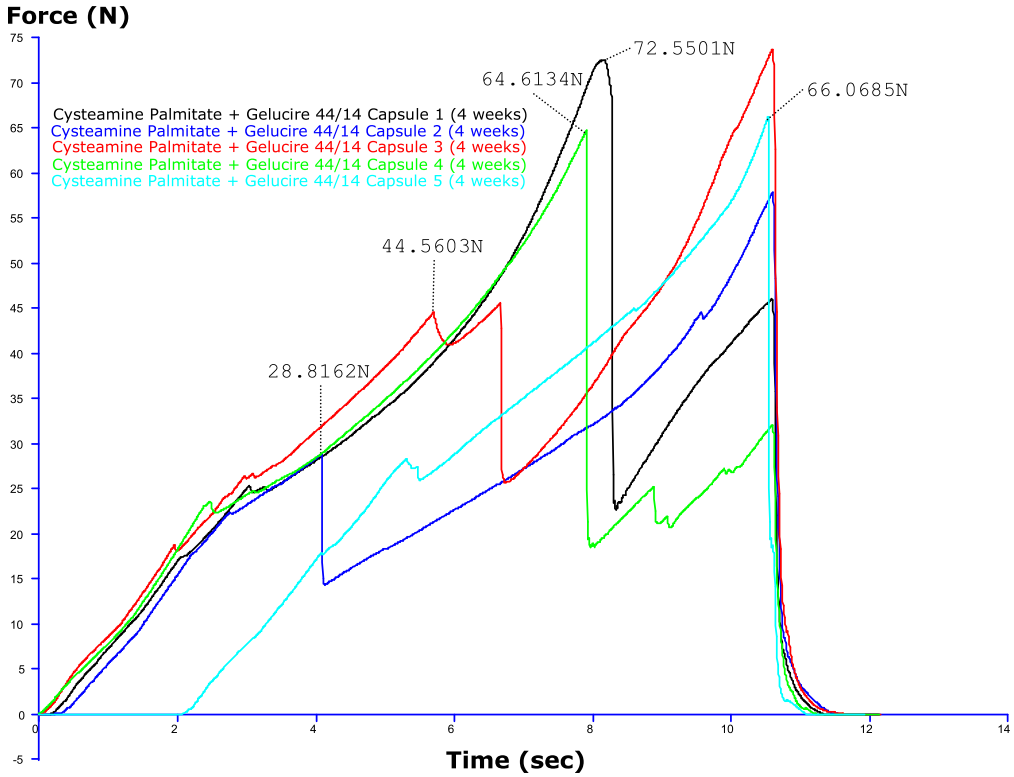


Figure 33: Determination of compressive strength (N) using the TA compression test for a size 0 gelatin capsule containing Gelucire 44/14 + cysteamine palmitate, after 4 weeks' storage at 40°C/75%RH.

Table 9: Determination of compressive strength (peak force (N)) at T_0 (initial) and after 4 weeks storage at 40°C/75%RH, using the TA compression test for a size 0 gelatin capsule containing Gelucire 44/14 + cysteamine palmitate

Capsule	Peak Force (N)	
	Initial	4 weeks
1	218.19	72.55
2	204.74	28.82
3	145.10	44.56
4	137.38	64.61
5	205.86	66.07
SD	37.91	18.15
Mean	182.26	55.32

The compressive strength peak force (N) recorded for a selected excipient, namely Gelucire 44/14 and CP in figure 32 and 33 is summarised in Table 9. The Force (N) required to break a filled capsule is represented by the first peak to be recorded for each capsule. The mean peak force for a sample group of 5 capsules at T0 is 182.26 N. There is a high variability (SD 37.91) in the individual results potentially due to capsule movement on the testing platform. After 4 weeks of accelerated conditions at 40°C/75%RH, the average peak force recorded for another set of 5 capsules from the same sample group of capsules has reduced to 55.32 N and the SD has lowered to 18.15. Again the high degree of variability may have been caused by capsule movement during the compression test. The difference in force required indicates that 4 week storage at accelerated conditions has affected the capsules' physical strength.

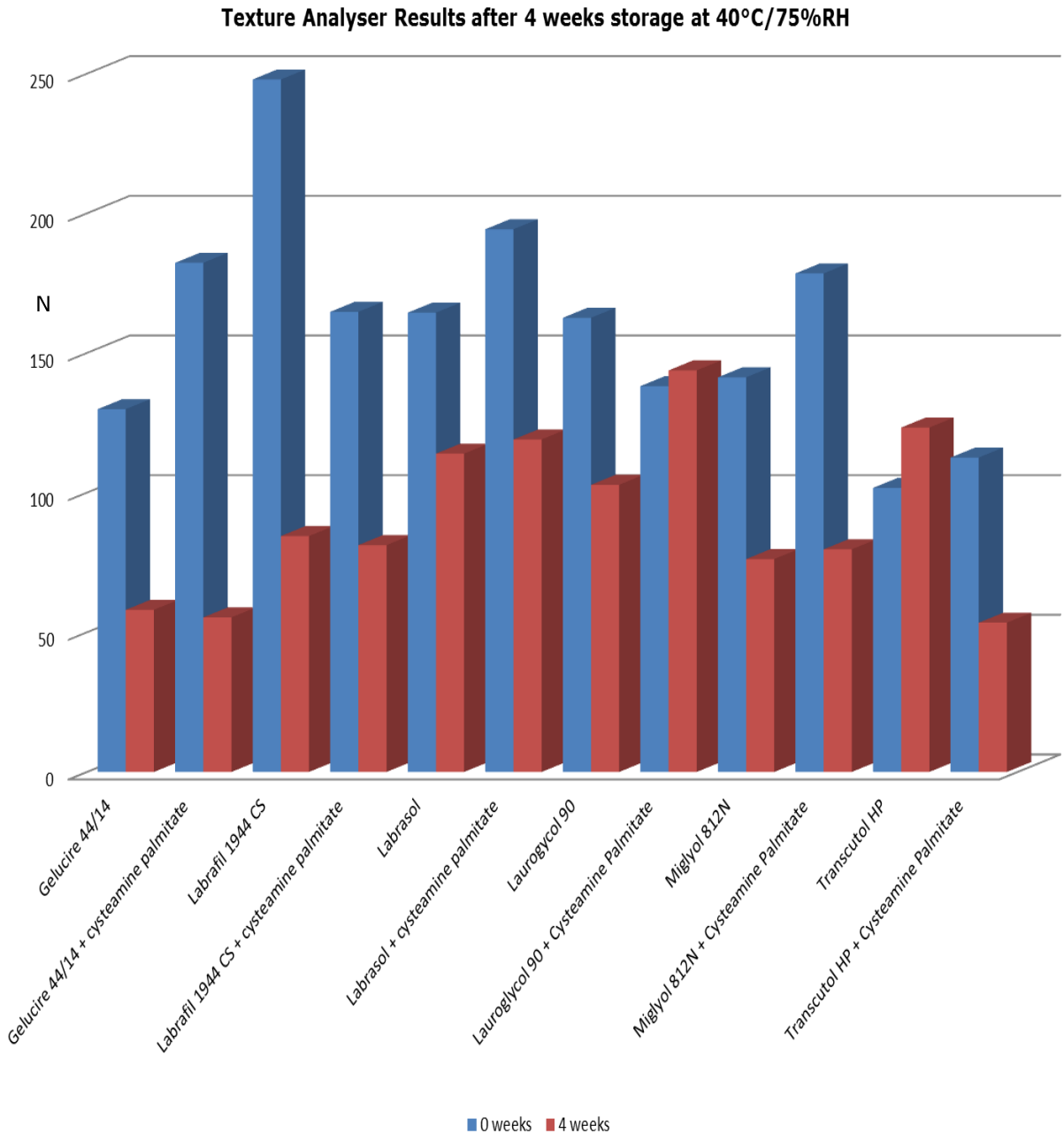


Figure 34: Determination of compressive strength (peak force (N)) at T_0 (initial) and after 4 weeks' storage at 40°C/75%RH, using the TA compression test for a size 0 gelatin capsule containing excipients and excipient and cysteamine palmitate combinations.

The texture analyser results are displayed in a bar chart in Figure 34. The graph illustrates clearly the difference in initial T0 results (blue) of capsules just manufactured compared to 4 weeks (red) at accelerated conditions.

