DEVELOPMENT OF NOVEL EXCIPIENT SCREENING PLATFORM FOR PAEDIATRIC ORAL FORMULATIONS

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Doctor of Philosophy

ASTON UNIVERSITY

Sep 2022

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Thesis summary

The paediatric regulation came into effect in 2007 and aimed to improve the health of children aged 0-17 in Europe by facilitating the development and availability of medicines. However, despite the regulatory incentives and recent advances regarding paediatric formulation development over the last two decades, the lack of integrated and suitable paediatric formulations still remains. The struggle in developing paediatric medicines is owing to many reasons including incomplete paediatric clinical datasets, and the lack of translatable dosage form development technologies and standardised formulation development guidance available. There is also a limitation of paediatric medicine development in that knowledge and principles from adult studies are directly applied to paediatric patients and treatment preferences and acceptability are often ignored. The current formulation development programme for children is based on an empirical, iterative process of trial and error, which results in significant delays in medicines reaching the market. The overarching hypothesis of the proposal is that the development of an evidence based decision making tool will facilitate accelerated product development, meeting both patient group and regulatory requirements.

Model drug compounds were selected from the inventory of the needs for paediatric medicines (EMA), with the selection criteria taking account of properties such as low aqueous solubility, stability challenges and absence of paediatric friendly dosage forms. Carvedilol is a weakly basic biopharmaceutical class II model drug. Orodispersible mini-tablets (ODMTs) were successfully developed, characterised and evaluated in paediatric specific biorelevant media, followed by long term stability studies. Furosemide exhibits extremely low aqueous solubility and sensitivities to light, air and acidic conditions. Two variations (preservative free and preservative) of an ethanol free oral solution of furosemide were prepared and evaluated for preservative efficacy and stability. Famotidine is a BCS class III drug, exhibiting high solubility-low permeability. Two strengths (2 mg and 10 mg) of famotidine ODMTs were developed, followed by evaluation in biorelevant media and stability studies. Regarding nifedipine formulation development as an age-appropriate formulation, for the first time, an attempt to integrate PBPK modelling to a paediatric specific formulation development approach to clinically inform an appropriate dosage form design and strategy was made. A 5 mg sustained release mini-tablet was successfully developed, with duration of release extending over 24 h and an informed optimised dosing strategy of 450 µg/kg twice daily.

Formulation developments within this research resulted in the development and validation of a novel decision making tool that informs appropriate paediatric dosage form designs and formulation optimisation opportunities by integrating fundamental pharmaceutics with analytical methods and a paediatric specific, bottom up formulation development approach. Additionally, the screening platform considers product scale up and manufacturing feasibility and embeds principles of Quality by design (QbD) and quality target product profile (QTPP). Application of this novel decision making tool accelerates and de-risks the development of paediatric oral formulations and directs the formulation scientist to develop a product which is clinically efficacious and patient centric.

Key words
Paediatric; Age-appropriate formulation; ODMT; Biorelevant; stability studies, fundamental pharmaceutics, PBPK, paediatric formulation decision making tool

Acknowledgments

In the name of Allah (God), The Most Gracious and The Most Merciful. I am forever grateful for all the blessing that Allah has bestowed upon me, verily all praise belongs to Allah.

I would like to start by expressing my gratitude to Professor Afzal R Mohammed for this cherished opportunity. His guidance, enthusiasm, trust and expertise have certainly brought the best out of me and shaped me into the researcher that I am now. I would like to extend my gratitude to my industrial sponsor Proveca for funding this invaluable opportunity and industrial supervisors Dr Simon Bryson and Dr Maryam Shah for their ongoing support, encouragement, kindness and motivation. I would also like to thank my second academic supervisor Dr Daniel Kirby for his valuable suggestions throughout the project and always being there to help. I could not have asked for a better supervisory team!

