LONDON METROPOLITAN UNIVERSITY

DEVELOPMENT OF PAEDIATRIC DOSAGE FORMS OF FUROSEMIDE USING THE PROBLEM STRUCTURING METHOD OF MORPHOLOGICAL ANALYSIS

A thesis submitted in partial fulfilment of the requirements of London Metropolitan University for the degree of

Doctor of Philisophy

Presented by

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ABSTRACT

The lack of age-appropriate (paediatric) authorised medicines is a long-standing problem amongst regulatory authorities, patients, parents and prescribers. This is driven by the paucity of information on clinical efficacy, deficiency in safety data (i.e. biopharmaceutics) and the lack of quality information such as palatability and acceptability data in children. To counteract this deficiency bespoke, unlicensed formulations are formulated by contract manufacturers, hospitals and dispensing pharmacists using a variety of 'recipes' and differing manufacturing protocols.

In this work, Morphological Analysis as a problem structuring method is deployed using key stakeholders of the problem complex. This method, developed from operational research and design thinking sectors, has the ability to structure and parameterise a complex problem to isolate a smaller subset of an internally consistent solution space for the design of experiments. Hence, Morphological Analysis is used experimentally to decide which pharmaceutical dosage forms of furosemide would be selected as a solution space for paediatric patients with low cardiac output syndrome. Morphological Analysis application resulted in the selection of two different dosage forms for further work (Microemulsion oral liquid dosage form and an Oro-dispersible Mini-tablet).

The furosemide microemulsion formulation was developed using ternary phase diagrams to isolate the efficient self-emulsification regions. A range of experimental techniques and instruments were used to characterise the system such as HPLC, phase stability studies, droplet size determination, surface tension measurement, drug-excipient compatibility studies using FTIR and NMR, viscosity determination, thermodynamic stability assessment and determination of shelf-life via accelerated and long-term stability studies. The optimum composition of the furosemide microemulsion consisted of: MCT Oil 14%, Labrasol 60%-Transcutol-HP 20% (3:1) and Water 6%.

A furosemide oro-dispersible mini-tablet formulation (ODMT) was also developed and analysed for quality assessment. The development approach for ODMT used factorial design at two levels with four factors. Pre-formulation studies included drug-excipient compatibility assessment using differential scanning calorimetry and powder flowability evaluation using angle of repose and Hausner's ratio techniques. For that, sixteen batches of ODMTs were manufactured using a Manesty F3 tablet press; Post-compression testing and characterisation processes were performed and this involved testing for weight variation, hardness assessment, friability, *in vitro* disintegration, wetting time, drug content analysis, dissolution time and stability of ODMTs under ICH conditions. The optimum composition of furosemide ODMT was found to be (ludiflash excipient + 0.6% magnesium stearate lubricant + 1mg/tablet furosemide API) with 10 minutes mixing time at value 19 compression force.

Dedication

*I wish to dedicate my research work to the **children of Syria** who have been suffering the brutal war.*

Syrian Camp, July 2012

"It is a poverty to decide that a child must die so that you may live as you wish"

Mother Teresa

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Hani Baghdadi

Declaration

I hereby declare that whilst studying for the Doctorate at London Metropolitan University, I have not been registered for any other award at another University. The work undertaken for this degree has not been submitted elsewhere for any other awards. The work contained within this submission is my own work and to the best of my knowledge and belief, it contained no material previously published or written by another person, except where due acknowledgement has been made in the text.

Mr Hani Baghdadi (2016)

Abbreviations

ADI: Acceptable Daily Intake

ADME: Absorption, Distribution, Metabolism and Excretion

AIDA: Analysis of Interconnected Decision Areas

API: Active Pharmaceutical Ingredient

AUC: Area under Curve

BBB: Blood Brain Barrier

BCS: Biopharmaceutical Classification System

BNF: British National Formulary

BP: British Pharmacopeia

BPCA: Best Pharmaceuticals for Children Act

CCA: Cross-Consistency Assessment

CHF: Congestive Heart Failure

C_{max}: Maximum plasma concentration

COPD: Chronic Obstructive Pulmonary Disease

CQA: Critical Quality Attributes

DCAV: Drug Content Acceptance Value

DH: Department of Health

DLS: Dynamic Light Scattering

DSC: Differential Scanning Calorimetry

EC: European Commission

EMA: European Medicine Agency

EMC: Electronic Medicines Compendium

EP: European Pharmacopeia

FAO: Food and Agriculture Organisation

FC: Formulation Code

FDA: Food and Drug Administration

FTIR: Fourier Transform Infrared Spectroscopy

GFR: Glomerular Filtration

GRAS: Generally Regarded As Safe

HLB: Hydrophilic-Lipophilic Balance

HPMC: Hydroxy propyl methyl cellulose

ICH: International Conference on Harmonization

IFM: Institute for Manufacturing

INR: International Normalised Ratio

JECFA: Joint FAO/WHO Expert Committee on Food Additives

LCOS: Low Cardiac Output Syndrome

LCT: Long Chain Triglycerides

MA: Morphological Analysis

MAO: Mono Amino Oxidase

MCT: Medium Chain Triglycerides

MHRA: Medicines and Healthcare Products Regulatory Agency

NAT: N-Acetyl Transferase

NCA: National Competent Authorities

NICE: The National Institute for Health and Care Excellence

NIH: National Institute of Health

NOAEL: No Observed Adverse Effect Levels

ODMT: Oro-dispersible Mini-Tablet

OR: Operational Research

PDCO: Paediatric Committee

PEG: Polyethylene Glycol

PIP: Paediatric Investigation Plan

PK: Pharmacokinetic

PMA: Postmenstrual Age

PSM: Problem Structuring Method

PVA: Polyvinyl alcohol

PVP: Polyvinyl pyrrolidone

QBD: Quality by Design

QTPP: Quality Target Product Profile

RP- HPLC: Reverse Phase High Performance Liquid Chromatography

RPM: Rounds per Minute

SCA: Strategic Choice Approach

SmPC: Summary of Product Characteristics

SODA: Strategic Options Development and Analysis

SSM: Soft Systems Methodology

TPP: Target Product Profile

TPQP: Target Product Quality Profile

USP: United States Pharmacopeia

UV: Ultraviolet

WHO: World Health Organisation

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CHAPTER 1

1.1 Introduction

The Complexity of Paediatric Formulation Development

"Children are the world's most important resource"

Prof. Bonita F. Stanton, 1990, Nelsons Textbook of Paediatrics

Paediatrics can be considered as the discipline concerned with all aspects of the well being of infants, children and adolescents, including their health and physical, mental and psychological growth and development and their opportunity to achieve full potential as adults. Healthcare professionals and formulators must be concerned not only with particular organ systems and biological processes but with overall well-being aspects from diagnosing an illness to administering an age-appropriate medicine aiming to provide the highest efficacy and lowest possible side effects with the maximum quality (e.g. smell, colour, texture and taste) from which have a major impact on the physical, emotional and mental health and social well-being of children and their families.[1]

One of the most challenging and critical areas in drug development is the pharmaceutical formulation of paediatric medicines. Children form a large percentage of patients' population but they have been a neglected group where medicines are concerned. It is not that children do not have access to medicines, but that few medicinal products have been designed and tested specifically for paediatric use.[2] Additionally, children can neither be regarded as small adults nor as a homogenous group amongst themselves. In fact, there are dramatic changes in physiology and pharmacology during the development to maturation meaning that the way children absorb, distribute, metabolise and eliminate drugs cannot often be extrapolated between different subsets or from adult

clinical data[3]. This got further complicated by the fact that there has been variation in the definition and classification of paediatric age groups between texts and studies. To overcome this limitation, the International Conference on Harmonization (ICH) has provided an agreed definition to paediatric groups for regulatory purposes (EMA, 2000). The definition and classification of age groups in paediatrics when provided was based on the major changes in physiological, pharmacokinetic (PK) and pharmacodynamics parameters occurring during development as defined in Table 1.

Table 1: Definition and age ranges of the paediatric population as per ICH[4]

Definition	Age group
Preterm newborn infants	<37 weeks' gestation
Term newborn infants (Neonates)	0 – 27 days
Infants and toddlers	28 days to 23 months
Children	2 – 11 years
Adolescents	12 – 16 or 18, depending on region

The crucial parameters applied in age-groups classification are discussed in further details to emphasise differences between paediatrics and adults and paediatrics amongst their different age groups specifically PK parameters for oral administration. Additionally, to develop an age-appropriate medicines for children, it is fundamental to understand all relevant variables and factors that differ between paediatric age groups and adults. Such variables include the pharmacological aspects, palatability and acceptability, regulatory requirements, excipients toxicity and commercial aspects behind paediatric medicines. In this chapter these variables will be discussed to simplify the objectives of the thesis.

Firstly, an illustration of the differences in body physiology and clinical pharmacology between paediatrics and adults is discussed including the pharmacokinetic and

pharmacodynamics changes during the maturation process.

1.2 Pharmacokinetics Aspects and their Differences between Paediatrics and Adults

Pharmacokinetics term is defined as the study of the kinetics of medicine including its absorption, distribution, metabolism and elimination/excretion which can be viewed simply as describing what happens to the drug within the body.[5]

In children, pharmacokinetic parameters change through the development and maturing processes, altering the disposition of drugs and bioavailability. Bioavailability is understood to be the extent and the rate to which a substance or its active moiety is delivered from a pharmaceutical form to become available in the general circulation.[6] These changes necessitates carrying out specific studies in the different paediatric ages to establish particular dosification steps in the paediatric population.[7]

Pharmacokinetic measures, such as area under the curve (AUC) and concentration at the maximum (C_{max}) are calculated from Absorption, Distribution, Metabolism and Elimination (ADME) measures, including clearance, half-life and elimination of a drug from the body. Generally, all drugs show inter- and intra-individual variance within bodies in pharmacokinetic measures and/or parameters. However, general variations are considered very substantial and significant within the different paediatric age groups and in comparison with adults. For instance, when administering a medicine, in order to achieve AUC and C_{max} values in children similar to values associated with effectiveness and safety in adults, it is important to evaluate the pharmacokinetics parameters of a drug over the entire paediatric age range in which the drug will be used[8]. Additionally, where growth and development are rapid, adjustment in dose within a single patient over time may be important to maintain a stable systemic exposure.[9]

Beside the fact that pharmacokinetics aspect are crucial for dosing, ADME data and parameters are also essential for a drug to gain licensing approval for paediatric use. Table 2 lists some cardiovascular drugs which have not been approved for paediatric use with either dosage forms by EMA due to lack of data on their ADME if medicines is taken orally, efficacy or safety in children for all routes of administration.

Table 2: List of some drugs not approved by EMA for paediatric use[10]

Medicine	Approved paediatric use	Needs
Amiodarone	> 3 years (United Kingdom)	Extension of the indication (efficacy, safety data and dose) < 3 years. Lower age limit to be defined in children due to iodine intoxication and thyroid adverse effects Age appropriate formulation (benzyl alcohol in current iv formulation)
Candesartan	Not approved	Extension of the indication (efficacy, safety data and dose) to all age groups Age appropriate formulation
Valsartan	Not approved	Extension of the indication (efficacy, safety data and dose) to all age groups Age appropriate formulation
Clopidogril	Not approved	Extension of the indication (efficacy, safety data and dose) in all age groups Age appropriate formulation
Prazocin	Children > 12 years	Extension of the indication to children < 12 years (efficacy, safety data and dose) Age appropriate formulation
Furosemide	Children (for treatment of oedema in Sweden, hypertension in France)	Indications for oedema and hypertension and age appropriate formulation to be made available in all Member States

1.2.1 Oral absorption of drugs in paediatrics

There are several routes of administration used in drug delivery for children, but as with adults, the most common and convenient route involves oral administration. A therapeutic agent administered by oral route must overcome chemical, physical, mechanical and biological barriers to be absorbed and achieve its targeted effect within the patient's body. The oral administration route has numerous factors and variables which can affect the drug absorption. These factors and variables include the developmental changes in the

gastrointestinal tract (gastric pH, volume and emptying rate) and at the absorptive surfaces in paediatrics which influence the rate and extent of the bioavailability of a drug.[11]

After oral administration, medicines can also get metabolised by intestinal enzymes which exert a large influence on drug absorption and bioavailability as well. For example, in neonates, there is underdeveloped enzymes production that causes a delay in the absorption rate[12]. Additionally, the bioavailability of some drugs can be influenced by intestinal microflora metabolism (hydrolysis and reductions), which is thereby different in infants, children and adults. For example, by two years of age there are bacteria in the intestine that are able to metabolise digoxin, however, it is not until adolescence when adult levels of metabolism are reached.[13]

1.2.2 Distribution of drugs in paediatrics

Following gastro-intestinal absorption, a medicinal drug is distributed to various body compartments according to its physicochemical properties, such as molecular size, ionisation constant and relative aqueous and lipid solubility. Several of the processes involved in the distribution of drugs are significantly different in neonates and infants when compared to adults. Factors including plasma protein binding and water partitioning are continuously fluctuating throughout the first few years of life, thus affecting the distribution of drugs. These factors are mainly affected by body water/ fat composition. There are age-related changes in body composition, protein binding and active transport mechanisms[14]. The most dramatic changes in body composition occur in the first year of life but changes continue throughout development towards puberty and adolescence, where observed particularly in the proportion of total body fat. This affects water soluble molecules such as aminoglycosides as will have a higher volume of distribution in the very young age group whilst fat soluble substances, such as diazepam may be expected to have their greater volume of distribution in older infants and toddlers where their body

fat composition increases in proportion to water.[14]

Plasma protein binding of compounds is another main factor as influential as body compositions in distribution of drug. This is dependent upon the amount of available binding proteins, the number of available binding sites, the affinity constant of the drug towards the protein and the presence of pathophysiological conditions or endogenous compounds that may alter the drug-protein binding interaction.[15]

1.2.3 Metabolism of drugs in paediatrics

Of all the body organs, the liver is quantitatively by far the most important site for paediatric and adults drug metabolism. At birth, the liver forms 5% of newborn's body weight approximately whereas, it constitutes only 2% of adult body weight[16]. There are numerous factors which can affect hepatic clearance of any medicinal product. These factors include blood flow, hepatic enzyme activities (intrinsic metabolism) and plasma protein binding, as discussed in the previous section. Blood flow and drug metabolising enzymes are considered to be at relatively low level in paediatric at birth and these two factors usually require more than one year of age for a child to reach adults rates or even slightly less.

Generally, drug metabolism involves two phases in the liver: phase I reactions such as oxidation, reduction and hydrolysis, and phase II reaction such as conjugation with glucuronic acid and sulphation. At birth, metabolic enzymes for both phases I and II in human liver are mostly immature. The different capacity to metabolise drug in paediatric patients may result in lower or higher plasma levels than these reached in adults which can cause over- or under-dosing of the intended dose, necessitating dose adjustment before administration.[17]

In phase I, metabolising oxidative reactions are the most important step, largely mediated by cytochrome P-450 (CYP)-dependent. The total cytochrome P450 content in the foetal

liver is between 30 and 60% of adult values and only approach adult values by 10 years of age[18]. CYP2C isozymes are barely detectable in newborns due to their very low concentration and existence. While, they represent one-third of average adult values one month after birth, remain unchanged until one year of age and then increase in proportion after that age[19]. Taking the anticonvulsant phenytoin as an example, in pre-term infants, the apparent half-life of phenytoin is prolonged (75h) relative to term infants <1 week after birth (20h) or term infants aged >2 weeks (8h).[20] To illustrate the variation in half-lives of drugs metabolised by CYP450 isoenzymes between neonates, infants, children and adults, an example of different medicines' half-lives is shown in Table 3.

Table 3: Different half-lives of drugs metabolized by CYP450 isoenzymes[21]

Isoenzyme	Drug	Neonate	Infant	Children	Adult
CYP1A2	Caffeine	95	7	3	4
	Theophylline	24-36	7	3	3-9
CYP2C9	Phenytoin	30-36	2-7	2-20	20-30
CYP2c19	Phenobarbital	70-500	20-70	20-80	60-160
	Diazepam	22-46	10-12	15-21	24-48
CYP3A	Carbamazepine	8-28	---	14-19	16-36
	Lidocaine	2.9-3.3	---	1-5	1-2.2

Phase II metabolic processes are more limited with methylation, acetylation and glucuronidation forming the principal types. Like phase 1 enzymes, there is age dependency in expression. For example, the activity of N-acetyltransferase, which involved in the metabolism of drugs such as hydralazine and isoniazid, is almost three-fold higher in adults compared to that in the foetal liver cytosol[22]. Given the enormity of this subject, more details on this type of metabolism and of other processes such as first

pass metabolism in the gastrointestinal cell wall, will not be described but concluded with examples as shown in Table 4.

Table 4: Isoenzymes activity in paediatric population compared to adults

Isoenzyme	Paediatric activity	Drug class	Examples
CYP1A2	↓ until 2 years	Antidepressant, Bronchodilator, Diuretic	Duloxetine Theophylline Triamterene
CYP2C9	↓ until 1-2 years	Anticoagulant, Antidepressant,	Warfarin Phenytoin
CYP2C19	↓until 10 years	Antidepressant, Benzodiazepine	Citalopram, Sertraline Diazepam
N-Methyltransferases	↓ until 7-10 years	Analgesic	Morphine
N-Methyltransferases	↓ until 7-10 years	Antiepileptic, Benzodiazepine	Lamotrigine, Clonazepam, Lorazepam
NAT2	↓ until 1-4 years	Antihypertensive, Anti-infectious	Hydralazine Isoniazid

In summary, it can be concluded that the metabolic variation between paediatrics and adults is of major significance as the drug metabolising rate varies significantly with children progressing through the 5 ICH age bands. Therefore, the medicinal dosing should always be associated with age, requiring adjustment of doses per kg body weight after considering the status of the metabolizing capacity. Subsequently, doses should be considered carefully in order to achieve equivalent therapeutic concentration and avoid any side effect and/ or toxicity caused by metabolic disorder and immaturation.

1.2.4 Renal elimination of drugs in paediatrics

In general, medicines are eliminated from the human body either unchanged as the parent drug or altered as metabolites. There are various means of drugs excretion and kidney is considered the principal organ involved in the elimination of drugs and their metabolites. Excretion of medicinal products by kidney is entirely dependent on three

processes; glomerular filtration, tubular secretion and reabsorption. These processes rely on renal blood and renal plasma flow which increases with aging as a result of an increase in cardiac contraction and cardiac output and a reduction in peripheral vascular resistance.

At birth, renal blood flow in a healthy paediatric is 5 to 6% of cardiac output, compared to 15 - 25% by one year of age and reaches adult values after approximately two years of age[23]. Therefore, paediatric patients with low cardiac output syndrome (the condition studied with its treatment options in this research) at birth develop slower renal filtration leading to conditions such as congestive heart failure and oedema which would have slower renal elimination as well. Previous studies have shown that during the neonatal period, the elimination of many drugs which get excreted in urine in unchanged form is restricted by the immaturity of glomerular filtration and renal tubular secretion. Other studies show that a similar or greater rate of elimination from plasma than in adults has been observed in late infancy and/or in childhood for many drugs including digoxin, phenytoin, carbamazepine, levetiracetam, chlorpheniramine and cetirizine[24]. Therefore, larger doses of these drugs (mg/kg) must be administered in children in order to achieve equivalent plasma concentrations and efficacy to adults.

Another factor affecting drug renal elimination is infant urinary pH values as they are generally lower than adult values[23]. In general, urinary pH may influence the reabsorption of weak organic acids and bases medicines and differences in renal drug elimination may reflect the discrepancy in urinary pH values which leads to variation in efficacy of administered drugs.

1.3 Pharmacokinetic of furosemide in adults and paediatrics

The preceding section on ADME highlighted the differences that drug in general undergo in adults and children. As this thesis will be using furosemide as the model drug in the design of age-appropriate formulations, it is pertinent to briefly discuss the differential

pharmacokinetic behaviour of furosemide between these two populations.

There is no data concerning the oral absorption of furosemide in very young children; additionally in neonates, furosemide is given via the intravenous route. Furosemide is metabolised into an inactive acidic metabolite (2-amino-4-chloro-5-sulfamoyl anthranilic acid) and is conjugated with glucuronic acid as a water-soluble entity for renal excretion[25]. However, glucuronyl transferase is poorly developed in children as seen observed from mid-gestation human foetal liver and kidney tissue samples. The metabolism of furosemide in neonates was studied by Aranda et al (1982).[26] Furosemide is metabolised into an inactive acidic metabolite (2-amino-4-chloro-5-sulfamoyl anthranilic acid) and is conjugated with glucuronic acid to give inactive furosemide glucuronide. Mean fractions of the total urinary excretion as unchanged furosemide ranged between 52.5 and 55.6%. The mean fractions of total urinary excretion as furosemide glucuronide and acidic metabolite ranged from 13.3 to 23.2% and from 20.9 to 29.3%, respectively.

In adults, average bioavailability of furosemide is 71 ± 35%. In neonates, mean bioavailability is 84.3% (range 56% to 106%) and time to peak effect when given intravenously is 1 to 3 h. There is a great inter-individual variability in the kinetic parameters of furosemide in neonates. The half-life (t½) is 6 to 20-fold longer, clearance is 1.2 to 14-fold smaller and volume of distribution (V_d) is 1.3 to 6-fold larger than the adult values.

In adults, renal elimination of furosemide occurs by glomerular filtration as well as by tubular secretion via a general organic anionic secretory pathway located in the proximal convolute tubule. In neonates, furosemide elimination is decreased because of a low rate of tubular secretion, and in infants with very low body weight, filtration is the major route of renal elimination.

Table 5 summarises the pertinent differences in metabolism and elimination of furosemide between paediatrics and adults.

Table 5: The main differences in furosemide metabolism and elimination between paediatrics and adults

Variable	Paediatrics	Adults
Bioavailability	Mean bioavailability is 84.3% (range 56% to 106%)	Average bioavailability is 71 ± 35%
Peak time	Time to peak effect when given intravenously is 1 to 3 h	Peak serum furosemide concentrations occur at approximately 1 to 1.5 hours
Half Life (t½)	The half-life (t ½) is 6 to 20-fold longer than adults	The half-life (t ½) is 1.3 h ± 0.8
Clearance (mL/h/kg)	Clearance is 1.2 to 14-fold smaller than adults	Clearance is 99.6 ± 34.8
Volume of distribution (V_d) (L/Kg)	Volume of distribution (V_d) is 1.3 to 6-fold larger than the adult values	Volume of distribution (V_d) is 0.13 ± 0.06
Elimination	Furosemide elimination is decreased because of a low rate of tubular secretion, and in infants with very low body weight, filtration is the major route of renal elimination.	Renal elimination of furosemide occurs by glomerular filtration as well as by tubular secretion via a general organic anionic secretory pathway located in the proximal convolute tubule.

1.4 Pharmacy Practice as it relates to Paediatrics

In the medicinal world, adults have better access to licensed medicines that have been tested and evaluated for efficacy, safety and quality than paediatrics. According to the Department of Health in the UK, in ideal circumstances children and young people should have access to licensed formulations which are appropriately evaluated for use in children. However, this is not the case in paediatrics practice as very few medicinal products have been designed, tested and licensed specifically for their use leading to difficulty in obtaining treatments. Therefore, it is relatively common for children to be treated with a medication for which there is no sufficient information in its prescribing label called off-label use[27]. This is currently defined by the Medicine and Healthcare products Regulatory Agency (MHRA) in the UK as medicines possessing UK/European

marketing authorisation but used outside of the indication(s) specified therein. Further, a medicine not possessing UK/European marketing authorisation is called an unlicensed medicine[28]. For instance, medicines authorised by the US FDA but which is imported into the UK or Europe.

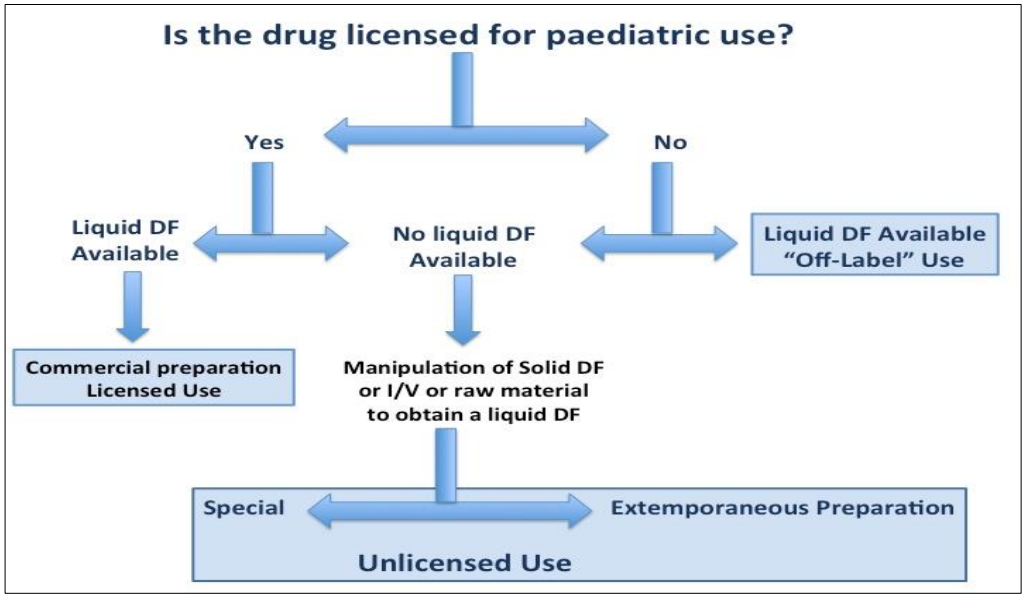
1.4.1 Off-label, extemporaneous, unlicensed medicines and special preparations

When dealing with paediatric patients' treatment options, it is important to distinguish between different types of preparations, not only with respect to regulatory aspects, but also with regards to the safety in paediatric settings. These types are explained in more details as the following:

- Unlicensed medicines are medicines administered via unlicensed dosage form obtained after manipulation of the original dosage form for example crushing or cutting tablets or opening a capsule. These are referred to as extemporaneous preparations. Ideally, extemporaneous products should be prepared from the active pharmaceutical ingredient (API), but frequently, commercial dosage forms are manipulated into a suitable form for administration to children. These preparations for paediatrics should usually take place in registered premises such as pharmacies, hospitals and health centres. In addition, they must be prepared under supervision of a pharmacist or qualified medical officer and in accordance with a prescription for administration to a particular patient. When larger amounts are prepared in bulk, the terms 'specials' is used. Specials have a similar status but are made in large volumes by licensed GMP manufacturers. The license is issued to manufacturers by specific national competent authorities (NCAs) such as MHRA in the UK. 2
- The term off-label medicine refers to any use of an authorised medicinal product not covered by the terms of its marketing authorisation. Examples would include using medicines for different non-authorised indication or at a different dose or dosage

frequency or for a patients group not specified on the label. The decision tree in Figure 1 describes the risk benefit of each option.

Figure 1: Decision pathway for providing oral doses to children for whom whole tablets/capsules are unsuitable



Recent hospital based studies within the European Union show that many drugs used in children are either not licensed or are prescribed outside their licensed indications (off-label). On general paediatric surgical and medical wards, 36% of children receive at least one drug that is either unlicensed or off label during their inpatient stay. In paediatric intensive care the figure is around 70% rising to 90% in neonatal intensive care settings. Another study on children's wards in five European countries found that almost half of all prescriptions were either unlicensed or off-label medicines. This suggests that many children in hospital are exposed to drugs without the guarantees the regulatory process should ensure. There is now evidence that the incidence of adverse drug reactions in hospitalised children is higher for unlicensed or off label drugs than licensed preparations[29]. Another study in the United States showed that four out of every five children hospitalized are treated with drugs that have never been tested on them and outside the hospital one third of all children take such medications [30] . These aforementioned studies reinforced the findings of a previous study carried out by Conroy

et al (2003), which estimated, depending on the condition, that up to 90% of medicines given to children in hospital are not licensed for use in the paediatric population.[31]

Unlicensed medicines have not been tested to define safety, efficacy and correct dosing. As a result, in a large specific study of children admitted to a paediatric hospital, adverse reactions and side effects were associated with 112 (3.9%) of the 2881 licensed drug prescriptions and 95 (6%) of the 1574 unlicensed or off- label drug prescriptions (35% of all prescriptions). The American Academy of Paediatrics sums up the situations with the following statement: “unapproved use does not imply an improper use and certainly does not imply an illegal use, but it has been recognised that off-label use and unlicensed medicines use is not ideal”.

In summary, it can be concluded that unlicensed and off-label medicines are not ideal option to treat paediatrics and can form major impact on their health. Therefore, it is very necessary to develop paediatric-friendly licensed formulations in order to achieve the right therapeutic concentration, avoid any side effect and enhance patient’s compliance.

1.5 Development of Paediatric-Friendly Oral Formulation

The ICH classification of paediatric population into five distinctive age groups reflects biological and physiological changes from birth towards adult maturity. Each of the differences and changes discussed previously should significantly impact on the choice of oral dosage form which may be used in each subset. In developing paediatric formulations, particularly those suitable for newborn and infants, there are issues and obstacles encountered concerned with the uncertainty of clinical knowledge with this subpopulation. The selection of the dosage form has its constraints on the acceptance and ability of administration to paediatrics especially the young ones in order to generate compliance and acceptability. Table 6 shows a summary of the preferred dosage forms

for drug delivery to different paediatric age groups according to Food and Drug Administration (FDA).

Table 6: Preferred dosage forms for paediatric age groups[32]

Age group	Recommended Dosage form
Newborn	Has not been identified (EMA suggests drops, intravenous and subcutaneous injections and suppositories as the oral is not feasible in most cases)
Infants	Liquid Small volumes (Syrups, Solutions)
Children: 2-5 years	Liquids (Liquids and effervescent tablets dispersed in liquids for administration)
Children: 6-11 years	Solids (Chewable tablets, orally disintegrating tablets)
Adolescents: 12-18	Solids (Typical adult dosage forms – tablets, capsules)

For instance, six year old children are generally considered the age at which oral solid dosage form can be safely swallowed, although this varies based on the child's acceptance[14]. In addition, the dose volume is a major consideration for the acceptability of a liquid formulation. Typical target dose volumes for paediatric liquid formulations are ≤ 5 ml for children under 5 years and ≤10 ml for children of 5 years and older.

Palatability and acceptability are another element considered of paramount importance in the development of paediatric medicines. For drugs to be accepted by children, they should have acceptable taste, smell, colour and texture. Therefore, it is recommended to sweeten and flavour paediatric medicines within acceptable limits and to avoid unusual flavours and complex taste mixtures in order to increase the chance of formulation acceptance by children[14]. Approaching a specific target paediatric population will also affect the flavour as cultural and social factors can have strong influence on children's attitudes and preferences. In general, children usually prefer a higher level of sweetness than adults. Additionally, they prefer brightly coloured preparations such as yellow or

bright pink colours that are associated with banana and strawberry flavours respectively and favourable smells which matches with the flavour if possible. Besides that, paediatric patients also prefer a smooth texture, thus the viscosity should be minimised to an optimal level in solutions and suspensions by dilution using aqueous diluents, and grittiness should be minimised in solid oral dosage forms by using high content of water-soluble granule diluents.[33]

To sum up, there are many important factors to address and consider when developing paediatric-friendly age-appropriate dosage form which adds further constraints and complexity to paediatric drug development and makes it more difficult to achieve the ideal composition and formula.

1.6 Formulation Design Considerations

In addition to pharmacokinetic parameters and palatability, excipients form a major concern in oral paediatric formulations. These 'inert' ingredients are essential in formulations to improve solubility, stability, quality and manufacturability. Although, a common misconception, excipients can be pharmacologically active[34]. Certain excipients have been found to cause adverse effects on children and particularly in newborns and infants due to their underdeveloped physiological characteristics (e.g. metabolism) and age-dependent maturation of organ function[35]. With regards to adverse effects, the addition of preservatives in formulations is seen as one of the main concerns to paediatric patients. For example, widely used preservatives such as benzyl alcohol, benzoic acid and hydroxybenzoates should be avoided in newborn, infants and children up to 3 years old due to their immature metabolism, as they can lead to toxic reactions caused by the accumulation of benzoates.[14]

Co-solvents, such as propylene glycol, are often used for substances which are not highly soluble in water such as diazepam and phenytoin[36]. However, paediatric patients below

four years of age have a limited expression of alcohol dehydrogenases leading to the accumulation of aldehyde intermediates and toxic effects which cause depression of the central nervous system and nephrotoxicity[37]. Ethanol is another common example of an excipient widely used as a solvent to aid solubility of active pharmaceutical ingredients. Studies have found that ethanol may cause adverse symptoms of intoxication, lethargy, stupor, coma, respiratory depression and cardiovascular collapse in children.[38]

Diluent and filler excipients can also become an issue in some conditions when used as excipients in pharmaceutical formulation. As a diluent and/ or filler, lactose is widely used in tablets and capsules, in lyophilised powders and as a carrier in dry power inhalation pharmaceutical products. Lactose intolerance occurs when there is a deficiency in intestinal enzyme lactase in the gastrointestinal tract. This enzyme is normally present in high levels at birth, declining rapidly in early childhood to lower levels than required. With respect to sweeteners, aspartame is used as an intense sweetener as it enhances flavour systems and can mask unpleasant tastes such as bitterness to improve palatability. In literature, a number of adverse events have been reported following the consumption of large quantities of aspartame such as hyperactivity in children.[38]

In conclusion, it is seen that excipients are not always inert and have associated toxicological risks if their intake by paediatric patients exceeds the acceptable daily intake (ADI), which is presently not known for all excipients in children). Therefore, WHO has indicated the ADI values of some commonly used excipients in paediatric oral dosage forms with warnings and restrictions on each type as shown in Table 7.

Table 7: WHO ADI values of commonly used excipients in paediatric oral dosage[39]

Excipient	ADI (mg/kg/day)	Warning/Restriction
Saccharin	5mg	Carcinogenic potential (banned in Canada)
Sucralose	15	None
Aspartame	40	Phenylalanine source, Phenylketonuria
Cyclamate	11	Unconfirmed carcinogenic potential Banned in US, permitted in EU & China
Acesfulme	15	None
Polysorbate	10	None
Azo dyes	2.5	Allergenic potential, avoid in children
Sucrose	Not specified	Causes dental caries and tooth decay

1.7 The Need for Pharmaceutical Regulations in Paediatrics

Licensed paediatric dosage forms, especially liquid formulations, are considered the best treatment options for paediatric patients as there is less difficulty in swallowing ensuring better compliance[40]. As described, licensed medicines differ from unlicensed forms in their efficacy and safety that is supported by clinical trial data. Generally, unless an illness largely affects infants and children, medicines remain unlicensed or not labelled for use in paediatric populations. The reasons behind this include a relatively small market size for children. This limits the return of investment by causing a potential delay in licensing a drug for adults. Consequently and according to Department of Health in the UK (DH), almost three quarters of medicines prescribed in neonatal intensive care units are prescribed outside of licensed indications. On children's wards, around one quarter of medicines are prescribed outside licensed indications ("off-label") in the UK and over one third of children received at least one of such medicines. As a consequence and for illustration purpose, warfarin as an example is not licensed in liquid form and is available

as a special but the expense and short shelf life along with the drug's water solubility means that it is usually administered as tablets crushed and dispersed in water. In a cohort study of paediatric patients receiving warfarin therapy, children under 1 year took significantly longer to achieve the target international normalised ratio (INR), needed more tests per month and required more dose changes per month compared with other age groups.[40]

From a financial point of view, there is also major concern in the cost of special preparations due to their short shelf lives, requiring more frequent preparations. In a BBC news report "Drugs 'specials' cost NHS £160m", it was stated that according to internal NHS sources, a saving of nearly £72m could be made if all specials were limited to £75 an item. This would indicate that the cost of specials could exceed that value probably because they are often prepared in small volumes due to the stability issues discussed previously[41]. Table 8 shows price list for some special preparations made by Aclardian Specials Laboratory in the UK for March 2014.