Table 10: Determination of compressive strength (peak force (N)) at T_0 (initial) and after 4 weeks storage at 40°C/75%RH, using the TA compression test for a size 0 gelatin capsule containing excipients and excipient and cysteamine palmitate combinations

Formulation	Peak Force (N) Initial	SD	4 weeks	SD
Labrafil 1944 CS	247.91	27.02	84.43	31.94
Labrafil 1944 CS + cysteamine palmitate	164.71	30.91	81.21	37.77
Labrasol	164.42	36.62	57.93	29.25
Labrasol + cysteamine palmitate[1]	194.29	56.34	119.02	48.16
Lauroglycol 90[1]	162.59	26.57	102.79	88.85
Lauroglycol 90 + Cysteamine Palmitate[1]	138.11	63.23	143.74	63.44
Miglyol 812N	141.26	34.99	76.21	36.80
Miglyol 812N + Cysteamine Palmitate	178.47	33.39	79.69	34.01
Transcutol HP	101.66	38.21	123.29	102.44
Transcutol HP + Cysteamine Palmitate	112.53	47.67	53.42	34.15

[1]The capsules moved from their centralised position on the texture analyser platform, during run and caused significant variance in the compressive force (N) required to break the test capsule.

The compressive strength peak force (N) recorded for selected excipients and selected excipients mixed with CP is summarised in Table 10. Labrafil 1944CS, Labrasol, Lauroglycol 90, Miglyol 812N all show a reduction in overall peak force (N) after 4 weeks storage at accelerated conditions (40°C/75%RH). The same was true for formulations composed of excipients and CP; however Lauroglycol 90 containing CP and Transcutol HP showed an increase in the compressive force after storage. Transcutol HP capsules showed extreme variation in the compressive force (N) required to break the test capsule and produced an SD of 123.3 and therefore results were not considered meaningful. Again the impact of capsule movement or a leak from the capsule cannot be ruled out at this stage without further studies being conducted. An immobilisation structure has now been employed to immobilise each capsule during testing. This system was developed in response to the difficulties observed during testing.

4.0 Discussion

4.1 Differential Scanning Calorimetry (DSC)

The DSC results generated provide information on the prodrug stability and crystal morphology. The results clearly identified significant thermal transitions in CS (figure 18) and CD, (figure 19) [84]. The main events identified were, phase changes, melting and crystallisation. The ramp rate was selected at 10°C/min and would allow for greater control and reflect the ramp rate of the corresponding TGA.

The main thermal events were identified during a three step temperature cycle. The prodrugs (CS and CD) were heated from room temperature (approx. 20°C) up to 200°C and 2 distinct endothermic peaks indicated that two crystal structures were present in both prodrugs. Sharp crystallisation was observed when cooling back to room temperature. Both prodrugs were reheated up to 200°C and a new endothermic event, with a significantly lower onset at 96.5°C (CS) and 79.6°C (CD) was detected, however the main melting event reappeared at approximately the same temperature. For CS and CD the two distinct endothermic peaks were separated by approximately 31.5°C and 42°C, respectively. The lower thermal event is thought to be as a result of microphase separation between the long-chain alkyl (soft segments) and cysteamine moieties (hard segments) [85]. As there was no change in the main melt it can be concluded that there is no mass loss, hence no water elimination or chemical degradation was observed [94]. This was supported by the TGA plots (figures 22 and 23) as there was no weight loss observed within the region of interest (RT-200°C).

Polymorphic behaviour in the crystalline domains and their interaction may also be at play but such detailed studies were out of the scope of this project. More important was the fact that this unique thermal behaviour was identical in all stability samples tested (Figure 18, 19, 22 and 23) suggesting that there had been absolutely no change in the conformation of these insoluble prodrugs during the stability study.

4.2 Hot Stage Microscopy (HSM)

Hot-stage microscopy was used to confirm the phase transitions of CS (Figure 20 and 21), as a function of temperature. Polarized light was used to distinguish crystalline from non-crystalline materials [28], and to enhance the differences between different crystalline domains (Figure 21) [94, 95].

The hot stage microscopy phase transitions (Figure 20 and 21) corresponded to the DSC results (Figure 18 and 19). The first heat cycle shows the appearance of cysteamine steramide at room temperature and the onset of melt at approximately 130°C and the prodrug completely melted (liquid phase) at 131.4°C. The cool cycle shows large crystals beginning to form at approximately 128°C and the prodrug was completely crystallised (solid phase) at room temperature. The second heat cycle (reheat) shows the onset of melt at approximately 130°C and the prodrug completely melted (liquid phase) at 131.8°C.

During the heating stage in the 2nd heat cycle, internal movement was observed in the crystals from around 85°C, which indicated a partial melt, possibly the alkyl groups, but the majority of the melt occurred at 131°C [85, 87]. This

supports the theory of a phase separated or mesophasic structure. Only cysteamine steramide was evaluated using HSM as there was limited prodrug available.

4.3 Thermogravimetric Analysis (TGA)

TGA analysis assesses the prodrugs (CS and CD) thermal stability. The sample had been stored for approximately 9 months at accelerated conditions (40°C/75%RH). The plots confirmed that there were no residual solvents from the synthetic process and were not linked to the lower thermal events identified in the DSC thermogram. The DSC results (Figure 22 and 23) showed that there was no removal of solvent, given the absence of a broad endothermic transition. As the weight loss was not observed in the TGA plot for either CS (Figure 22) or CD (Figure 23), solvates were ruled out, supporting the given explanation of mesophasic separation of distinct microcrystalline domains [95]

The sample in figure 22 and 23 showed slight mass loss at 100 °C associated to retained water when analysed by TGA which means that samples were correctly prepared and adequately dried. Therefore it can be concluded that the endotherms observed in the DSC thermograms were associated with the main function groups, including disulphide bonds (S-S), thiols (S-H), amine (N-H) and carbonyl groups (C=O) identified in FT-IR (ATR) spectra.

4.4 Fourier Transform Infrared (FT-IR)

FT-IR (ATR) spectroscopy was used for the detection of functional groups of the newly synthesized prodrugs (CS and CD). FT-IR spectra indicated that all disulphide bonds (S-S), thiols (S-H), amine (N-H) and carbonyl groups (C=O) were present at almost the same absorption wavenumbers after 9 months storage (Figure 24, 25 and Appendix 1 and 2) at accelerated conditions (40°C/75%RH) [6]. CS and CD showed no visible increase, reduction or disappearance of any peaks. Therefore this data indicates that CS and CD have not degraded on stability testing.

4.5 Qualitative study of prodrug solubility in a wide range of known solubilisers

A wide range of potential solubilisers were screened using two prodrugs (CS and CD). The prodrug solubility results indicate that only Gelucire 44/14 (API-60mg/ml) and propylene glycol (API-40mg/ml) appear to form a solid solution [36, 37] with CS and CD prodrugs. The levels of solubilisation were not sufficient in order to match the current commercially available cysteamine bitartrate (Cystagon™) dose of 50mg and 150mg [15]. This indicates that prototype suspensions (solid dispersions [39]) containing self-emulsifying excipients [23, 24] may be more suited to prodrug development. Miglyol 812 demonstrated visually poor solubility for both prodrugs of less than 10mg/ml and therefore would be an ideal excipient to use to form a solid dispersion.

The insoluble prodrugs could form amorphous/crystalline combinations of solid dispersions on their own or in combination with surfactants like Labrafil M1944CS to enhance API release, long term stability and improve bioavailability [39, 41]. This technique could also potentially reduce the particle size of the

prodrugs. As a carrier such as Gelucire 44/14 containing insoluble prodrug dissolves, the insoluble prodrug would be delivered as very fine particles to dissolution medium resulting in an increase in both surface area and solubilisation, resulting in faster absorption and dissolution.

4.6 Release studies on tramadol HCl/excipient combinations

The solubility screening section 3.2 showed Gelucire 44/14 effectiveness for solubilising cysteamine prodrugs. Gelucire 44/14 was selected as a capsule diluent for its non-ionic water dispersible surfactant [59] properties and was mixed with various ratios of Gelucire 50/02 to demonstrate how effective Gelucires were at modifying dissolution rates.