The technical team and members of staff of the Pharmaceutical/Pharmacy department must also be acknowledged for their assistance and support. A special thanks goes out to Dr Raj Badhan for his assistance with the pharmacokinetic paper. I would also like to thank my amazing colleagues and friends at Aston for their support and true friendships and making my time at Aston extremely enjoyable. I am fortunate to have spent the last couple of years with you all where we have shared some wonderful memories.

Although words will never be enough, I am extremely grateful to my family; my elder brother and my parents for all that they have done and constantly still do. Without their love, prayers, sacrifices, guidance, care and concern I would not have found myself in such a fortunate position. I love you Mom and Dad! And I humbly pray that: 'My Lord, have mercy on them as they nourished me in my childhood.' (The Holy Quran, 17:24). This thesis has been dedicated to my beloved Mother and Father. A special feeling of appreciation must be made to my wife for always standing beside me and being extremely patient with me throughout this journey.

I would like to write off with a reminder to all that are in hardships and difficulties that: 'for indeed, with hardship [will be] ease' (The Holy Quran, 94:5) and to never give up and always keeping believing. We are in this all together!

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Abbreviations

Aw – Water activity

BBB – Blood Brain Barrier

BCS – Biopharmaceutical classification system

BD – Twice daily

BNFc – The British national formulary for children

BP – British Pharmacopeia

CCS – Croscarmellose sodium

CD – Cyclodextrins

CFU – Colony forming units

CI – Carr's Index

CNS – Central Nervous System

CPV – Visual predictive checking

CQA– Critical quality attributes

CYPs – Cytochromes P450

DSC – Differential Scanning Calorimetry

EMA – European Medicines Agency

FaSSGF – Fasted- state Simulated Gastric Fluid

FaSSIF – Fasted- state Simulated Intestinal Fluid

FDA – The United States Food and Drug Administration

FeSSGF – Fed- state Simulated Gastric Fluid

FeSSIF – Fed- state Simulated Intestinal Fluid

GFR – Glomerular filtration rate

GI – Gastrointestinal

GORD – Gastroesophageal reflux disease

GRAS – Generally Recognised as Safe

HCl – Hydrochloric acid

HPLC – High Pressure Liquid Chromatography

HPMC – Hydroxypropyl methylcellulose

HP-β-CD – Hydroxypropyl-β-cyclodextrins

HR – Hausner ratio

ICH – International Conference on Harmonisation

IT – Intestinal transit

Log P – Log Partition coefficient

MDF – Multi-particulate dosage forms

MIC – Minimum inhibitory concentration

NaOH – Sodium hydroxide

NICE – The National institute for health and care excellence

OD – Once daily

ODMT – Orally disintegrating mini-tablets

ODT – Orally disintegrating tablets

OTT – Oesophageal transit time

PBPK – Physiologically based pharmacokinetics

PET – Preservative efficacy test

P-gp – P-glycoprotein

PK – Pharmacokinetic

PUMA – The paediatric use marketing authorisation

QbD – Quality-by-Design

QTPP – Quality target product profile

SFB – Segmented Filamentous Bacteria

SNS – Sympathetic nervous system

SSG – sodium starch glycolate

STEP – Safety and Toxicity of Excipients for Paediatrics

TDS – Three times a day

TFA – Trifluoroacetic acid

USP – United States Pharmacopeia

WHO – World Health Organisation

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Publication list

Peer Reviewed Articles:

Khan, D.; Kirby, D.; Bryson, S.; Shah, M.; Mohammed, A.R. Development of an Age-Appropriate Mini Orally Disintegrating Carvedilol Tablet with Paediatric Biopharmaceutical Considerations. Pharmaceutics 2021, 13, 831.

Khan, D.; Kirby, D.; Bryson, S.; Shah, M.; Mohammed, A.R. Paediatric specific dosage forms: Patient and formulation considerations." International Journal of Pharmaceutics: 121501.

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Khan, D.; Kirby, D.; Bryson, S.; Shah, M.; Mohammed, A.R. Accelerating and de-risking the production of paediatric oral formulation. APS International PharmSci, 2019. London, England United Kingdom.