Table 8: Price list of some special preparations from a UK licensed lab[42]

Product Name	Pack Size	Price
Cyclizine oral solution 50mg/ 5ml	100ml	**£131**
Diaxozide oral suspension 250mg/ 5ml	30ml	**£115**
Tacrolimus oral suspension 2.5mg/5ml	100ml	**£162**
Hydrocortisone oral suspension 10mg/5ml	100ml	**£103**

To conclude, all formerly studied problems and issues encountered with unlicensed preparations and their consequences were the focus of many drug regulatory bodies worldwide. In order to overcome these limitations and problem complexes, drug regulations were recently developed and established which aimed on establishing better

regulations, recommendations and incentives on paediatric drugs for safer and more effective licensed paediatric formulations with higher quality and lower cost. Therefore, regulatory bodies have tried to resolve this by bringing appropriate regulations and directives to address this situation. On 26 January 2007, the new regulation of the European Union (EU) on medicinal products for paediatric use[43] came into force to improve the availability and access of licensed medicines for paediatrics. Its main objectives were to facilitate the development and accessibility of medicinal products for use in paediatric populations and to ensure that medicinal products that are used to treat paediatric populations are subject to ethical research of high quality. It is worth mentioning that the EU regulations came after almost 10 years of US regulations on paediatric drugs (initiated by section 111 of the 1997 US Food and Drug Administration Modernization Act) which has very similar aims and focus as the EU[44]. The EU regulations have also introduced the key measures and incentives to create and encourage paediatric drug development, which include a reward for studying medicines for children of 6 months extension to the supplementary protection certificate, in effect 6 months patent extension for the product including adult use and for the patent medicines, 8 plus 2 years of data exclusivity on paediatric use of the product for new studies awarded via a Paediatric Use Marketing Authorisation (PUMA). These incentives are very similar to those in USA but the EU proposal is more robust as it requires the sponsor to market the paediatric medicine for the approved indication within 12 months, thus speeding up the availability for patients.

The European Medicine Agency has also set the Paediatric Investigation Plan (PIP) which is considered the fundamental instrument of the new regulation related to paediatric formulations. A pharmaceutical company that intends to apply for marketing authorisation within the EU must submit a PIP that contains detailed information on the planned developments and clinical investigations in all subsets of the paediatric populations[45]. According to the EU regulation, the PIP shall specify the timing and the

measures proposed to access the quality, safety, and efficacy of the medicinal product in all subsets of paediatric population that may be concerned. In addition, it shall describe any measures to adapt the formulation of the medicinal product so as to make its use more acceptable, easier and safer or more effective for different subsets of the paediatric population [46]. Thus, the drug development section in the PIP must include the development strategy for *age-appropriate* formulations as well. A similar legislation has also been established by FDA called (Best Pharmaceuticals for Children Act, BPCA).

1.8 Research Aims

The aim of this research is to develop age-appropriate paediatric oral pharmaceutical dosage forms of furosemide for the treatment of low cardiac output syndrome. The problem-structuring approach “Morphological Analysis” is applied to address and parameterise the uncertainties encountered in paediatric drug development and to isolate key critical paths for the selection in order to develop novel paediatric-friendly formulations using stakeholders.

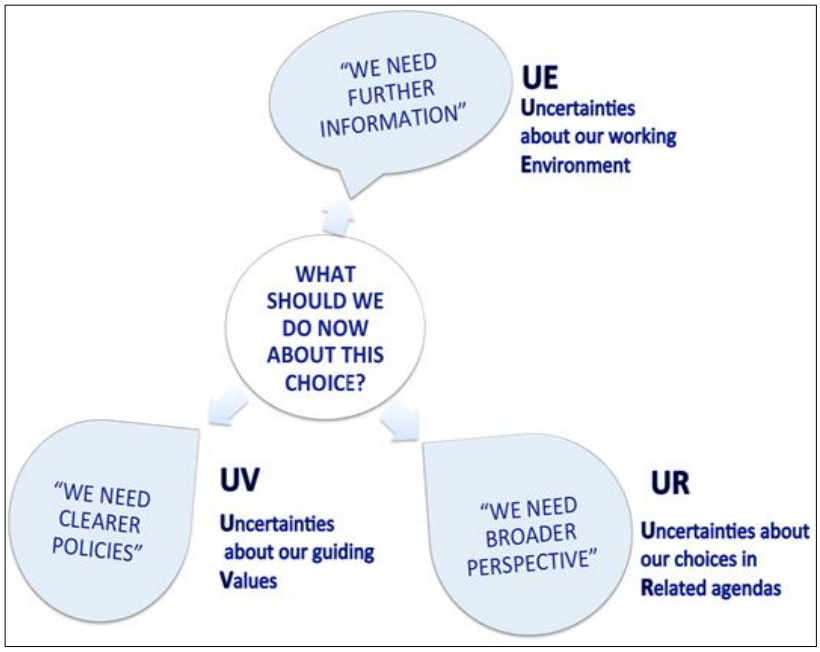
1.9 Research Objectives

1.9.1 Uncertainties within paediatric pharmaceutical formulation space

In the light of the reviewed literature from the preceding sections, significant number of variables and problems (or a system of problems) are associated with paediatric pharmaceutical development. These variables and problems arise from the lack of information in the clinical efficacy, pharmaco-toxicological safety data, concerns with excipients selections and the deficiency in access to licensed formulations can all be merged under a the term of ’uncertainties’.

In developing paediatric formulations, tackling uncertainties often reveals or generates unintended consequences such as toxicological effects, under-over-dosing, adverse drug reactions and lack of compliance. As such, paediatric drug development is a multidimensional problem, ambiguous and strongly stakeholder oriented situation with many different competing thoughts. Analysing and modelling such problems presents formulators and other stakeholders with a number of difficulties and methodological problems. Firstly, many of the factors involved in paediatric formulations are not meaningfully quantifiable, since they contain strong physiological, psychological, toxicological, practice-based and pharmacological dimensions (also referred to as parameters or factors). Secondly, the uncertainties inherent in such problem complexes are in principle non-reducible and often cannot be fully described or delineated due to the large number of parameters and values (or options) within each parameter. Finally, the extreme non-linearity (non-uniformity) of paediatric development factors means that literally everything depends on everything else such as co-morbidity, duration of treatment and paediatric cultural differences etc. Therefore, an alternative form of decision support modelling is required which is rarely used in the natural sciences, termed problem structuring methods. These methods, as reported by the Institute for Manufacturing (based at the University of Cambridge), are able to address three broad types of uncertainties as shown in Figure 2.

Figure 2: Elements used to structure a problem[47]



1. *Uncertainties about our working environment (UE):*

   This usually requires further information to be gathered. For example, the biological effect of a medicine in children and adverse events due to excipients. This information can often be obtained from technical expert opinion e.g. healthcare professionals, specialists working groups of regulatory agencies etc.

2. *Uncertainties about our guiding values (UV):*

   This is where clearer policies for overall understanding of the problem are needed. This type of information usually relates to the perspectives of other stakeholders such as regulators, industry executives and patient support groups.

3. *Uncertainties about our choices in related agendas (UR):*

   This requires a much broader perspective, which is usually obtained from higher level ministerial bodies such as Department of Health and international organisations (e.g. EMA, WHO etc.).

In designing paediatric formulations the type of uncertainties include the following:

- Clinical uncertainties such as:
    - Use of potentially toxic excipients such as benzyl alcohol, propylene glycol, ethanol etc.
    - Juvenile toxicity studies availability as related to changing ADME in children (e.g. appropriate animal models)
- Quality associated uncertainties such as
    - Practice related (i.e. palatability, acceptability and administration issues e.g. nasogastric tube administration)
    - Development of novel dosage forms and devices with attendant stability and manufacturing concerns

1.9.2 Problem structuring methods

Uncertainties inherent in paediatric drug development can be tackled using problem structuring methods (PSMs) which has not been employed in drug development before in the design of pharmaceutical formulations. PSMs are a branch of Operational Research (applied mathematics for real life problems such as logistics, optimisation and scheduling) that provides a more 'softer' human-centric approach to address' messy problems'. These methods are designed to achieve to clarify issues amongst a wide group of relevant stakeholders, generate group consensus, have the ability to combine qualitative and quantitative data and commit the working group to an agreed action plan of work.

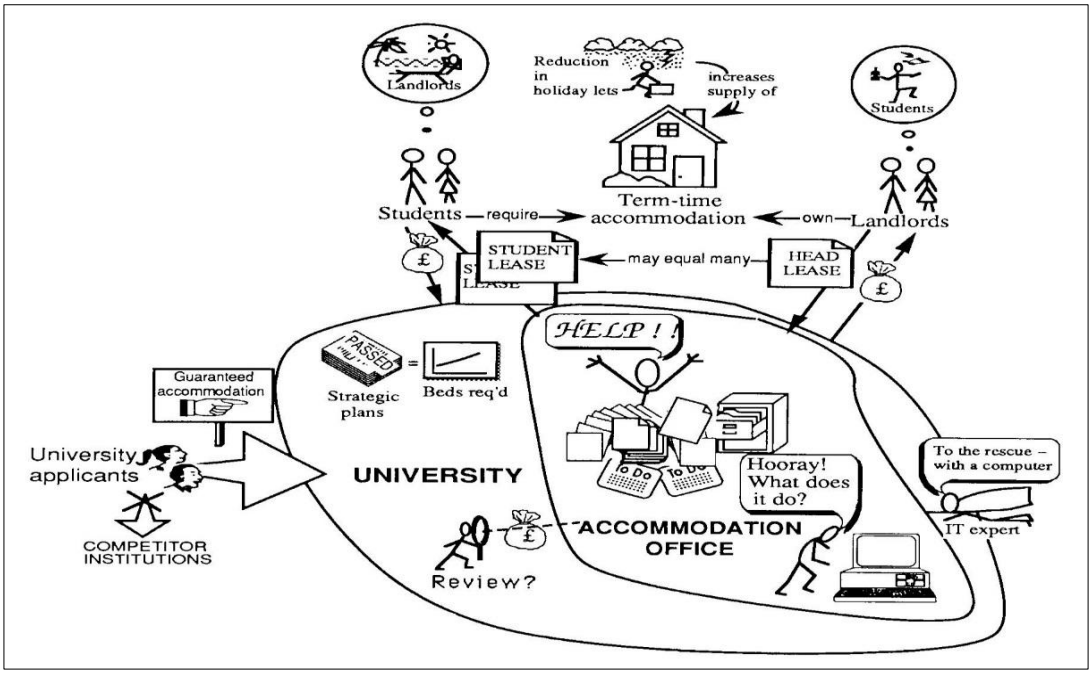
In short, PSMs are a collection of participatory modelling approaches that aim to support a diverse collection of stakeholders in addressing a problematic situation of shared concern. Often the situation is characterised by high levels of complexity and uncertainty, where differing perspectives, conflicting priorities, and prominent intangibles are the norm

rather than the exception.

In general, there are four main PSMs:

- *Soft Systems Methodology* (SSM): SSM was developed by Peter Checkland[48] in the late 1960's at University of Lancaster whilst seconded from ICI. As an industrial chemist he was puzzled that despite the plant operating at 'maximum' capacity it did not always achieve optimum production efficiency, which was later attributed to human factors. Each stakeholder in the system had an individual worldview of how the plant should be running and these confluences of thoughts needed to be disentangled to achieve a holistic and consensual approach amongst these actors (aka stakeholders). Like many other systems approaches, SSM is a comparison between the world, as it is, and some models of the world as it might be. Out of this comparison arises a better understanding of the world ("research"), and some ideas for improvement ("action"); as such the term 'action research' is used for real time intervention. Initial work involves interviews and meetings to gain an understanding of the problem situation, which is represented by the use of 'rich pictures' of diagrams drawn by the participants as visualised (Figure 3).

Figure 3: Example of a rich picture diagram in SMM to describe the problem of students' accommodation[49]

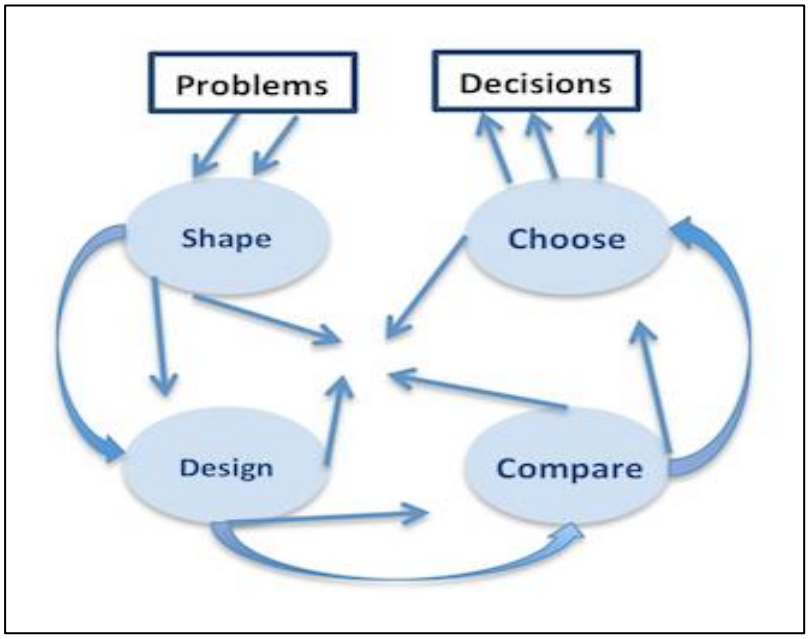


Systems' thinking uses the concepts of hierarchy, communication, control, and emergent properties to identify 'relevant systems' (drawn by participants as a rich picture) which can provide useful insights. The method is particularly useful when there are deep divisions as to what is the problem. Since the problem is known in paediatric drug development, SSM was not applicable as a PSM in this thesis mainly because it is a resource intensive and there is no known software for capturing the discussions or transcribing the diagrams.

- *Strategic Choice Approach:* Unlike SSM that tries to define the nature of the problem as seen through the lens of the various actors in the system, Strategic Choice Approach (SCA) is an analytical perspective, based on choice models that focuses on strategies for shaping the context of decision-making. Friend and Hickling are the originators of the method developed at UCL's Tavistock Institute in the mid-1960s[50].

In this method, there are four components: shaping, designing comparing and choosing as shown in Figure 4.

Figure 4: Four components of the strategic choice approach



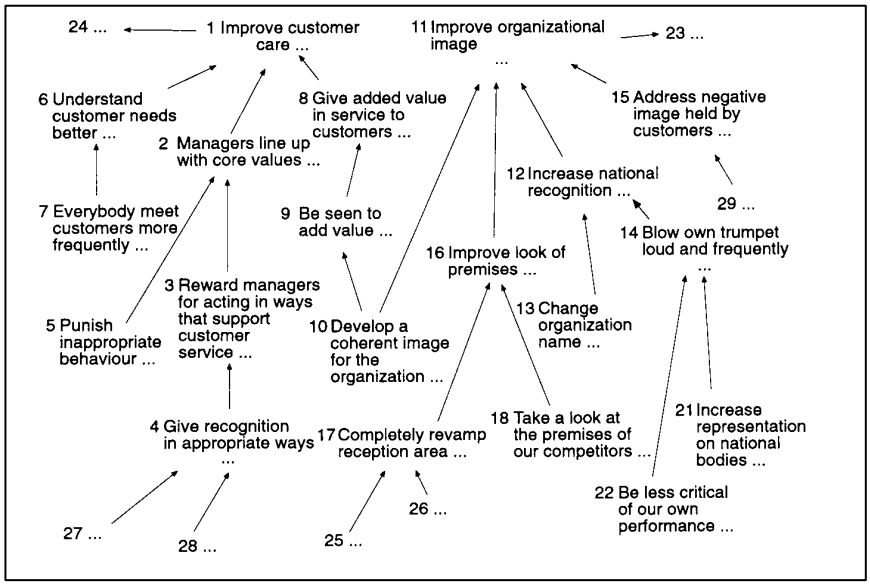
The shaping mode considers the nature of the problems, their inter-linkages and the focus. In the designing mode, one considers the options and how they may be linked to form alternative solutions. The comparison mode evaluates these alternatives and the benefit-risk profile of each proposed solution. Finally, in the choosing mode criteria are developed by the working group to evaluate the alternative solutions (weighted) to yield the most optimal solution and the way forward.

Unlike SSM, rudimentary software is available however it cannot run on modern operating systems (i.e. beyond Windows XP). It shares some features with Morphological Analysis in that it parameterises the problem and then links the options under each parameter using a procedure similar to the cross consistency analysis known as analysis of interconnected decision areas (AIDA). However, there remain two important differences in that SCA tends to focus on no more than four or five parameters (i.e. the decision areas) and three to four options as a maximum. Further differentiating feature is

that during the AIDA procedure, criteria are developed and weighted, whereas in morphological analysis no such weighing takes place as one is concerned with exploring what is possible and not what is probable – the latter requiring some form of probability distribution associate with each variable of the problem complex, which usually does not exist. SCA gets around this problem by asking the group to make a judgement call by using qualitative scales such as the Likert scale.

- Strategic Options Development and Analysis (SODA): SODA was originally developed by Colin Eden at the University of Bath[51]. This PSM, also known as cognitive mapping, uses interview and mental maps (captured as 'bubbles' or thoughts) to capture individual views of an issue. Group maps are constructed through the aggregation of individual cognitive maps then the various paths from the problem to the solution give the *options*. An example of a SODA's group maps is shown in Figure 5.

Figure 5: Example of group maps in SODA

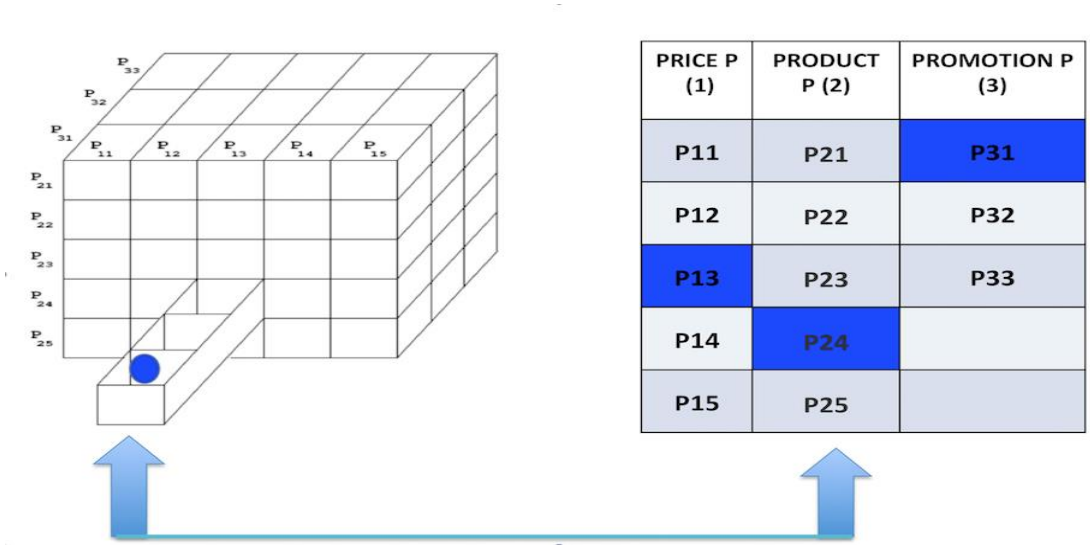


In the group format, the process uses two personal computers, special software, one (preferably two) large monitor, blank wall space and flipcharts. It is managed by two

facilitators: one to focus on the content and one to guide the process. A group map can become enormously large (several hundred bubbles or thoughts on a single cognitive map are not uncommon). In the opinion of the author and his supervisors, SODA was not considered an option to tackle the uncertainties in paediatric drug development for several reasons. Firstly, this approach results in an output that is not visually appealing and is not well received by readers as it displays a complicated problem in a rather complicated fashion. Secondly, the method is resource intensive requiring specialist software and additional equipment as mentioned above. Finally, the necessity of having two facilitators, interviewing individual stakeholders and convening them over several plenary sessions was not feasible in terms of logistics, timing and expense.

- *General Morphological Analysis* or simply Morphological Analysis (MA) is a method for structuring and investigating the total set of relationships contained in multidimensional, usually non-quantifiable problem complexes. MA was used as the PSM in the research described in this thesis and will be described in more detail in chapter 3. Briefly, it involves constructing a series of dimensions of an uncertain problem complex and various options within each dimension. As problems with *more* than three dimensions cannot be visualised on a 2-dimensional format, they are simply constructed as a table as shown in Figure 6. MA was selected in this research as it can cover any number of dimensions, and allows inclusion of both qualitative and quantitative dimensions. Furthermore, the method has seen widespread applications in both social and engineering sectors[52].

Figure 6: Three dimensions and options (cells) in a typological format containing 75 possible configurations. This can be re-formatted as a table. The blue marked cell (blue dot) represents the blue-shaded configuration



PRICE P (1)	PRODUCT P (2)	PROMOTION P (3)
P11	P21	P31
P12	P22	P32
P13	P23	P33
P14	P24	
P15	P25	

1.10 Morphological Analysis

"Every problem interacts with other problems and is therefore part of a set of interrelated problems, a system of problems…. I choose to call such a system a mess"

Russell Ackoff, Redesigning the Future, 1974

Typically, typology as a simple structuring method addresses uncertainties by combining possible combinations obtained between a few (usually two to three) variables or dimensions. Each variable contains a range of values or conditions and each of the possible combinations of variable-values in the typological field is called a constructed type. As exemplified in Figure 6 in the introduction section, a hypothetical problem involving three variables (Price P1, Product P2 and Promotion P3) gives rise to many constructed types of solutions. Visually, a typology uses the Cartesian dimensions of a physical space to represent the variables (e.g. P1, P2 and P3) with their values containing 75 cells (5x5x3). However, the number of coordinates that can be represented in Cartesian display is limited to three dimensions. Typologies of greater dimensions (4, 5,

6 etc.) usually get around this problem by embedding variables within each other. But, such formats quickly become very difficult to interpret, prone to errors and add another layer of complexity to an already existing complex situation such as the case of paediatric drug development. Thus, Morphological Analysis (MA) as a PSM was selected to liberate such spatial constraints of 3-dimensional space and allows the researcher to allocate any number of dimensions (aka variables). In Addition, it is the first application of its kind in the pharmaceutical or drug development field.

1.10.1 Background to morphological analysis

MA is a method for structuring and investigating the total set of relationships contained in multidimensional, usually non-quantifiable problem complexes. MA was first applied to the aerospace industry by Fritz Zwicky, a professor at the California Institute of Technology in the 1930s and 1940s. Zwicky chose to analyse the structure of jet engine technology (amongst other application such as the design of telescopes etc.). His first task was to define the important parameters of jet engine technology, which include thrust mechanism, oxidiser, and fuel type. He continued, in turn, to break each of these technologies down into its component parts. Having exhausted the possibilities under each parameter (i.e. dimensional) heading, alternative approaches were assembled in all possible permutations: for example, a ramjet that used atmospheric oxygen and a solid fuel. For some permutations, a jet engine system already existed; for others, no systems or products were available. Zwicky viewed the permutations representing "empty cells" as stimuli for creativity and for each asked, "Why not?" For example, “Why not a nuclear-powered ceramic fan-jet?”[53]

Although Zwicky coined the term Morphological Analysis, the technique predates him and can be traced back to Ramón Lull (1235-1315), according to Lucien Gerardin[54]. Zwicky was the first to use the technique in modern-day applications. The primary use of MA has been in technological forecasting and new product ideation. Historically,

scientific knowledge develops through cycles of analysis and synthesis; every synthesis is built upon the results of a proceeding analysis, and every analysis requires a subsequent synthesis in order to verify and correct its results. The process of MA is composed of the cycles of analysis and synthesis in number of iterative steps. In order to perform these steps, the problem space is developed (i.e. analysed) and structured by identifying the most important dimensions and underlying options that have an influence on furosemide formulation and its administration in paediatrics. This type of analysis will be used to assess on the design considerations in the development of an age-appropriate furosemide dosage form.

MA relies on a constructed parameters space, linked by way of logical relationships, rather than on causal relationships and a hierarchal structure. Analysing and modelling critical conditions in pharmaceutical formulations for paediatrics with different age groups having their physiological development presents number of difficult problems and uncertainties. Firstly, many of the factors and parameters involved in such conditions are not meaningfully quantifiable. Secondly, the uncertainties encountered in the problem complexes and issues in paediatric formulations can often not be fully assessed as explained previously.

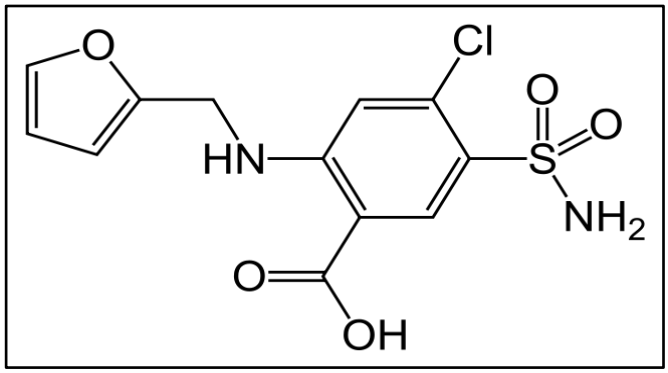
Therefore, using MA was essential to facilitate a graphical (visual) representation for the systematic exploration of a solution space using a combination of literature searches and assisted by subject matter experts such as paediatricians, pharmacists, formulators and regulators to name a few stakeholders in the problem complex.

1.11 Furosemide Drug and its Application in the Treatment of LCOS

Furosemide is a potent loop diuretic classified as class IV according to Biopharmaceutical Classification System (BCS) with low solubility and low permeability. It is chemically designed as 4-Chloro-2-[(furan-2-ylmethyl) amino]-5-sulfamoylbenzoic acid (Figure 7). It is a white or almost white, odourless crystalline powder, practically insoluble in water

(10µg/ mL & 73.1 mg/L at 30°C), soluble in acetone, sparingly soluble in ethanol (96%) and freely soluble in dilute alkali solutions. It has the molecular formula of $C_{12}H_{11}ClN_2O_5S$, a molecular weight of 330.7 and melting point of 295°C[55].

Figure 7: Furosemide chemical structure[56]



Furosemide, an off-patent drug first synthesised in 1962, is primarily used for the treatment of oedematous states associated with cardiac, renal and hepatic failure. It is also used in the treatment of hypertension and widely used in paediatric patients with low cardiac output syndrome (LCOS). LCOS in paediatrics is caused by a transient decrease in systemic perfusion secondary to myocardial dysfunction. The outcome is an imbalance between oxygen delivery and oxygen consumption at the cellular level which leads to metabolic acidosis. In children, LCOS is most often caused by congenital heart disease and cardiomyopathy. There are few therapy principal options clinically indicated for LCOS treatment such as furosemide, digoxin and dopamine. Furosemide is the most widely used agent in cardiac failure and LCOS and for this reason it is included in the WHO's Essential Medicines List for Children.

However, furosemide's low solubility and permeability plus the lack of age-appropriate oral dosage form for paediatrics is a major problem. Thus, one of the aims of this research is to contribute knowledge to the field by using morphological analysis as a decision making tool for the development and selection of age-appropriate dosage forms of

furosemide and to formulate the dosage forms suggested as solutions by morphological analysis.

1.12 Development of Microemulsion as Paediatric-Friendly Liquid Formulation

Microemulsion is defined as a system of water, oil and an amphiphile which is a single optically isotropic and thermodynamically stable liquid solution[57]. There are significant differences between microemulsions and ordinary emulsions. In particular, in emulsions the average droplet size grows continuously with time so that phase separation ultimately occurs under gravitational force. Therefore, emulsions are thermodynamically unstable and their formation requires input of work whereas a microemulsion droplet tends to be more stable. Another major difference is that the droplets of the dispersed phase when just formulated are generally larger in an emulsion (>0.1µm) such that they often take on a milky rather than a translucent appearance.[58]

Microemulsions as drug delivery tool show favourable properties such as spontaneous formation without the use of any high-shear equipment, thermodynamic stability (long shelf-life), easy formation (up to zero interfacial tension), optical isotropy, ability to be sterilised by filtration, high surface area (high solubilisation capacity) which is very favourable for low solubility drugs such as furosemide molecule. Another main advantage of microemulsions is that they are considered as age-appropriate liquid dosage form offering dose flexibility and can be easily administered to children and paediatric patients. Microemulsion offer high solubilising potency without using solvents that can be harmful to paediatrics such as solution which would require co-solvents such as ethanol and propylene glycol. In addition, it has smooth texture with zero grittiness, which forms no risk of blocking the nasogastric tube in paediatric treatment. Microemulsion is composed of oil, surfactant, co-surfactant and has the ability to form water-in-oil w/o microemulsion when dispersed with aqueous phase under gentle agitation[59]. The nanosized droplets in microemulsion have very high surface to volume ratios what makes them capable to

efficiently solubilise the drug. The drug is released in a more reproducible manner, which normally becomes less dependent on the GI physiology and the fed/fasted state of the patient. However, since the drug delivery system should be mild and biocompatible, the choice of excipients can be relatively limited and this will be discussed in further detail.

1.12.1 Microemulsion's structure

Microemulsions are dynamic systems in which the interface is continuously and spontaneously fluctuating[60]. Structurally, they are divided into three types: oil-in-water (o/w), water-in-oil (w/o) and bicontinuous microemulsions. In w/o microemulsion, water droplets are dispersed in the continuous oil phase while o/w microemulsion is formed when oil droplets are dispersed in the continuous aqueous phase. In systems where the amounts of water and oil are similar, a bicontinuous microemulsion may result. In all three types of microemulsions, the interface is stabilized by an appropriate combination of surfactants and co-surfactants by reducing the surface tension between the two phases. The mixture of oil, water and surfactants is able to form a wide variety of structures and phases depending upon the proportions of these components. Hence, the flexibility of the surfactant film is an important factor in this regard. For instance, a flexible surfactant film will enable the existence of several different structures like droplet like shapes, aggregates and bicontinuous structures and therefore broaden the range of microemulsion existence. A very rigid surfactant film will not enable existence of bicontinuous structures, which will impede the range of existence.[61]

1.12.2 Components of microemulsion formulation

There are numerous types of oils, surfactants and co-surfactants that can be used as components for the formulation of microemulsion systems. However, their toxicity, irritation potential and unclear mechanism of action put more restrictions on their applications. Generally, formulators must choose materials that are biocompatible, non-toxic, clinically acceptable, and use emulsifiers in an appropriate concentration range that

will result in mild and non-aggressive microemulsions. The emphasis is, therefore, on the use of generally regarded as safe (GRAS) excipients within their Acceptable Daily Intake (ADI) values.

Oil Phase

The oil component influences curvatures by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chain oils penetrate the tail group region to a greater extent than long chain alkanes, and hence swell this region to a greater extent, resulting in increased negative curvature[62]. There are two types of fatty acids that are used as oily phase:

- Saturated fatty acids (for example, lauric, myristic and capric acid)
- Unsaturated fatty acids (for example, oleic acid, linoleic acid and linolenic acid).

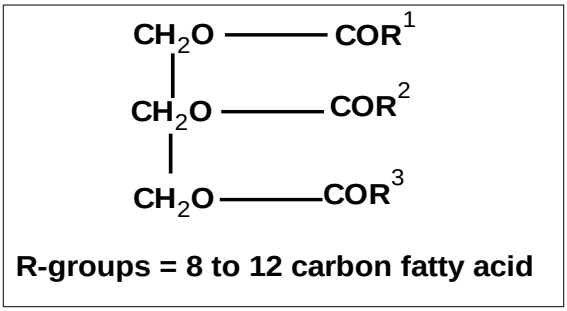
The main criterion for selecting the oil phase in microemulsions formulation is that the drug should have high solubility in the selected oil. Therefore, lipophilic drugs are preferably solubilized in o/w microemulsions. This will minimise the volume of the formulation to deliver the therapeutic dose of the drug in an encapsulated form[63] or in microemulsion oral drug delivery form for paediatric patients. Medium chain triglycerides are an example of oily phases in microemulsion formulation.

Medium Chain Triglycerides (MCT) - Labrafac Lipophile

Medium chain triglycerides are a medium triacylglycerol of saturated fatty acids with a chain length of 6–10 carbons, i.e., hexanoic acid (C6:0, common name carbonic acid), Octanoic acids (C8:0, common name acrylic acid) and decanoic acid (C10:0, common name capric acid). Sometimes, dodecanoic acid (C12:0, common name lauric acid) is included as well[64]. MCTs are defined a class of lipids in which three saturated fats are bound to a glycerol backbone. The only way to distinguish between MCTs from other

triglycerides is the fact that each fat molecule in MCTs is between six and twelve carbons in length as shown Figure 8.

Figure 8: MCT chemical structure[65]



Unlike Long Chain Triglycerides (LCT), significant absorption of MCT occurs in the absence of bile acids and pancreatic lipase rending them more advantageous in formulating a bioavailable dosage form. Thus, the uptake and absorption of MCTs is more rapid than LCT and hence the intestinal uptake of the API in microemulsion containing MCT as an oily phase[66]. MCTs are oxidized rapidly in the organism and they have very low tendency to deposit as a body fat in addition to that MCTs are considered a source of abundant and rapidly available energy. These particular physicochemical properties of MCTs make it a valuable tool in the dietetic management of a number of disorders of lipid metabolism.[67]

Surfactants

The surfactant used in microemulsions must be able to lower the interfacial tension to a very small value. This type of excipient also facilitates the dispersion process during the preparation of a microemulsion and provides a flexible film that can readily deform around the droplets and can be of the appropriate lipophilic character to provide the correct curvature at the interfacial region. There are many types of surfactants available such as labrafil and labrasol.

Labrafil

Labrafil also known as Linoleoyl macrogol-6 glycerides, Linoleoyl polyoxyl 6 glycerides or Corn oil PEG-6 esters with a molecular formula $C_{43}H_{88}O_{10}$ and a molecular weight (765.15282).[68] Labrafil if taken orally would be in the form of water dispersible surfactant composed of well-characterized PEG-esters and a glycerides fraction. Labrafil is primarily used as solubiliser to improve the solubility of active pharmaceutical ingredients *in vitro* and *in vivo*. Its effect to improve the solubility gives it another property as a bioavailability enhancer especially for low solubility drugs. Increased oral bioavailability is potentially associated with the long chain triglyceride composition and selective absorption of highly lipophilic APIs by the lymphatic transport system reducing hepatic first-pass metabolism which enhances the administered API efficacy.