Gelucire 44/14 was used at 100% and tramadol hydrochloride achieved approximately 100% release in less than 1 hour. A ratio of 50:50 of Gelucire 44/14 and Gelucire 50/02 achieved full dissolution release after approximately 12 hours. Alternatively, using 100% Gelucire 50/02 the plot shows a steady release of tramadol hydrochloride in the dissolution medium with just over 70% released after 14 hours.

A large range of different dissolution profiles, from fast to very slow, can be achieved by simply varying the Gelucire 44/14 to Gelucire 50/02 ratios without the need for additional excipients due to its non-ionic water dispersable surfactant properties and its ability to self-emulsify on contact with aqueous media forming a microemulsion [23, 24]. Gelucire 44/14 has a HLB of 14 which means that it is hydrophilic (water soluble) and Gelucire 50/02 (oil soluble). Therefore Gelucires amphiphilic nature is attributed to alcohol moieties and a

long hydrocarbon chain that make these bases suitable as a lipid carrier for both hydrophilic and lipophilic API's [96]. Tramadol HCl is a hydrophilic API and these types of API tend to release by diffusion compared to lipophilic derivatives of cysteamine or prodrugs which would tend to disperse within an aqueous environment. Other excipients screened in the solubility section, e.g. Lauroglycol 90, could also be combined with Gelucires to refine release profiles further and inhibit enterocytic enzymes to increase bioavailability [61]. Labrasol [60] or Labrafil M1944CS [58] would reduce the interfacial tension between API and formulation, thus enhancing wettability and therefore accelerates dispersal during dissolution [39, 41].

4.7 Capsule shell compatibility study

The capsule shell compatibility results were a manual physical test and all gelatin capsules, HPMC and V-caps plus capsules were deemed compatible after 9 months storage at 40ºC/75%RH. The program could not support additional time points due to the limited amount of capsules available as a result of the small quantities of prodrugs supplied.

At the time of analysis there was no texture analyser available onsite at Encap and therefore a manual pressure test and visual test was performed only. Once the texture analyser became available at Encap, additional prodrug was requested. The prodrug provided was cysteamine palmitate (CP). Therefore all texture Analysis data has been generated on CP only and not CS or CD, due to a lack of availability from RGU.

4.8 Texture Analyser

The texture analysis method is extremely sensitive in terms of monitoring small changes of the mechanical properties of capsules [91, 92] over a short period of time (4 weeks).

Capsules were filled with one excipient or one excipient and CP to test mechanical strength and all gelatin capsules with the exception of those containing Lauroglycol 90 and CP and Transcutol HP, showed an increase in the force required to break the capsule after four weeks storage (Table 10) at 40ºC/75%RH. However due to the large SD observed for Transcutol HP and CP, the results were not considered as meaningful. An example of one of the formulation which reduced in mechanical force was Labrafil 1944 CS and CP. The average maximum force peak for Labrafil 1944 CS at 0 weeks was 164.7 N, with a standard deviation of 30.9 N. After 4 weeks storage the average maximum peak was 81.2N with a standard deviation of 37.8. This reduction in capsule strength of approximately 50% requires to be confirmed using a larger sample group, and this conclusion is supported by the large variation between data sets for each formulation tested (Table 10). Some of the variation can be explained by capsule movement or a band breaching allowing for the internal formulation to leak slightly and allow for full compression (e.g. Labrasol).

An increase in the number of capsules to be tested would also provide more reliable results and compensate for the variability observed by only testing 5 capsules per time point. Even though the data (Table 10) shows that the force required to rupture the capsule has decreased it may still be within the limits of manual handling and bottle packaging. Blister packing might need to be

extensively evaluated on stability to determine if the capsule ejection force (N) required exceeds the capsules tolerance.

Another potential factor could be water exchanges between fill mass and the capsule shell at different rates (1-2 weeks), unlike other excipients with lower diffusion coefficients (e.g. low molecular weight Labrasol), and a much longer period of time might be required in order to reach equilibrium. The migration of water can also be impacted by the presence of other hydrophilic excipients [97].

5.0 Future work

5.1 DSC

Further DSC work could be undertaken on prototype formulations to determine product stability, cure/cure kinetics and oxidative stability [84, 85]. It was also noted that the impact of testing prodrugs to below 0°C was not evaluated. With enough of each prodrug (CS and CD), samples could be re-tested and cooled to sub-zero temperatures (e.g. -20°C) to determine the impact of freezing both prodrugs, specifically their morphology. This is useful to do as part of any DSC evaluation to detect small traces of water which may be encased within the crystal structure [93].

5.2 HSM

Additional work could be carried out to assess cysteamine decanamide and cysteamine palmitate if sufficient quantities were available, however the primary information in regards to significant thermal events are available from DSC.

5.3 TGA

TGA could be used to screen prototype formulations to determine their thermal stability [87]. This information could be useful in selecting the most stable formulations for progression.

5.4 FT-IR

FT-IR could be used to determine the physical and chemical reaction between prodrug and excipient. Prototype formulations based on the excipient screening section could potentially use FT-IR to investigate the intermolecular interaction between the prodrug and excipient to determine product compatibility and therefore stability (e.g. solid dispersions).

5.5 Capsule shell compatibility

The shell compatibility study may be repeated with a higher dose as the dose selected was due to the limited amount of prodrug available. Therefore it would be useful to understand the effect of other storage conditions (e.g. 25ºC/75%RH and 2-8ºC).

5.6 Further Characterisation of Prodrugs

X-ray crystallography could be used as a "fingerprint" for further prodrug characterisation and identification. Once the material has been identified, X-ray crystallography could be utilised to determine how the atoms pack together in the crystalline state and what the interatomic distance and angle for the cysteamine prodrugs and X-ray powder diffraction could be used as additional confirmation of the two polymorphs.

If sufficient quantities of cysteamine palmitate were available then DSC, TGA, HSM, FTIR could be performed to compare all prodrugs supplied to date.

If sufficient quantities of all prodrug were available then DSC, TGA, FTIR thermoanalytical techniques could be used to determine product stability of prototype formulations.

5.7 Continuation of liquid fill technology evaluation

Additional texture analyser studies could be performed on the two original prodrugs of cysteamine steramide and cysteamine decanamide to compare the compressive strength of capsules.

Further prototype formulations could be evaluated using different levels of bioavailability enhancers such as low molecular weight polyethylene glycol (PEG) or propylene glycol by moving to soft gel capsules or muco adhesives dissolvable discs.

6.0 Conclusion

Characterisation of cysteamine prodrugs, particularly cysteamine decanamide and steramide, suggested that both insoluble derivatives of cysteamine had a mesophasic crystalline morphology. The possibility of solvate formation was discounted based on the results of thermogravimetric analysis.

One proposed theory to explain the unknown thermal event and appearance of a previously undetected peak at approximately 80°C may be explained by microphase separation of the alkyl groups (soft segment) from the main disulphide unit (hard segment). Once the separation has occurred during the heat and/or cool cycle the alkyl groups are able to realign and cause a separate

melt on reheating prior to the main melt at 131°C for steramide and 125°C for decanamide. This is known as 'mesophasic' behaviour i.e. the existence of more than one crystalline phase within a pure sample. No variation in the thermal properties was detected at all-time points in the stability study.

Solubilisation of these prodrugs for improved treatment of cystinosis was difficult to achieve at clinically relevant prodrug contents. Gelucire 44/14 and propylene glycol, however, did appear to dissolve 60 and 40 mg/ml (CS and CD) respectively. Formulations were prepared using tramadol hydrochloride and mixes of Gelucire 44/14 and 50/02 to demonstrate the effectiveness of this excipient in modifying release. The results generated indicated that immediate release (< 1 hour) to delayed release (70% release within 14 hours).

From this point on, these studies focussed on the effect of suitable excipients for cysteamine palmitate prototype suspensions, and revealed that all of the chosen excipients weakened the integrity of gelatin capsules on storage but were still considered physically viable under conditions of accelerated stability, with the exceptions of Lauroglycol 90 and Transcutol HP (Table 10) which had little effect on capsule integrity.

It is concluded that liquid fill encapsulation may have the potential to produce a dosage unit with enhanced capability for the delivery of cysteamine prodrugs but additional studies are required to confirm this theory.