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Khan, D.; Kirby, D.; Bryson, S.; Shah, M.; Mohammed, A.R. Optimising the process of blending for carvedilol to develop a paediatric specific formulation. EuPFI 2020 Virtual Conference.

Oral Presentations

Khan, D.; Kirby, D.; Bryson, S.; Shah, M.; Mohammed, A.R. Integrated Paediatric Formulation Development – role of the excipient screening tool. EuPFI 2021 Virtual Conference.

Chapter 1: Introduction

Work from this chapter has been published in International Journal of Pharmaceutics - Khan, D., et al. (2022). "Paediatric specific dosage forms: Patient and formulation considerations." International Journal of Pharmaceutics: 121501.

Abstract

The total number of paediatric formulations available only account for a small proportion of the full therapeutic plethora required to effectively treat paediatrics and, therefore, the availability of high quality medicines designed specifically for children remains an ongoing challenge. Currently, the World Health Organisation (WHO) report that around 50% of medication issued for long-term conditions are not taken as advised, whilst it has also been established that, in general practice, around one tenth of medicines prescribed for children are either off-label or unlicensed. Such off-label and unlicensed use is owing to the considerable anatomical and physiological differences observed between paediatric subsets. Identifying such differences, is essential for better informing paediatric drug development and assisting regulatory reviews, whilst ensuring safe and effective therapeutic concentrations of pharmacological substances.

Points covered: The chapter (review) discusses factors affecting the safety, toxicity and efficacy of paediatric drug delivery systems. The research highlights features of the gastrointestinal tract and reports anatomical and physiological differences between paediatrics and adults. Additionally, differences observed in paediatric pharmacokinetic profiles (absorption, distribution, metabolism and elimination) due to physiological differences are also discussed. Furthermore, this review considers the advantages and limitations of current paediatric specific dosage forms available and assesses the acceptability of innovative small flexible solid oral dosage forms. Lastly, this review highlights factors affecting paediatric medicine adherence and acceptability and discusses the techniques available to overcome barriers associated with non-adherence.

Keywords: age-appropriate; pediatrics; medicine adherence; pharmacokinetics; anatomy; physiology

1.1 Current landscape of paediatric medicines and initiatives to support formulation developments

Within the late 1990s, a number of legislative and regulatory reforms were introduced by the National Institutes of Health (NIH) and the Food and Drug Administration (FDA) in recognition of the lack of paediatric specific pharmacological data on medicines prescribed to children (1). In 1997, the FDA Modernization Act (FDAMA) was issued and aimed to encourage research that would enable the FDA to label drugs for use by children (2). Similarly, Europe also introduced the paediatric regulation in 2007 with an objective to improve the health of children in Europe by facilitating the development and availability of medicines for children aged 0 to 17 years. Ever since the introduction of the paediatric regulations, great collaborative effort has been made to better the health of children by encouraging the development and availability of medicines (3). Furthermore, the regulations aims to ensure medicine intended for children are safe, effective, high quality and appropriately authorised.

Despite global efforts to increase the availability of paediatric specific age appropriate formulations, the challenge still remains to accelerate the development and increase the availability of paediatric formulations. At present, the (World Health Organisation) WHO report that around 50% of medication issued for long-term conditions are not taken as advised, whilst it has also been established that, in general practice, around one tenth of medicines prescribed for children are either off-label or unlicensed (4, 5). In the UK, around 1% of medicines prescribed are ‘specials’ and account for more than 75,000 different formulations (6). The term ‘specials’ refers to unlicensed medicines that are manufactured, imported, or distributed in order to meet the individual clinical needs of patients without an EU Marketing Authorisation or a UK Marketing Authorisation (7). Specials are often prescribed where a licensed medicine might not be available or where an unlicensed preparation my better suit the needs of the patient. For example, transforming tablets into a liquid format to achieve the lower doses required and/or overcome swallowability issues. Nonetheless, Specials can only be manufactured in the UK by companies who have been granted a 'Specials Manufacturer's Licence' by the Medicines and Healthcare products Regulatory Agency (MHRA).