Labrasol

Labrasol is another surfactant known by various names such as Caprylocaproyl macrogol-8 glycerides, Caprylocaproyl polyoxyl-8 glycerides or PEG-8 Caprylic/Capric Glycerides. It is available in a liquid form and can be administered orally as a non-ionic water dispersible surfactant composed of well-characterised polyethylene glycol (PEG) esters, a small glyceride fraction and free PEG. Labrasol is able to self-emulsify on contact with aqueous media forming a fine dispersion such as microemulsions. It is mainly used as a solubiliser and wetting agent, to improve the solubility and wettability of active pharmaceutical ingredients *in vitro* and *in vivo*. It also enhances the bioavailability and the increased bioavailability is reported to be associated with strong inhibition of the enterocytic efflux transporter (known as P-glycoprotein inhibition).[69] Labrasol contains polyethylene glycol in a liquid form and most polyethylene glycols are absorbed when taken orally.[70]

Co-surfactants

In most cases, single-chain surfactants alone are unable to reduce the o/w or even w/o interfacial tension sufficiently to enable a microemulsion to form[71]. The presence of a co-surfactant in a formulation allows the interfacial film sufficient flexibility to take up different curvatures required to form microemulsion over a wide range of composition[72]. If a single surfactant film is desired, the lipophilic chains of the surfactant should be sufficiently short, or contain fluidising groups (e.g. unsaturated bonds). Short to medium chain length alcohols (C3-C8) are commonly added as co-surfactants which further reduce the interfacial tension and increase the fluidity of the interface[73]. Transcutol-HP is an example of novel co-surfactant that can be used in microemulsion formulations.

Transcutol-HP

Transcutol-HP as a co-surfactant is a high purity solvent and solubiliser for poorly water-soluble active pharmaceutical ingredients. It's associated with improved drug penetration; permeation and drug depot effect what makes it ideal option for low permeable molecules such as furosemide. Transcutol-HP co-solvent is approved as safe to use in the preparation and formulation of pharmaceutical products[74]. It has the chemical name of Diethylene glycol monoethyl ether and available in a liquid form with mild and pleasant odour hydroscopic.[75]

1.12.3 Acceptable daily intake values of microemulsion excipients

The concept of the Acceptable Daily Intake (ADI) for humans was originally developed between 1956 and 1962 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and defined as "an estimate of the amount of a food additive, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk".

Generally, results from studies in humans, experimental animals and *in vitro* are used in deriving and forming the ADI values. The standard toxicity data set should include acute, sub-acute (28-90 days), chronic toxicity and carcinogenicity studies. Combined chronic toxicity and carcinogenicity studies are often used in the testing of food additives. The toxicity tests also include studies on reproductive toxicity/teratogenicity covering at least exposure in utero, neonatally (via mothers milk) and up to weaning. In addition studies on metabolism and kinetics (preferably also in humans) as well as short-term *in vitro* studies of mutagenicity/ clastogenicity are required.[76]

An alternative method to study excipients intake is the “no observed adverse effect level” (NOAEL) principle. NOAEL is determined from the most sensitive study in the most sensitive species tested. The NOAEL is thus found by study or observation and is expressed as the highest dose level producing no detectable adverse alterations of morphology, functional capacity, growth, development or life span. The ADI is usually established from the NOAEL by dividing it by a safety factor. When the database is considered adequate a factor of 100 is used by default, but may be modified when adequate human data are available or based on comparative pharmacokinetic/ pharmacodynamics data. In some cases where the database is defective, safety factors larger than 100 are used if it is found appropriate to establish a temporary ADI.

1.12.4 Toxicological aspects of microemulsion excipients

The use of lipid base oral formulations such as microemulsion is of growing interest and particularly in paediatric patients. However, the excipients used in formulating microemulsion need to be assessed for safe use especially if paediatrics are the targeted patients. For instance and based on previous literature, labrasol and transcutol-HP excipients cannot be considered as inert compounds in pharmacology and toxicology studies especially if administered orally.

Labrasol is classified under the name of Caprylocaproyl polyoxylglycerides. Generally, Polyoxylglycerides including labrasol are regarded as non-irritant and nontoxic material[77]. In previous tests, labrasol showed high tolerance and very low toxicity in rats with a LD_{50} of 22g/kg as well as led to both the alteration of the membrane permeability and the inhibition of the secretory systems in the intestinal epithelium.[78]

Transcutol-HP, as a co-surfactant excipient, in early investigations was used as a solubiliser and absorption promoter. It has been screened for acute toxicity, influence on the behaviour and on the sedative and muscle relaxant properties in mice. Since drug safety research is frequently faced with the challenge of the selection of appropriate vehicles for use in *in-vivo* non-clinical safety and toxicity assessment studies with poorly water soluble drugs and as the safety profile of Labrasol and Transcutol-HP is not well documented with enough knowledge, both Labrasol and Transcutol-HP were tested for 4 weeks by the oral route in Wistar rats. Both excipients were well tolerated at 5ml/Kg/day. However, changes in appearance and behaviour were observed from 10ml/kg/day with volume related incidence, severity and duration. In addition, it was summarized that Labrasol and Transcutol-HP as 5ml/kg/day were considered as an acceptable level for oral administration use as a vehicle for poorly water-soluble drugs.[79]

Medium chain triglycerides (MCTs) are widely used for parenteral nutrition in individuals requiring supplemental nutrition and are being more widely used in foods, drugs and cosmetics. MCTs are essentially non-toxic in acute toxicity tests conducted in several species of animals. MCTs exhibit no capacity for induction of hypersensitivity. Ninety-day toxicity tests did not result in notable toxicity, whether the product was administered in the diet up to 9375mg/kg body weight/day or by intramuscular (IM) injection (up to 0. 5ml/kg/day, on rabbits). There was no evidence that intravenous (iv) or dietary administration of MCTs adversely affected the reproductive performance of rats or resulted in maternal toxicity, foetal toxicity or teratogenic effects at doses up to 4.28g/kg body weight/day (iv) or 12,500mg/kg body weight/day (dietary). In addition, the safety of

human dietary consumption of MCTs, up to levels of 1g/kg, has been confirmed in several clinical trials.[80]

1.13 Development of Oro-Dispersible Mini Tablets as Paediatric-Friendly Solid Formulation

When considering dosage forms, tablets remain the most conventional and cost effective route of administration for most of medicines. However, approximately, one-third of the patients' population encounters difficulty in swallowing or dysphagia[81]. According to World Gastroenterology Organisation (WGO), Dysphagia refers either to the difficulty someone may have with the initial phases of a swallow or to the sensation that food and/ or liquid are somehow being obstructed in their passage from the mouth to the stomach[82]. Consequently, paediatric patients encompass the largest portion of the population that demonstrates swallowing difficulty and challenges such as risk of aspiration and choking causing asphyxia. As solution to this issue, alternative solid dosage forms have been investigated for helping to minimise these difficulties such as orally disintegrating tablets. Orally disintegrating tablets were developed as a novel dosage form to play a vital role in drug delivery to this unique subset of paediatric patients groups as well as those who have an aversion to swallowing tablets in general such as geriatric patients without the risk of aspiration.

Over the past three decades, orally disintegrating tablets (ODTs) have gained more attention as a preferred alternative mean of administration to conventional oral solid dosage forms such as standard tablets and capsules. According to the European Pharmacopoeia (EP), an ODT is defined as tablet that can be placed in the mouth where it disperses rapidly before swallowing[83]. The FDA however defines them as a "solid dosage form containing medicinal substances, which disintegrates rapidly, usually within a matter of seconds, when placed upon the tongue.[84]

1.13.1 Excipient considerations in oro-dispersible mini tablets

Oro-Dispersible Mini-Tablets (ODMTs) are small ODTs and the only difference they have is their diameter size, usually 2-4mm whereas with conventional ODTs they are usually between 6-12mm diameters. When formulating Oro-Dispersible Mini-tablets, it is important that the ingredients used in the formulation support and contribute towards a rapid release of the drug content, resulting in faster disintegration and dissolution. The drug content includes the pharmacologically active ingredients and the excipients. Therefore, designing an ODMT formulation requires a unique selection of specific excipients. Each of these excipients has its role towards formulating stable, palatable and fast melting tablets and has specific criteria to be considered in selection. For example, s*uperdisintegrants* are agents to aid the rapid ingress of surrounding water to aid the break-up of the solid dosage form. If used at high percentage they can adversely affect mouth feel (grittiness) and tablet quality such as low hardness and poor friability.

1.13.2 The selection ODMT excipients

One of the principle challenges encountered by formulators is how to design formulations that are stable, of high efficacy and safety and support patient compliance. The latter consideration is a key factor when it comes to long-term treatment of chronic diseases such as LCOS. However, hard-to-swallow and/ or unpleasant tasting tablets and large volume oral liquids are known to be key barriers for medication adherence.

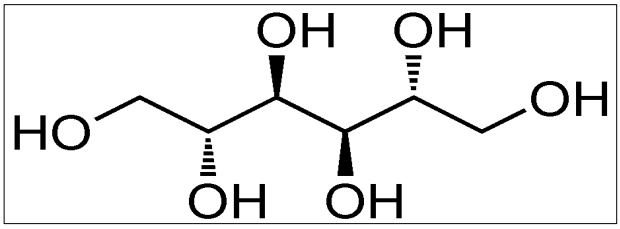
In formulating tablets, flowability, compressibility, hardness, stability and compatibility are principal factors to take into consideration. There are some ready-to-compress mixtures containing ODMT excipients such as Ludiflash. Ludiflash as an example is a co-processed excipient with super-disintegrating and palatable properties. Its composition supports the quality factors (i.e. processability, palatability etc.) substantially and

complies with all leading pharmacopoeia monographs. Ludiflash consists of the following[85]:

- **90% Mannitol**

  Mannitol is an excipient widely used in pharmaceutical formulations and food product preparations. In pharmaceutical formulations, it is primarily used as a diluent (10–90% w/w) when formulating tablets and it gains a particular value since it is not hygroscopic and may be used with moisture-sensitive active pharmaceutical ingredients. Mannitol is a hexahydric alcohol related to mannose and isomeric with sorbitol. It occurs as a white, odourless, crystalline powder, or free-flowing granules and has a sweet taste, approximately as sweet as glucose and half as sweet as sucrose and it imparts a cooling sensation in the mouth[77]. It can be used in direct-compression tableting formulation for which the granular and spray-dried forms are available or in wet granulations where granulations containing mannitol have the advantage of being easily dried. Mannitol is commonly used as an excipient in the manufacture of chewable tablet formulations because of its negative heat of solution (endothermic, hence cooling sensation), sweetness, and 'mouth feel'. It is also used as a diluent in rapidly dispersing oral dosage forms such as (ODMTs). If administered orally, mannitol is not absorbed significantly from the gastrointestinal tract, but in large doses (more than 10g per dose for a 70kg adult) it can cause osmotic diarrhoea. From a chemistry aspect, mannitol's empirical formula is $C_6H_{14}O_6$ and has a molecular weight of 182.2. The chemical structure of mannitol is shown in Figure 9.

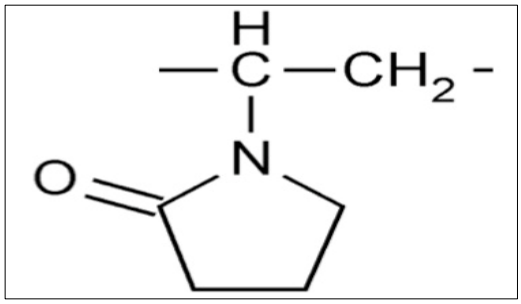
Figure 9: Mannitol Structural Formula[86]



- **5% crospovidone**

  Crospovidone is an insoluble polymer of *N*-vinyl-2-pyrrolidone used as a superdisintegrant in pharmaceutical tablets [87] . Crospovidone works as a superdisintegrant by quickly wicking saliva into the tablet to generate the volume expansion and hydrostatic pressures necessary to provide rapid disintegration in the mouth, unlike other superdisintegrants, which rely principally on swelling for disintegration. When examined under a scanning electron microscope, crospovidone particles appear granular and highly porous. This unique porous particle morphology facilitates wicking of liquid into the tablet and particles to generate rapid disintegration. In addition, crospovidone with its high crosslink density swells rapidly in water without gelling thus avoiding the rubbery and grittiness sensation. Gelling is usually observed with sodium starch glycolate and croscarmellose sodium superdisintegrants because they have a lower crosslink density. In contrast, crospovidone superdisintegrants exhibit virtually no tendency towards gel formation, even at high use levels. The formerly mentioned disintegrants if used in ODMT and chewable products can result in gel with an unpleasant and gummy texture[88]. Crospovidone disintegrants are highly compressible materials because of their unique particle morphology as compared to other superdisintegrants which are either poorly compressible or non-compressible. The chemical structure of crospovidone is shown in Figure 10.

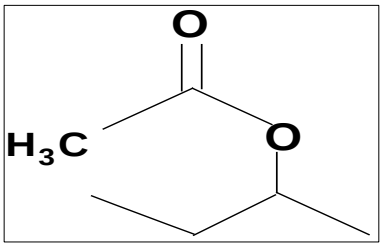
Figure 10: Crospovidone structural formula[89]



- **5% Kollicoat SR 30D (polyvinyl acetate)**

  Kollicoat SR 30D is a polyvinyl acetate dispersion (Figure 11) stabilised with povidone and sodium lauryl sulphate. This dispersion is suitable for the manufacture of pH-independent sustained-release formulations and consists of about 27% polyvinyl acetate, 2.7% povidone and 0.3% sodium lauryl sulphate. Kollicoat SR 30D is miscible with water in any ratio while retaining its milky-white appearance.[77]

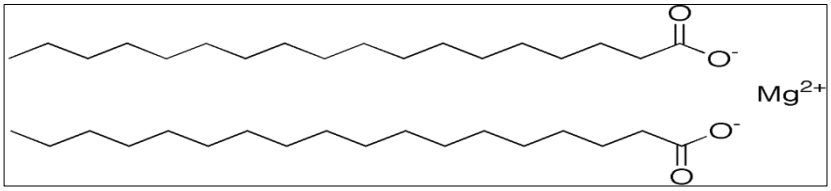
Figure 11: Chemical structure of Kollicoat SR 30D[90]



As with all oral solid doses formulation, a lubricant is essential to prevent adherence of granule/powder to punch die/faces and promote smooth ejection from the die after compaction. Magnesium stearate (Figure 12) is by far the most extensively used tableting lubricant due to its optimal effect and low cost. Lubricants tend to be hydrophobic, so their levels (typically 0.3 –2%) need to be optimised and right lubricating proportion has to be selected as under-lubricated blends tend to flow poorly showing compression sticking problems, weight and content variation, and over-lubricated blends can

adversely affect tablet hardness and dissolution rate (due to the hydrophobic barrier around the tablet) causing fraction, cracking and capping respectively.

Figure 12: Magnesium stearate chemical structure[91]



1.13.3 Available techniques for the formulation of ODMTs

There are two types of techniques that can be followed in formulating ODMTs. These are:

- Conventional techniques such as freeze drying, spray drying, moulding, phase transition process, melt granulation, sublimation, mass Extrusion, and Direct compression.[92]
- Patented techniques have been developed and patented on the basis of formulation aspects and processes. Each patented technique has its characteristics that vary on several parameters like mechanical strength, porosity, dose, stability, taste, mouth feel, dissolution rate and overall bioavailability to other techniques. Examples include Zydis, Orasolv and Ziplet techniques.[93]

1.13.4 Quality-By-Design (QBD) approach in pharmaceutical formulation

According to the EMA, any medicine needs to be designed to meet (paediatric) patients' needs and to consistently deliver the intended product performance. When QBD is applied, the quality target product profile (QTPP) gets established taking into consideration the specific needs of the paediatric population users[94]. QTPP is a major shift from the traditional *Quality by Testing* approach which relies on checking product quality against the approved regulatory specifications at the end of manufacturing stream at great effort and cost[95]. The QTPP as a systematic approach to development begins

with pre-defined objectives and emphasises product and process understanding and process control based on sound science and quality risk management[96]. This approach is particularly relevant to morphological analysis, which defines the overall problem landscape before isolating a subset of possible solutions, thereby defining the boundary conditions of the experimental work.

Chapter 2

2. MATERIALS AND METHODS

2.1 The Application of Morphological Analysis in Paediatric Formulation Design

This step of the research was carried out by conducting literature exercise followed by speaking with and interviewing subject matter specialists such as paediatricians, chemists, clinical pharmacists, formulators, regulators, paediatric nurses and parents. Morphological Analysis consists of two phases as described in the following:

A- Analysis Phase

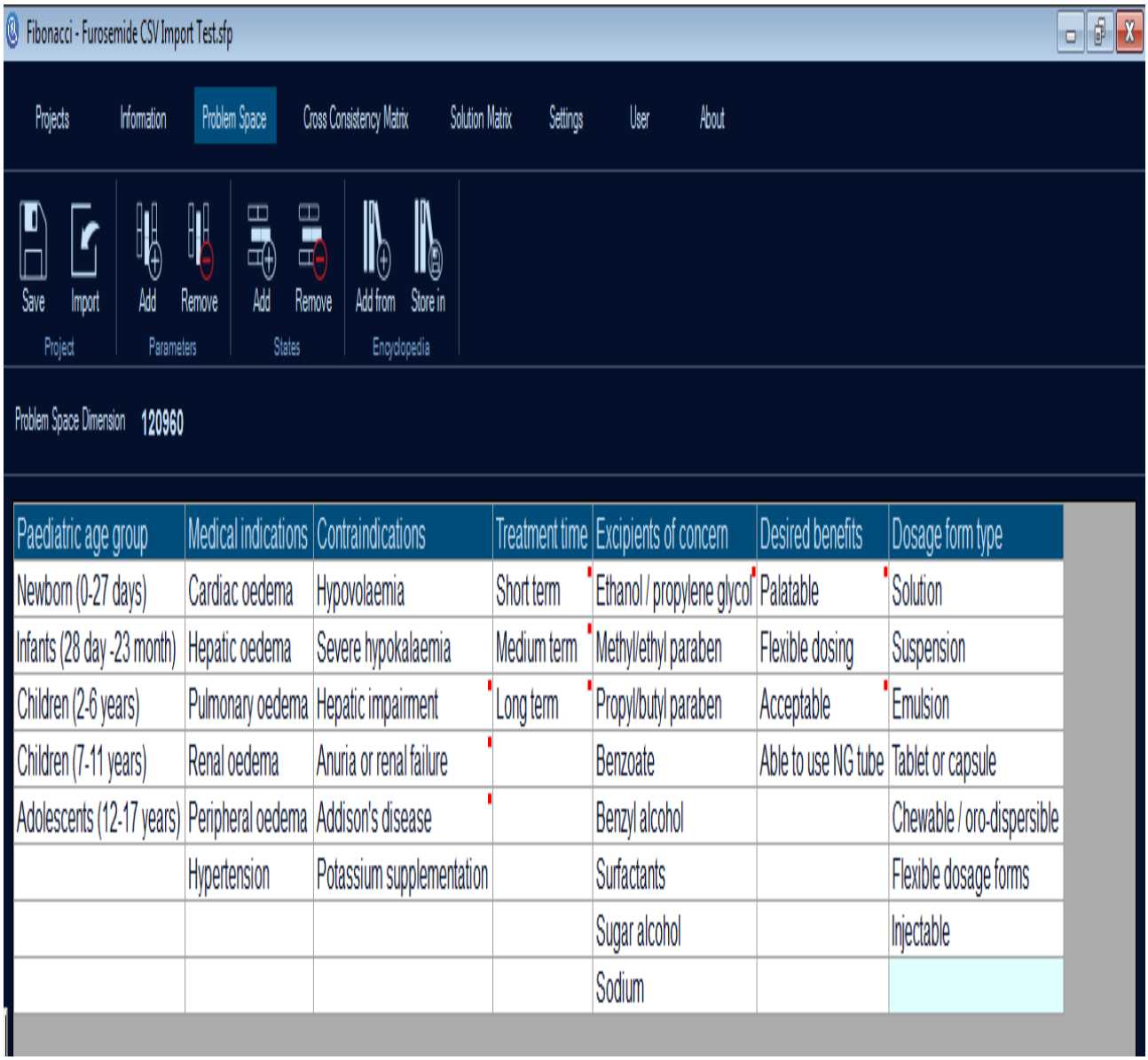
Analysis phase involves the identification of dimensions (parameters) mainly concerned when using furosemide by paediatric patients alongside with values or options of each parameter. Based on the reviewed literature and subject matter specialists, analysis phase was performed and the problem space was constructed as shown in Table 9.

Table 9: Factors and conditions involved in using furosemide in paediatric patients. In this analysis phase, there are 120,960 possible options in this problem space

Paediatric Age Group	Indications		Contra-indications	Treatment Duration	Administration Concerns	Problematic Excipients	Dosage form Type
Newborn (0-27days)	Oedema associated with:	Congestive Heart Failure	Hypokalaemia	Short-Term use (Acute)	Compliance and Adherence	Ethanol/ Propylene Glycol	Solution
Infants (28days-23months)		Renal Disease	Severe Hyponatraemia	Medium-Term Use	Large Dosage Form Size or Volume	Methyl/ Ethyl Parabens	Suspensions
Children (2-6years)		Hepatic Disease inc. nephritic syndrome	Hepatic impairment	Long-Term Use (Chronic)	Palatability and acceptance	Sugar-Alcohols	Microemulsions
Children (7-11years)		Pulmonary Disease due to left ventricular failure	Anuria or renal failure		Dosing Flexibility and/ or Accuracy	Surfactants	Standard Tablet or Capsule
Adolescents (12-18years)	Peripheral Oedema		Addison's Disease		Physiological Concerns	Benzyl Alcohol	Chewable/ Orodispersible / Wafer
	Hypertension		Potassium supplements			Propyl/ butyl Parabens	Flexible Solid Dosage Forms
						Benzoate	Injectable (IM/ IV
						Sodium containing excipients	

The analysis as shown in Table 9 was input into a proprietary MA software (Fibonacci™) as shown in Figure 13.

Figure 13: The morphological field (problem space) of furosemide drug for paediatric patients as seen in Fibonacci™ proprietary MA software



Paediatric age group	Medical indications	Contraindications	Treatment time	Excipients of concern	Desired benefits	Dosage form type
Newborn (0-27 days)	Cardiac oedema	Hypovolaemia	Short term	Ethanol / propylene glycol	Palatable	Solution
Infants (28 day -23 month)	Hepatic oedema	Severe hypokalaemia	Medium term	Methyl/ethyl paraben	Flexible dosing	Suspension
Children (2-6 years)	Pulmonary oedema	Hepatic impairment	Long term	Propyl/butyl paraben	Acceptable	Emulsion
Children (7-11 years)	Renal oedema	Anuria or renal failure		Benzoate	Able to use NG tube	Tablet or capsule
Adolescents (12-17 years)	Peripheral oedema	Addison's disease		Benzyl alcohol		Chewable / oro-dispersible
	Hypertension	Potassium supplementation		Surfactants		Flexible dosage forms
				Sugar alcohol		Injectable
				Sodium		

For each dimensional heading and option cells underneath, comments and definitions as agreed by the subject matter specialists are entered (denoted as a red square symbol).

B- Synthesis Phase

The 'synthesis phase' in MA is also known as the Cross Consistency Assessment process (CCA). This step is performed because the morphological field of furosemide generates 120,960 possible configurations. A configuration is a point in a multi-dimensional space represented as a path cutting each axis at the appropriate value. The total number of configurations is calculated by multiplying the number of options from each dimensional heading (i.e.5x6x6x3x8x4x7). CCA is used to reduce this vast number of configurations in the problem space to those which are feasible or not in order to generate the solutions space.

Cross-Consistency Assessment

CCA is based on examining and assessing the pair wise relationship of options (as shown in Figure 14). For instance, a newborn cannot be administered with chewable tablets dosage form as this is considered "be a logical constraint" or an incompatibility – this is denoted by placing X in the CCA matrix[97]. These types of judgements are made by subject matter specialists or informed by data from the literature as whether the option pair can co-exist[98] or not. Figure 14 displays how the problem space matrix (morphological field) has been formatted to generate the CCA matrix and the entry of Xs. When consistency checks are performed, internally inconsistent configurations are deducted to form the solution space.[99]

The CCA matrix comprises of 73 logical constraints and 52 empirical constraints. The total number of pair wise cells evaluated was 1088. [X] Represents a logical constraint, [P] is an empirical constraint and [–] is no constraint under all conditions. An empirical constraint is an option that can is possible under some circumstances (unlike X which is impossible). The grey shaded cells represent no direct relationship between the two options (i.e. they do not impact each other, often referred to as a 'different universe' in problem structuring). It also can mean non-redundant, non-overlapping or irrelevant. However, the orthogonal definition

also has been extended to this use, meaning the characteristic of something being independent (relative to something else).

Figure 14: The CCA matrix which simply pits each option from its dimension against other options derived from other dimensions. This screenshot was taken from the Fibonacci MA software

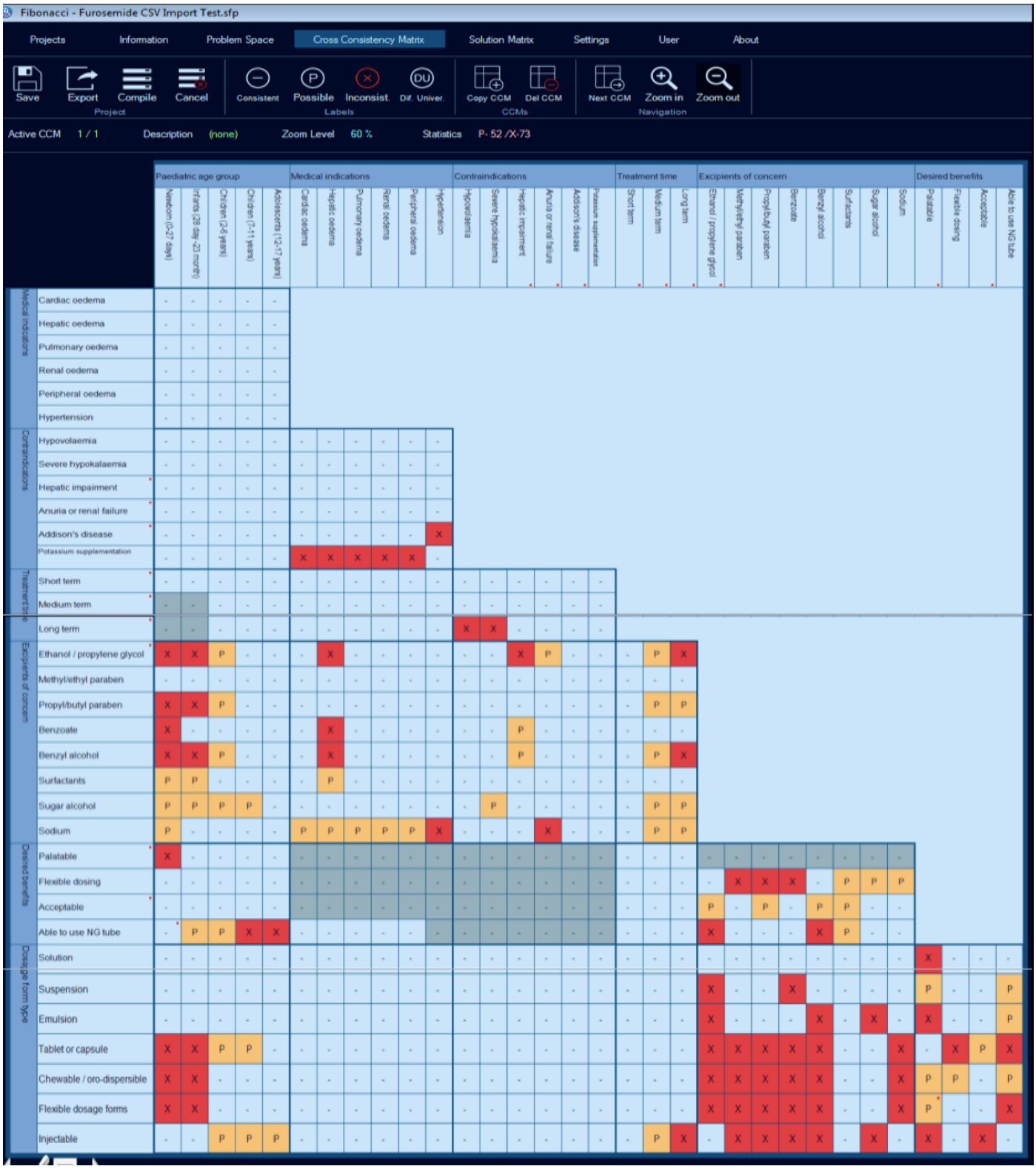


Figure 15: The CCA matrix shown in Excel for clarification

		Paediatric Age Group					Indications (BNFc,MIMS,SMPC)						Contraindications (BNFc,MIMS,SMPC)						Treatment Duration			Excipients of Concern								Desired Benifits				Dosage form Type							
							Oedema associated with:																																		
		Newborn(0-27days)	Infants (28days-23months)	Children (2-6years)	Children (7-11years)	Adolescents (12-18years)	Congestive Heart Failure	Hepatic Disease inc. nephrotic syndrome	Pulmonary Disease due to left ventricular failure	Renal Disease	Peripheral Oedema	Hypertension	HypoKalaemia	Severe Hyponatraemia	Hepatic impairment	Anuria or renal failure	Addison's Disease	Potassium suppliments	Short-Term use (Acute)	Medium Term	Long-Term Use (Chronic)	Ethanol/ Propylene Glycol	Methyl/ Ethyl Parabens	Propyl/ butyl Parabens	Benzoate	Benzyl Alcohol	Surfactants	Sugar-Alcohols	Sodium	Palatable	Flexible dosing	Acceptable	Able to use NG tube	Solution	Suspensions	Microemulsions	Standard Tablet or Capsule	Chewable/ Orodispersible/ Wafer	Flexible Solid Dosage Forms	Injectable (IM/ IV)
Indications	Oedema associated with: Congestive Heart Failure	-	-	-	-	-																																	
	Oedema associated with: Hepatic Disease inc. nephrotic syndrome	-	-	-	-	-																																	
	Oedema associated with: Pulmonary Diisease due to left ventricular failure	-	-	-	-	-																																	
	Oedema associated with: Renal Disease	-	-	-	-	-																																	
	Oedema associated with: Peripheral Oedema	-	-	-	-	-																																	
	Oedema associated with: Hypertension	-	-	-	-	-																																	
Contraindications (BNFc,MIMS, SMPC)	HypoKalaemia	-	-	-	-	-	-	-	-	-	-	-																											
	Severe Hyponatraemia	-	-	-	-	-	-	-	-	-	-	-																											
	Hepatic impaiment	-	-	-	-	-	-	-	-	-	-	-																											
	Anuria or renal failure	-	-	-	-	-	-	-	-	-	-	-																											
	Addison's Disease	-	-	-	-	-	-	-	-	-	-	X																											
	Potassium suppliments	-	-	-	-	-	X	X	X	X	X	-																											
Treatment Duration	Short-Term use (Acute)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																					
	Medium Term	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																					
	Long-Term Use (Chronic)	-	-	-	-	-	-	-	-	-	-	-	X	X	-	-	-	-																					
Problomatic Excipients	Ethanol/ Propylene Glycol	X	X	P	-	-	-	X	-	-	-	-	-	-	X	P	-	-	-	P	X																		
	Methyl/ Ethyl Parabens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																		
	Propyl/ butyl Parabens	X	X	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P																		
	Benzoate	X	-	-	-	-	-	X	-	-	-	-	-	-	P	-	-	-	-	-	-																		
	Benzyl Alcohol	X	X	P	-	-	-	X	-	-	-	-	-	-	P	-	-	-	-	P	X																		
	Surfactants	P	P	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-																		
	Sugar-Alcohols	P	P	P	P	-	-	-	-	-	-	-	-	P	-	-	-	-	-	P	P																		
	Sodium	P	-	-	-	-	P	P	P	P	P	X	-	-	-	X	-	-	-	P	P																		
Desired Benifits	Palatable	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
	Flexible dosing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	X	X	-	P	P	P										
	Acceptable	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	P	-	P	P	-	-										
	Able to use NG tube	-	P	P	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	X	P	-	-										
Dosage form Type	Solutions	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-							
	Suspensions	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X	-	-	-	P	-	-	P							
	Microemulsions	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	X	-	X	X	-	-	P							
	Standard Tablet or Capsule	X	X	P	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	X	X	X	X	-	-	-	X	P	X							
	Chewable/ Orodispersible/ Wafer	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	X	X	X	X	-	-	X	P	P	-							
	Flexible Solid Dosage Forms	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	X	X	X	X	-	-	X	P	-	-							
	Injectable (IV,IM)	-	-	P	P	P	-	-	-	-	-	-	-	-	-	-	-	-	-	P	X	-	X	X	X	X	-	X	-	X	-	X							

2.2 Methods and Materials Used in Formulation and In-vitro Evaluation of Furosemide Microemulsion

The studies described in this section aimed to formulate a ready-to-use microemulsion of furosemide as an age-appropriate dosage form for paediatric oral administration. Before formulating the microemulsion, the ADI values of its components are calculated based on paediatric oral administration. In order to calculate the ADI values of furosemide Microemulsion's excipients, previous literature was searched and Gattefosse Company (excipients manufacturers) was contacted. As per Gattefosse detailed response and data collected, each excipient intended for use in the formulation has its ADI value as following:

2.2.1 Transcutol-HP

Oral route administration has a NOAEL of 1000mg/kg/day obtained from a 3 months oral toxicology study in dogs by Gattefosse in 2007. Based on available toxicological data, literature and history of use, Transcutol-HP (Diethylene glycol monoethyl ether) can be administrated safely to humans at the following proposed dose levels (Table 10).

Table 10: ADI level for Transcutol-HP

Acceptable DAILY INTAKE (ADI) (PROPOSED BY GATTEFOSSE)		
Route of Administration	PDE (mg/kg/day)	PDE for 1 adult, 60kg (mg/day)
Oral	10	600

2.2.2 Labrasol

According to Gattefosse, labrasol can be administered safely to humans by oral route at dose levels up to 1800mg/ day (Table 11).

Table 11: ADI level for labrasol

Acceptable DAILY INTAKE (ADI) for Labrasol		
Route of Administration	ADI (mg/kg/day)	ADI for 1 adult, 60kg (mg/day)
Oral	30	1800
ADI based on a safety factor of 100 and NOAEL of 3000mg/kg/day		

2.2.3 Labrafac (Medium Chain Triglycerides)

Based on available toxicological data, literature, history of use and manufacturer data, labrafac has no toxicological effects reported and is considered safe. Therefore, it has no limits to volume administered per day.

2.3 Solubility Screening

Solubility screening test was conducted in order to choose the right surfactant (labrasol and labrafil) with optimal solubilising ability of furosemide API. The test was performed by taking an equal volume of both surfactants and saturating each sample with furosemide API. Both mixtures were mixed using vortex mixer and sonicated then left for enough time to solubilise the maximum concentration of the drug. Thereafter, both samples were filtered, diluted and analysed for quantitative purpose to assess their solubilising capacity using HPLC.

2.3.1 Materials and reagents used in solubility screening test

Furosemide active pharmaceutical ingredient was bought from Sigma-Aldrich suppliers Company. Labrafil and labrasol surfactants were provided by Gattefosse pharmaceutical manufacturers and suppliers. The reagents used for the experiment were of high purity and with analytical grade. Stock solutions and aliquots of the furosemide API were prepared in methanol at room temperature. The water used in mobile phase preparation was double-deionized water and Fresh working solutions were prepared daily. Furosemide API was dissolved with two equal volumes of labrafil and labrasol surfactants till saturation and the mixtures were degassed by sonicating them for half an hour and filtered using syringe filter before injection.

Apparatus

The analytical separation and quantification processes of the method were performed with HPLC system. The HPLC system was Dionex ultimate 3000 at LPG-3400A quaternary pump, VWD-3100 UV detector and a dimension 15X4.6 mm column with Eurospher ACE5 C18 packing. The sample injection was manual, the operating software was Chromeleon and the separation was carried out at room temperature.