References

1. McCaughan, B., Kay, G., Knott, R.M., Cairns, D. (2008) A potential new prodrug for the treatment of cystinosis: Design, synthesis and in-vitro evaluation. Bioorganic & Medicinal Chemistry Letters, 18, 1716-1719.

2. Lim, J.C., Lam, L., Li, B., Donaldson, P. J. (2013) Molecular identification and cellular localization of a potential transport system involved in cystine/cysteine uptake in human lenses. Experimental Eye Research, 116, 219–226.

3. Patient, D., Ainsworth, P. (1994) The Chemistry of Flour and Bread. Nutrition & Food Science, 94, 22-24.

4. Besouw, M., Tangerman, A., Cornelissen, E., Rioux, P., Levtchenko, E. (2012) Halitosis in cystinosis patients after administration of immediate-release cysteamine bitartrate compared to delayed-release cysteamine bitartrate. Molecular Genetics and Metabolism, 107, 234–236.

5. Dohil, R., Fidler, M., Barshop, B.A., Gangoiti, J., Deutsch, R., Martin, M. Schneider, J.A. (2006) Understanding intestinal cysteamine bitartrate absorption. The Journal of Pediatrics, 148, 764-769.

6. Besouw, M., Blom, H., Tangerman, A., Graaf-Hess, A., Levtchenko, E. (2007) The origin of halitosis in cystinotic patients due to cysteamine treatment. Molecular Genetics and Metabolism, 91, 228–233.

7. Jaradat, S., Al-Rababah, B., Hazza, I., Akl, K., Saca, E., Al-Younis, D. (2015) Molecular analysis of the CTNS gene in Jordanian families with nephropathic cystinosis. Nefrología, 35, 547–553.

8. Ullman, S., Nelson, L.B., Jackson, L.G. (1985) Chorionic villus sampling Survey of Ophthalmology. Prenatal diagnostic techniques, 30, 33-40.

9. Wilmer, M.J., Kluijtmans, L.A.J., Van der Velden, T.J., Willems, P.H., Scheffer, P.G., Masereeuw, R., Monnens, L.A., Van den Heuvel, L.P., Levtchenko, E.N. (2011) Cysteamine restores glutathione redox status in cultured cystinotic proximal tubular epithelial cells. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1812, 6, 643–651.

10. Thoene, J.G. (2007) A review of the role of enhanced apoptosis in the pathophysiology of cystinosis. Molecular Genetics and Metabolism, 92, 292-298.

11. Swaminathan, K., Jeyaraman, M. (2014) Cystinosis: An "eye opener" Apollo Medicine, 11, 1, 43–45.

12. Forestier, L., Jean, G., Attard, M., Cherqui, S., Lewis, C., van't Hoff, W., Broyer, M., Town, M., Antignac, C. (1999) Molecular Characterization of *CTNS* Deletions in Nephropathic Cystinosis: Development of a PCR-Based Detection Assay, 65, 353-359.

13. Tsilou, E., Zhou, M., Gahl, W., Sieving, P.C., Chan, C. (2007) Ophthalmic Manifestations and Histopathology of Infantile Nephropathic Cystinosis: Report of a Case and Review of the Literature. Survey of Ophthalmology, 52, 1, 97–105.

14. Bozdağ, S., Gümüş, K., Gümüş, O., Ünlü, N. (2008) Formulation and in vitro evaluation of cysteamine hydrochloride viscous solutions for the treatment of corneal cystinosis. European Journal of Pharmaceutics and Biopharmaceutics, 70, 260-269.

15. Dohil, R., Gangoiti, J.A., Cabrera, B.L., Fidler, M., Schneider, J.A., Barshop, B.A. (2010) Long-Term Treatment of Cystinosis in Children with Twice-Daily Cysteamine. The Journal of Pediatrics, 156, 823–827.

16. Omran, Z., Kay, G., Di Salvo, A., Knott, R.M., Cairns, D. (2011) PEGylated derivatives of cystamine as enhanced treatments for nephropathic cystinosis. Bioorganic & Medicinal Chemistry Letters, 21, 45–47.

17. Aultons, M.E., (2007) The design and manufacture of medicines. In: Aultons [Ed.] *Aulton's Pharmaceutics*, 337.

18. Atkins, P. (2003) The Elements of Physical Chemistry. In: *Atkins* [3rd Edition], page 406.

19. Mahmood, Q., Khan, A.F., Khan, A. (2014) Chapter 25 – Colloids in the Environmental Protection—Current and Future Trends. The Role of Colloidal Systems in Environmental Protection, 25, 635–677.

20. Talegaonkar, S., Azeem, A., Ahmad, F.J., Khar, R.K., Pathan, S.A. and Khan, Z.I. (2008) Microemulsions: a novel approach to enhanced drug delivery, 2, 238-257.

21. Eastoe, J., Yan, C., Mohamed, A. (2012) Microemulsions with CO_2 as a solvent, Current Opinion in Colloid & Interface Science, 17, 266–273.

22. Spernath, A. and Aserin, A. (2006) Microemulsions as carriers for drugs and nutraceuticals. Advances in Colloid and Interface Science, 128-130, 47-64.

23. Levi-Schaffer, F., Bernstein, A., Meshorer, A. and Arnon, R. (1982) Reduced toxicity of daunorubicin by conjugation to dextran. Cancer Treatment Reports, 66, 107-114.

24. Hornig, S., Bunjes, H. and Heinze, T. (2009) Preparation and characterization of nanoparticles based on dextran–drug conjugates. Centre of Excellence for Polysaccharide Research, 338, 56-62.

25. Bootz, A., Vogel, V., Schubert, D. and Kreuter, J. (2004) Comparison of scanning electron microscopy, dynamic light scattering and analytical ultracentrifugation for the sizing of poly(butyl cyanoacrylate) nanoparticles. European Journal of Pharmaceutics and Biopharmaceutics, 57, 369–375.

26. Xu, Z., Jin, J., Zheng, M., Zheng, Y., Xu, X., Liu, Y., Wang, X. (2016) Co-surfactant free microemulsions: preparation, characterization and stability evaluation for food application, Food Chemistry. Press release – Available online.

27. Hiubers, P.D.T. and Shah, D.O., (1997) Evidence for synergism of non-ionic surfactant mixtures: enhancement of solubilisation in water-in-oil microemulsions, Langmuir 13, 5762–5765.

28. Asakawa, H., Nishida, K., Yamamoto, J., Inoue, R., Kanaya, T. (2012) Heating rate effects on the crystallization behavior of isotactic polypropylene from mesophase – A de-polarized light transmission study. Polymer, 53, 2777–2782.

29. Aultons, M.E., (2007) The design and manufacture of medicines. In: Aultons [Ed.] *Aulton's Pharmaceutics*, 588.

30. Schlatt-Masut, B. Quartz-resonator-biosensor for medical application. Available from http://www.unimagdeburg.de/imos/mea_sen/docs_en/ profile. html.

31. Watwe, R., Bellare, J. (1995) Manufacture of liposomes: a review, Current Science, 68, 715–724.

32. Kozubek, A., Gubernator, J. Przeworska, E, Stasiuk, M. Liposomal drug delivery, a novel approach: PLARosomes. Department of Lipids and Liposomes, Institute of Biochemistry and Molecular Biology, University of Wrocaw, [cited: 2000 August 10].
Available from http://www.actabp.pl/pdf/3_2000/639-649s.pdf

33. Risch, S., Reineccius, G. (1995) Liposomes for controlled release in the food industry: Encapsulation and controlled release of food ingredients, American Chemical Society, 113–131.

34. Singh, H., Thompson, A., Liu, W., and Corredig, M. (2012) Liposomes as food ingredients and nutraceutical delivery systems. A volume in Woodhead Publishing Series in Food Science, Technology and Nutrition, Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals, 11, 287-318.

35. Riedera, A.A., Kollera, D., Lohnera, K., Pabsta, G. (2015) Optimizing rapid solvent exchange preparation of multilamellar vesicles. Chemistry and Physics of Lipids, 186, 39–44.

36. Pritula1, I., Sangwal, K. (2015) Fundamentals of Crystal Growth from Solutions, A volume in Handbook of Crystal Growth. Handbook of Crystal Growth (Second Edition), 29, 1185-1227.