In Europe, a product licence ('marketing authorisation') is required to market a medicinal product, that specifies the agreed terms of use, including medical indication, target age, dose and route of administration (8). However, many medicines intended for and used within the paediatric population do not have a product licence and, therefore, are often subjected to 'off-label' and unlicensed use. Indeed, owing to insufficient age-appropriate formulations that enable paediatric dosing and administration, many medicines are manipulated prior to use (e.g. crushing of tablets, emptying content of capsules and sprinkling over food, or changing the dosage form type), with an aim to improve patient compliance and adherence (9). Although this serves as a vital option to deliver medicines to children when needed, many safety-related incidents have been reported, due to contamination, and purity and potency inconsistencies (10, 11). On account of such limitations and to limit the potential of unknown adverse effects of unlicensed and 'off-label' use, the primary expectation and preference with paediatric pharmacotherapy is to develop an age-appropriate and ready to administer dosage form that is licensed and commercially available (12). This provides regulatory safeguards and ensures the formulation intended has thoroughly been evaluated in all aspects of safety and efficacy and is appropriate for paediatric use.

In 2017, the European Commission published a ten-year report on implementation of the Paediatric Regulation that showed considerable impact on the development of paediatric medicines in the EU. In addition, the report highlights promising new development paradigms within paediatric medicines. Since the implementation of the paediatric regulation, the number of new marketing authorisations and new indications of medicines for paediatric use stands above 260, whilst the number of agreed paediatric investigation plans (PIPs) is upwards of 1000 in 2017 (13). However, only 6 paediatric use marketing authorisations (PUMAs) have been granted till date. These include Buccolam, Hemangiol, Sialanar, Alkindi, Kigabeq and Slenyto.

Although, legislative and regulatory frameworks for paediatric formulation developments in the US and Eu are well established, there still lacks a systematic and holistic paediatric formulation development approach that encompasses and considers the differences between adults and children, as well as the differences among paediatric subsets. It is clear that children are not 'small' adults and that the differences observed are significant and influence the development and effectiveness of medicines. Thus, the drug development community needs to work within specific guidelines and continually optimise the existing frameworks based on evidence to make available more age-appropriate medicines that children deserve.

1.2 Anatomy and physiology of the paediatric gastrointestinal (GI) tract

Directed and effective pharmacotherapy has a significant impact on disease outcomes, where patients benefit from improved prognosis, better quality of life and fewer health related complications. Paediatrics are a distinct population, with differences observed between each subsets. Many factors differentiate children from one another and include anatomical and physiological changes and differences, as well as evolving competencies. Such features are inherent to the child and present many challenges in regards to medicine safety, toxicity and acceptability. It is therefore essential for formulators and researchers to have a good understanding of such variations in order to predict the fate of administered dosage forms within and across the paediatric age range, thereby limiting the potential of medicine related adverse effects.

Several routes of administration are possible within the paediatric population; however, the oral route is the most preferred, as it is simple, convenient and non-invasive. After oral administration, the drug/pharmacological substance is subjected to several processes before being eliminated from the body; the gastrointestinal (GI) tract is an organ system, composed of the oral cavity, oesophagus, stomach and intestines that serves to transport, digest, absorb and expel food and pharmacological substances (14). In medicine, identifying the anatomical and physiological differences of components of the GI system between paediatrics and adults is paramount to achieve safe, non-toxic and effective therapeutic concentrations of pharmacological substances. Furthermore, the effect of physiological differences shall better inform paediatric drug development and assist regulatory reviews (15) (Table 1, Figure 1).

1.2.1 Oral cavity

The first stage of digestion starts from the mouth, where saliva is excreted to moisten the mouth and aid swallowability. In newborns, the tongue is short and broad, descending into the oropharynx by the age of 4 years (16). The larynx is situated at a higher position, while the soft palate touches the epiglottis. During development, this contact is lost, the larynx moves downwards and the pharynx associates with both the food way and airway, increasing the possibility of aspiration (17). This developmental change, coupled with motor skill deficits, limits the use of solid oral dosage forms in the younger subsets of the paediatric population (18). Additionally, the size of the oral cavity would limit the size/volume of dosage possible.