Chromatographic conditions

The mobile phase used was H_3PO_4 (0.5% in aqueous solution) – Methanol (60:40) and the pH of the H_3PO_4 (0.5% in aqueous) mobile phase was adjusted to 3.2 with orthophosphoric acid. The analysis was carried out under isocratic conditions H_3PO_4 (0.5% aqueous) – methanol using flow rate of 1ml per minute at room temperature with equilibrating time set to 5 minutes. Chromatograms were recorded at 236nm using VWD-3100 UV detector and the total run time was for 15 minutes. Injector with 20µl sample loop introduced the samples into the HPLC machine.

Analytical procedure

Three millilitres of each selected surfactant (labrafil & labrasol) were added into glass vial containing excess amount of furosemide (510mg/vial). Both vials were sealed and mixed for 10 minutes with the help of vortex mixer and placed into sonication bath for half an hour at 35°C temperature to optimize the dispersion and dissolution of furosemide API into each surfactant. The vials were then foiled to avoid light degradation as furosemide is photosensitive molecule and left for one week to pseudo-equilibrate prior to HPLC analysis. After one week a fresh standard stock solution of furosemide API was prepared by weighing 25 mg of furosemide standard and transferring it into 25ml volumetric flask; around 15ml of methanol (mobile phase) was added to the content in volumetric flask and sonicated for 10 minutes for complete solubility then made up to volume with methanol to obtain a final concentration of 1mg/ ml of furosemide standard. From the stock solution, the aliquots of desired concentration of furosemide stock solution were prepared by six serial dilutions (50µg/ml, 100µg/ml, 150µg/ ml, 200µg/ml, 250µg/ml, 300µg/ml). The mixture of each vial was then filtered prior to injecting using Nalgene 0.45µm syringe filter to obtain clear filtrate for HPLC injection. Although the filtrates were of high purity, they had high viscosity due to the oily nature of these surfactants and the high concentration content of furosemide. Each filtrate was diluted with a compatible solvent, for instance, labrasol was diluted with 5ml of methanol and labrafil was diluted with chloroform. The pH of mobile phase was adjusted with orthophosphoric acid using pH meter. Mobile phase was then fitted in the HPLC machine and 20µl of each standard was injected to obtain the calibration curve. After calibration was obtained, 20µl volume of each diluted surfactant filtrate was injected and the injected samples were chromatographed under above conditions and chromatograms were obtained.

2.3.2 Method development

The aim of the method development was to develop a rapid and accurate HPLC method for the quantification of dissolved furosemide API into two different surfactants for solubility screening purpose using easily available reagents. Prior to the chromatographic method development, the detection wavelength was determined by obtaining the UV spectrum of furosemide reference solution in order to identify λ_{max} which was determined at 236 nm wavelength.

The chromatographic detection was investigated at 236 nm wavelength using different mobile phases consisting of acetonitrile, methanol, H_3PO_4 (0.5% aqueous) and double-deionised water on C_{18} column. The mobile phase was chosen only after numerous trials in order to achieve good results with clear and sharp peaks. For instance, a composition of acetonitrile and water (50:50) mobile phase with their normal pH was applied to the method. No resolution was produced and the results weren't clear as acetonitrile interfered with the furosemide peak and has left doubled-headed peak with tailing due to the pH value and column affinity. A trial with methanol and water (50:50) mobile phase with pH adjusted to 3.2 reduced the peak interference with the mobile phase. However, elution time was short, the peak was tailed and there was obstructing solvent peak observed. Water mobile phase was applied as 60% initially and the analysis was performed under isocratic elution condition using a flow rate 1ml/ min at room temperature. Unfortunately, the solvent peak was still visible and the furosemide peak was not sharp enough. Further trials with H_3PO_4 (0.5% aqueous) and methanol were made under isocratic conditions. Under these modifications better peak was observed and longer elution time was achieved.

Again, the furosemide API peaks were saturated. This was obviously due to the high concentration of the furosemide API dissolved in both surfactants. Appropriate solvents were used to dilute each of the surfactants to reduce the concentration to be within the detectable limits and to reduce the viscosity of the surfactants as high

viscosity was another concern before injecting into the column. Initially methanol was used to dilute both surfactants; it formed a good diluted clear solution by mixing it with labrasol filtrate. However, it was immiscible with labrafil filtrate and formed a white cloudy and turbid mixture of two different colours as white drops in bright yellowish oily phase. After trying different solvents, chloroform was found to be the most miscible and compatible solvent, forming an injectable, clear and less viscous solution with labrafil filtrate. Using these specifications on the C_{18} column, the retention time of 11:71 minutes was reliably produced.

2.3.3 Phase diagram construction for furosemide microemulsion

Ternary Phase Diagram was constructed to identify the efficient self-emulsification region for furosemide microemulsion. With the help of CHEMEX software, the pseudo-ternary phase diagram of medium chain triglycerides (MCT) oil, surfactant: co-surfactant (Labrasol:Transcutol-HP) and water was developed. Ternary phase diagram represents the equilibrium between the various phases that are formed between the three components, as a function of temperature[100]. This was developed using water titration method and the pseudo-ternary phase diagrams were constructed at three different ratios of surfactant/co-surfactant (Labrasol:Transcutol-HP) mixtures [S/Co-S= 1:1 (v/v), 2:1 (v/v) and 3:1 (v/v)] in order to identify the self-emulsifying regions. Firstly, the mixtures of MCT oil, surfactant and co-surfactant at certain volume ratio were prepared in the presence of furosemide 10mg/ ml. Then, these mixtures were diluted with water in a drop wise manner using burette till a translucent and homogenous mixtures contain furosemide were formed under constant mixing using magnetic stirrer as shown in Figure 16.

Figure 16: W/O Microemulsion formulation using dropper and magnetic stirrer

❖ **Finalised Method of the Preparation of Furosemide Microemulsion**

Furosemide microemulsion with fixed ratio of surfactant: co-surfactant (3:1) was prepared after series of trials to select the optimum ratio of oil, surfactant: co-surfactant and water. In all formulations, the concentration of furosemide was chosen to be 10mg/ml. This concentration allowed the maximal amount of furosemide to be contained in an age-appropriate dose volume for young children and infants. The formulation methods was performed as the following:

1) Furosemide API was dissolved in the surfactant using hot plate and magnetic stirrer.

2) Medium chain triglycerides oil (MCT) and Transcutol-HP co-surfactant were mixed together using gentle agitation via the vortex mixer.

3) The two mixtures from steps 1 and 2 were mixed together by gentle stirring at room temperature until furosemide was completely dissolved and a stable, single phase was formed.

4) Water was then added to mixture in drop wise manner using a burette under continuous stirring condition via a small magnetic stirrer until a clear and transparent mixture was formed.

5) The mixtures were then poured into child resistant amber coloured glass bottle as furosemide is light-sensitive and the preparation was stored at room temperature.

❖ **Methods of Characterisation and Evaluation of Furosemide Microemulsion**

2.3.4 Drug content analysis using RP-HPLC

100µl of the prepared furosemide microemulsion equivalent to 1 mg concentration was diluted in chloroform (compatible solvent) and mixed well using vortex mixer before analysis. The diluted sample of furosemide microemulsion was then filtered and injected after serial standard dilution injections for calibration were collected using RP-HPLC. The sample injection was manual and the separation was carried out at room temperature. A mobile phase of phosphoric acid (0.5% in aqueous) with methanol was pumped under isocratic conditions at a flow rate of 1ml per minute. A 20µl volume of each dilution was injected into the column and the effluent was monitored at 236nm detecting wavelength.

2.3.5 Phase separation study

0.05 ml of furosemide microemulsion was added to 5ml mixture of 0.1N HCL and distilled water mixture in a clear glass test tube. The tube was inverted 4 times up and down then left to observe any phase separation. Thereafter, the sample was monitored visually for any phase separation every hour for 6 -15 hours.

2.3.6 Droplet size determination of furosemide microemulsion

The size distribution of droplets formed in the microemulsion after dispersing the aqueous phase into the oily phase was measured with Zetasizer (Malvern) at 25°C. Zetasizer instrument detects particles and droplets sizes using Dynamic Light Scattering (DLS) of a laser

beam at 633 nm wavelength. DLS measures Brownian motion and relates this to the size of the particles by illuminating the particles with the laser and analysing the intensity fluctuations of the scattered light.

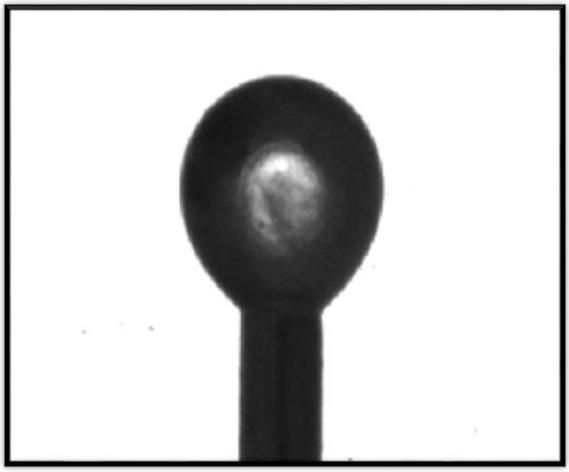
2.3.7 Viscosity determination of furosemide microemulsion

The viscosity of furosemide formulation was determined using HAAKE Viscometer C. Spindle number 3 was used for the test at 100 rpm and the viscosity was measured in centipoise (cP) unit twice (at 0min & 30min).

2.3.8 Dynamic surface tension measurement

This test was conducted in order to evaluate the influence of surfactant on reducing the surface tension formed between the two immiscible phases (water and oil). The surface tension (ST) was determined by Attension Theta Optical Tensiometer at 25°C using hooked needle method by passing aqueous droplet into dispersant filled quartz cuvette as seen in Figure 17.

Figure 17: Droplet shape through hooked needle into dispersant



The examinations were performed on the day of preparation and one week after later.

Water/ MCT Oil Surface Tension

The test was carried out on the aqueous droplet into MCT oil dispersant phase before adding the surfactant to the oily phase in order to determine the formed surface tension value.

Water/ Oil and Surfactant Surface Tension

At this stage, MCT oil was mixed with Labrasol surfactant at the right proportion forming dispersant and the water droplet was inserted into the dispersant phase using hooked needle technique and the surface tension value was measured.

2.3.9 Furosemide microemulsion conductivity measurements

Conductivity measurement was conducted to monitor percolation or phase inversion phenomena of furosemide microemulsion. Percolation is often referred to transition of isolated water droplets to an interconnected bi-continuous structure (phase inversion)[101]. Electrical conductivity "σ" was measured using Jenway 4510 conductivity meter at ambient temperature (228/°C) as a function of water content (H2O %) for MCT oil and S/Co-S mixture. In general, the electrical conductivity of water is very high (7μS) compared to that of MCT oil and labrasol surfactant.

2.3.10 Drug-excipient compatibility studies using ftir

In order to assess the stability of furosemide and evaluate any interaction might occur between the API and microemulsion excipients. Pure furosemide powder and the microemulsion mixture were run using FTIR spectroscopy and the spectra for both samples were collected for compatibility assessment.

2.3.11 Furosemide drug-excipients compatibility studies using NMR

Nuclear magnetic resonance spectroscopy (NMR) was selected as an alternative method to FTIR in order to validate the stability of microemulsion and ensure the compatibility between

furosemide and other microemulsion excipients. The NMR spectrum of furosemide API was obtained after dissolving it in acetone using Bruker NMR. The microemulsion mixture was diluted using acetone as well and the both spectra were collected for analysis purpose.

2.3.12 Visualisation of furosemide microemulsion using light microscopy

A sample of the prepared microemulsion was analysed visually using an Olympus IX81 microscope. The micelles or droplets seemed very well distributed and integrated into the whole texture of the formulation. In order to visualise the aqueous phase dispersion onto the oily phase of furosemide microemulsion, deionised water was again added drop wise to a freshly prepared sample of the microemulsion and the sample was stirred continuously using magnetic stirrer. Straight after, the sample was scanned with microscopy at 20, 40 and 60x.

2.3.13 Temperature stability studies

The objective of the thermodynamic stability test was to evaluate the effect of temperature variation on the furosemide microemulsion formulation. Furosemide microemulsion was centrifuged at 15,000 rpm for 15 minutes and the formulation was then investigated visually for any phase separation. Furosemide microemulsion was also subjected to freeze-thaw cycle (-20°C for 3 days followed by +40°C) for 2 days. The sample was then observed visually to assess the stability of microemulsion to withstand different temperatures and the ability of its spontaneous emulsification property through these temperature cycles.

2.3.14 Palatability testing

Palatability is one of the main important elements that contribute toward patient drug acceptance and compliance. It's defined as the overall appreciation of a medicine towards its smell, taste, aftertaste and texture[102]. Therefore, palatability assessment was a prioritised test for microemulsion. From testing regime, there are two types of tasting drug palatability, *in vivo* and *in vitro* testing. *In vivo* testing required an ethical approval and had many constraints;

therefore it was not performed in this research. Instead, in vitro testing using E-tongue was planned tried.

2.3.15 Shelf life

According to the International Conference on Harmonization (ICH), the purpose of stability testing is to provide evidence on how the quality of an active pharmaceutical ingredient such as furosemide or a pharmaceutical formulation such as microemulsion varies with time under influence of a variety of environmental factors such as temperature, humidity, and light. This is often designed to establish a re-test period for the active pharmaceutical ingredient or a shelf life for the medicinal product and recommended storage conditions.[103] The stability testing and shelf life recommendations of ICH were followed in this test and both Accelerated and long-term shelf life studies were conducted on the furosemide microemulsion formulation.

Stability studies under stress temperature conditions

In regards to the accelerated studies, microemulsion preparations were kept for 0-60 days at temperature 30, 40 and 50°C and the formulation was assayed to quantify the amount of the furosemide and to assess if any degradation occurred under the stress conditions. Serial dilutions from furosemide reference stock solution were prepared and calibration curve was plotted after areas of injected aliquots were recorder. Once calibration was obtained, 20 µl of furosemide microemulsion was injected after diluting it with chloroform and filtering the diluted sample. The chromatograms of each sample exposed to stress conditions were collected and analysed for quantity and purity purposes.

Long term stability studies

Furosemide Microemulsion was left at room temperature in an amber glass container for 12 months and the content was analysed again for stability and degradation studies using HPLC.

2.3.16 Long Term Phase Separation Study

Phase separation and precipitation were inspected after 12 months of furosemide microemulsion preparation in order to evaluate its phase and content stability and dissolvability. A 5ml mixture of 0.1N HCL and distilled water was prepared and 0.05 ml of furosemide microemulsion was added to the mixture in glass test tube. The tube was inverted four times up and down then left to observe any phase separation. The sample was then monitored visually for any phase separation every hour for 6 continuous hours.

2.3.17 Long term viscosity determination of furosemide microemulsion

The viscosity of furosemide microemulsion formulation was measured again after 12 months of preparation and storage on shelf at room temperature. This test was conducted in order to evaluate any texture changes which may have an effect to the formulation viscosity. The measurement was carried out using HAAKE Viscotester C and spindle number 3 was used for the test at 100 rpm.

2.4 Materials Used and Methods Followed in Formulation and Evaluation of Furosemide Oro-Dispersible Mini Tablets

In order to implement the QbD system and based on QTPP, the Critical Quality Attributes (CQAs) of the product was identified as well as the formulation and process parameters that may affect the attributes of furosemide ODMTs dosage form. QTPP is divided into two parts: Target Product Profile (TPP) which embodies the overall *objectives* of safety and efficacy of (furosemide) pharmaceutical development program and Target Product *Quality* Profile (TPQP) which involves the performance attributes and specifications that furosemide ODMT should possess. The QTPP, quality attributes and criticality for furosemide ODMTs listed are in Table 12.

Table 12: QTPP of furosemide ODMT's

Attributes	QTPP		Criticality
	TPP	TPQP	
Drug-excipient compatibility	Stable	No interaction should occur	Compatibility is assured, for example ,by using DSC
Dosage form	Orodispersible Mini-tablet	ODMT (<40seconds), Dissolution (not less than 90% in 20 minutes in pH 6.8 buffer medium)	Ensures complete dispersion, release of furosemide drug, efficacy and ease of administration
Appearance	Uncoated tablets	Immediate-Release (IR) Round tablets	Smooth texture without any cracks
Strength	2mg and 5mg	Identification (positive), Assay (±5%), content uniformity (complies)	content uniformity of analysis as determined by the European Pharmacopeia (*Ph. Eur*)
Route of Administration	Oral	Palatable to paediatrics	Acceptability and palatability to the paediatric population
Proposed indication	Treatment of Low Cardiac Output Syndrome	Dissolution, bioavailability and bioequivalence to any existing products	Ensure therapeutic efficacy
Mechanical Strength	To be determined	Sufficient to ensure good handling stability and short disintegrating time	Ensures good post-compression performance balanced by complete dispersion
Impurity	To be determined	Quality to meet International Conference of Harmonisation (ICH) Q3B and Q6A regulatory guidelines	Safety is assured by controlling any impurity using HPLC after identifying limits of impurity based in qualified toxicological studies.
Packaging	Blister packaging	Enough to prevent environmental humidity and breakage during handling	Stability is assured by HPLC and/or FTIR and visual inspection

After designing the performance objectives and quality attributes, the practical part of formulation was performed based on factorial design at 2 levels and the methods applied in evaluation of ODMTs formulations are as described in the next sections.

Factorial design at two levels

Factor (variable) 1: Formulation Strength (amount per each ODMT):

Level1: (-) 1mg and Level2: (+) 3mg

Factor (variable) 2: lubricant (magnesium stearate) concentration per ODMT:

Level1: (-) 0.3mg % and Level2: (+) 0.6mg %

Factor (variable) 3: Mixing time

Level1: (-) 5min and Level2: (+) 10min

Factor (variable) 4: Compression force

Level1: (-) 18 compression force and Level2: (+) 19 compression force

Note: Manesty F3 (tablet machine) had empirical compression force with no unit.

16 different batch designs or runs were thus developed as shown in Table 13.

Table 13: Factorial design of furosemide ODMTs formulation

Batch Number	1	2	3	4
1	_	_	_	_
2	+	_	_	_
3	_	+	_	_
4	+	+	_	_
5	_	_	+	_
6	+	_	+	_
7	_	+	+	_
8	+	+	+	_
9	_	_	_	+
10	+	_	_	+
11	_	+	_	+
12	+	+	_	+
13	_	_	+	+
14	+	_	+	+
15	_	+	+	+
16	+	+	+	+

2.4.1 Formulation technique followed in furosemide ODMTs preparation

To formulate furosemide ODMTs, direct compression method was followed. Direct compression is considered by far the most common and simple technique that usually involves the use of conventional pressing equipment, commonly available excipients and a limited number of processing steps making it a time saving and cost effective tablet manufacturing technique.

Initially, placebo batch (control batch with no API) was formulated to estimate the average weight of ODM tablet using Ludiflash mixture. Once the weight was evaluated, the excipients (Ludiflash and magnesium stearate) were weighed in the right proportions/ratios according to the established factorial design and mixed thoroughly with the addition of furosemide API. The final powder mixture was then compressed using Manesty F3 single punch machine with custom-made 3mm diameter punch and die station. However, a full pre-compression characterisation was carried out before each formulation in order to evaluate the fluidity and compressibility of the mixed powder before compression. In addition, a Quality by Design approach was applied to pre-define the quality parameters.

2.4.2 Preliminary work to establish process parameters (pre-formulation studies)

2.4.3 Drug-excipient compatibility studies

These studies were conducted to determine any physical and/or chemical interaction that might occur between furosemide and formulation excipients. The compatibility of furosemide with excipients chosen to formulate ODMTs was evaluated using Differential Scanning Calorimetry (DSC-60, Shimadzu, Japan) instrument. DSC is a thermo-analytical technique that measures the difference in heat flow rate (mW = mJ/sec) between a sample and inert reference as a function of time and temperature[104]. Furosemide has a polymorphic form and it exists in two forms usually. A Polymorphism is the ability of solid materials to exist in two or more crystalline forms with different arrangements or conformations of the constituents in the crystal lattice[105]. These polymorphic forms of a furosemide drug differ in the physicochemical properties which have an effect on its stability and compatibility with other chemicals. Using DSC, each excipient was analysed individually then samples of furosemide drug-excipients were prepared in 1:1 ratio and analysed. Individual samples of (drug - excipient) were weighed to 5mg in the DSC aluminium pan. The sample pan was then crimped for effective heat

conduction and scanned in the temperature range of 25-300°C. Heating rate of 20°C/ min was used and the thermogram obtained was analysed for any evidence of any interaction.

2.4.4 Production of a preliminary batch of furosemide ODMTs

This batch included the following parameters developed by aforementioned factorial design at two levels:

Run 1	_	_	_	_

1: Furosemide strength 1mg

2: Magnesium stearate concentration 0.3mg

3: Mixing time 5min

4: Compression force 18N

2.4.5 Powder flowability tests

The fluidity of powder mixtures of furosemide API and excipients is crucial towards a successful ODMT formulation. Poor powder flowability largely influences the tablet characteristics such as: content uniformity, inconsistent hardness, varying in disintegrating time and dissolution rate. There are several tests to characterise powder flowability and compressibility and the most frequently employed methods are the one followed in this research.

- **Angle of repose (ϕ)**

  Angle of repose was determined using funnel method where the blend of the final powder mixture intended for compression was poured through funnel until a maximum

pile height (h) was obtained. Radius of the heap (r) was measured and the angle of repose value was calculated using the formula:

$$\phi = \text{Tan}^{-1} (\text{Height}/ \text{radius})$$

Flow properties as defined by the EU Pharmacopeia are shown in Table 14.

Table 14: Flow properties for angle of repose[106]

Flow Property	Angle of Repose
Excellent	25 - 30
Good	31 - 35
Fair - aid not needed	36 - 40
Passable - may hang up	41 - 45
Poor - must agitate, vibrate	46 - 55
Very poor	56 - 65
Very, very poor	> 66

- **Compressibility index (Hausner's ratio)**

  Hausner's ratio is an indirect index of ease of powder flow. It's applied to measure the bulk density, size and shape, surface area and cohesiveness of materials by determining the flowability of the powder. It is calculated using the ratio: Tapped Density/Bulk Density = P_t / P_d. These parameters were calculates as the following:

  - Bulk density (P_d): apparent bulk density was determined by pouring the powder mixture of ODMT into a graduated cylinder. The bulk volume (Vb) and weight of powder (M) were determined. The bulk density was calculated using the following formula: Pb= M/Vb
  - Tapped density (P_t): the plastic measuring cylinder containing known mass of ODMT blend was tapped to solid surface for 750 taps within a fixed time. The minimum volume Vt occupied in the cylinder and weight (M) of the blend was measured. The tapped density was calculated using Pt= M/ Vt

Table 15: Flow properties for Hausner's ratio

Flow character	Hausner's ratio
Excellent	1.00 - 1.11
Good	1.12 - 1.18
Fair	1.19 - 1.25
Passable	1.26 - 1.34
Poor	1.35 - 1.45
Very poor	1.46 - 1.59
Extremely poor	> 1.60

2.4.6 Post-compression evaluation and characterisation tests

This involved a variety of tests. Each one is described below:

Weight variation evaluation

The purpose of this test is to assure the uniformity of weight among furosemide ODMTs. For that, twenty tablets from the formulated batch were selected randomly and the average weight was determined. Then each individual tablet was weighed and compared with the average weight of tablet to calculate the standard deviation.

Standard deviation for each tablet weight was calculated using the following formula:

Random tablet weight X 100/average tab weight

Tablet dimensions

Thickness determination is usually applied to evaluate the influence of thickness on disintegration time and the uniformity in thickness amongst the same batch tablets. For that, five random tablets from the batch were picked and measured for thickness determination using Mitutoyo Digimatic Caliper (CD-12"C) and the measures obtained were recorded.

Hardness measurement

Tablets must be able to withstand the rigors of handling and transportation experienced in the manufacturing environment, in the drug distribution system and tablet handling by the consumer. Ideally, orally disintegrating tablets have a hardness of 20-30 N.

The hardness test was used to determine crushing strength of furosemide ODMT's. For that, Schleuniger-4M tablet hardness tester was used and 10 tablets were selected randomly and their crushing strengths were recorded.

Friability

Friability test is applied to measure the tendency of furosemide ODMT to chip, crumble or break while handling. This test was determined using Copley Friabilator FR-1000. The device subjects the tablets to the combine effect of abrasion and shock in the plastic chamber revolving at 25 rounds per minute (RPM) for 4 minutes. For this test, ten tableted were selected randomly, weighed and then placed in the friabilator. The tablets were collected after 100 rounds (4min), de-dusted using fine sieve and reweighed. Using the formula W1-W2 x 100/W1, where: (W1) is the weight of 10 tablets before the test and (W2) is the weight of 10 tablets after the test, friability will be calculated. According to Ph. Eur., weight loss of not more than 1% is acceptable for most tablets.

***In vitro* disintegration time**

This test is used to determine the time required for each ODMT to disintegrate into small particles when contacting with an aqueous surface (e.g. saliva). Disintegration testing was performed by placing a tablet on wet tissue saturated with saliva solution. The test was conducted at room temperature and the tablet will pass the test after a full disintegration occurs and it becomes small particles.

Wetting time

Wetting time is relative to the inner structure of tablets and the hydrophilicity of the excipients used in the formulation. This test was conducted to measure the time required for an aqueous phase to penetrate into furosemide ODMT reaching its centre and surface. This was performed by placing a tablet gently into direct contact with wet tissue in petri dish and the aqueous phase was dyed blue in order to help distinguishing once the wetness reaches the tablet surface.

Uniformity of dispersion

This test was obtained by placing 3 random tablets of each batch in 25 ml of water and a gentle stirring was applied until the 3 tablets were completely dispersed. The dispersion was then passed through sieve screen with a nominal mesh aperture of 1mm (BS410/Endoecotts) to check if full dispersion was achieved.

Visual characterisation of batches 1-16

The 16 formulated batches were inspected visually by using high-resolution optical zoom camera.

Disintegration step analysis

This process was performed by placing one furosemide ODMT into an aqueous droplet and the tablet was filmed during the disintegration process which involved wicking and swelling mechanisms then a full dispersion was observed.

Drug content analysis

This test was performed to ensure the uniformity of furosemide API content amongst all formulated ODMTs. Three tablets were selected at random from each batch and dissolved in methanol using 10ml volumetric flask. The mixture was then sonicated for

10 minutes and made up to mark with the addition of deionized water to form 100µg/ml concentration. A stock solution of furosemide pure standard powder was prepared at (1mg/ml) with methanol to obtain serial dilutions. Six serial dilutions (aliquots) were prepared from stock solution and diluted in deionised water ranging from 25 to 150 µg/ml. Once calibration curve obtained, the ODMTs sample were injected and their areas were collected in order to quantify the content of furosemide in each tablets.

Quality Control test of Furosemide Oro-dispersible Mini tablets

For that, the dissolution test was conducted if tablets were swallowed instead of being dispersed on tongue. Dissolution test is the process by which the molecules transform from solid phase state and enter into solution state. This test was performed to analyse by quantifying the proportion of furosemide drug released from furosemide ODMT into a simulated gastric fluid under controlled conditions such as temperature and simulated gastric motility (mixing by paddle). Dissolution test was performed using a *Hanson Research SR8-Plus* Dissolution Test Station, which consisted of paddle with 1-litre glasses. The dissolution medium was 900ml of aqueous solution (pH4) and no dissolution accelerating components were added. The dissolution experiments were conducted at 37ºC ±0.5 ºC at a paddle rate of 50 rpm for 15 minutes. At pre-determined intervals 5ml of gastric medium was sampled at 5, 10 and 15 minutes filtered and injected for analysis using HPLC (20µl).

2.4.7 Analysis of the design of experiments

Using the Design Expert™ software (Stat-Ease Inc., Minneapolis, USA), the main interaction effects of the four factors on the seven response levels (e.g. tablet hardness, friability etc.) were determined by analysing the experiments design.

2.4.8 Furosemide ODMT stability testing

This test was conducted to evaluate the stability, impurity and assay of furosemide ODMTs under ICH conditions (humidity, temperature and light). The stability study was conducted on one batch only (Batch 15) since all batches contained same ingredients. However, before the stability test was performed the furosemide ODMT's were packaged in a container closure system that is very similar to the blister packaging proposed for storage and distribution.

Furosemide ODMTs were packaged using blister packing and each package box contained two tablets of furosemide ODMT's where the packaging was composed of transparent plastic body sealed with an adhesive laminated paper as displayed in figure 18.

Figure 18: Furosemide ODMT Packaging

The stability testing was performed according to ICH conditions so called accelerated stability testing principle at 50ºC ±2ºC temperature and 75±5% relative humidity using an incubator (Sanyo MLR-351H).

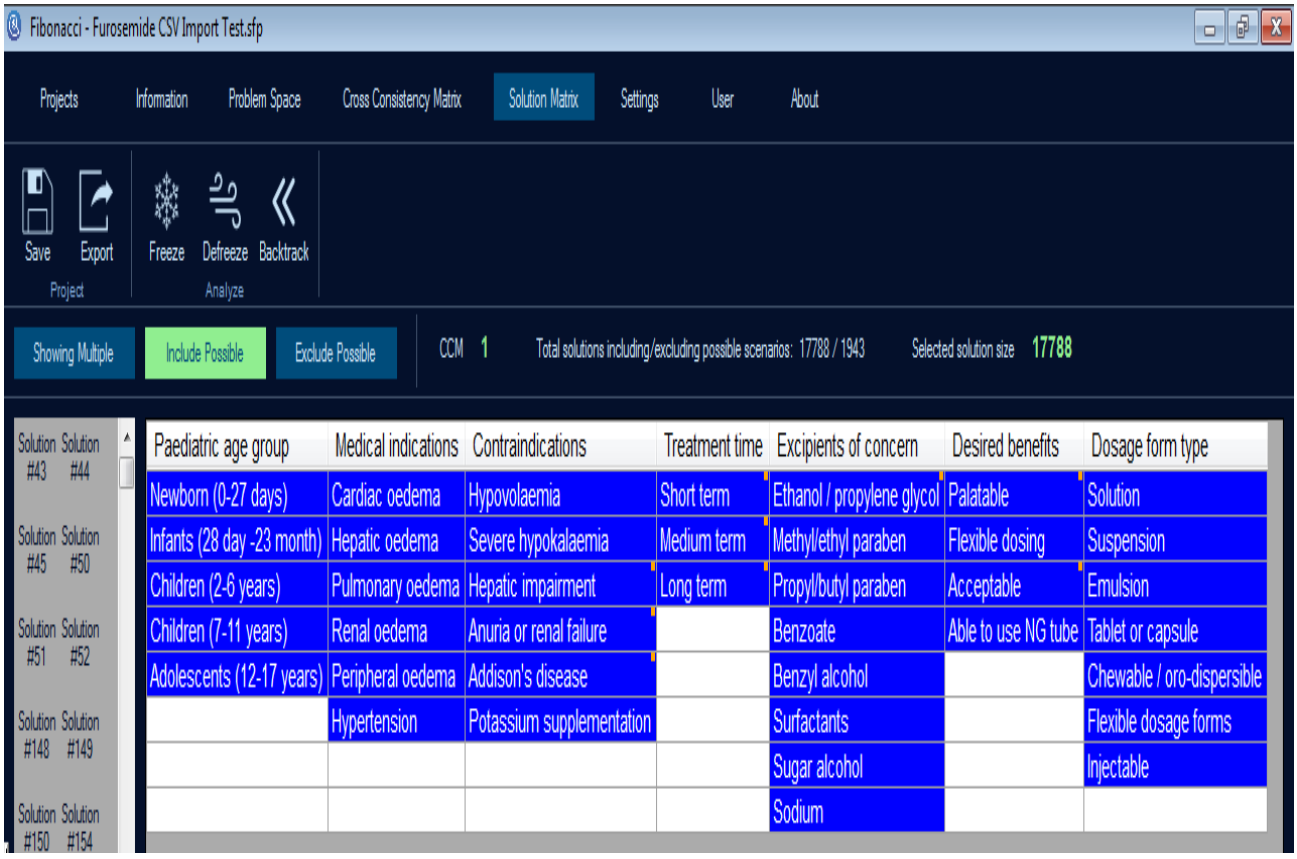
A packaged batch of furosemide ODMTs was left in incubator for 12 weeks then analysed for any degradation using RP-HPLC.

Chapter 3

3.1 Morphological Analysis Results

Based on the cross-consistency assessment, the reduced problem space consists of 17,788 feasible options taking into account of only the logical constraints, which means a reduction of 85% of the problem space was achieved as seen in Figure 19.

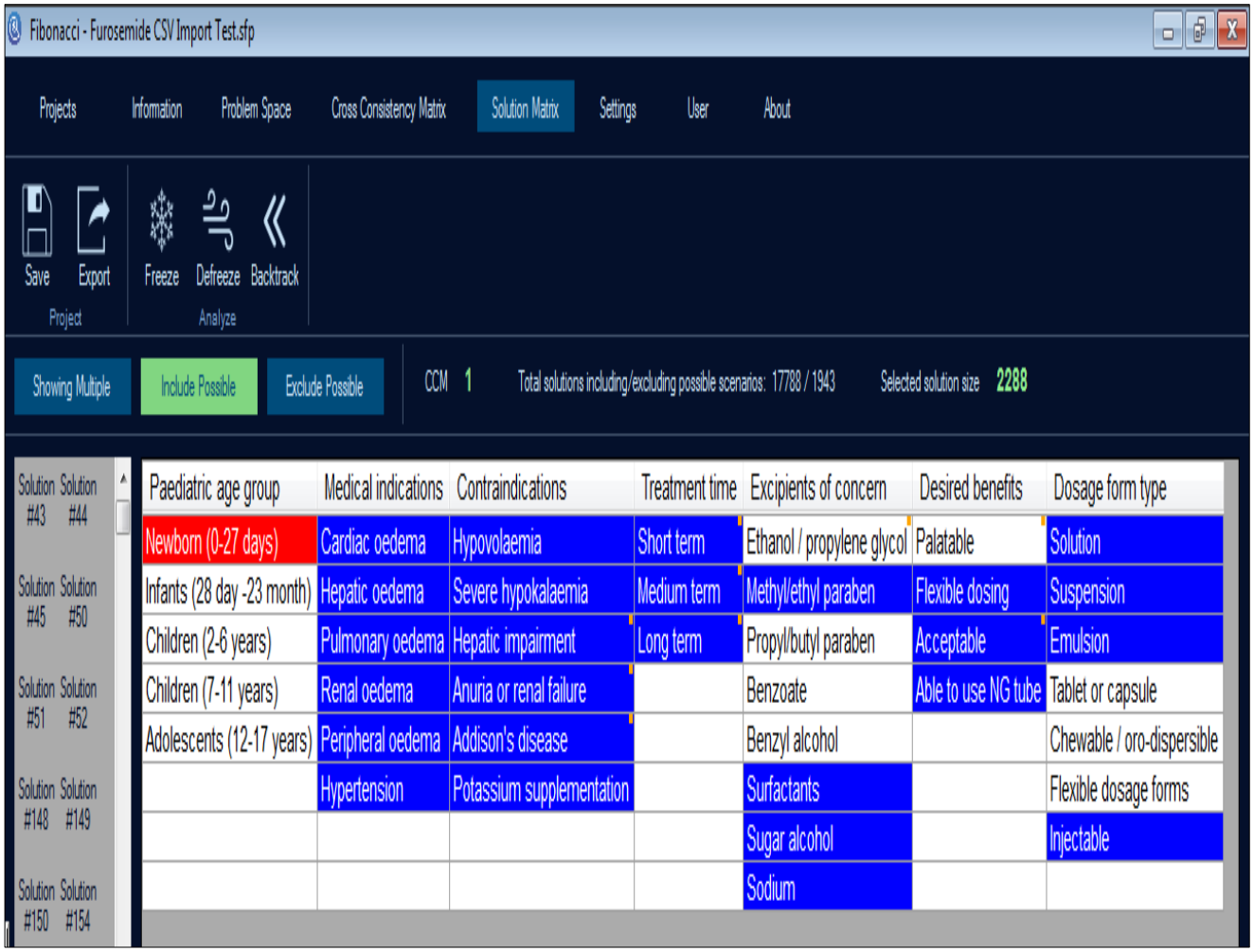
Figure 19: Solution Space showing feasible options listed in the left hand column



From Figure 19, it is seen that for newborn babies, there are 2288 options, including oral solution; suspension, emulsion and injectable dosage form that can be used (taking into account clinical indications, contraindications, practice considerations as assessed by the CCA matrix). Oral liquids (unlike solid dosage forms such as tablets and capsules) do give concerns to palatability as constituents of the formula can diffuse easily to taste receptors. For

injectable, palatability is clearly not a concern as this dosage form is not given orally. The blue shaded cells represent what is compatible with the red input cell. Thus, certain excipients should ideally not be used in newborns such as ethanol or propylene glycol (CNS depressants), propyl or butyl hydroxybenzoates as preservatives (oestrogenic effects) and sodium benzoate or benzoic acid and benzyl alcohol also as preservatives (now contraindicated according to the EMA). [107]

Figure 20: Display field showing output (solutions) in blue for given input condition in red



By using this interactive and dynamic decision support matrix, the numbers of options that are possible for each paediatric age group and pharmaceutical dosage form are shown in Table 16.