37. Goldberg, A.H., Gibaldi, M., Kanig, J.L (1965) Increasing Dissolution Rates and Gastrointestinal Absorption of Drugs Via Solid Solutions and Eutectic

Mixtures I: Theoretical Considerations and Discussion of the Literature, Journal of Pharmaceutical Sciences, 54, 1145–1148.

38. Vo, C.L., Park, C. and Lee, C.B. (2013) Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. European Journal of Pharmaceutics and Biopharmaceutics, 85, 799–813.

39. Huanga, Y., Daib, W. (2014) Fundamental aspects of solid dispersion technology for poorly soluble drugs. Drug Delivery System and Pharmaceutical Technology. Acta Pharmaceutica Sinica B, 4, 18-25.

40. Baghel, S., Cathcart, H., O'Reilly, N.J. (2016) Polymeric Amorphous Solid Dispersions: A Review of Amorphization, Crystallization, Stabilization, Solid-State Characterization, and Aqueous Solubilization of Biopharmaceutical Classification System Class II Drugs. Journal of Pharmaceutical Sciences. Available online at http://www.sciencedirect.com/science/article/pii /S002235491500009X.

41. Vasconcelos, T., Sarmento, B., Costa, P. (2007) Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discovery, 12, 1068–1075.

42. Kaur, J., Aggarwal, G., Singh, G., Rana, A.C. (2012) Improvement Of Drug Solubility Using Solid Dispersion. International Journal of Pharmacy and Pharmaceutical Sciences, 4, 2012.

43. Upadhyaya, P., Panditc, J.K., Wahic, A.K. (2014) Studies on biological macromolecules lipid-Gelucire based gastroretentive multiparticulate, International Journal of Biological Macromolecules, 67, 463–477.

44. Karataş, A. Yüksel, N. and Baykara, T. (2005) Improved solubility and dissolution rate of piroxicam using Gelucire 44/14 and Labrasol. Il Farmaco, 60, 777–782.

45. Jannin, V., Musakhanian, J., Marchaud, D. (2008) Advances in Drug Delivery: Review, 60 (2008), 734–746.

46. Cole, E.T., Cadé, D., Benameur, H. (2008) Challenges and Opportunities in The Encapsulation of Liquid and Semi-Solid Formulations into Capsules for Oral Administration Advanced Drug Delivery Reviews, 60, 747-756.

47. Cadé, D., Cole, E.T., Mayer, J.P, Wittwer, F. (1987) Liquid filled and sealed hard gelatin capsules, Acta Pharm. Technology. 33 (1987) 97–100.

48. Bowtle, W.J. Burrows, S.L., Holmes, E.D., Jones, B.E. (1989) S.S.M. formulations and capsules: an improved way to handle toxic compounds, AAPS annual meeting and exposition, Pharm. Res. 7. 612.

49. Silva Iahnke, A.O., Costa, T.M.H., Oliveira Rios, A. Hickmann, S. (2015) Residues of minimally processed carrot and gelatin capsules: Potential materials for packaging films. Industrial Crops and Products, 76, 1071–1078.

50. Bowtle, W.J. (1999) Recent developments in oral multiphase and targeted release products. Pharmaceutical Science & Technology Today, 2, 32–36.

51. Al-Gousous, J., Penning, M., Langguth, P. (2015) Molecular insights into shellac film coats from different aqueous shellac salt solutions and effect on disintegration of enteric-coated soft gelatin capsules. International Journal of Pharmaceutics, 484, 283–291.

52. Kelemen, A., Szalay, A., Sovány, T., Pintye-Hódi, K. (2015) Role of the particle size of sorbitol during the compression of common tablets and prediction of mini-tablet compression parameters. Chemical Engineering Research and Design, 104, 814–818.

53. Gao, Z., Crowley, W.R., Shukla, A.J., Johnson, J.R., Reger, J.F. (1995) Controlled release of contraceptive steroids from biodegradable and injectable gel formulations: in vivo evaluation. Pharm. Res., 12, 864–868.

54. Zhang, H., Wang, Z. Oscar Liu, O. (2016) Simultaneous determination of kolliphor HS15 and miglyol 812 in microemulsion formulation by ultra-high performance liquid chromatography coupled with nano quantity analyte detector. Journal of Pharmaceutical Analysis, 6, 11-17.

55. Ishikawa, T., Watanabe, Y., Takayama, K., Endo, H., Matsumoto, M. (2000) Effect of hydroxypropylmethylcellulose (HPMC) on the release profiles and bioavailability of a poorly water-soluble drug from tablets prepared using macrogol and HPMC. International Journal of Pharmaceutics, 202, 173–178.

56. Hiemenz, P.C. and Rajagopalan, R. (1997) Electrostatic and Polymer-Induced Colloid Stability. Principles of Colloid and surface chemistry, 13, 575-614.

57. Qi, X., Wang, L., Zhu, J., Hu, Z. and Zhang, J. (2011) Self-double-emulsifying drug delivery system (SDEDDS), A new way for oral delivery of drugs with high solubility and low permeability, International Journal of Pharmaceutics, 409, 245–251.

58. Available from http://www.gattefosse.com/en/applications/labrafil-m1944cs.html?administration-route,oral or technical data sheet.

59. Available from http://www.gattefosse.com/en/applications/Gelucire-4414.html?administration-route,oral or Technical data sheet.

60. Available from http://www.gattefosse.com/en/applications/labrasol. html? administration- route,oral or technical data sheet.

61. Available from http://www.gattefosse.com/en/applications/lauroglycol-90.html?administration-route,oral or technical data sheet.

62. Available from http://www.gattefosse.com/en/applications/transcutol-hp.html?administration-route,oral or technical data sheet.

63. Ogony, J., Suneetha Mare, S., Wu, W., Ercal, N. (2006) High performance liquid chromatography analysis of 2-mercaptoethylamine (cysteamine) in biological samples by derivatization with N-(1-pyrenyl) maleimide (NPM) using fluorescence detection, Journal of Chromatography B, 843, 57–62.

64. Shen, H., Howles, P., Tso, P. (2001) From interaction of lipidic vehicles with intestinal epithelial cell membranes to the formation and secretion of chylomicrons, Supplement: Oral Drug Absorption: A Renaissance, Advanced Drug Delivery Reviews, 50, 103–125.

65. Bunjes, H. (2011) Structural properties of solid lipid based colloidal drug delivery systems. Current opinion in Colloid and Interface Science, 16, 405-411.

66. Dissolution comparison of a self-emulsifying formulation vs. lipid formulation using an insoluble model compound.
Available from http://www.encapdrugdelivery.com/library.html.

67. Liu, J., Zhang, L., Hu, W., Tian, R., Teng, Y., Wang, C (2012) Preparation of konjac glucomannan-based pulsatile capsule for colonic drug delivery system and its evaluation in vitro and in vivo, Carbohydrate Polymers, Carbohydrate Polymers, 87, 377–382.

68. Rao, A.S., Nayeemuddin, M., Hadi, M.A. (2013) Formulation and evaluation of a novel capsule-in-a-capsule technology for biphasic delivery of lornoxicam in the treatment of migraine. International Journal of Pharmaceutical And Biomedical Research, 4, 170-176.

69. Capsule within a capsule. Image modified from http://www.capsugel.com/ihc/duocap.

70. Multiphase release diagram. Image modified from http://www.worldpharmaceuticals.net/contractors/drug-delivery systems/qualicaps1/.

71. Ku, M.S., Li, W., Dulin, W., Donahue, F., Cade, D., Benameur, H., Hutchison, K. (2010) Performance qualification of a new hypromellose capsule: Part I. Comparative evaluation of physical, mechanical and processability quality attributes of Vcaps Plus®, Quali-V® and gelatin capsules. International Journal of Pharmaceutics, 386, 30–41.

72. Jones, B.E., Basit, A.W., Tuleu, C. (2012) The disintegration behaviour of capsules in fed subjects: A comparison of hypromellose (carrageenan) capsules and standard gelatin capsules. International Journal of Pharmaceutics, 424, 40– 43.

73. Mei, X., Etzler, F.M., Wang, Z. (2006) Use of texture analysis to study hydrophilic solvent effects on the mechanical properties of hard gelatin capsules. International Journal of Pharmaceutics, 324, 128–135.