1.2.2 Oesophagus

The oesophagus is a fibrous passageway that allows the transport of food from the mouth and into the stomach. The digestive process continues within the oesophagus through contractions. In neonates, the length of the oesophagus is 18 cm, increasing a further 2 cm by the age of three years and reaching adult measurements by the age of 10 years (25 cm)

(19). Following the transportation through the oesophagus, the contents then enter the stomach where it is subjected to further digestion. The main physical oesophageal differences observed between children and adults is the length and diameter of the oesophagus, where size increases as the child gets older. Such differences may impact the total transit time, where content is emptied into the stomach quicker in younger children due to the shorter oesophagus. This may have a more significant affect when the patient is in a specific anatomical position, since it has been established that the oesophageal transit time (OTT) varies when in different anatomical positions (90º, 45º and 0º, respectively) (20). It would also be important to note that, although peristatic movements are present by the second trimester, the spread of peristalsis and the lower oesophageal sphincter is immature at birth, resulting in frequent symptoms of Gastro oesophageal Reflux Disease (GORD) during neonatal age (21). This may not only vary transit time but also alter the total amount of drug actually reaching the stomach as some contents may be expelled out of the mouth when regurgitating.

1.2.3 Stomach

The stomach continues to digest and break the food down into a more liquid state before transferring the contents into the intestines; it is at this point where differences in stomach physiology (gastric pH, fluid volume and gastric emptying time) between paediatrics and adults may affect the absorption of pharmacological substances, especially those that are absorbed in the stomach (theoretically, weakly acidic drugs). Gastric acid secretion begins shortly after birth, gently increasing over several hours. In preterm infants, gastric acid secretion occurs more slowly, with the highest concentration observed by the fourth day of life (22). The secretion of gastric acid during infancy is lower compared to adults, resulting in a higher gastric pH. At birth, gastric pH is neutral but drops to pH 1-3 within 24 to 48 hours. The pH then slowly returns to neutral by day 8, thereafter gradually declining and reaching adult values only after the age of 2 years (23). Therefore, drugs (e.g. phenytoin and phenobarbital) that would fully be in its un-dissociated form and readily absorbed in the acidic gastric contents, may result in decreased bioavailability in children due to the higher gastric pH levels (23). In contrast, increased pH values may provide a protective effect on acid-labile drugs and encourage increased bioavailability of weak bases such as penicillin and ampicillin (23).

Although some absorption takes within the stomach, majority of the absorption takes place within the small intestines and therefore, gastric emptying and intestinal motility are rate limiting steps for absorption. Compared to adults, gastric emptying in new-borns and neonates is reduced and variable. This increase in gastric emptying time, alongside a shorter gut transit time and reduced intestinal absorption surface area may result in delayed absorption within the neonatal population (23). Furthermore, the duration of a drug's exposure to the highly

acidic gastric environment is dependent on the gastric emptying time, this may also potentially alter the total drug absorption depending on its physicochemical properties.

In addition, the capacity of the stomach also increases with age from 10-20 mL in neonates, 200 mL by the age of 2 years and 1500 mL by the age of 16 years. This would be significant for BCS Class II and IV drugs that exhibits low solubility, since larger gastric fluid volumes result in enhanced dissolution values (22, 24).

1.2.4 Intestines

Post gastric digestion, the contents then enter the small intestines, where further digestion takes place before the nutrients/drug is absorbed into the systemic blood stream. Within the intestine, intestinal transit time, intestinal permeability, bile secretion, intestinal microflora and active transport process are all physiological factors in which paediatrics differ from adults, leading to varied drug absorption capabilities between the two populations (25). The small intestine is classified into three parts; this includes the duodenum, jejunum and ileum. The duodenum makes up the first part of the small intestines and serves to combine food/nutrients with digestive enzymes from the pancreas and bile from the gallbladder. Moving forward, the jejunum is responsible for absorbing nutrients into the bloodstream, while the ileum (last section) connects to the large intestine and also contributes in absorbing nutrients into the bloodstream. At birth, the small intestine measure in around 300 – 350 cm, gradually increasing to 500 cm at age 10 and reaching adults level by the age of 20 years (26).