Table 16: Potential oral dosage forms for different paediatric age groups as informed by morphological analysis

Pharmaceutical dosage form							
Paediatric age group	Solution	Suspension	Emulsion	Tablet or capsule	Chewable or orodispersible	Flexible dosage forms	Injectable
Neonates	774	774	540	0	0	0	200
Infants	904	1058	670	0	0	0	200
Young children	1240	1442	826	312	624	468	240
Older children	813	1080	477	312	468	468	140
Adolescents	813	1080	477	312	468	468	140

The greatest need for age-appropriate dosage form for furosemide is for neonates, infants and young children. Using MA, this decision support tool suggests that that a suspension could cover all three age groups taking into account the uncertainties, as assessed in the CCA matrix. However it was decided to concentrate the development on the following two dosage forms:

- Emulsion: furosemide is a poorly-water soluble drug that can be made as a suspension. However nasogastric tube administration, often needed in neonates with LCOS, can be a problem due to blockage from suspension particulates and complex viscosity patterns of suspensions through narrow-bore tubes. In regards to solution, it's known not to form any risk on nasogastric tube. However, solutions do not enhance solubility unless a solvent such as ethanol is added which is banned for infant. Emulsion instead can enhance the solubility of poor water-soluble molecule such as furosemide if surfactants are used and some of these surfactants are safe to use in infants. In addition, there is a paucity of

current research in the science of emulsion dosage form design despite the development of novel emulsifiers. As such, this gave an opportunity to explore this avenue of research.

- Oro-dispersible mini-tablets (ODMT): these are flexible dosage forms in that 2 - 4 mm tablets can be made incorporating lower doses of furosemide (or any other APIs). Contemporary research[108] indicates that young children are able to use ODMT thus affording dose titration and achieving the correct maintenance dose without resorting to breaking large adult strength tablets. By using acceptable excipients such as mannitol, these mini-tablets can afford rapid disintegration in the mouth, further minimising problems of choking. For parents and caregivers, there is the additional advantages of portability and spitting difficulty compared to emulsions and better long-term stability. Therefore ODMTs were selected as a second solution space to be investigated practically for viability.

3.2 Discussion

According to previous published literature, neither MA nor any other forms of PSMs have been used in drug development or developing pharmaceutical formulations, let alone in the design of paediatric medicines. However, there have been few applications of PSMs in improving healthcare services such as the development of modelling method selection tool for health services management[109], agreeing a local paediatric care strategy[110]. Nevertheless, Parallel Coordinate Geometry (PCG), which shares some similarities in principle with MA, has been employed in in the design of pharmaceutical formulations. PCG is a graphical methodology that enables multidimensional data to be displayed in two dimensions. There is a paucity of research published in literature using PCG with the principal paper entitled 'Graphical representation of formulation data for analysis and optimisation'[111] being one of the few articles describing the process in any great detail. The critical difference of PCG compared to MA is that the former uses quantifiable data whereas the latter is capable of incorporating qualitative (i.e. descriptive) data. Additionally, PCG creates a factorial design and performs it

prescriptively. However, MA is a prospective method in that it creates a theoretical framework by analysing the problem and synthesising via the CCA process to yield a solution space for experimental testing.

MA by offering the ability to cover any number of dimensions (variables) of a complex problem and by allowing the inclusion of qualitative and quantitative data created the most optimal decisions, resulting in the selection of a microemulsion and an orodispersible mini-tablet dosage form (ODMT). Unfortunately, with MA some limitations were encountered. For example, each subject matter specialist had to be consulted individually rather than the preferred group format approach. This was principally due to the fact that a number of workshops session are needed (3-4 days), making it difficult to convene such specialists. The only way to collect and assess data was by interviewing each stakeholder individually and this posed a limitation on the scope of action and influenced the synthetic phase of MA including i.e. CCA. It is hoped that future work can explore the use of technology like real-time decision conferencing to elicit a group-based problems structuring approach.

Chapter 4

4. Furosemide Microemulsion Formulation Results

4.1 Acceptable Daily Intake Values for Microemulsion Excipients

4.1.1 Transcutol-HP

The average weight for paediatric age groups varies and so does the ADI value. Therefore, the ADI was calculated for Transcutol-HP and other excipients after evaluating the maximum daily dose of furosemide for each age group and their average weight as shown in Table 17.

Table 17: Calculated dose for Transcutol-HP in different age groups

Age	Weight (KG)	Max daily dose by mouth (BNF-c)	Theoretical max daily dose (mg)	Actual Max dose (BNF-c)/ mg	Furosemide Microemulsion 10mg/ ml	Transcutol-HP limit mg (ADI= 10mg/kg/day)	Transcutol-HP (mg) from furosemide Microemulsion
1m	3.5	12mg/kg	42	42	4.2	**35**	**0.84**
3m	6.1	12mg/kg	73.2	73.2	7.32	**61**	**1.464**
6m	7.6	12mg/kg	91.2	80	8	**76**	**1.6**
1y	9	12mg/kg	108	80	8	**90**	**1.6**
3y	14	12mg/kg	168	80	8	**140**	**1.6**
5y	18	12mg/kg	216	80	8	**180**	**1.6**
7y	23	12mg/kg	276	80	8	**230**	**1.6**
10y	32	12mg/kg	384	80	8	**320**	**1.6**
12y	39	12mg/kg	468	80	8	**390**	**1.6**
14y	49	120mg	120	120	12	**490**	**2.4**
18y F	58	120mg	120	120	12	**580**	**2.4**
18y M	68	120mg	120	120	12	**680**	**2.4**

It can be concluded that the maximum dose administered of furosemide microemulsion gives 2.4mg of Transcutol-HP/ day which is below the ADI (10mg/kg/day). Thus, the safe administration of Transcutol-HP and its maximum dose falls within the ADI acceptable values.

4.1.2 Labrasol

Labrasol can be administered safely to humans by oral route at dose levels up to 1800mg/ day. Based on that, the labrasol limits of administration were calculated, shown in Table 18.

Table 18: Calculated dose for labrasol in different age groups

Age	Weight (KG)	Max daily dose by mouth (BNF-c)	Theoretical max daily dose (mg)	Actual Max dose (BNF-c)/ mg	Furosemide Microemulsion 10mg/ ml	Labrasol limit (mg) ADI(= 30mg/kg/day)	Labrasol (mg) from furosemide Microemulsion
1m	3.5	12mg/kg	42	42	4.2	**105**	**2.52**
3m	6.1	12mg/kg	73.2	73.2	7.32	**183**	**4.392**
6m	7.6	12mg/kg	91.2	80	8	**228**	**4.8**
1y	9	12mg/kg	108	80	8	**270**	**4.8**
3y	14	12mg/kg	168	80	8	**420**	**4.8**
5y	18	12mg/kg	216	80	8	**540**	**4.8**
7y	23	12mg/kg	276	80	8	**690**	**4.8**
10y	32	12mg/kg	384	80	8	**960**	**4.8**
12y	39	12mg/kg	468	80	8	**1170**	**4.8**
14y	49	120mg	120	120	12	**1470**	**7.2**
18y F	58	120mg	120	120	12	**1740**	**7.2**
18y F	68	120mg	120	120	12	**2040**	**7.2**

By comparing the date obtained from ADI which indicates the Labrasol limits/kg/day to the maximum Labrasol administered from the formulated furosemide microemulsion, it can be clearly observed that the maximum amount of Labrasol administered falls within the safe limits of the acceptable daily intake values and hence forms no harm being used in furosemide microemulsion formulation.

4.1.3 Labrafac (Medium Chain Triglycerides)

Labrafac has no toxicological effects reported and is considered safe. Furthermore, it can be administered safely to humans. Thus, the amount of Labrafac used in doses of furosemide microemulsion is calculated as shown in Table 19.

Table 19: Calculated dose for Labrafac in different age groups

Age	Weight (KG)	Max daily oral dose	Theoretical max daily dose (mg)	Actual Max dose (BNF-c)/ mg	Furosemide Microemulsion 10mg/ ml	Labrasol limit (mg) ADI(= 30mg/kg/day)	Labrasol (mg) from furosemide Microemulsion
1m	3.5	12mg/kg	42	42	4.2	**Not Limited**	**0.588**
3m	6.1	12mg/kg	73.2	73.2	7.32	**Not Limited**	**1.0248**
6m	7.6	12mg/kg	91.2	80	8	**Not Limited**	**1.12**
1y	9	12mg/kg	108	80	8	**Not Limited**	**1.12**
3y	14	12mg/kg	168	80	8	**Not Limited**	**1.12**
5y	18	12mg/kg	216	80	8	**Not Limited**	**1.12**
7y	23	12mg/kg	276	80	8	**Not Limited**	**1.12**
10y	32	12mg/kg	384	80	8	**Not Limited**	**1.12**
12y	39	12mg/kg	468	80	8	**Not Limited**	**1.12**
14y	49	120mg	120	120	12	**Not Limited**	**1.68**
18y (F)	58	120mg	120	120	12	**Not Limited**	**1.68**
18y (M)	68	120mg	120	120	12	**Not Limited**	**1.68**

To conclude, all furosemide microemulsion excipients used in the proposed formulation were within their respective ADI limits. This assures the administration's safety aspect of the furosemide microemulsion and justifies its formulation and development.

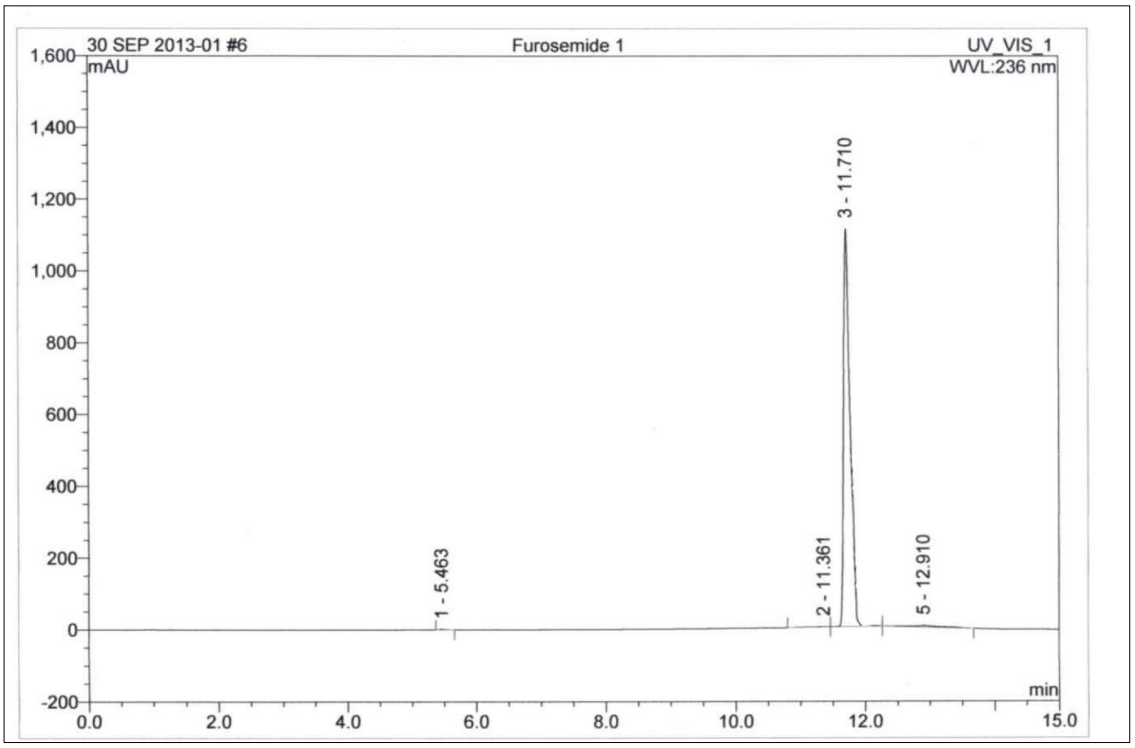
4.2 HPLC Method development Results

The method developed consisted of the mobile phase of H_3PO_4 (0.5% aqueous) with pH 3.2 and methanol under gradient conditions. Under these conditions a clear peak was observed with elution time achieved at 11.71 minutes.

4.2.1 HPLC Injections Results

After injecting the serial dilutions standards, chromatograms were obtained and the data (areas) were collected. Once the calibration was achieved, diluted labrasol and labrafil filtrates were injected separately and their chromatograms were obtained. Figure 21 represents a typical chromatogram obtained from standard furosemide sample injected into HPLC.

Figure 21: The typical chromatogram representing the standard furosemide



4.2.2 Linearity Results

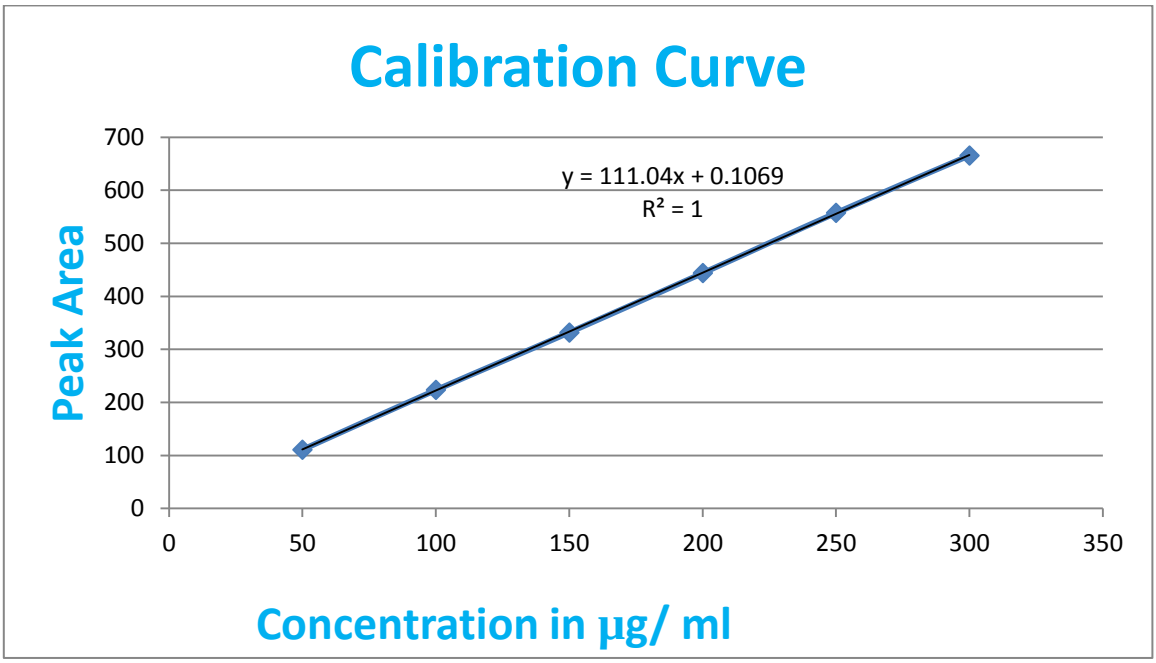
Serial concentrations of furosemide API were used to measure the linearity plot and areas were recorded as shown in Table 20.

Table 20: Areas recorded for aliquots using HPLC

Concentration		Area
1	50 µg/ml	110.941
2	100 µg/ml	223.306
3	150 µg/ml	331.856
4	200 µg/ml	443.686
5	250 µg/ml	557.229
6	300 µg/ml	665.52

The plot of peak areas responses against concentrations of furosemide created the calibration curve with excellent linearity (figure 22).

Figure 22: Linearity plot for furosemide drug substance



4.2.3 Concentration calculation of furosemide in the surfactants

- Area of furosemide in Labrasol surfactant was 4998.827 units

Concentration found = 45.02 mg/ml

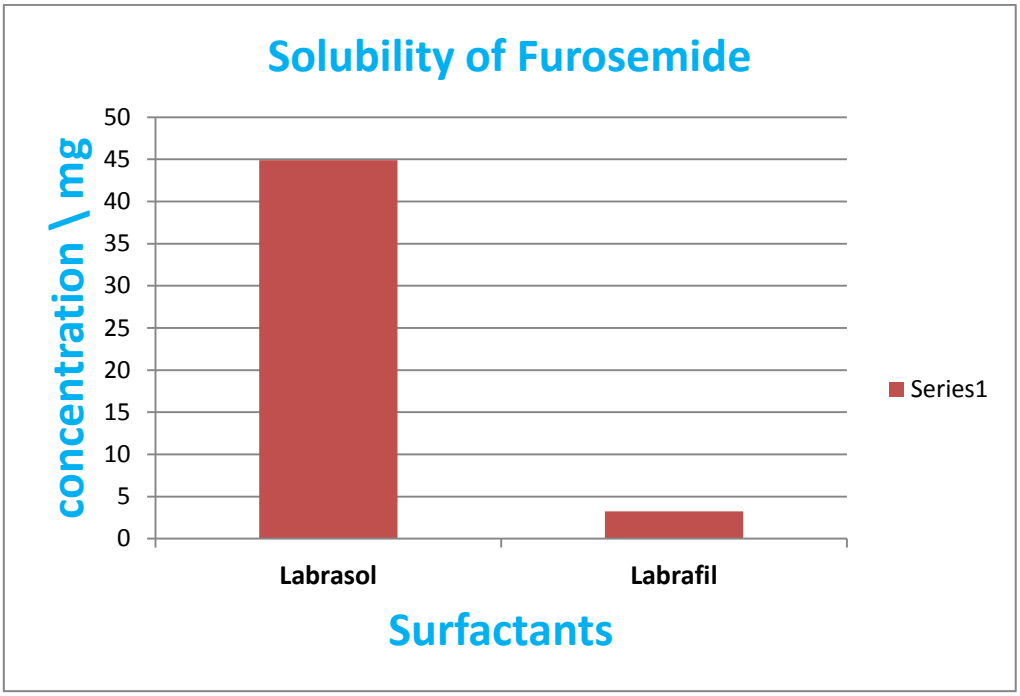
- Area of furosemide in Labrafil surfactant was 371.5 units

Concentration found = 3.34 mg/ml

4.2.4 Solubility study

According to the data obtained from analysing both surfactants and quantifying the amount of dissolved furosemide using HPLC, furosemide showed far higher solubility rate in labrasol surfactant than labrafil (Figure 23). Hence labrasol was selected to formulate the microemulsion.

Figure 23: The solubility of furosemide in Labrasol and Labrafil Surfactants



4.2.5 Phase diagram construction of Furosemide microemulsion

A series of microemulsions were formed, prepared and their self-emulsifying properties were observed visually as shown in Figures 24, 25 and 26.

Figure 24: Pseudo-ternary phase diagram indicating the efficient self-emulsification region at [S/Co-S= 1:1(v/v)]

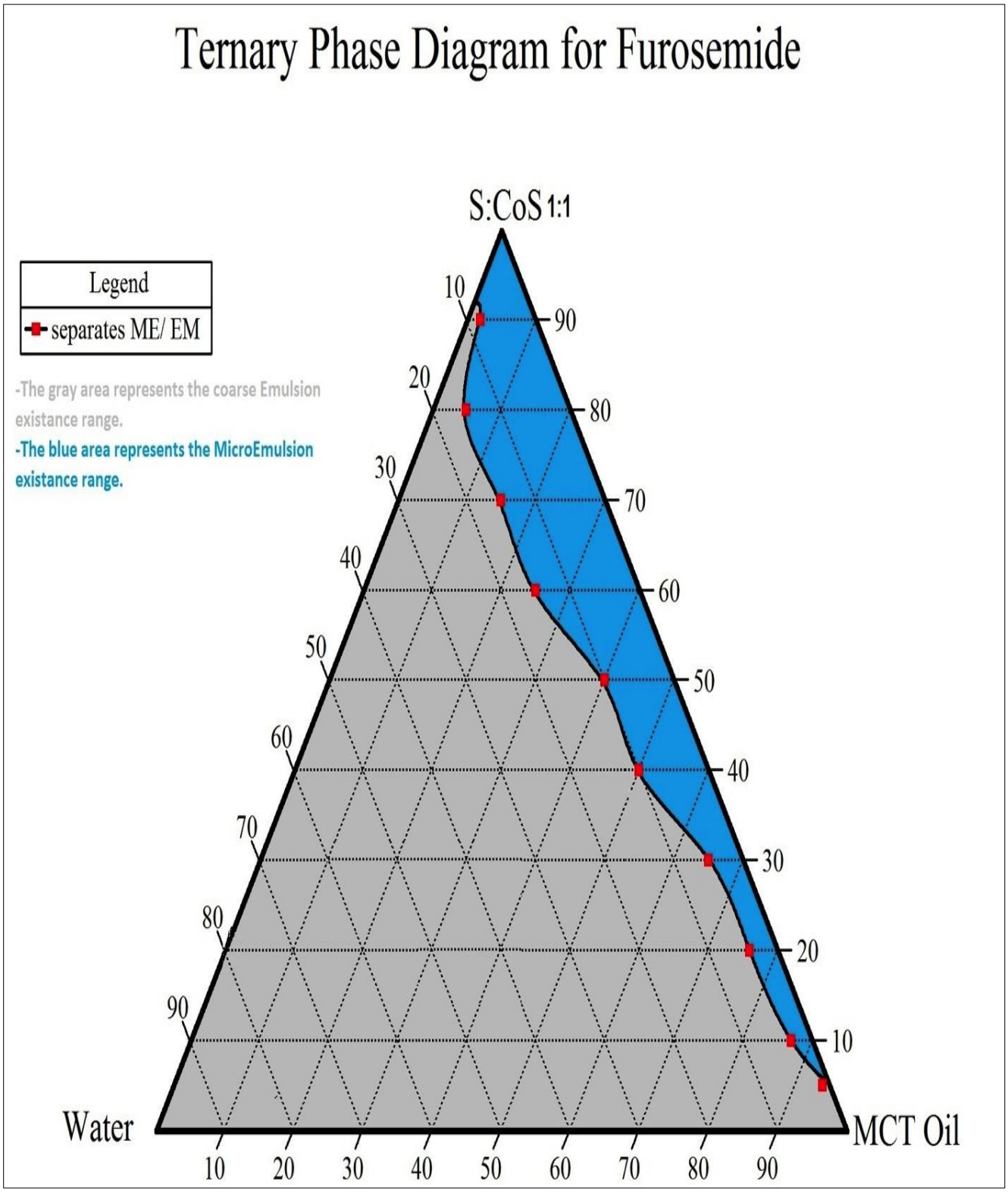


Figure 25: Pseudo-ternary phase diagram indicating the efficient self-emulsification region at [S/Co-S= 2:1(v/v)]

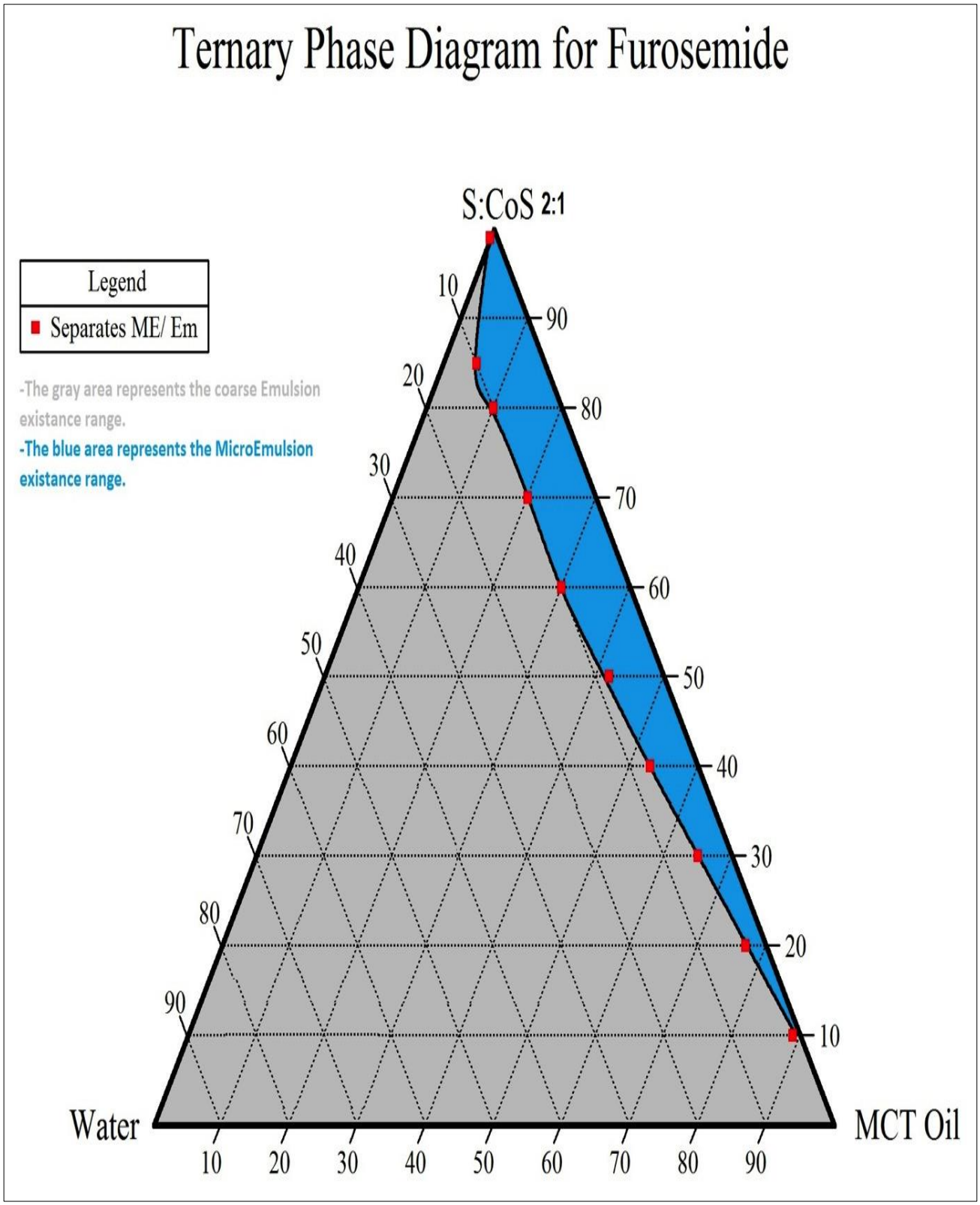
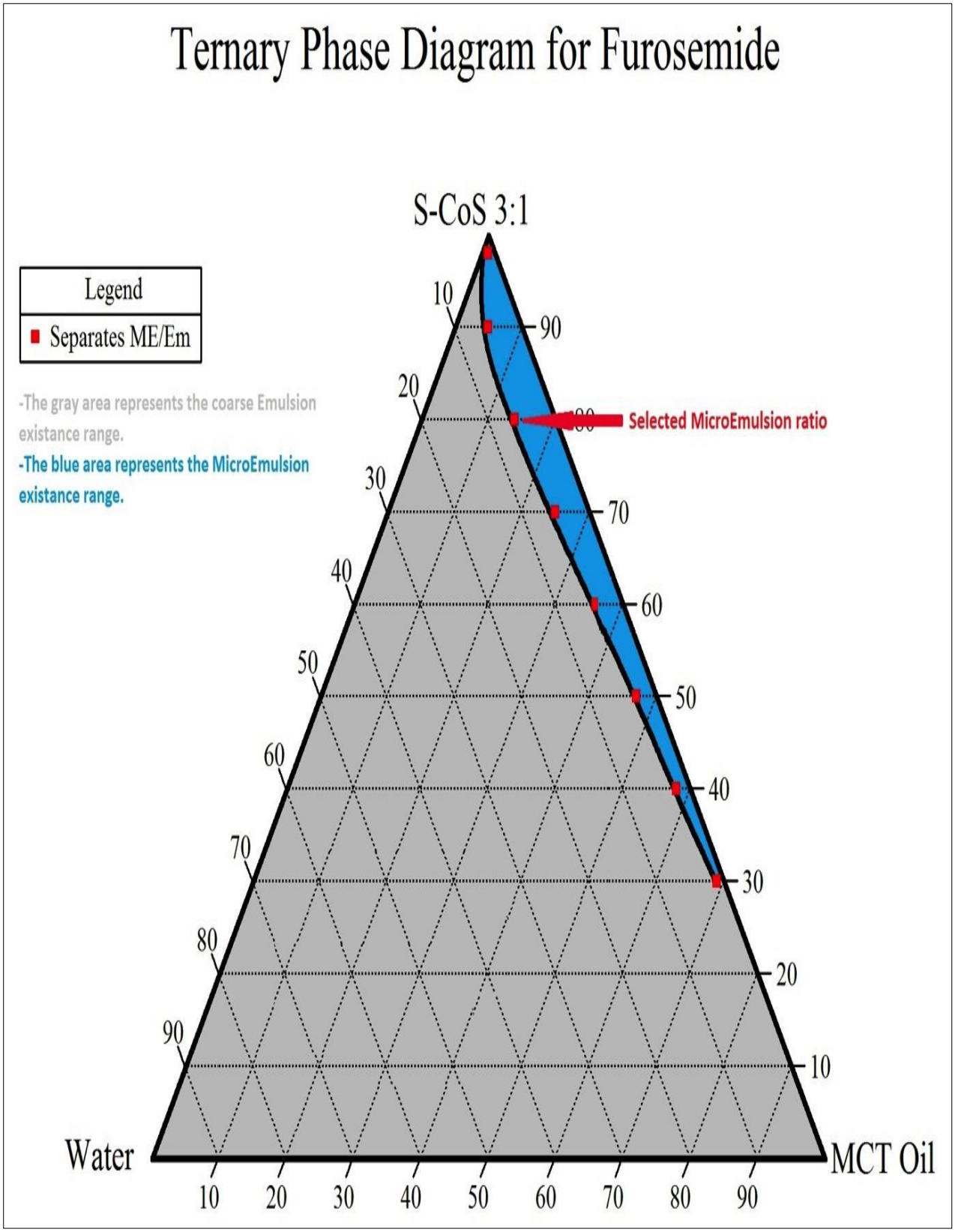


Figure 26: Pseudo-ternary phase diagram indicating the efficient self-emulsification region at [S/Co-S= 3:1(v/v)]



It was observed that the efficiency of emulsification improved when the proportion of Labrasol: Transcutol-HP S/Co-S concentration contained at least 30% of the total composition. In addition, it was noticed that increasing the concentration of Transcutol-HP co-surfactant in the composition increased the spontaneity of self-emulsification region. Therefore, the higher concentration of Transcutol-HP co-surfactant S/Co-S= 1:1(v/v), the larger is the self-emulsifying region in phase diagrams (Figure 24). However, the stability of the self-emulsifying formulations with the ratios of S/Co-S= 2:1 and S/Co-S= 1:1 was decreased because of a slight precipitation observed visually after 24 hours. This could be due to the dilution of labrasol surfactant which showed an optimum solubilising ability to furosemide API. Meaning, the higher the labrasol concentration, the greater is the solubility and hence is the stability. Thus, the ratio of Labrasol:Transcutol-HP S/Co-S 3:1 was chosen for the composition of furosemide microemulsion as shown by the red arrow in Figure 26.

4.2.6 Furosemide Microemulsions Compositions

The series of furosemide microemulsion formulations obtained were prepared as shown in Table 21 and each concentration ratio was given a formulation code (FC).

Table 21: Composition of furosemide microemulsions

Formulation Code (FC)	Medium Chain Triglycerides (MCT) %	Water	(S/Co-S) Labrasol/ Transcutol-HP	Furosemide concentration mg/ ml	Visual Observation	Inference
FC 1	5%	5%	90%	10mg/ ml	Transparent	Stable
FC 2	14%	6%	80%	10mg/ ml	Transparent	Stable
FC 3	25%	5%	70%	10mg/ ml	Transparent	Stable
FC 4	36%	4%	60%	10mg/ ml	Transparent	Stable
FC 5	47%	3%	50%	10mg/ ml	Transparent	Stable
FC 6	58%	2%	40%	10mg/ ml	Transparent	Stable
FC 7	69%	1%	30%	10mg/ ml	Transparent	Stable

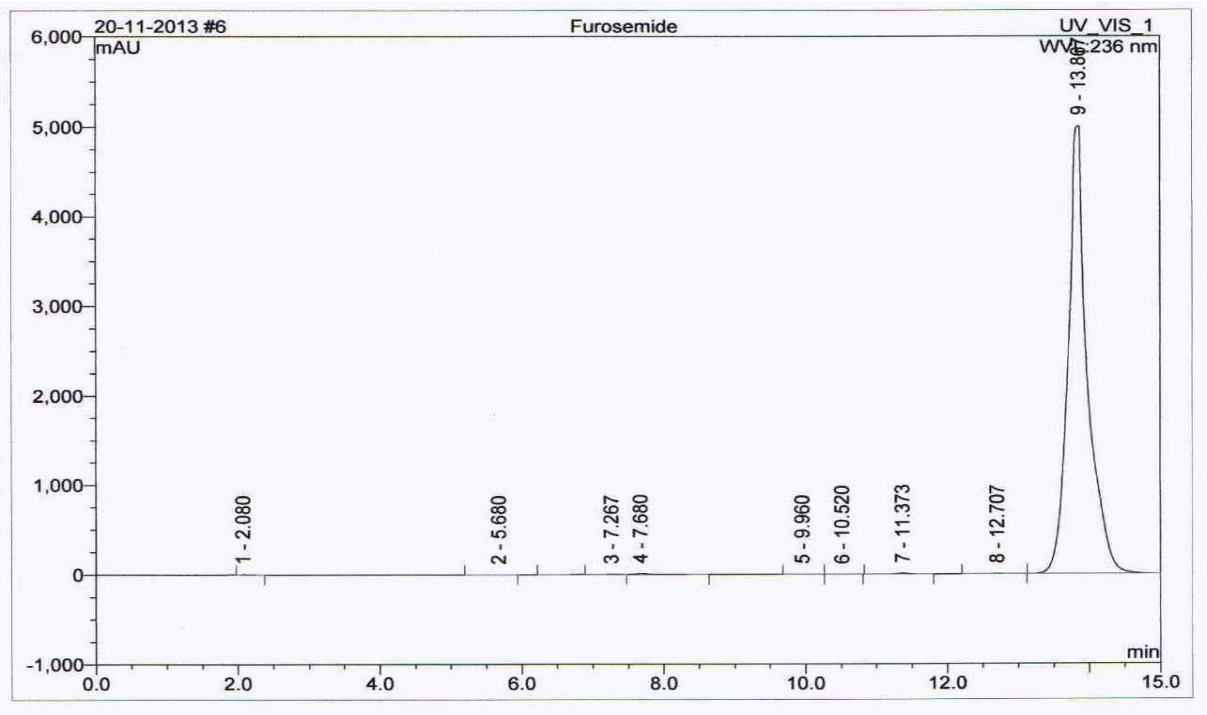
Although, all above formulations showed good physical stability, the second formulation (FC 2) was chosen, containing MCT Oil 14%, Labrasol 60%, Transcutol-HP 20% and Water 6% for further testing.