74. Nazzal, S., Wang, Y. (2001) Characterization of soft gelatin capsules by thermal analysis. International Journal of Pharmaceutics, 230, 35–45.

75. Moreton, R.C., Armstrong, N.A. (1998) The effect of film composition on the diffusion of ethanol through soft gelatin films. International Journal of Pharmaceutics, 161, 123–131.

76. Moreton, R.C., Armstrong, N.A. (1995) Design and use of an apparatus for measuring diffusion through glycerogelatin films. International Journal of Pharmaceutics, 122, 79-89.

77. Technical data available from http://www.pharmaceutical-technology.com/contractors/process_automation/pharmagel/.

78. Technical data available from http://phaseonemachining.com/index.php?page=rotary-gel-dies.

79. Technical data available from http://www.catalent.com/index.php/offerings/A-Z-Offerings/vegicaps?gclid=CNXo1NX_9MoCFbYV0wodpSQEtQ.

80. Seager, H. (1985) Soft gelatin capsules: A solution to many tableting problems. Pharmaceutical Technology, 9, 84–104.

81. Ebert, W.R. (1977) Soft elastic gelatin capsules: A unique dosage form. Pharmaceutical Technology, 1, 44–50.

82. Mallis, G.I., Schmidt, D.H., Lindenbaum, J. (1975) Superior bioavailability of digoxin solution in capsules (article), Clinical Pharmacology and Therapeutics, 18, 761-768.

83. Li, J., Shao, S., Solorzano, M., Allmaier, G.J., Kurtulik, P.T. (2009) Determination of the residual ethanol in hydroalcoholic sealed hard gelatin capsules by static headspace gas chromatography with immiscible binary solvents, Journal of Chromatography A, 1216, 3328–3336.

84. Wilson, P.W., Arthur, J.W., Haymet, A.D.J. (1999) Ice Premelting during Differential Scanning Calorimetry, Biophysical Journal, 77, 2850–2855.

85. Riga, A., Collins, R. and Mlachak, G. (1998) Oxidative behavior of polymers by thermogravimetric analysis, differential thermal analysis and pressure differential scanning calorimetry. Thermochimica Acta, 324, 135-149.

86. Vitez, I.M., Newman, A.W., Davidovich, M. and Kiesnowski, C. (1998) The evolution of hot-stage microscopy to aid solid-state characterizations of pharmaceutical solids. Thermochimica Acta, 324, 187-196.

87. Kaur, G., Karir, G., Mehta, S.K. (2013) Studies on thermogravimetric analysis and solvophobic interactions of micellisation of Pd (II) complex in non-aqueous solvents. Colloids and Surfaces A: Physicochemical and Engineering Aspects Volume 434, 25–34.

88. Cross, W.M., Ma, S., Winter, R.M., and Kellar, J.J. (1999) FT-IR/ATR and SEM study of colloidal particle deposition. Colloids and Surfaces A: Physiochemical and Engineering Aspects, 154, 115-125.

89. Calvo, N.L., Kaufman, T.S., Maggio, R.M (2016) Mebendazole crystal forms in tablet formulations. An ATR-FTIR/chemometrics approach to polymorph assignment. Journal of Pharmaceutical and Biomedical Analysis, 122, 157–165.

90. FT-IR Spectroscopy: Attenuated Total Reflectance (ATR). Technical summary from Perkin Elmer. http://www.utsc.utoronto.ca/~traceslab /ATR_FTIR.pdf.

91. Estellé, P., Lanos, C., Mélinge, Y., Servais, C. (2006) On the optimisation of a texture analyser in squeeze flow geometry. Measurement, 39, 771–777.

92. Mei, X., Etzler, F.M. and Wang, Z. (2006) Use of texture analysis to study hydrophilic solvent effects on the mechanical properties of hard gelatin capsules. International Journal of Pharmaceutics, 324, 128–135.

93. Wilson, P.W., Arthur, J.W., Haymet, A.D.J. (1999) Ice Premelting during Differential Scanning Calorimetry, Biophysical Journal, 77, 2850–2855.

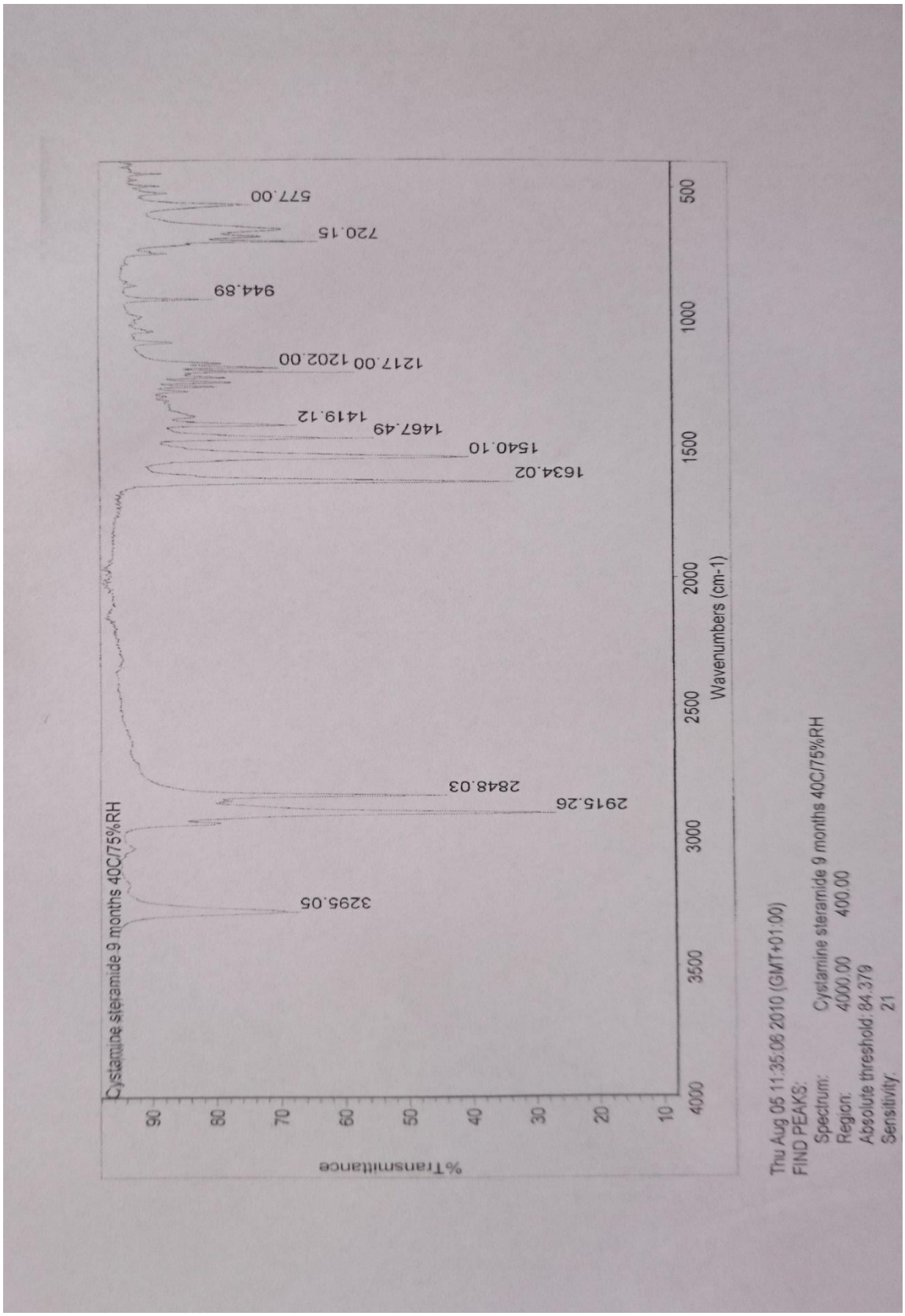
94. Diogo,H. P., Moura Ramos, J.J (2014) Study of the thermal behavior of choline ibuprofenate using, differential scanning calorimetry and hot-stage microscopy, Journal of Molecular Structure, 1078, 174–180.

95. Asrar, J., Toriumi, H., Watanabe, J., Krigbaum, W.R., Ciferri, A. (1983) Thermotropic Homopolyesters. I. The preparation and properties of polymers based On 4, 4 Prime –dihydroxybiphenyl. Journal of polymer science, Part A-2, Polymer physics, 21, 1119-1131.

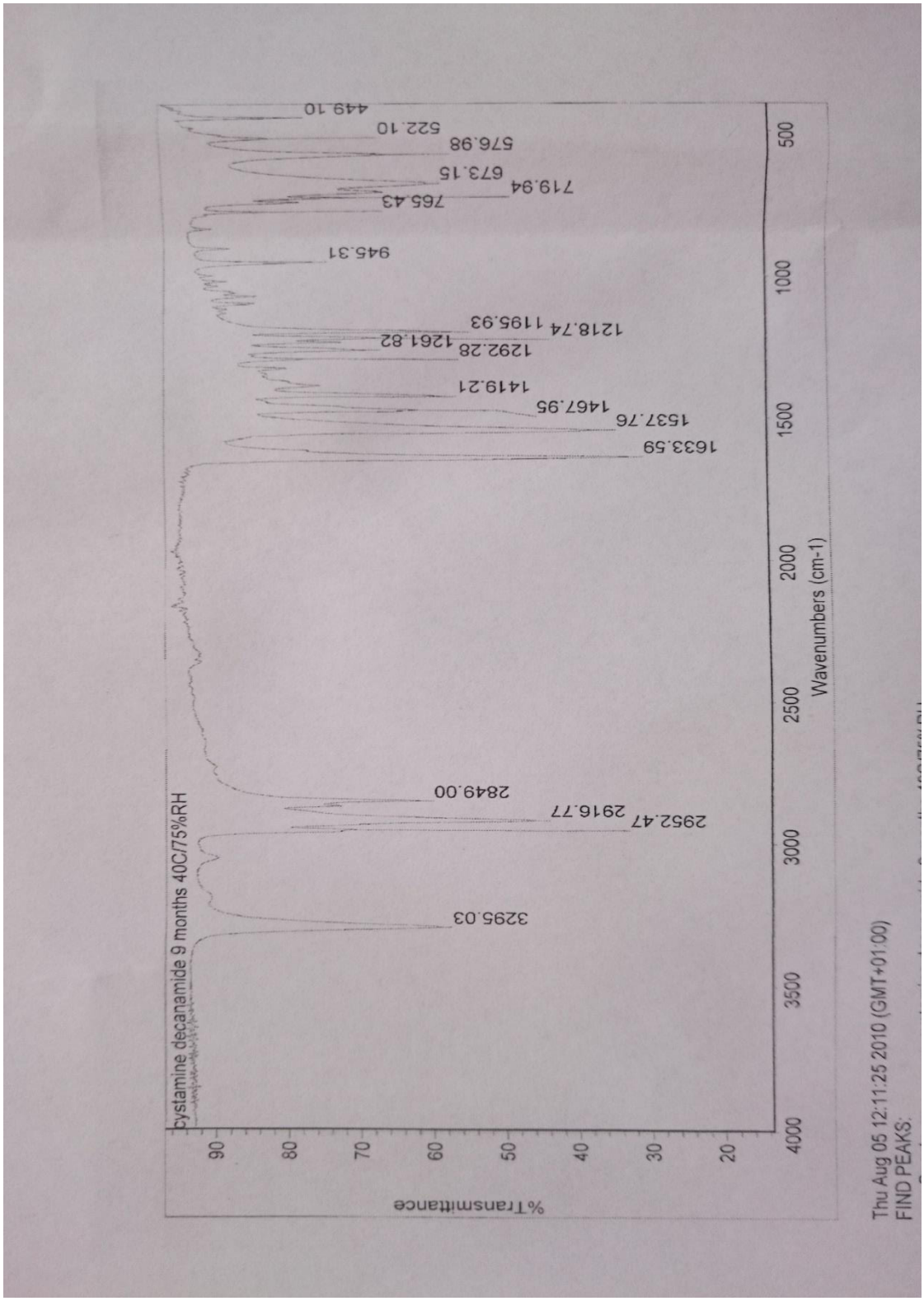
96. Sutananta, W., Craig, D.Q.M, Newton, J.M. (1995) An evaluation of the mechanisms of drug release from glyceride bases. Journal of Pharmacy and Pharmacology, 47, 182–187.

97. Barham, A.S., Tewes, F., Healy, A.M. (2015) Moisture diffusion and permeability characteristics of hydroxypropylmethylcellulose and hard gelatin capsules, International Journal of Pharmaceutics, 478, 796–803.

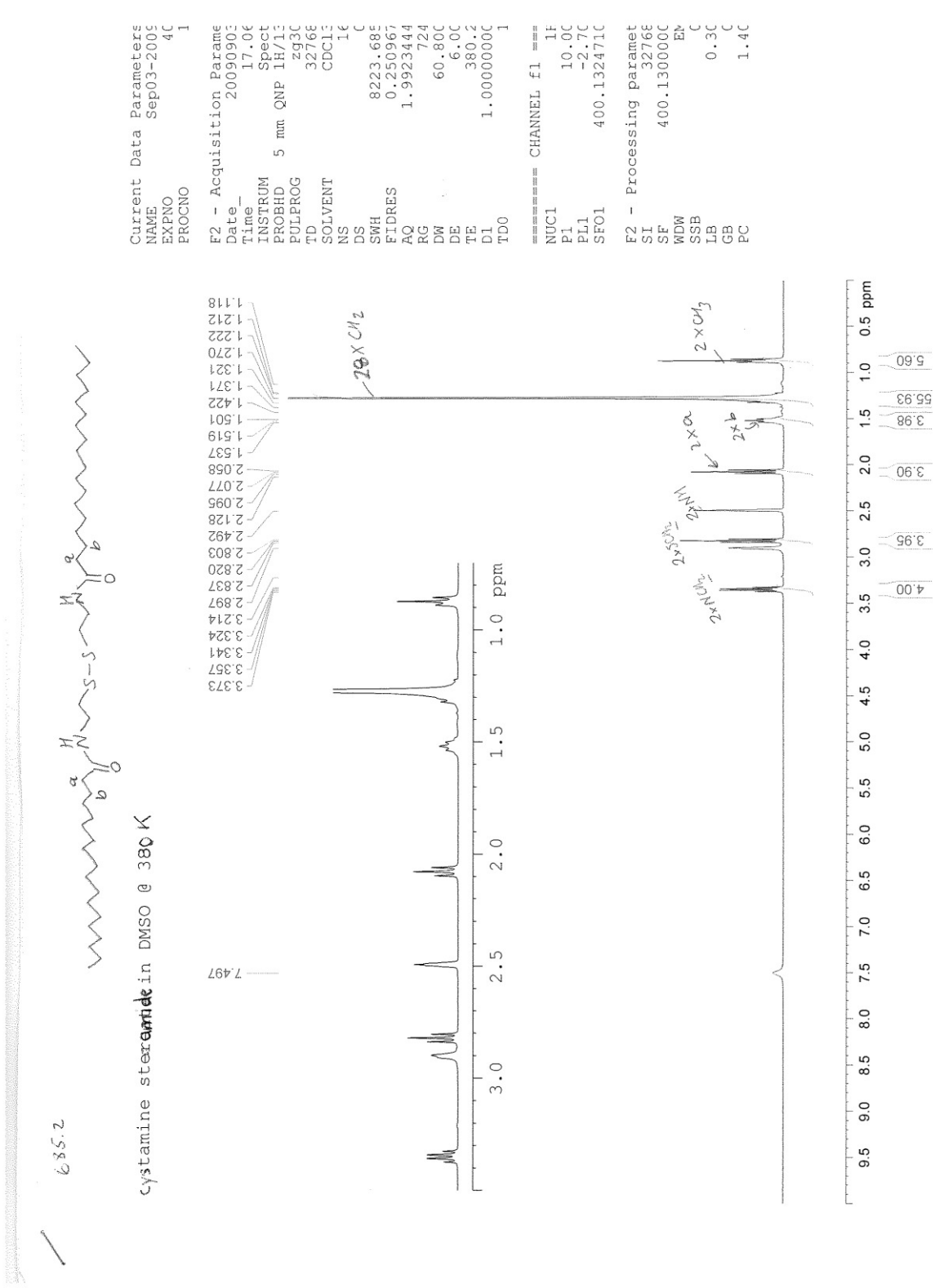
Appendix 1 FT-IR scan of cysteamine steramide and corresponding wavenumbers (cm^{-1}) at 9 months