❖ **Characterisation and Evaluation of Furosemide Microemulsion**

4.2.7 Drug content analysis using RP-HPLC

The drug content in furosemide microemulsion sample (60µl) was found to be 597.8µg. The percentage of furosemide drug content was calculated to be 99.6%, which is considered acceptable according to Drug Content Acceptance Value (DCAV) of the British Pharmacopeia (BP)[112]. A typical chromatogram is shown in Figure 27 representing an injected furosemide microemulsion sample.

Figure 27: Chromatogram of furosemide microemulsion



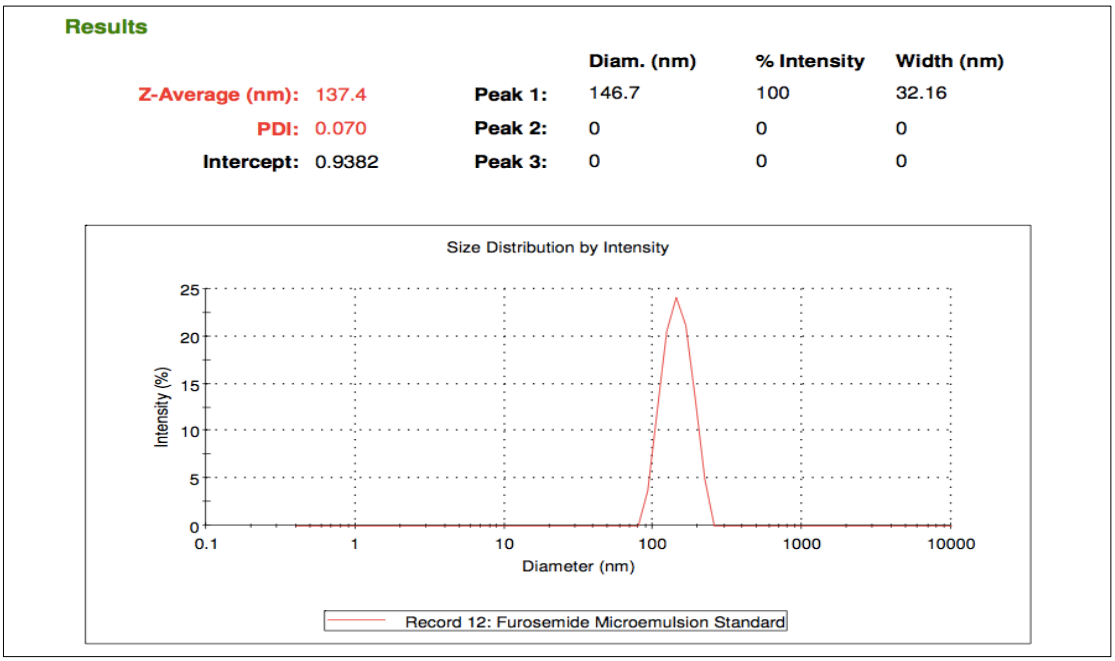
4.2.8 Phase separation study

There was no sign of precipitation observed for the whole period of monitoring and the sample remained clear and stable throughout the test indicating good phase stability with no sign of separation or precipitation.

4.2.9 Droplet size determination of furosemide microemulsion

The droplet size measured was found to be 137.4nm with a low polydispersity index (PDI) of 0.07 as seen the Figure 28.

Figure 28: Droplet size determination using dynamic light scattering



4.2.10 Viscosity determination of furosemide microemulsion

The viscosity results are shown in Table 22.

Table 22: Viscosity determination of furosemide microemulsion

1) Furosemide microemulsion formulation viscosity measurement at 1 minute:	**61.0 cP**
2) Furosemide microemulsion formulation viscosity after 30 minutes from first reading:	**61.1 cP**

4.2.11 Dynamic Surface Tension Measurement

The examinations were performed on the day of preparation and one week after later. Results obtained at different times showed same values and the data were recorded.

Water/ MCT oil surface tension

The surface tension measured of W/O was 86.3 - 88.3 mN/m as presented in Table 23.

Table 23: Water/ MCT oil surface tension reading

Detailed results		
Time [s]	ST [mN/m]	Volume [ul]
0.00	87.44	27.04
0.08		
0.17	87.62	27.04
0.25	87.60	27.04
0.33	88.27	27.01
0.42		
0.50	87.91	27.01
0.58		
8.08	87.26	27.05
8.17	86.48	27.10
8.25	86.83	27.06
8.34	87.47	27.06
8.42	87.60	27.11
9.58	86.80	27.07
9.66	86.03	27.11
9.75	86.51	27.07
9.84	86.70	27.12
9.91	87.93	27.10
10.00	86.88	27.07

Water/ Oil and surfactant surface tension

The surface tension value of W/O+surfactant dropped as expected and was found to be 30.2 – 31.0 mN/m as shown in Table 24.

Table 24: Water/ Oil and Surfactant surface tension reading

Detailed results		
Time [s]	ST [mN/m]	Volume [ul]
0.00	30.92	2.15
0.08	30.92	2.15
0.17	30.95	2.15
0.26	30.95	2.15
0.35	30.88	2.15
0.42	30.96	2.15
0.50	30.91	2.17
0.58	30.84	2.15
0.67	30.96	2.15
0.75	30.91	2.14
0.83	30.89	2.15
0.92	30.95	2.17
1.00	30.85	2.15
1.08	30.80	2.15
8.08	30.56	2.14
8.16	30.43	2.14
8.25	30.49	2.14
8.33	30.41	2.14
8.41	30.23	2.14
8.50	30.38	2.14
8.58	30.45	2.14
8.66	30.47	2.14
8.75	30.49	2.14
8.85	30.80	2.14
8.93	30.44	2.14
9.00	30.59	2.14
9.08	30.21	2.14
9.16	30.68	2.17
9.25	30.53	2.14
9.33	30.58	2.14
9.42	30.54	2.14
9.50	30.52	2.14
9.58	30.55	2.14
9.67	30.60	2.14
9.75	30.47	2.14
9.83	30.47	2.13
9.91	30.41	2.13
10.00	30.55	2.14

By comparing both surface tension values, Labrasol showed great reduction to surface tension of w/o emulsion from 88.27 to 30.21 mN/m which confirmed the important role labrasol plays as an interfacial surface tension reducer. This is expected to enhance the stability of the microemulsion preventing phase separation.

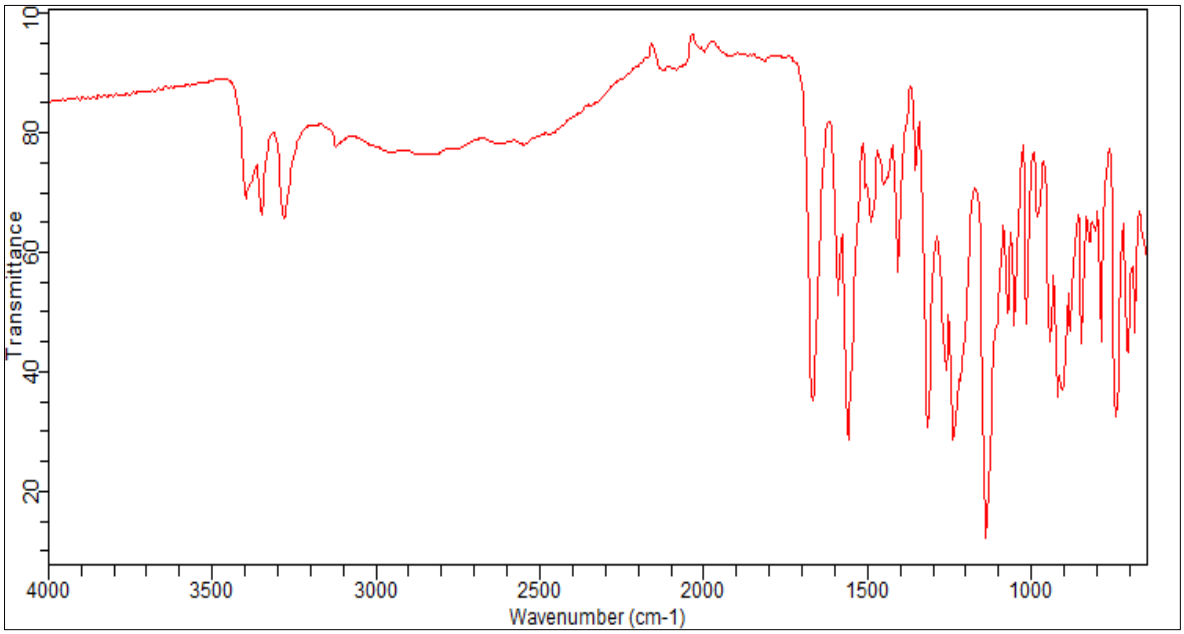
4.2.12 Furosemide microemulsion conductivity measurements

The conductivity of furosemide microemulsion was found with 0.53µS value and stayed the same after similar trials which confirmed the stability of microemulsion with no phase inversion observed.

4.2.13 Drug-excipient compatibility studies using FTIR

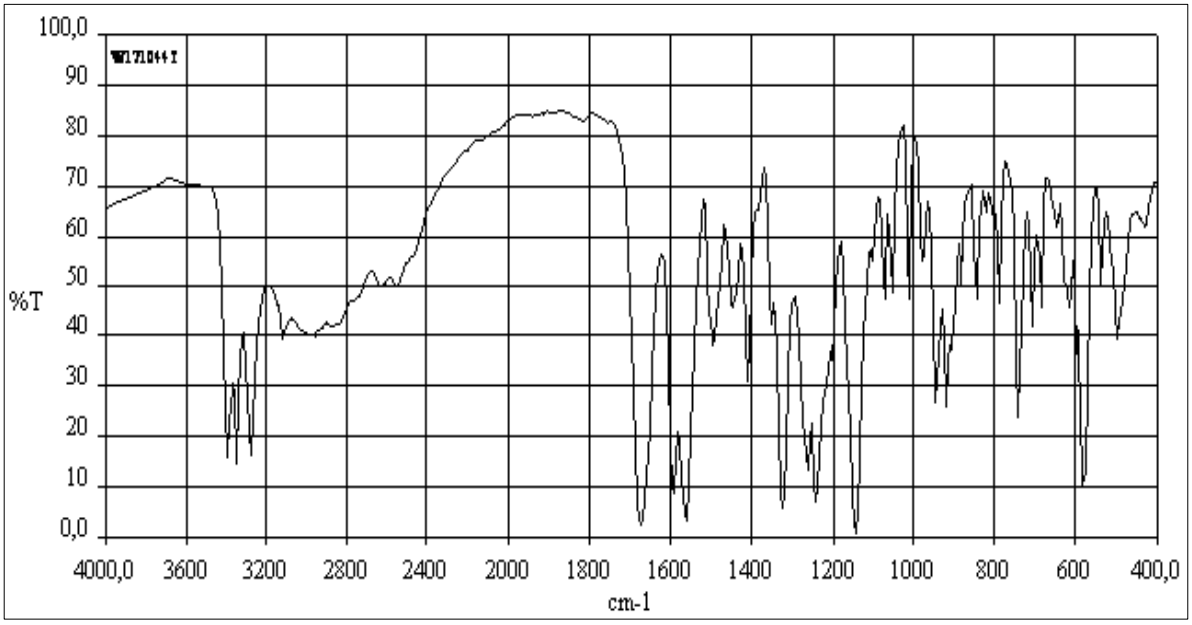
The FTIR spectrum of furosemide was obtained from a solid phase state using Agilent FTIR spectroscopy as shown in Figure 29.

Figure 29: Furosemide standard powder FTIR spectrum



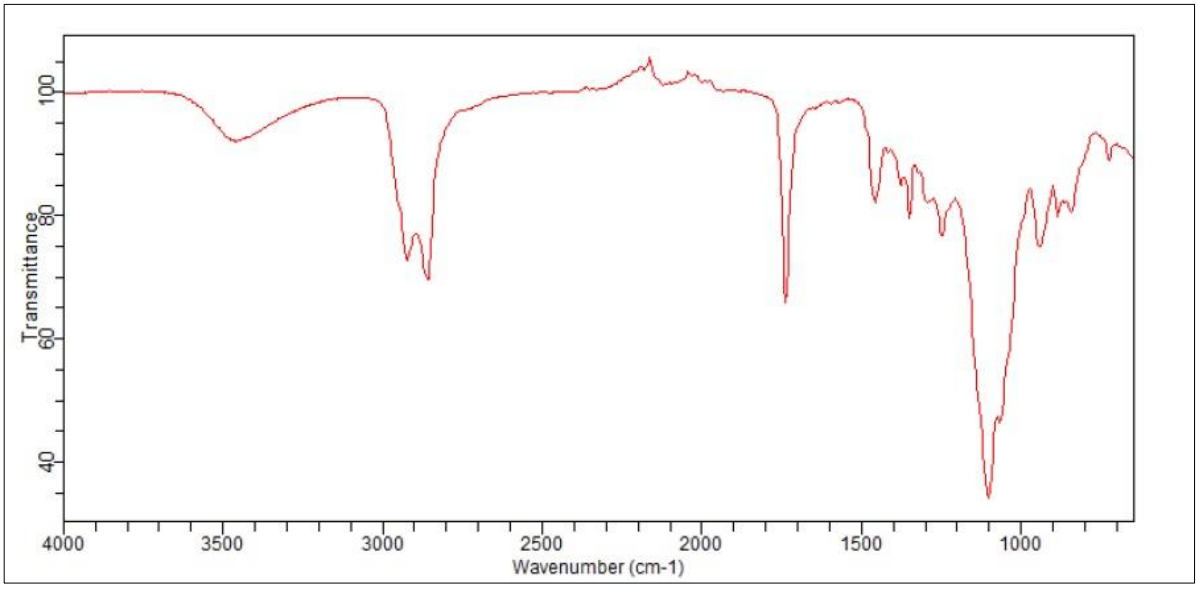
The spectrum obtained in Figure 29 shows well-defined bands with relatively high intensity in the 1700-700 cm^{-1} wavenumber range. A comparison of the furosemide FTIR spectrum versus furosemide reference spectrum (Figure 30) from the International Pharmacopeia reference showed a similar frequency correlation.

Figure 30: Furosemide reference spectrum (the international pharmacopeia) [113]



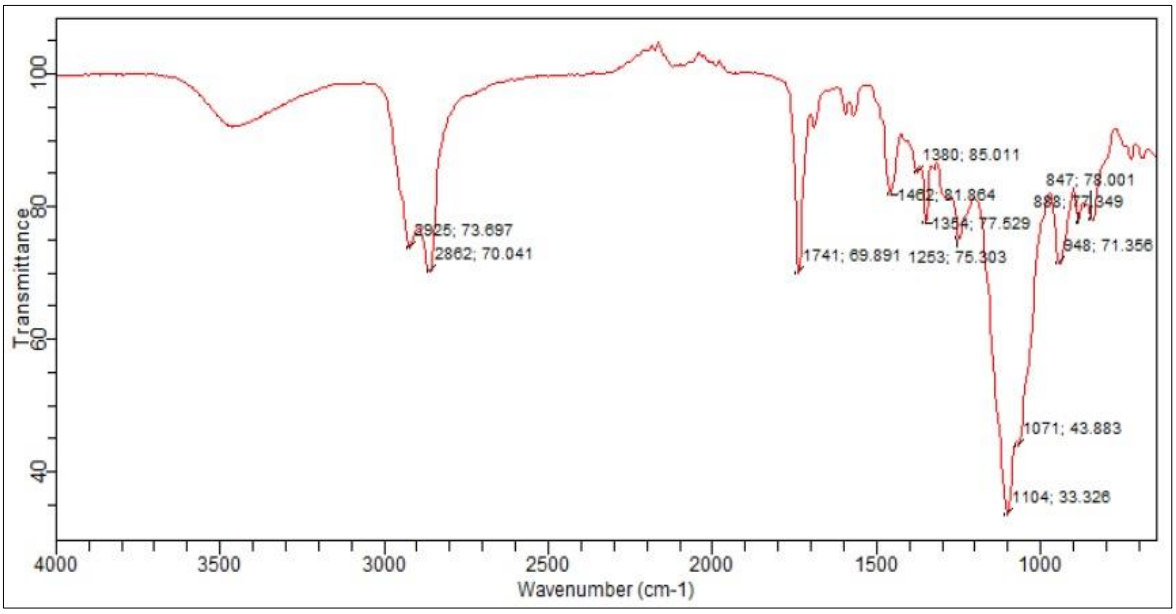
The chemical sulphur group gives specificity to the furosemide chemical structure. Indeed, SO_2 and SO sulphur compound groups produced strong IR bands in the 1400-1100cm^{-1} with two main wavelengths at 1141 and 1320 cm^{-1}. Amine shows stretching band for NH and NH_2 in the range of 3200 and 3400cm^{-1} with three main peaks at 3398, 3350 and 3283cm^{-1}. The C=O peak was observed at 1670cm^{-1} and the OH band was in the range of 2400-3150cm^{-1}. The FTIR spectrum of microemulsion was then collected as shown in Figure 31.

Figure 31: FTIR spectrum of furosemide microemulsion



Unfortunately, the obtained microemulsion spectrum did not show the furosemide characteristic peaks in order to identify them and hence the furosemide. Placebo microemulsion (drug-free formulation) was analysed used FTIR to compare it to furosemide microemulsion IR spectrum (Figure 32).

Figure 32: Placebo microemulsion FTIR Spectrum

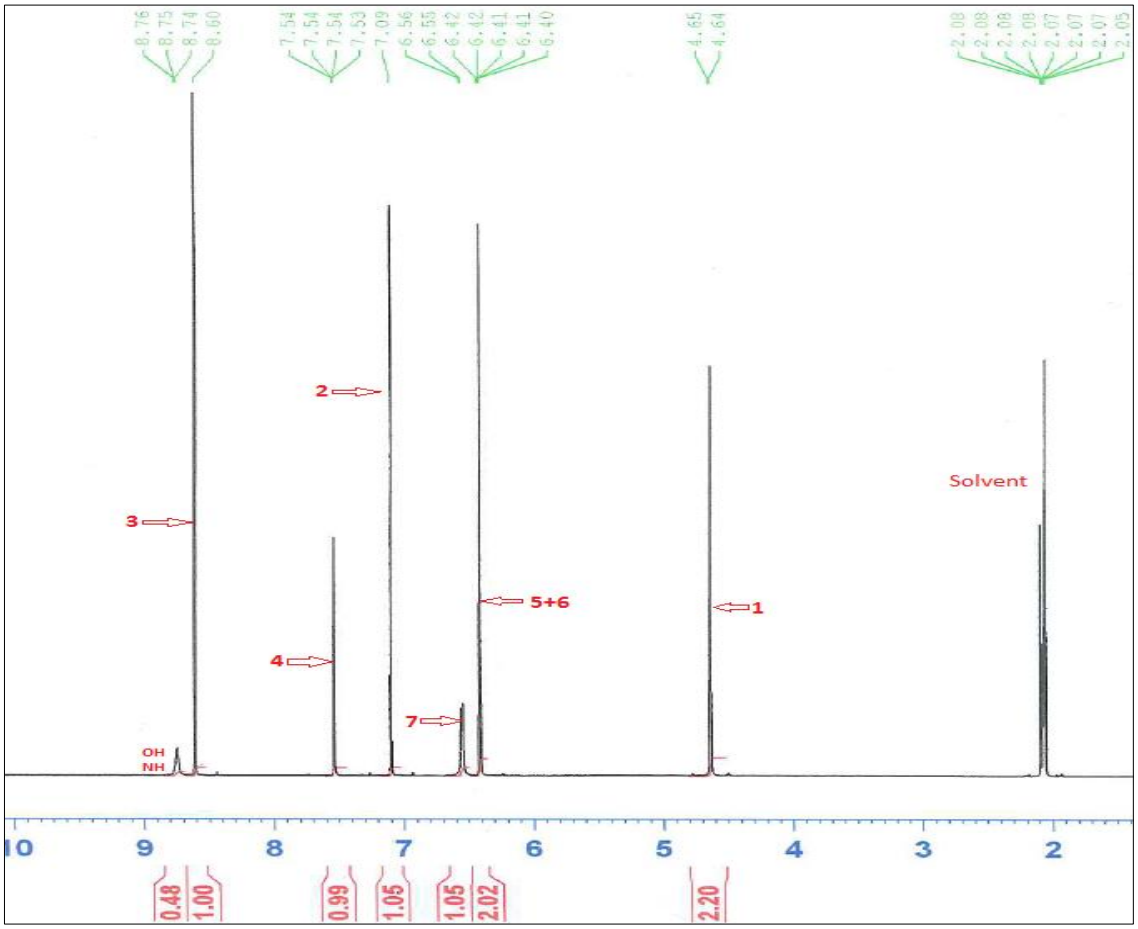


Furosemide peaks could not be identified and had no difference when its IR microemulsion spectrum was compared with placebo microemulsion spectrum. In fact, there was an appearance of two minor peaks in placebo's spectrum at 1660 & 1600 cm^{-1} but they had no significance toward furosemide's identification. Furosemide concentration was quadrupled in microemulsion and a sample was analysed using FTIR but again no difference was observed. Therefore, it is was concluded that the disappearance of furosemide peaks was caused by the overlapping of microemulsion excipients spectral bands over furosemide API bands and led to the obscuration of the furosemide API peaks by surfactants and co-surfactants stretching bands. This concluded that FTIR could not be used for drug-excipient compatibility and stability analysis of furosemide microemulsion and therefore, HPLC and NMR tests were applied for this purpose.

4.2.14 Furosemide Drug-excipients compatibility studies using NMR

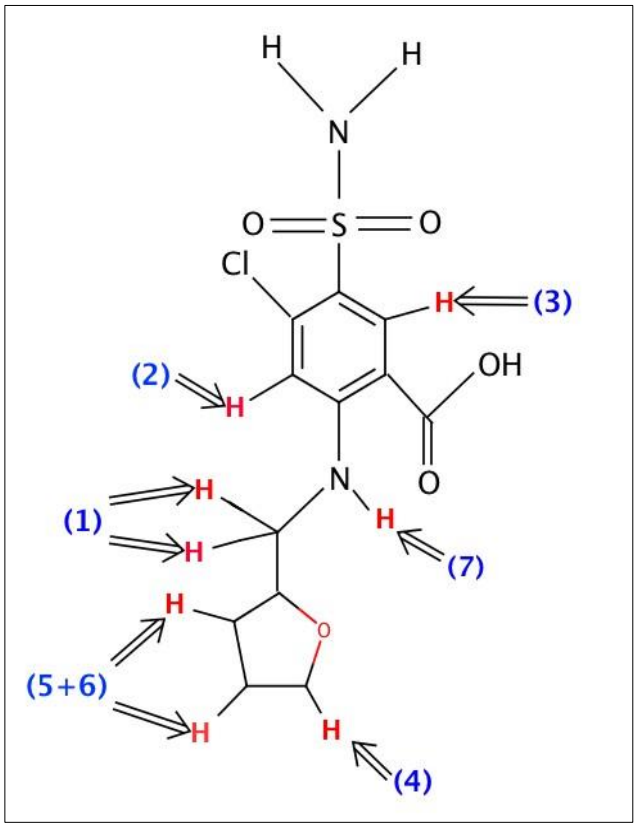
The NMR spectrum of furosemide API was obtained after dissolving it in acetone using Bruker NMR as shown in Figure 33.

Figure 33: ^{1}H NMR spectrum of Furosemide using acetone solvent



By analysing the proton peaks of furosemide NMR spectrum, the correlated hydrogen atoms have been assigned (1-7) as shown in Figure 34.

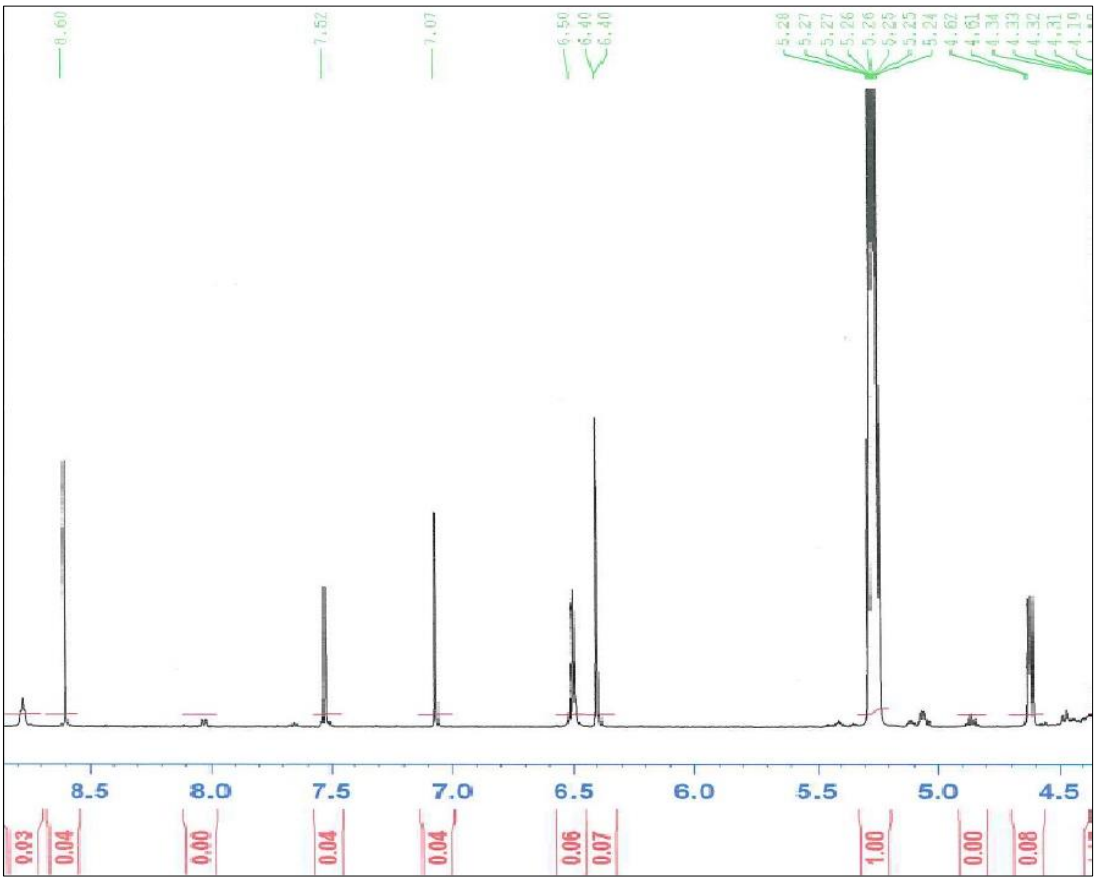
Figure 34: Furosemide structure with numbers of hydrogen types identified



Furosemide microemulsion was then diluted using acetone solvent and analysed using NMR.

Figure 35 shows diluted furosemide microemulsion spectrum.

Figure 35: Furosemide microemulsion spectrum using acetone solvent

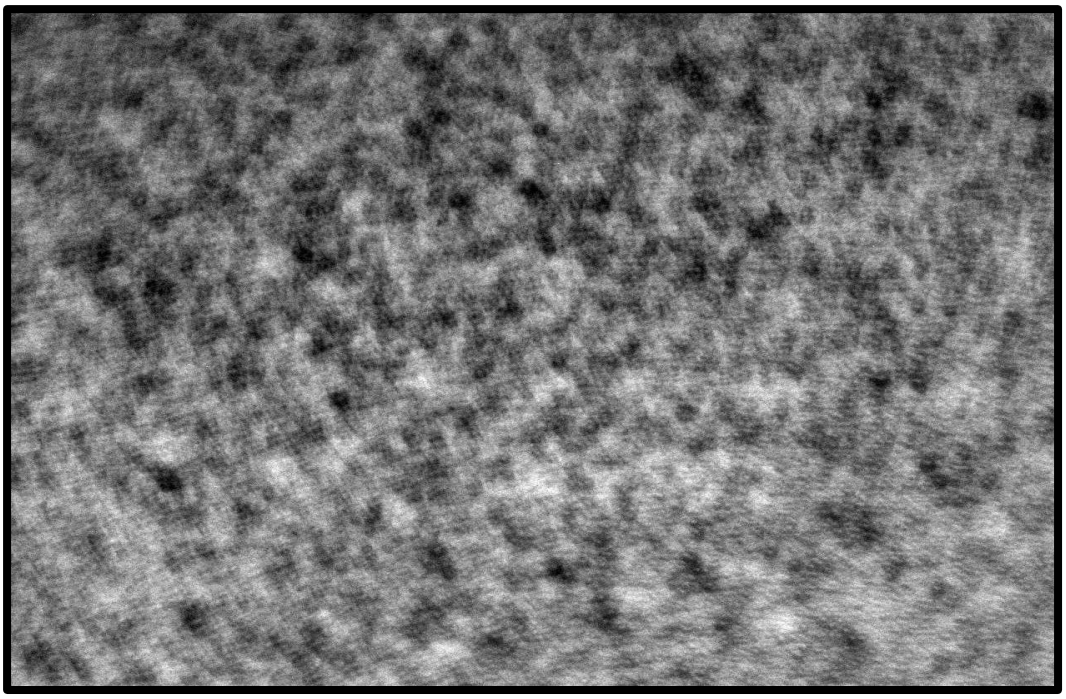


By comparing both spectra of pure furosemide standard and furosemide microemulsion, it was observed that proton peaks' places and shifts are identical with no difference. This showed that no interaction had been exerted on furosemide molecule following formulation of the microemulsion and proved a good compatibility between furosemide and microemulsion's excipients.

4.2.15 Visualisation of furosemide microemulsion using light microscopy

It was barely possible to spot the water droplets dispersed into the oily phase at 20X magnification where furosemide drug is dissolved as shown in Figure 36.

Figure 36: Microscope image of furosemide microemulsion magnified to 20X



The conjugated aqueous droplets were then gradually disintegrated into discrete, seemingly spherical droplets. Higher magnifications were applied and figures 37 and 38 are pictures captured showing the aqueous content and distribution over the oily phase at 40X and 60X respectively.

Figure 37: Microscope image of furosemide microemulsion at 40X with excess aqueous droplets added to the furosemide microemulsion preparation

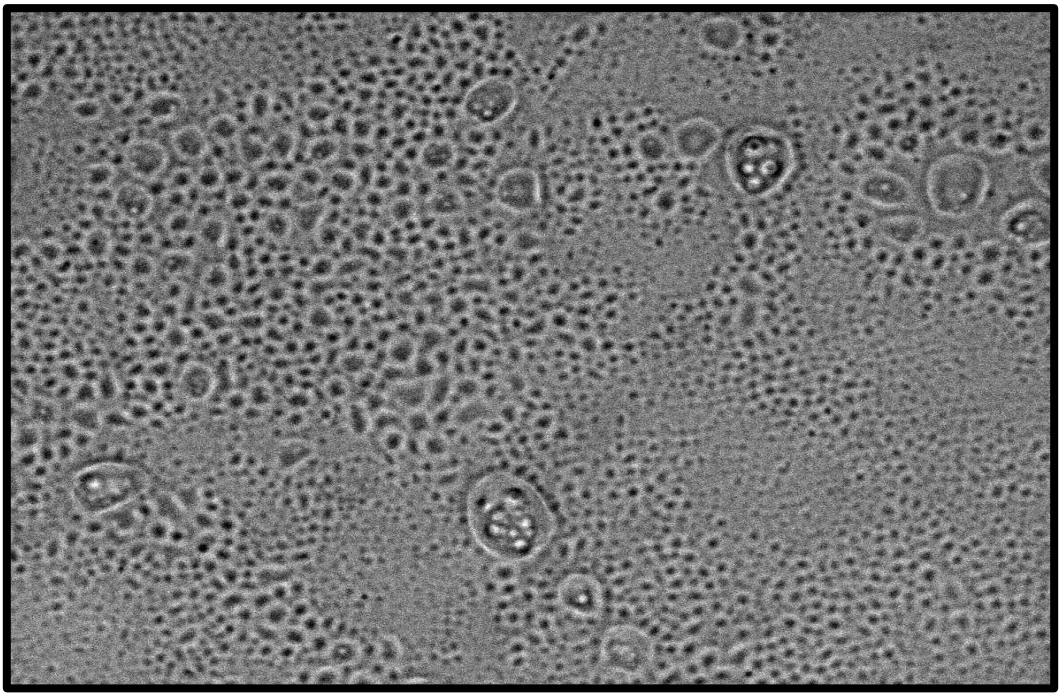
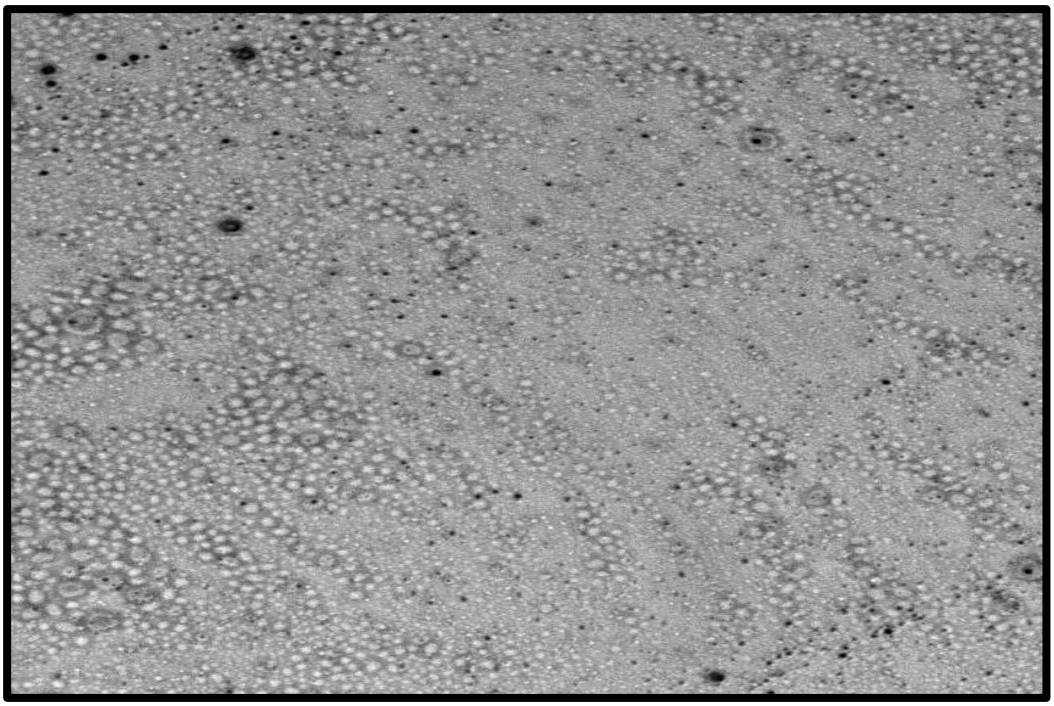


Figure 38: Microscope image of furosemide microemulsion at 60X shows disintegrated aqueous droplets onto the oily phase of the microemulsion



4.2.16 Temperature stability studies

Furosemide microemulsion was found to be stable in the freeze-thaw cycle and there were no signs of phase separation or precipitation observed. The results obtained were summarized in Table 25.

Table 25: Thermodynamic stability results

Thermodynamic stability and precipitation studies for furosemide microemulsion							
Centrifugation test	**Freeze thaw cycle**	**Precipitation after 1-6 hours**					
		1 h	2 h	3 h	4 h	5 h	6 h
No phase separation	No phase separation	Clear	Clear	Clear	Clear	Clear	Clear

4.2.17 Palatability testing

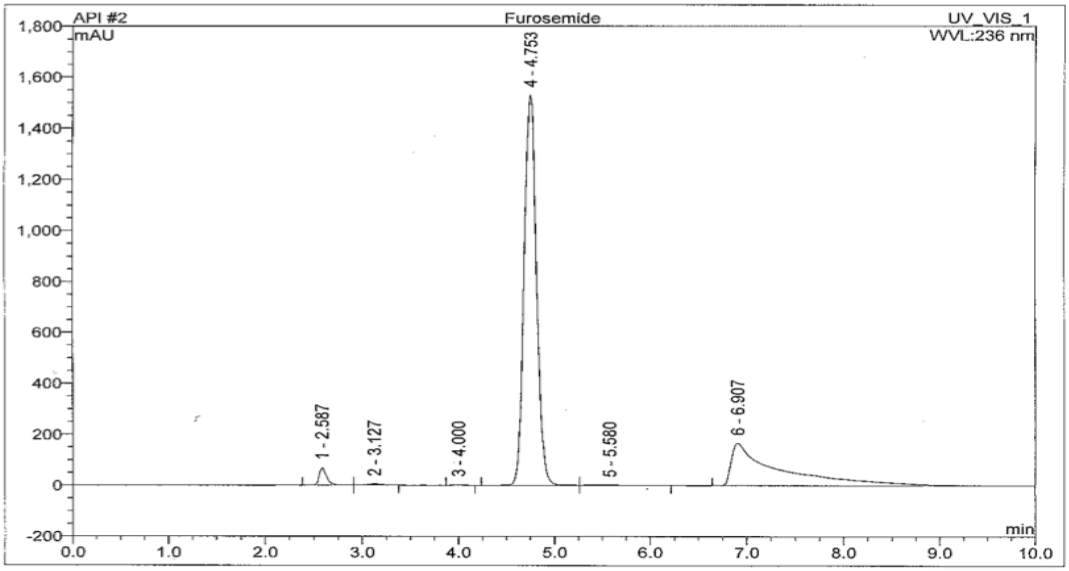
Unfortunately, the palatability tests were not successful because the sample damaged the sensors of E-tongue. The E-tongue manufacturers were contacted and the company stated that C00 sensors could be damaged if exposed to surfactants e.g. labrasol as they get adsorbed onto the sensors surface, which in fact, caused the damage to sensors.

4.2.18 Shelf Life Studies

Stability studies under stress temperature conditions

The output of injected furosemide microemulsion showed chromatogram with a clear peak as shown in Figure 39.

Figure 39: Furosemide microemulsion chromatogram



The furosemide microemulsion areas were recorded at 2381, 2379 and 2383 from 30, 40 and 50°C samples respectively. Using the calibration equation, the concentration of microemulsion samples were calculated and the values were summarised Table 26.

Table 26: Concentrations obtained at different temperatures

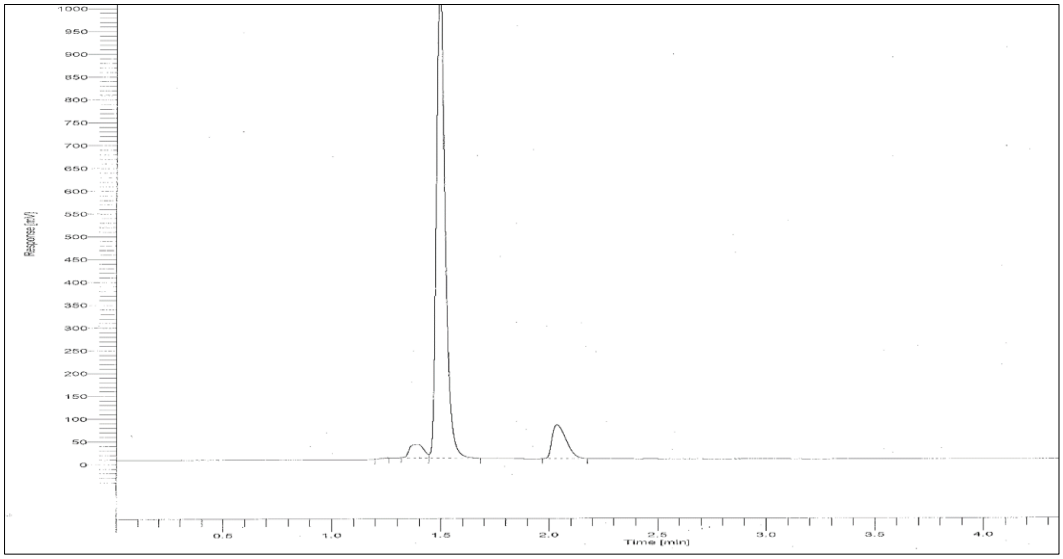
Sample record	Area	Concentration
Sample 1 at 30 °C	2381	50.18 µg/ ml
Sample 2 at 40 °C	2379	50.13 µg/ ml
Sample 3 at 50 °C	2383	50.22 µg/ ml

By comparing the results obtained with the expected concentration of injected microemulsion samples (50µg/ml), it can be observed that the samples concentrations remained the same under stress conditions. Moreover, the results indicated a good stability of furosemide in the microemulsion dosage form at these conditions over 60 days as no degradation of the API was observed.

Long term stability studies

The concentration obtained was 50.4µg/ml with clear sharp peak indicating great stability as shown in Figure 40.

Figure 40: Furosemide microemulsion chromatogram recorded after 12 months shelf life storage



4.2.19 Long term phase separation study

After 12 months of preparation, there was no sign of precipitation observed during the 6 hours monitoring and sample remained clear throughout the test indicating good phase stability with no sign of separation or furosemide precipitation.

4.2.20 Long term viscosity determination of furosemide microemulsion

After 12 months of preparation, the measured viscosity remained similar to the initial viscosity measured at time of preparation as presented in Table 27.

Table 27: Viscosity determination of furosemide microemulsion

1) Furosemide microemulsion formulation viscosity measurement at 1 minute:	**62.0 cP**
2) Furosemide microemulsion formulation viscosity after 30 minutes from first reading:	**59.0 cP**

4.2.21 Discussion

Furosemide is considered as an age-appropriate oral dosage form, preferred for neonates, infants and young children. The fact that microemulsion as a dosage form is almost absent on the commercial market, has good solubilising property compared to other dosage forms for poorly water soluble molecules and the novel application of surfactants, such as Labrasol, Transcutol-HP and Labrafac has contributed to new knowledge to the field of paediatric formulation design.

According to the published literature, no microemulsion has been manufactured incorporating furosemide. The majority of microemulsions that have been formulated were designed for transdermal routes (e.g. tretinoin [114], aceclofenac [115] and nimodipine [116]) and for nasal administration (carbamazepine[117]). However, there are a few reports that detail the oral route for molecules such as biphenyl dimethyl dicarboxylate (BDD) for the treatment of liver disease and clinidipine for hypertension[118].

The published article on clinidipine (BCS class II) microemulsion stated that it has droplet size (globule) of 13.3nm[119]. However, furosemide microemulsion in this research had droplet size of 137.4nm. According to microemulsion field, droplet size can range between 20-200nm, so it is unclear if clinidipine in fact was swollen micelle. With reference to the Handbook of Microemulsions science and technology[120], droplet size depends on interfacial tension and entropy of mixing. The variation in droplet size is also a function of concentration, which means larger amounts incorporated in microemulsion will yield larger droplet size. Chemically,

furosemide's partition coefficient log P value is 3 with pKa value of 3.9. Itraconazole microemulsion with a log P of 5.7 and pKa value of 3.7 exhibits a droplet size of 150nm[121] while glimepiride microemulsion (log P 3.5 and pKa 3.7) gave a value of 176.1nm[122]. Thus, it would appear that the variation in the physico-chemical properties of the drug results in the differing sizes, not to mention the type and level of surfactant(s) used. Table 28 summarises the pertinent details of microemulsions as reported in the literature. It is difficult to ascertain the relationship between droplet sizes with the varying parameters such as HLB value and water level.

Table 28: Examples of oral microemulsion reporting the excipient type and level used

Microemulsion	Oil	Surfactant	Co-surfactant	Water level	Drug Ratio	Droplet size
Atorvastatin[123] (W/O) Log P: 5.7 pKa: 4.3	Castor oil 13%	Labrafil 61% HLB: 4	Transcutol-HP HLB: 4.2 20%	6%	10mg/ml	28.27nm
Itraconazole (O/W) Log P: 5.7 pKa 3.7	Oleic acid	Tween 80 HLB: 15	Ethanol HLB: 4.2	Unknown	Unknown	150nm
Glimepride (O/W) Log p: 3.5 pKa 3.7	Cremphore RH 40 (32%)	Labrafil HLB: 4 8%	Transcutol-P HLB: 4 8%	52%	20mg/ml	176.1nm
Clinidipine (O/W) Log p: 5.42 pKa 2.61	Tocotrienol	Tween 20 HLB: 16.7	Transcutol-HP HLB: 4.2	Unknown	Unknown	13nm
Furosemide (W/O) Log P: 3 pKa: 3.9	MCT 14%	Labrasol HLB: 14 60%	Transcutol-HP HLB: 4.2 20%	4%	10mg/ml	137.4nm

In terms of drug-excipient compatibility studies, majority of published papers on microemulsions prioritised physical stability studies and did not conduct compatibility assessments. However, few articles such as simvastatin[124] and telmesart[125] microemulsions investigated the excipient compatibility using FTIR. Unfortunately, using FTIR for furosemide-excipient compatibility was not successful because furosemide bands were obscured by excipient absorption. Proton NMR, however, appeared to indicate good excipient compatibility

as proton peaks places and shifts were identical between reference furosemide and furosemide microemulsion. In reviewing previous literature on microemulsions this type of analysis has not been performed before and the only published source citing NMR explored the characterisation of vinpocetine microemulsion for transdermal delivery to assess the correlation between the transdermal permeation rate and structural characteristics of vinpocetine.[126]

It is also worth noting that majority of research conducted on microemulsion focused on the method of formulation without indicating the intended patient population. Therefore, no toxicity studies or data on microemulsion excipients were mentioned. Additionally, no attention was given to paediatric patients except for a single source from Wisch (2000) that reported a microemulsion for the treatment of paediatric patients with tuberculosis[127]. Again no toxicity values or studies were given in the thesis and only the age-appropriateness of the microemulsion as a dosage form was discussed as the main concern. In this research by contrast, excipient toxicity concerns and ADI values were assessed for all paediatric age groups based on body weight and therapeutic doses to give a patient-centric approach.

A major concern with liquid dosage forms is palatability and taste masking. Unfortunately, no studies were conducted on palatability for furosemide microemulsion as *in vivo* testing had ethical constraints and *in vitro* testing could not be performed because the surfactants in the furosemide microemulsion damaged the E-tongue electrode. However, this is a critical quality attribute and it is hoped that alternative methods can be developed in the near future to overcome such constraints.

For the sake of completeness, there is a single published report of a furosemide self microemulsifying drug delivery system (SMEDDS).[128] However, SMEDDS formulation exhibits significant differences to the ready-to-use microemulsion developed in this work. For instance, SMEDDS is an oil and surfactant single-phase mixture and only becomes dispersed once it

encounters the gastric liquid content (chime) under gentle agitation (motility) of the intestine (i.e. a pre-concentrate is formed and administered as the dosage form). In contrast for microemulsions, the dispersed phase (water in this research) is dispersed in continuous phase (e.g. MCT) and stabilised with surfactant and co-surfactant combination before administration.

Finally, despite the significant advantages of microemulsions, these dosage forms have some drawbacks and limitations. For example:

- Microemulsions require large concentration of surfactant and co-surfactant to form stabilisation[129].
- Many of surfactants and co-surfactants are new on to the market and not widely used, therefore there is no long-term clinical safety data.
- Small number of pharmaceutically acceptable excipients (classified as generally regarded as safe) are available compared to other dosage forms.[130]
- An obstacle is usually met when developing a microemulsion is the lack of good predictive *in vitro* models for palatability assessment of the formulation.[131]
- Microemulsion stability is influenced by environmental parameters such as temperature and pH value[132]. These parameters change upon patient handling and home storage, which can lead to a stability issue.

Chapter 5

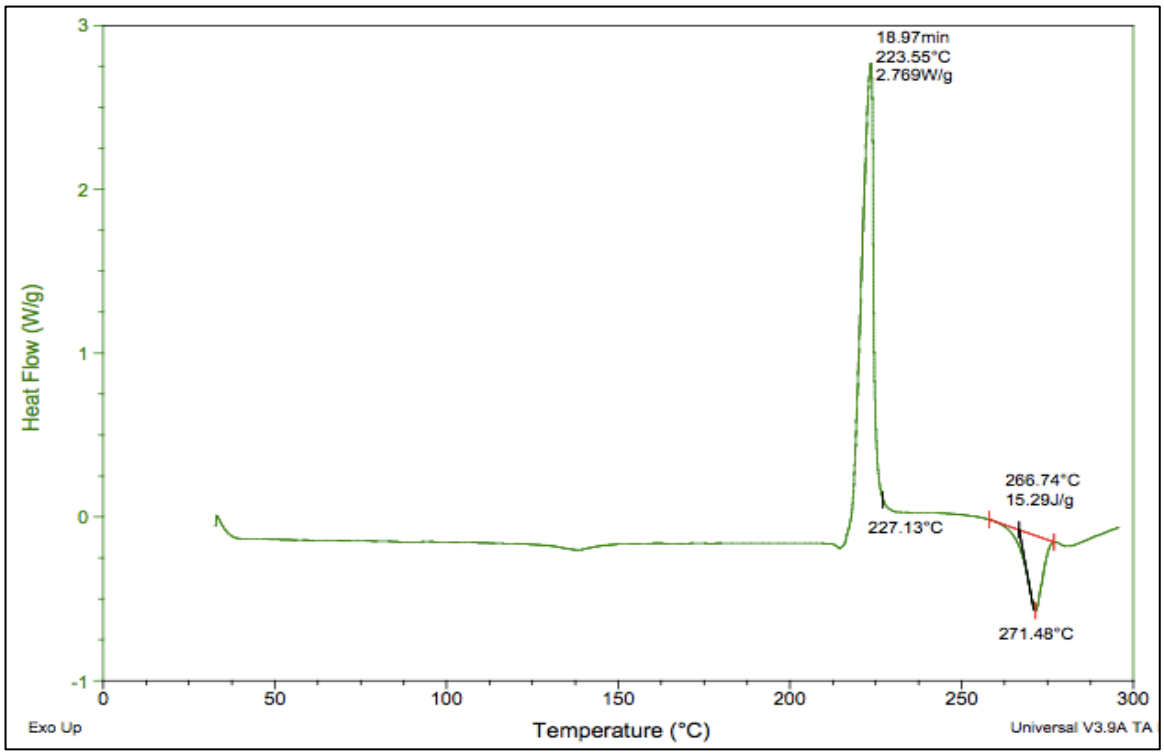
Furosemide Oro-Dispersible Mini Tablets Formulation Results

5. Preliminary Work Results for Establishing Process Parameters (pre-formulation studies)

5.1 Drug-Excipient Compatibility Studies

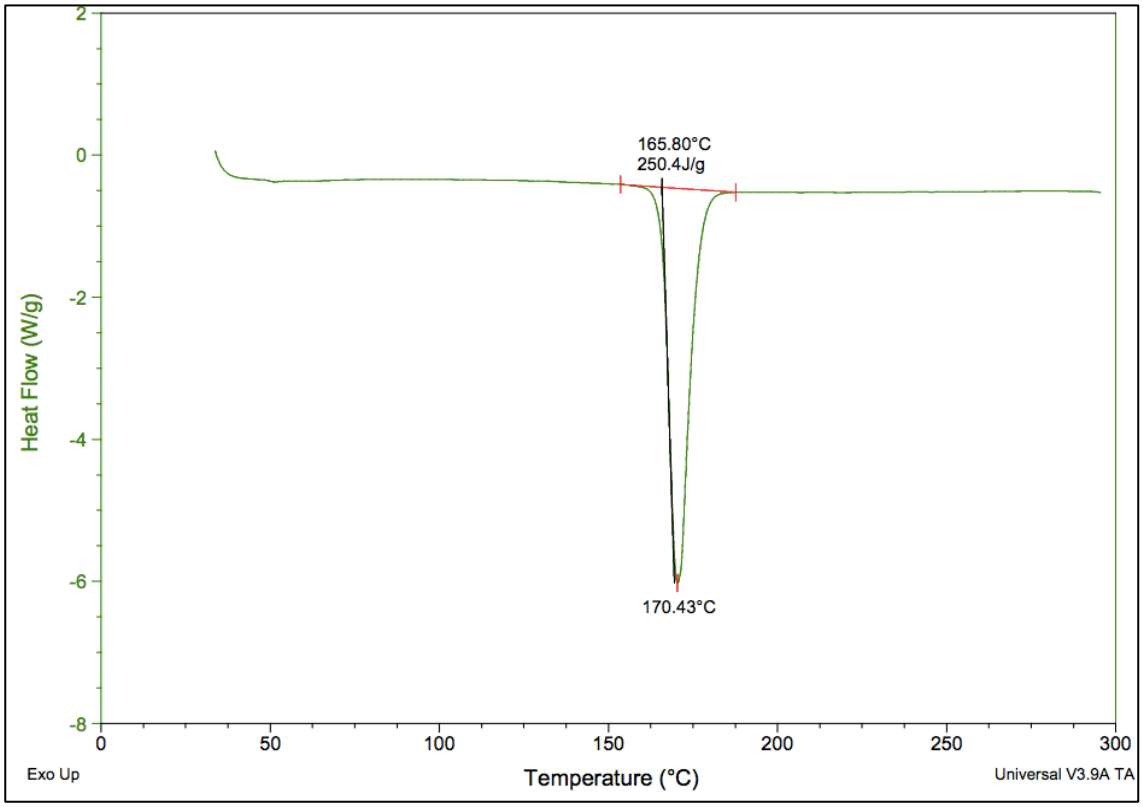
The DSC thermogram of furosemide sample obtained (Figure 41) showed an exothermic peak of recrystallization at 223.55°C (18.97min) and endothermic peak with melting point observed at 271.48°C.

Figure 41: Furosemide Thermogram Using DSC



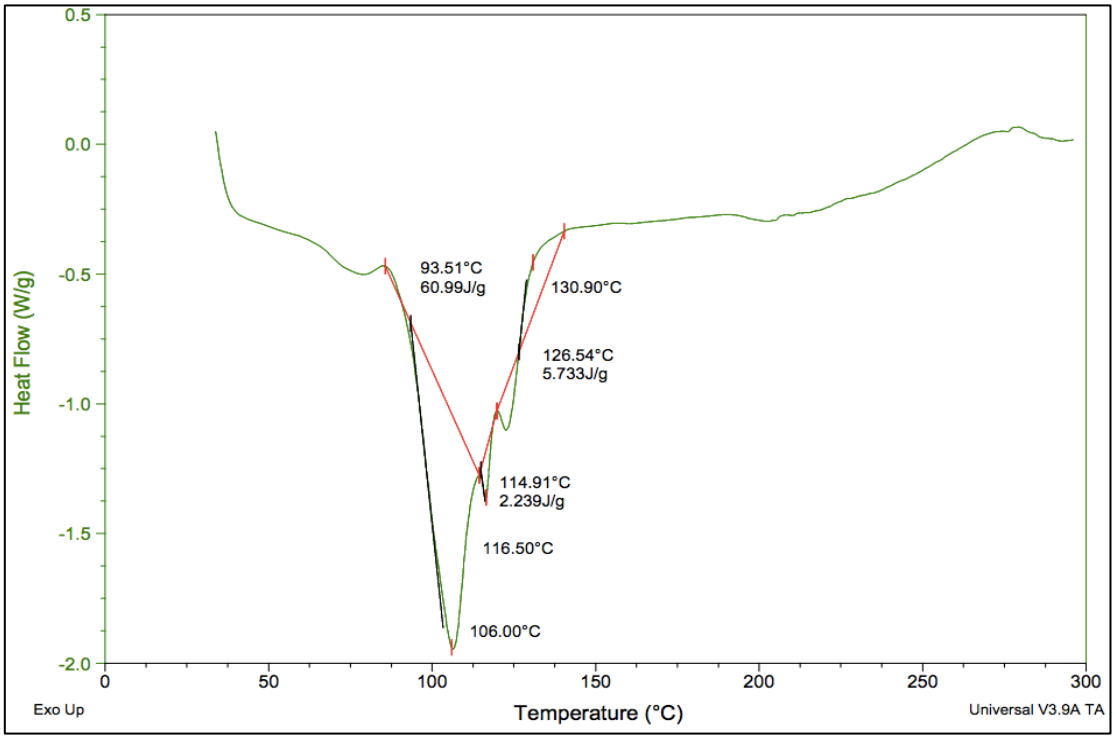
The ludiflash DSC thermogram obtained showed an endothermic peak only with melting point noticed between 0-300°C range observed at 170.43°C as shown in Figure 42.

Figure 42: Ludiflash Thermogram Using DSC



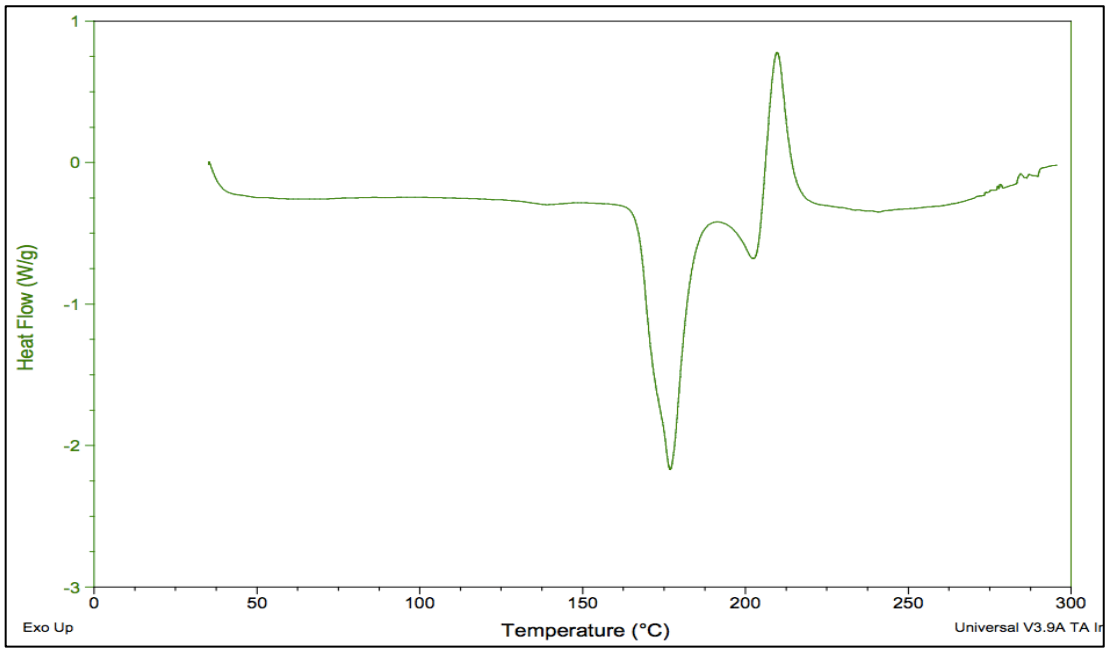
Magnesium stearate gave a thermogram showing two endothermic peaks with different melting points (106 and 114.9°C). This usually happens when a combination or mixture of two or more compounds are scanned together such as magnesium stearate. European Pharmacopeia describes magnesium stearate as a mixture of magnesium salts of different fatty acids consisting mainly of stearic acid and palmitic acid and in minor proportions of other fatty acids[133]. Figure 43 shows the thermogram of magnesium stearate.

Figure 43: Magnesium stearate thermogram using DCS



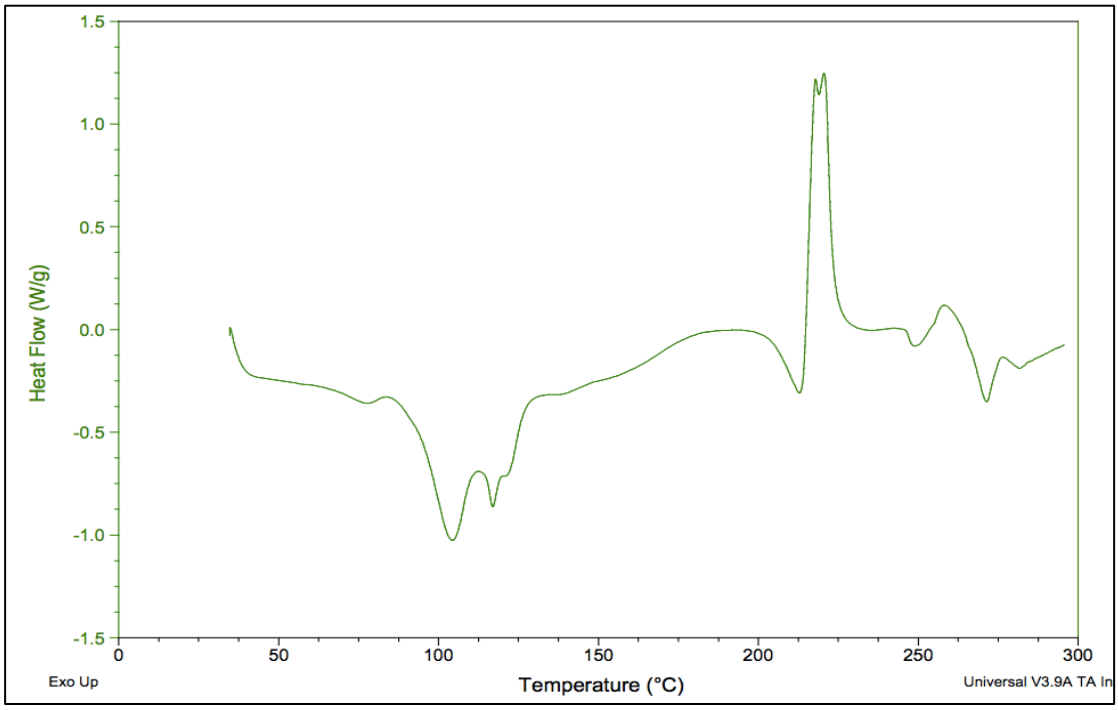
The DSC thermogram of Furosemide-Ludiflash sample showed an endothermic peak of Ludiflash and exothermic peak of furosemide confirming compatibility as a mixture with no signs of interaction between both compounds as seen in Figure 44.

Figure 44: Furosemide-Ludiflash mixture Thermogram Using DCS



Magnesium stearate and furosemide mixture sample gave a DSC thermogram showing multi-endothermic melting peaks of the excipient and a sharp exothermic peak of furosemide confirming the compatibility between the two compounds with no observed interaction as shown in Figure 45.

Figure 45: Furosemide-magnesium stearate mixture thermogram using DCS



Overall, furosemide drug showed no significant interaction or any signs of incompatibility with Ludiflash and magnesium stearate excipients for the intended ODMT formulation.

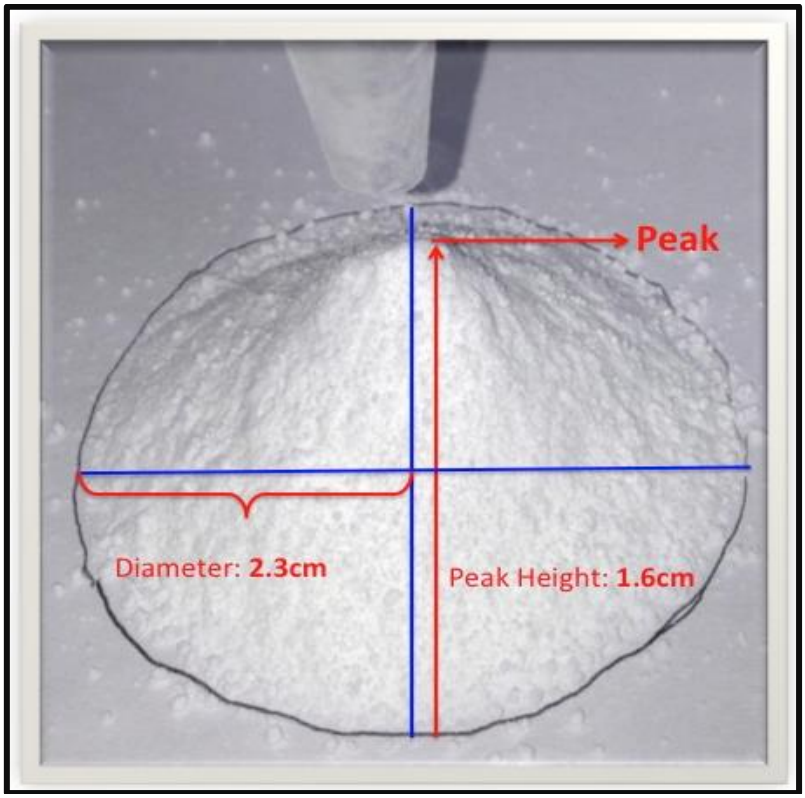
The pre-compression tests results of batch one will be discussed in details whereas the rest of the batches will be summarised bearing in mind that identical characterisation steps were followed for all batches.

5.2 Batch One Pre-Compression Tests Results

- **Angle of repose (ϕ)**

  Figure 46 displays the pile obtained with the height and diameter values.

Figure 46: Batch one pile using funnel method



Height of pile: 1.6 cm

Diameter: 2.3 cm; radius= diameter/2= 1.15 cm

Thus, angle of repose = $\text{Tan}^{-1}(0.69) = 34.6$

Based on flowability index range, the results were found to be good.

- **Compressibility index (Hausner's ratio)**

The values were obtained for Hausner's ratio were:

Bulk density= 54.23/100=0.5423

Tapped density =54.23/86=0.63

Yielding Hausner's ratio value of =0.63/0.5423 = 1.16

From Table 15, it is seen that the blend exhibits a good flow

5.3 Post-Compression Evaluation and Characterisation Results

This involved a variety of tests. Each test's results are described separately.

Weight variation evaluation

The average weight per tablet was found to be = 27.37mg

Standard deviation for each tablet weight was calculated as shown in Table 29.

Table 29: Individual tablet weight of batch one and their SD to average

Tablet	Weight	SD
1	27.1	99.01%
2	28.1	102.6%
3	27.9	101.9
4	27.2	99.37
5	27.3	99.7%
6	27.3	99.7%
7	27.28	99.6
8	27.27	99.63
9	27.67	101%
10	27.13	101%
11	27.09	98.9%
12	27.23	99.48%
13	27.40	100.1%
14	27.15	99.19%
15	27.25	99.56%
16	27.19	99.3%
17	27.51	100.5%
18	27.40	100.1
19	27.92	102%
20	27.13	101%

Tablet dimensions

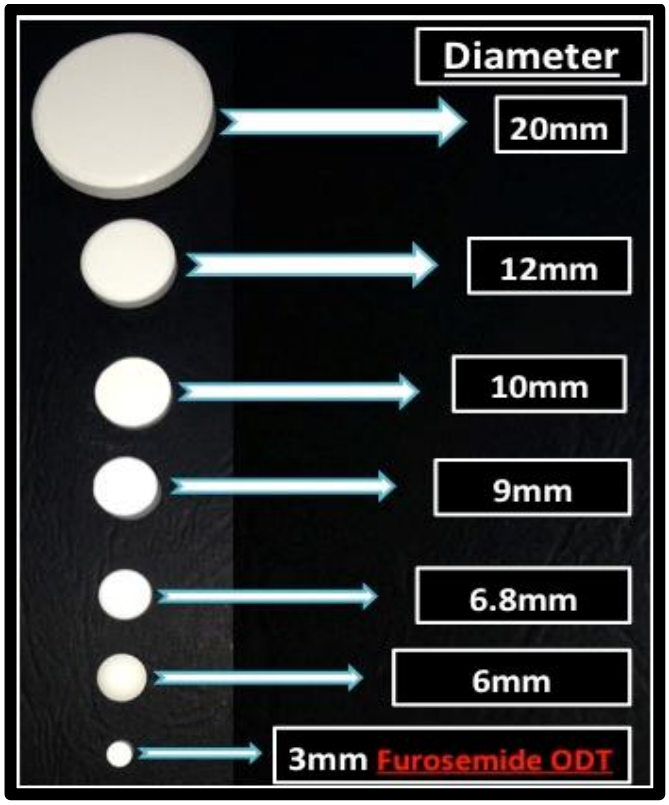
The measures obtained for 5 random tablets were recorded in Table 30.

Table 30: Shows thickness values of 5 random tablets from batch 1

Tablet	Thickness	Diameter
1	3.15mm	3mm
2	3.17mm	3mm
3	3.18mm	3mm
4	3.15mm	3mm
5	3.15mm	3mm

Figure 47 shows the formulated furosemide ODMT size in this research compared to other existing tablets on the market of different sizes.

Figure 47: Furosemide ODMT size compared to other sizes



Hardness measurement

The hardness of the 10 randomly selected tablets was recorded in Table 31.

Table 31: Crushing strength values of 10 tablets from batch 1

Tablet	Crushing Strength (N)
1	29N
2	23N
3	28N
4	21N
5	27N
6	29N
7	28N
8	33N
9	29N
10	21N

Friability

The friability for batch one was found 0.3%. According to Ph. Eur., weight loss of not more than 1% is acceptable for most tablets and thus the friability of batch one (<1%) was considered acceptable.

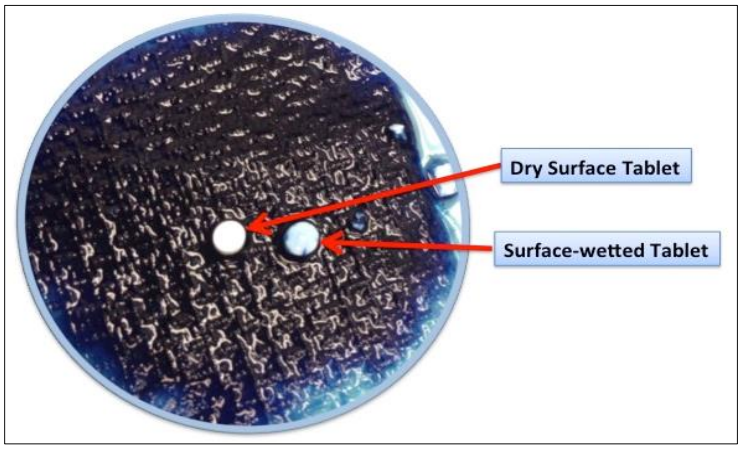
***In vitro* disintegration time**

Disintegration time for batch 1 ODMT was found to be 19 seconds.