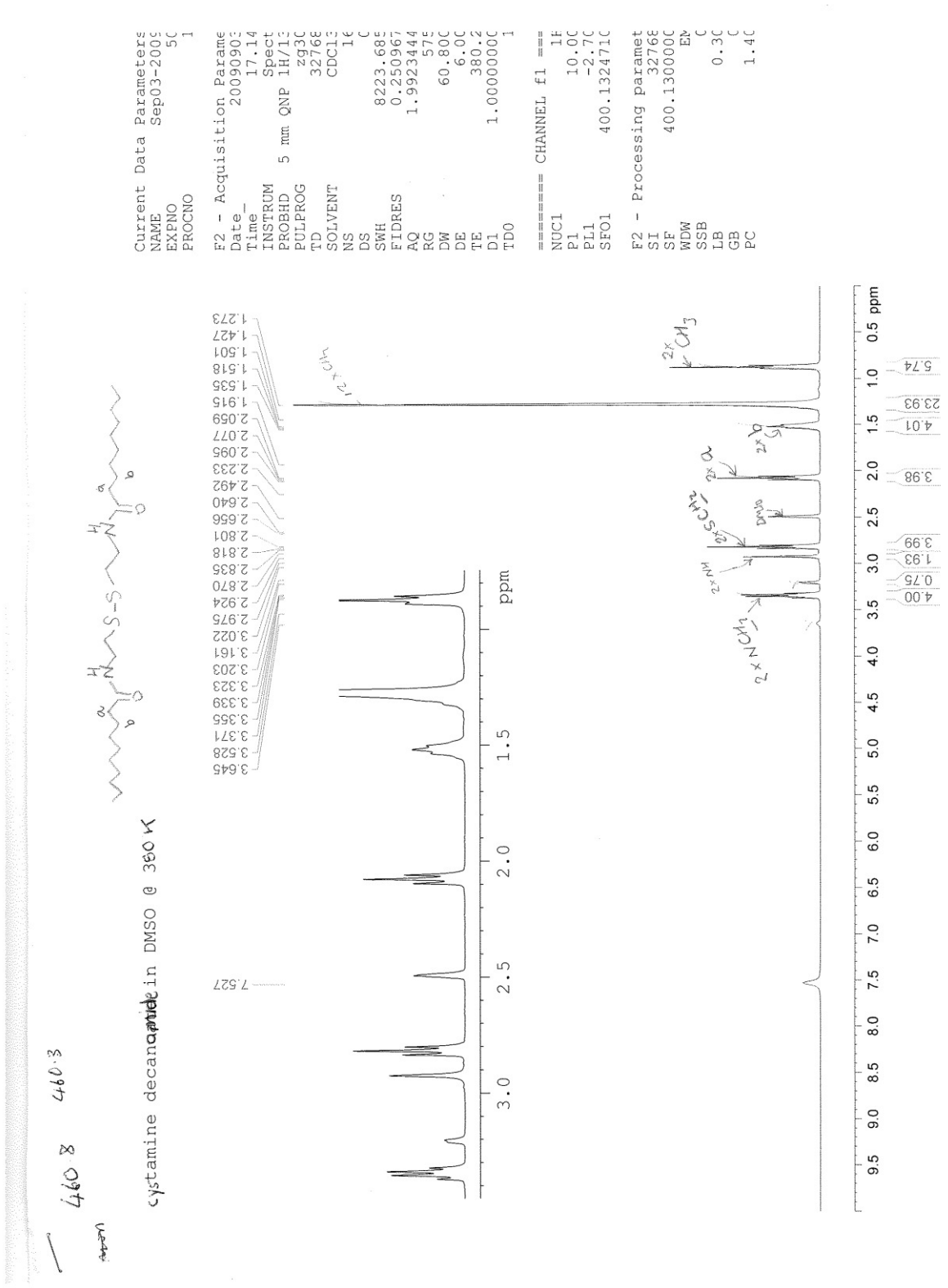
Appendix 2 FT-IR scan of cysteamine decanamide and corresponding wavenumbers (cm^{-1}) at 9 months



Appendix 3: NMR scan of cysteamine steramide in DMSO



Appendix 4: NMR scan of cysteamine Decanamide in DMSO



Appendix 5: Statistical analysis of capsule compressive strength at initial and after 4 weeks storage at 40°C/75%RH

Gelucire 44/14

Descriptive Statistics: Initial, 4 weeks

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial	5	0	129.9	15.5	34.7	93.0	101.7	119.9	163.0	182.9
4 weeks	5	0	58.0	12.0	26.8	33.8	34.2	48.3	86.7	86.7

Gelucire 44/14 + Cysteamine Palmitate

Descriptive Statistics: Initial_1, 4 weeks_1

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_1	5	0	182.3	17.0	37.9	137.4	141.2	204.7	212.0	218.2
4 weeks_1	5	0	55.32	8.11	18.15	28.82	36.69	64.61	69.31	72.55

Labrafil 1944 CS

Descriptive Statistics: Initial_2, 4 weeks_2

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_2	5	0	247.9	12.1	27.0	210.3	222.6	253.5	270.4	282.5
4 weeks_2	5	0	84.4	14.3	31.9	50.4	55.6	81.0	115.0	130.6

Labrafil 1944 CS + Cysteamine Palmitate

Descriptive Statistics: Initial_3, 4 weeks_3

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_3	5	0	164.7	13.8	30.9	128.5	138.2	157.1	195.1	207.8
4 weeks_3	5	0	81.2	16.9	37.8	27.1	45.5	83.3	115.9	121.5

Labrasol

Descriptive Statistics: Initial_4, 4 weeks_4

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_4	5	0	164.4	16.4	36.6	124.2	130.9	154.8	202.7	203.1
4 weeks_4	4	0	113.9	57.3	114.5	31.0	36.7	71.4	233.7	282.0

Labrasol + Cysteamine Palmitate

Descriptive Statistics: Initial_5, 4 weeks_5

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_5	5	0	194.3	25.2	56.3	128.3	133.6	221.6	241.3	248.3
4 weeks_5	5	0	119.0	21.5	48.2	59.1	71.1	121.3	165.8	167.9

Lauroglycol 90

Descriptive Statistics: Initial_6, 4 weeks_6

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_6	5	0	162.6	11.9	26.6	141.1	147.1	154.7	182.0	209.0
4 weeks_6	5	0	102.8	39.7	88.8	28.9	49.9	75.9	169.1	257.4

Lauroglycol 90 + Cysteamine Palmitate

Descriptive Statistics: Initial_7, 4 weeks_7

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_7	5	0	138.1	28.3	63.2	45.1	78.0	159.4	187.6	212.7
4 weeks_7	4	0	143.7	31.7	63.4	50.9	77.7	166.0	187.5	192.0

Miglyol 812N

Descriptive Statistics: Initial_8, 4 weeks_8

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_8	5	0	141.3	15.6	35.0	92.9	109.5	143.8	171.8	187.0
4 weeks_8	5	0	76.2	16.5	36.8	36.1	46.3	72.6	107.9	134.4

Miglyol 812N + Cysteamine Palmitate

Descriptive Statistics: Initial_9, 4 weeks_9

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_9	5	0	178.5	14.9	33.4	123.0	149.3	186.4	203.7	208.0
4 weeks_9	5	0	79.7	15.2	34.0	50.8	58.5	69.8	105.8	138.6

Transcutol HP

Descriptive Statistics: Initial_10, 4 weeks_10

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_10	5	0	101.7	17.1	38.2	46.4	68.9	94.7	137.9	138.0
4 weeks_10	3	0	123.3	59.1	102.4	21.8	21.8	121.4	226.7	226.7

Transcutol HP + Cysteamine Palmitate

Descriptive Statistics: Initial_11, 4 weeks_11

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Initial_11	5	0	112.5	21.3	47.7	54.7	76.7	108.1	150.6	186.9
4 weeks_11	5	0	53.4	15.3	34.2	24.4	27.6	48.5	81.7	110.8