Wetting time

The wetting time for this batch (1) was found to be 16 seconds. Figure 48 was captured when wetting was observed at one of the tablets surface compared to other tablet placed later.

Figure 48: Wetting test of furosemide ODMT from batch 1



Uniformity of dispersion

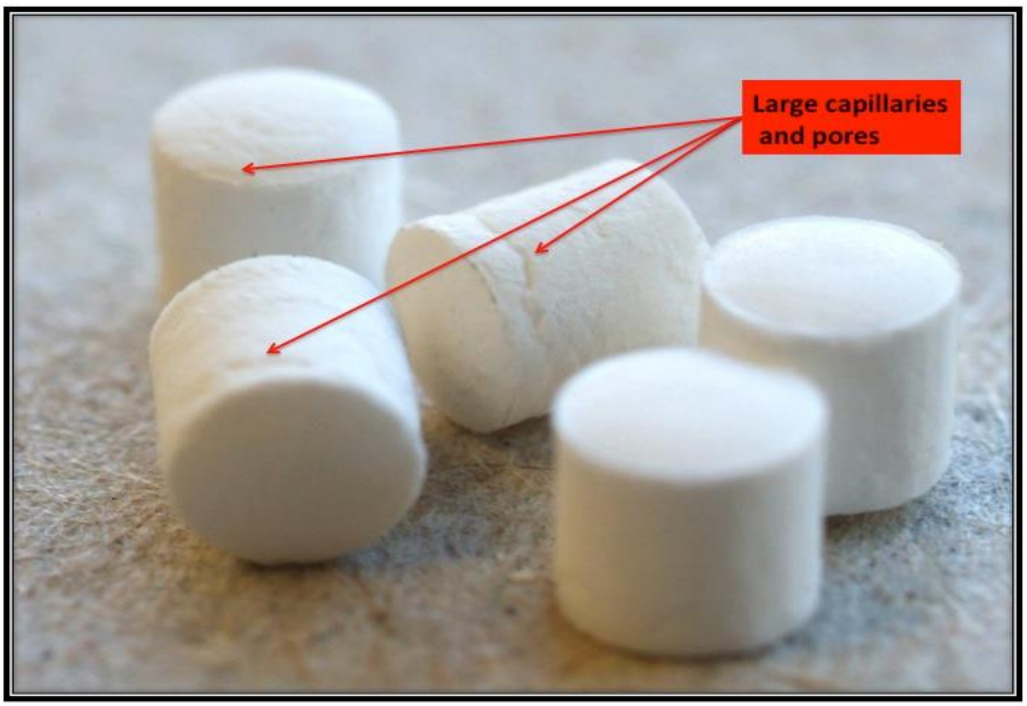
It was observed that there was no retention of any particles or residues on sieve surface and the results complied with uniformity of dispersion expectation.

Visual characterisation of batches 1-16

Three main types were visually observed as the following:

Type 1: This type of visualisation was noticed with batches compressed at low compression force. It can be seen that the tablet surface was rough and the thickness was higher with capillaries, abrasion and large pores on the tablet body (Figure 49).

Figure 49: Type one furosemide ODMT's inspected visually

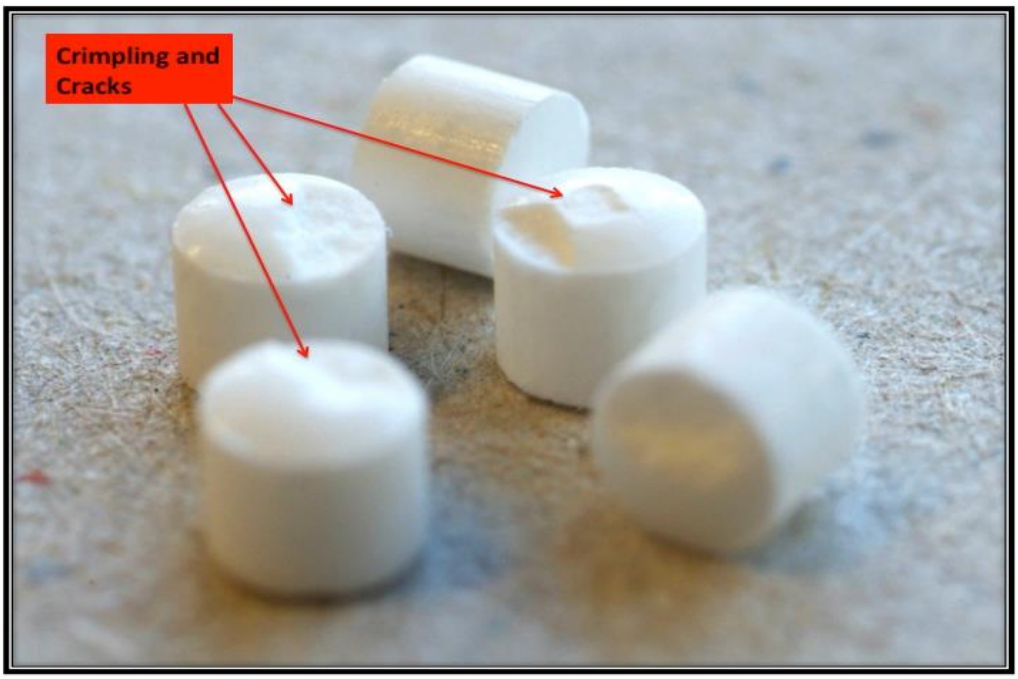


Type 2: This type was observed at high compression force and mainly at low concentration of API and lubricant. It was characterised with a smooth surface, lower thickness than type one and shiny non-porous body surface (Figure 50).

Figure 50: Type 2 furosemide ODMT's inspected visually

Type 3: This particular type was observed at high compression force where lubricant and furosemide were used at their maximum concentration 3mg and 0.6% respectively. The tablets were characterised by very smooth surface but with clear chipping, cracks and crumples were observed in some tablets (Figure 51).

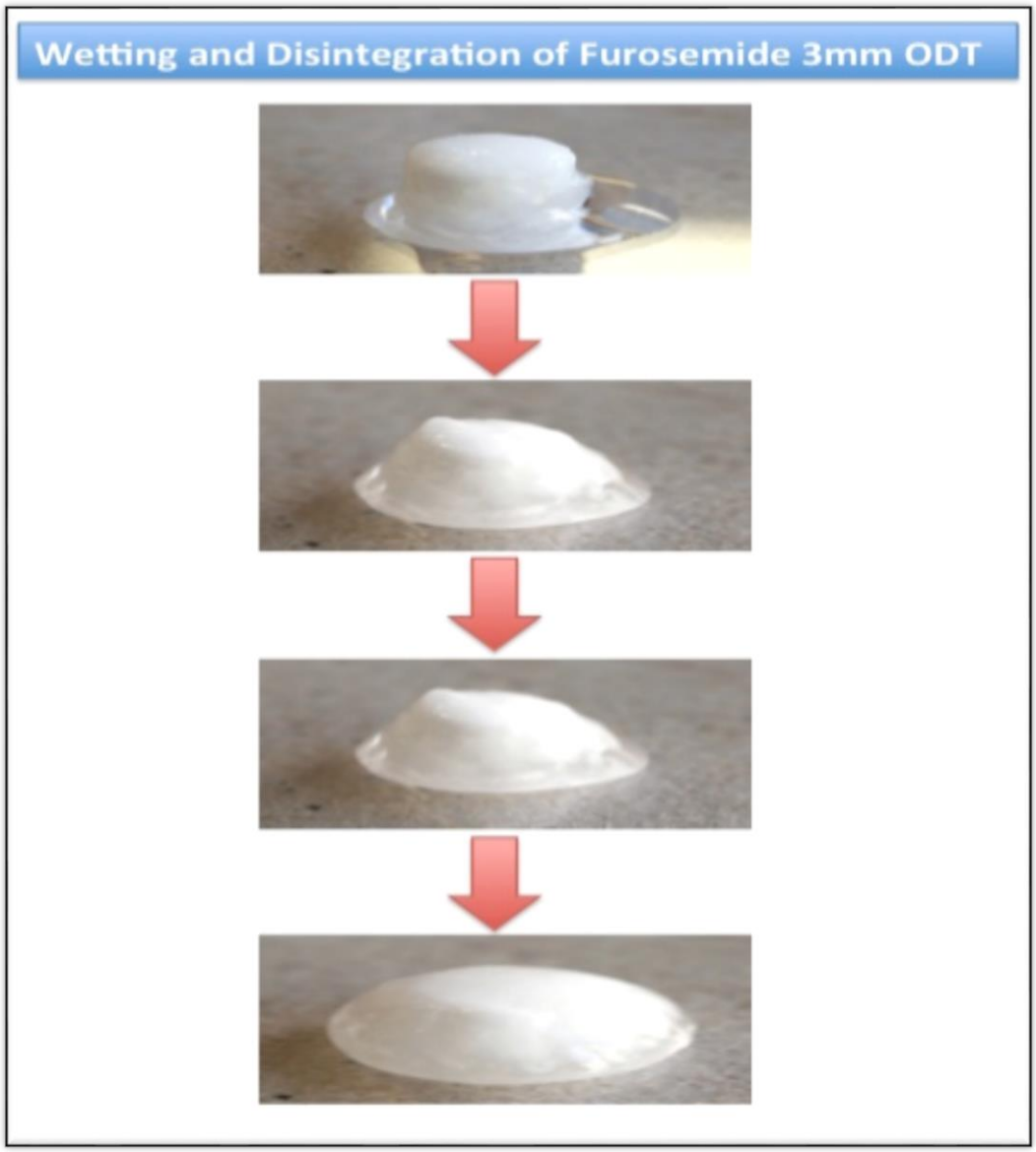
Figure 51: Type 3 furosemide ODMTs inspected visually



Disintegration step analysis

The disintegration processes, which involved wicking and swelling mechanisms then a full dispersion, were captured as shown in Figure 52.

Figure 52: Dispersion process of furosemide ODMT



Drug content analysis

Using the previously developed HPLC method, an assay values between 103.2 and 107.1µg/ml were obtained.

Quality control test results of furosemide ODMTS in dissolution test

Although the standard furosemide concentration of Batch 1 ODMT in simulated gastric juice was expected to be 1.11µg\ml, it was noticed that the samples contained a slightly

higher concentrations than expected as shown in Table 32. This is explained as drug content had up to 8% extra in some tablets analysed for content from batch 1.

Table 32: Dissolution samples concentrations at different times

Dissolution test of Furosemide Oro-dispersible Mini Tablets	
Time	**Concentration (pH4)**
At 5 minutes	1.15 µg (103.6%)
At 10 minutes	1.19 µg (107.2%)
At 15 minutes	1.19 µg (107.2%)

Overall, the dissolution rate showed acceptable results and the cumulative release of furosemide not only reached standard acceptable range (90-100%) but exceeded it after the first 5 minutes of dissolution and only a slight increase was observed over the 2nd and 3rd intervals samplings which indicated an excellent dissolution rate within short period of time.

5.4 Tests Results of Batches 1-16

The tests results of pre and post-compression batches 2-16 were conducted and evaluated as described in the preceding tests. The combined results are shown in Tables 33 and 34.

Table 33: Pre-compression parameters for 1-16 batches

Formulation batch	Angle of repose		Hausner's ratio	
	Value	Level	Value	Level
B1	34.6	Good	1.16	Good
B2	40.59	Fair	1.14	Good
B3	29.6	Excellent	1.17	Good
B4	34.6	Good	1.17	Good
B5	39.35	Fair	1.16	Good
B6	40.82	Fair	1.14	Good
B7	29.6	Excellent	1.17	Good
B8	32.21	Good	1.17	Good
B9	34.6	Good	1.16	Good
B10	40.59	Fair	1.14	Good
B11	34.6	Good	1.17	Good
B12	34.99	Good	1.17	Good
B13	40.8	Fair	1.13	Good
B14	39.69	Fair	1.14	Good
B15	35.75	Good	1.17	Good
B16	34.63	Good	1.17	Good

Pre-compression studies and assessments concluded good acceptability for the compression of all batches and therefore all batched were formulated using single punch Manesty F3 tablet machine with 3mm die diameter.

Table 34 shows results of compressed batches 2-16.

Table 34: Post-compression parameters for 1-16 batches (X refers to batches failed indicated tests)

Batch no.	Hardness Average (N)	Friability (%)		Weight variation			Disintegration Time (Sec)	Wetting time (Sec)	Accepted Batches
		Value	Acceptance (<1%)	Average	Max (%)	Min (%)			
B1	27	0.29	✓	27.37	+2.6	-1	19	16	✓
B2	2.2	41.79	X	21.46	+12.11	-21.63	13	13	X
B3	11.6	3.78	X	22.04	+4.99	-3.68	21	16	X
B4	5.8	26.51	X	21.35	+9.83	-11.5	29	21	X
B5	25.7	0.14	✓	27.2	+2.13	-2.8	16	11	✓
B6	8.4	5.2	X	25.84	+6.07	-7.36	18	13	X
B7	12.2	1.34	X	21.85	+4.25	-4.44	23	15	X
B8	3.4	26.98	X	21.43	+7.6	-9.44	27	21	X
B9	36	0.721	✓	23.42	+14.51	-8.25	34	31	X
B10	14.3	19.66	X	21.72	+7.96	-9.21	21	19	X
B11	23.3	0.82	✓	24.37	+2.74	-2.84	24	21	✓
B12	10.7	1.16	X	19.97	+5.7	-3.96	37	30	X
B13	41.6	0.69	✓	23.1	+6.27	-8.88	28	26	X
B14	23.1	1.85	X	22.47	+11.08	-16.11	28	17	X
B15	24.7	0.73	✓	24.45	+2.53	-2.05	27	20	✓
B16	11.7	1.08	X	19.12	+5.4	-2.74	38	29	X

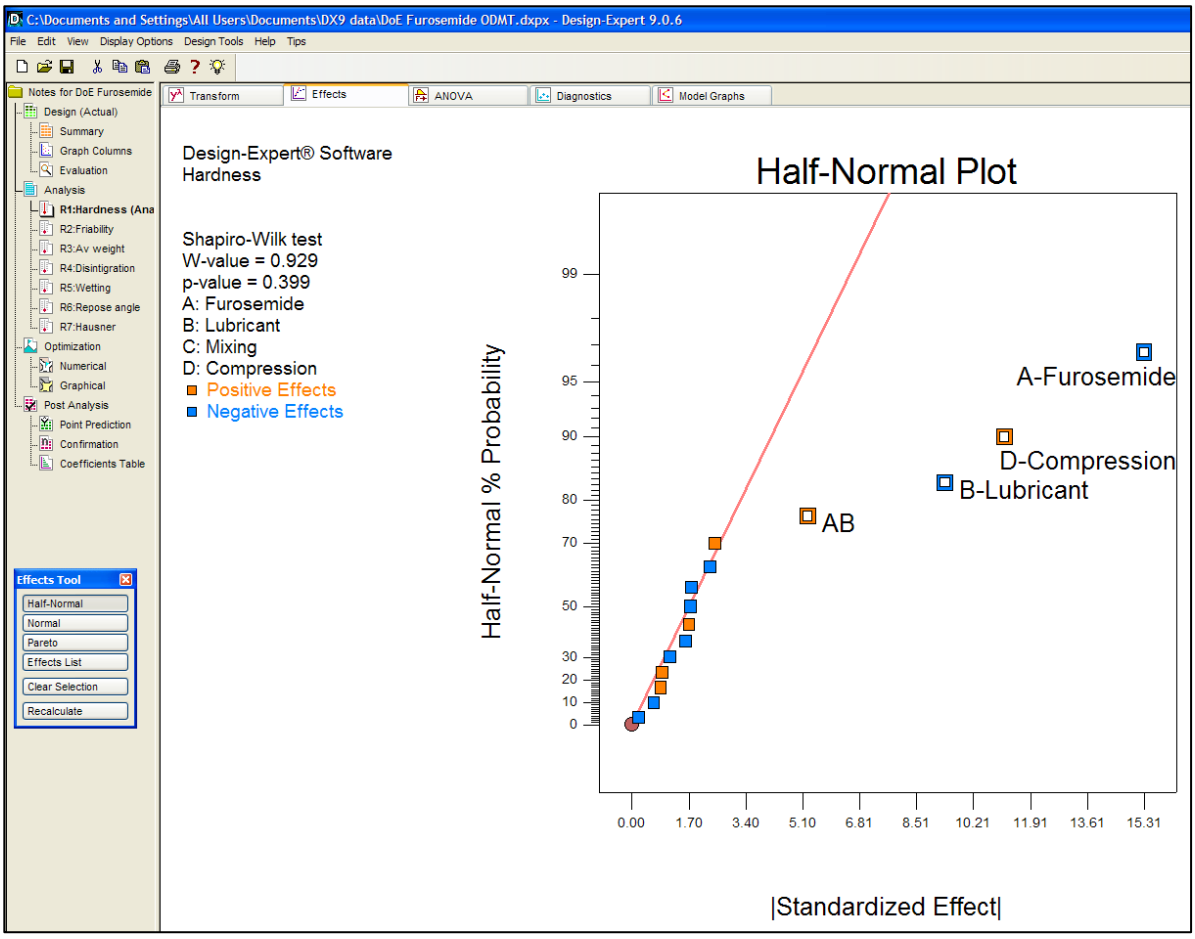
Post-compression tests results showed unacceptable values for some batches. These values were noticed with friability and weight variation tests and the batches which failed the tests were

excluded from further assessments and analysis. The batches that showed acceptable values were batches 1,5,11 and 15. Hence, they were selected for further studies and evaluation such as content uniformity, dissolution and stability tests (section 5.6).

5.5 Analysis of the Design of Experiments

Effect on tablet hardness response

Figure 53: Shows the factors effect on response levels



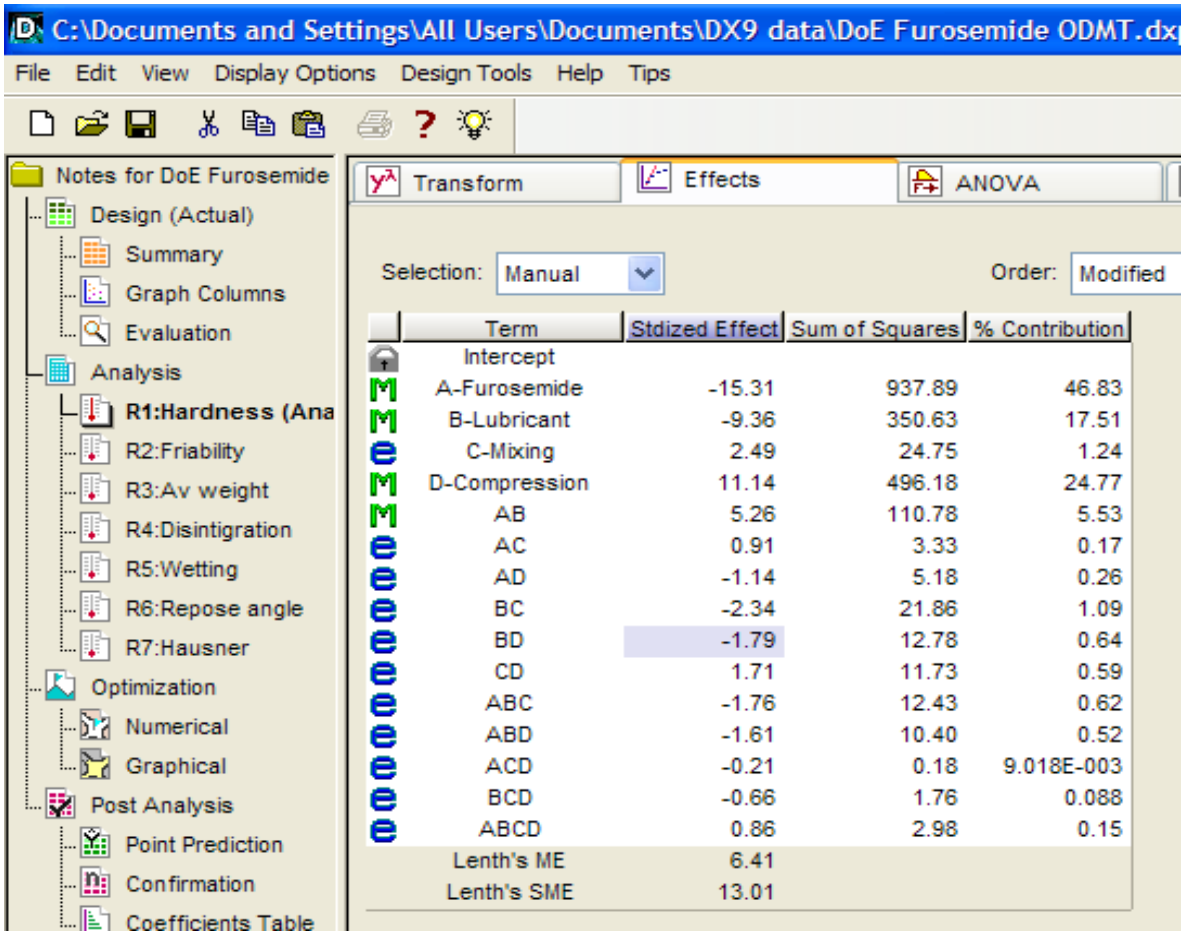
- Increasing the furosemide level from 1 to 3mg on average reduced the hardness by 15N

- Increasing the compression strength from 18 to 19 units increased the hardness by ~12N
- Increasing the lubricant level of magnesium stearate from 0.3 to 0.6% reduced the strength by 10N

Intuitively the above makes sense. Increasing furosemide load level within the small confines of a mint-tablet is likely to be detrimental, requiring either a higher compaction force or another diluent such as microcrystalline cellulose. The latter diluent cannot be used for ODMT as it does not render tablets dispersible. It is well known that increasing lubricant levels can lead to softer tablets and this has been borne out in these results. There is also a significant interaction between the furosemide level and lubricant amount, which reduces hardness by ~ 7N.

Figure 54 below lists the actual values of the main effects due to furosemide, compression and lubricant factors plus the aforementioned interaction effect.

Figure 54: shows the list of the actual values with main effects on furosemide ODMTs



Effect on friability

Using similar types of graphical analysis and calculations it was observed that surprisingly, increasing furosemide level reduced the level of friability by 15% whereas an increase in the mixing time and compression force reduced hardness by 7 and 10%, respectively. This is counterintuitive. A deeper analysis of the interactions revealed that in fact friability was increased 9% given the combination of furosemide and compression factors. This suggests that increasing compaction force may in fact push waken ODMT as furosemide is pushed towards the periphery or the surface.

Effect on average tablet weight

No major effects were observed.

Effect on disintegration time

The only factors of note, as expected, were that increasing compaction force and/or lubricant levels increased average increased disintegration time by 9s and 7s, respectively. Interestingly, however, there was little interaction between these two factors despite the fact that the hydrophobic magnesium stearate will repel water from the tablet surface.

Effect on wetting times

The major effect observed was the compression force, which going from 18 to 19 units on the Manesty F3 machine settings lead to an average increase of 8s (i.e. reduction in surface porosity, or water channels).

Effect of lubricant levels on powder flowability

Two response factors were used here: angle of repose and the Hauser ratio. The only affect observed of note was, as expected, was the increasing level of the lubricant, which on average increased the angle of repose by 5.6°. Lubricants are known to have poor glidant properties.

No significant effects were seen for Hausner's ratio simply because of its insensitivity to measurement *cf*. angle of repose response

Overall, these findings led to the selection of batches 1,3,5 and 11 for further characterisation.

5.6 Acceptable Batches (1, 5, 11, 15) Quantitative, Qualitative and Stability Tests Results

The drug content and dissolution rate results for the acceptable batches is concluded in table 35.

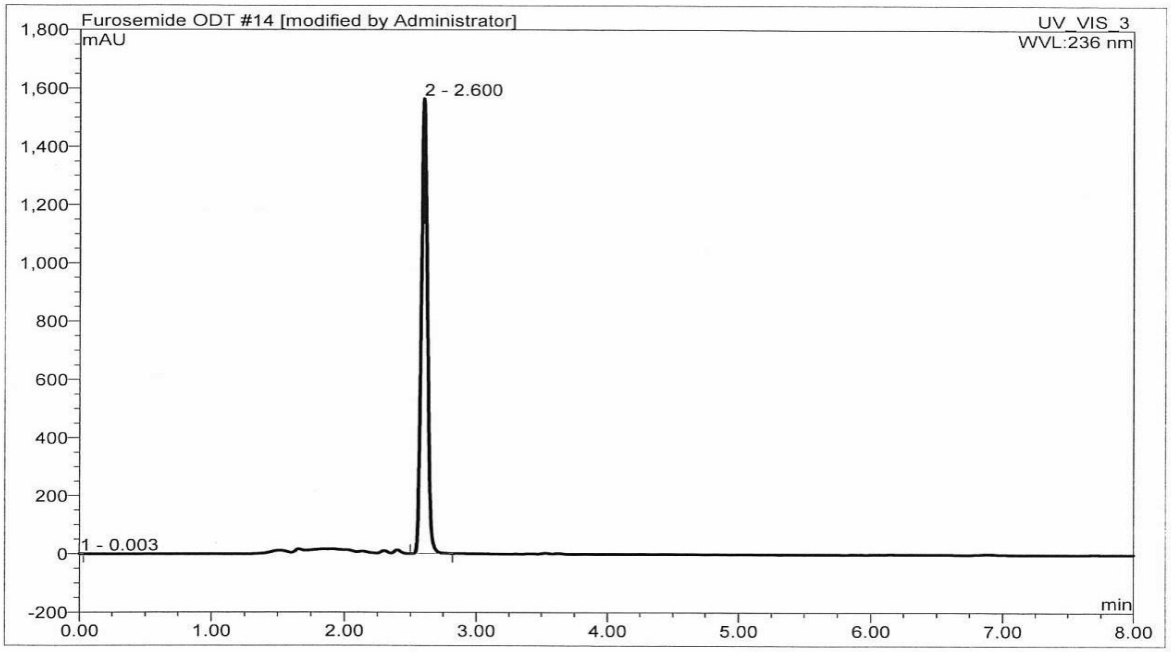
Table 35: Dissolution test results of accepted batches

Batch Number	Drug Content (%)		Dissolution rate (%) at:		
			5 Min	10 Min	15 Min
B1	3%	+8%	103%	107%	107%
B5	0	+3%	101%	102%	102%
B11	-2%	+3%	99%	101%	103%
B15	-2%	+2%	101%	101%	101%

5.7 Furosemide ODMT Stability Testing

The chromatogram for furosemide ODMT showed a clear peak (Figure 55) and no significant impurities or major peaks were detected.

Figure 55: Batch 15 HPLC chromatogram under incubating conditions



The assay content was found 97µg, which is close value with the expected content concentration of ODMT (100µcg/ml). This concludes a very slight degradation to furosemide drug molecules that may have occurred as tiny impurity peaks appeared between 1.5-2.5 minutes of chromatogram.

5.8 Discussion

MA suggested orally dispersible mini-tablets (ODMTs) as a flexible solid dosage form which is considered as age-appropriate for infants and children. ODMTs are a valuable alternative to liquid dosage forms in terms of good solid-state stability and ease of portability. Therefore, the second part of this research focused on the design and manufacturing of ODMTs. According to the published literature, there exists abundant information on oro-dispersible tablets, which are simply a large form of ODMTs. However, very few articles were specific to ODMTs intended for paediatric use e.g. risperidone oral disintegrating mini-tablets[134]. In that work, 15 risperidone batches were formulated and drug-excipient compatibility assessed using FTIR; no drug degradation was observed. However, with furosemide ODMT

formulation, the more accurate method of DSC was used; furosemide showed good compatibility with excipients with no sign of degradation. Powder fluidity assessment (angle of repose and Hausner's ratio) for risperidone and furosemide was accepted for all batches although risperidone used silicon dioxide glidant and furosemide used magnesium stearate lubricant, which has some minor glidant properties. However, powder flow is also dependent upon particle/granule size, granule shape, porosity and other mechanical factors such as the use of vibratory hoppers.

Of the 16 batches of furosemide ODMTs manufactured using a Manesty D3 machine only 6 batches passed the friability test compared to the 15 batches formulated for risperidone in which they all passed using Tablet-press II MT. According to publishers, the friability test was passed by all batches after a pre-treatment was applied to achieve 5 kN crushing force, which means the compression force was altered but its values were not mentioned. The design of furosemide experiments led to the selection of batches 1, 5, 11 and 15. This approach showed that increasing lubricant level and furosemide content led to poorer quality tablets when evaluated for friability and disintegration properties. These two factors are important as they relate to mechanical stability (e.g. handling, transport) and in vivo performance.

The disintegration time of furosemide ODMT batches varied between 13 - 38 seconds whereas with risperidone it ranged between 3 - 103 seconds. However, the disintegrating medium used in testing furosemide ODMTs was human saliva in small test tube; where with risperidone, skimmed milk was used. The variation in disintegrating time of furosemide ODMTs was explained with consideration of the lubricant level and compression force. With risperidone ODMTs, different superdisintegrants were used amongst the 15 batches (Plasdone XL, Explotab and AC-Disol) what explains the large variation in disintegration times.

In this research, furosemide ODMTs were packaged and tested for stability under accelerated conditions, which showed good stability with no degradation of API. No such studies were conducted on risperidone ODMTs and packaging type was not discussed either. The quality

target product profile (QTTP), as part of the Quality-by-Design approach, was partly established for furosemide ODMTs. Elements of the QTPP were incorporated in the manufacture of batches by using factorial design of experiments approach.

Overall, there are few limitations encountered with ODMTs. Firstly, high dose content cannot be incorporated. To circumvent this multiple number of mini-tablets can be taken, which a number of recently published studies have shown to be possible in young children e.g. 3 x 2mm tablets in a single dosing session[135]. Secondly, taste can be difficult to mask and for ethical reasons palatability testing is difficult to conduct. Thirdly, ODMTs are hygroscopic in nature and special packaging is required to ensure physical, chemical and mechanical stability. Despite these limitations, and based on the results obtained from furosemide and risperidone formulations, it is envisaged that the research described on ODMTs herein can contribute towards the development of this age-appropriate dosage form for paediatric patients.

Chapter 6

6. Conclusion and Future Work

Morphological analysis, as a problem structuring method, is based on the fundamental scientific method of alternating between analysis and synthesis phases. Given the large number of uncertainties present in the field of paediatric drug development, MA was chosen to cycle between the analytical phase of generating and refining the problem space (morphological field) and synthetic phase with the CCA processes. It was observed that CCA revealed certain conditions or values under one or more parameter that are not constrained at all (i.e. no X or P with any other value) thus identifying and removing an artefact in the system.

The application of MA offered the unearthing of new relationships and/or configurations which could have been overlooked by other less structured methods. And most importantly as indicated in this thesis, it encourages the identification and investigation of boundary conditions i.e. the limits and extremes of different contexts and factors found in paediatric drug development.

Overall, MA has definite advantages for scientific investigations by parameterising complex problems and finding possible solutions. This is achieved by the participation of stakeholders of different scientific background. For example, the method applied in this research demands that parameters, conditions and the issues underlying the uncertainties of paediatric formulation be clearly defined. Poorly defined parameters become immediately evident when they are cross-referenced and assessed for internal consistency (CCA). Additionally, both the construction of the morphological field itself and the assessments made in the CCA matrix, represent a clear ‘audit trail’, which makes the judgmental processes inherent in MA relatively traceable, and in a certain sense even reproducible.

Based on the MA solution space, microemulsion as an oral liquid dosage form was considered an age-appropriate dosage form for all paediatric age groups. Additionally, according to the ADI and NOAEL values and with reference to toxicological data on the excipients used, a non-toxic paediatric-friendly formulation was thus developed for all paediatric age groups. Full studies were conducted to optimise the furosemide microemulsion dosage formulation. These studies on microemulsion preparation revealed that the optimal microemulsion formulation containing furosemide at 10mg/ml for paediatric oral route of administration could be developed using the composition of:

(MCT Oil 14%, Labrasol 60%-Transcutol-HP 20% (3:1) and Water 6%)

This composition showed spontaneous emulsification, good thermodynamic stability and drug-excipient compatibility. The solubility screening studies showed good solubilising capability of labrasol surfactant over labrafil surfactant to dissolve sufficiently high concentration of furosemide.

To sum up, the study resulted in the development of a novel microemulsion containing labrasol surfactant and transcutol-HP co-surfactant and is believed to be a promising strategy for enhancing the solubility and subsequently the oral bioavailability of molecular entities with low solubility such as furosemide.

MA has also revealed orally disintegrating tablet dosage form as a novel age-appropriate dosage form that can be administered for infant and children. Orally disintegrating tablets of furosemide were successfully developed and formulated in this research. Prior to formulation, the selection of excipients was approved after the DSC thermograms indicated good compatibility between selected excipients and furosemide API. Pre-compression results showed good flowability and compressibility values of powder mixture at low and high concentration of magnesium stearate lubricant. Therefore, the total 16 batches resulted from factorial design were compressed. After compression, only four batches (**1**, **5**, **11**, and **15**) were found to be acceptable and passed all the post-compression characterisation tests

including assay analysis and stability studies. The discarded batches either failed friability or weight variation test. However, all batches showed very good wetting and disintegration times and the longest were13 and 38 seconds respectively. Based on QTPP design and out of the four acceptable batches, batch one failed content acceptability test (> 105%) and the rest passed the content analysis test with acceptable dissolution rate. One of these batches was packaged and tested for stability and shelf-life studies (Batch 15). Using RP-HPLC, the tablets of selected batch remained stable under stress conditions and gave assay values within the acceptable range of drug content (95-105%) with no significant decomposition or deterioration to furosemide.

The work carried out in this thesis indicates that MA offers a valuable tool for approaching multi-faceted problems. In this case the area of paediatric drug formulation has been looked at, suitable formulation approaches have been identified for a specific API and formulations have been developed which might be suitable for use. The ability of this system of problem-solving to incorporate numerical and non-numerical data in reaching decisions as to appropriate solutions makes it a useful approach to problems where there are impacts on society as well as scientific issues to be considered. MA could therefore have a significant role to play in development of drug formulations but also potentially in other areas of pharmaceutical and wider medical research.

6.1 Practical considerations and future perspectives on the use of morphological analysis to develop better medicines for children

MA, as a problem structuring method, has been shown to be a useful decision-support framework, leading to the isolation of a solution space when considering all the uncertain variables involved in deciphering the 'right' dosage form for paediatrics. However, MA requires further validation in terms of using the approach for other medicines for unmet paediatric clinical need, for example those listed on the EMA priority list of off-patent drugs used in children.

In addition, and in the context of the described herein, palatability testing of microemulsion and ODMT formulation needs to be performed to iteratively improve the MA approach. Secondly, extended stability studies need to be conducted for both dosage forms particularly for the presence, if any, of impurities that can be derived from the active, furosemide, and/or the excipients. Thirdly, the efficacy of furosemide at low doses needs to be assessed in order to evaluate the effect of API in clinical trials after enhancing the solubility using.

Finally, MA approach in paediatric formulation design should be refined and validated by involving subject matter specialists through a series of workshops facilitated by a neutral, objective facilitator. This can be performed by expanding the subject matter specialists and the stake holders lists to assure each parameter of the problem space is defined and the cross-consistency is look at after full consideration of entire dimensions.

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