In addition to the formation and secretion of faeces, the large intestine is also responsible for the absorption of water, electrolytes and vitamins. In children below the age of 2 years, the large intestine measures in around 52 cm, increasing to 73 cm at 4-6 years and 95 cm at 9-11 years (27). In newborns, the small intestine measures between 300-350 cm, with quantitatively significantly reduced circular folds (plicae circulares) (28). These folds increase surface area for absorption and increase intestinal transit time by retarding the movement of semi digested food, allowing for effective digestion and sufficient absorption to occur.

Intestinal permeability describes the passage of material from the intestines into the rest of the body. At birth, the intestinal permeability is high, with rates three to four fold higher compared to adults (29). This may be due to the immature intestinal mucosa that results in a defective mucosal barrier (30). Permeability then begins to decrease in infancy and is expected to reach levels comparable to adults early in childhood (31).

The GI tract is colonised by a wide range of microorganisms which affect various physiological process. Both metabolism and GI motility are under the influence of gut flora and changes in bacterial colonisation can result in altered bioavailability (32). The composition of microbiota found in paediatrics is significantly different to that of adults, where microbial quantities vary

as a result of physiological differences (33). A study by Hollister *et al*. (2015) concluded that the child's gut possessed greater quantities of microbes supporting the functions of development, where microbes relating to inflammation and obesity, such as Segmented Filamentous Bacteria (SFB) and bacterial species from the Firmicutes phylum were found at a higher concentration in adults (34-36). Levels of intestinal microbiota in paediatrics were thought to reach adult levels in between the ages of 1 to 3 years; however, more recent studies suggest otherwise and indicate an adult-like gut flora environment to establish at a later age of 4 years (34, 37).

Active transport systems involves the movement of substances across membranes and determines the absorption of molecules. At birth, these transport systems are immature, resulting in variable absorption values. Both active and passive transport systems completely mature at around the age of 4 months (38). P-glycoprotein (P-gp) is a plasma membrane protein that pumps drugs/substances out of the cell. P-gp is accountable for restricting cellular uptake and distribution of toxic substances; hence, its influence on drug absorption, metabolism, distribution and elimination is substantial (39). It has been reported that intestinal and hepatic p-gp expression at birth is limited, intensifying during the first few months of life and reaching adult levels by the age of 2 years (23, 40). However, within the paediatric population, the origin and development of P-gp expression is speculative, where contrasting results have emerged in which intestinal and hepatic P-gp expression values reaching adult levels are reported to be at the age of 0 and 12 months, respectively (41, 42).

Table 1: An overview of the anatomical and physiological differences of components of the gastrointestinal tract observed between paediatrics and adults.

Anatomical and physiological differences	Paediatrics	Adults
Oral cavity	Tongue: short and broad in new-borns Tongue: Proportionally larger in young children Soft palate in contact with epiglottis Epiglottis: longer, floppy and U shaped Larynx: situated more anterior and superior	Tongue: proportionally smaller Soft palate and epiglottis contact lost Epiglottis: shorter and stiff Larynx: posterior and lower
Oesophagus	Neonates: measures at ≈ 18 cm Age 3: measures at ≈ 20 cm Vertebral column location: C4 – T9	Measures at ≈ 25 cm Vertebral column location: C6 – T11
Stomach		
Anatomical	Neonatal capacity: 10-20 mL Age 2 capacity: 200 mL	≥ 16 years capacity: 1500 mL
Physiological	Gastric pH: neutral at birth Gastric emptying rate: ↓ and linear (until 6-8 months)	Gastric pH: 2-3 Gastric emptying: ↑ and bi-phasic
Small intestine		
Anatomical	At birth: measures ≈ 300-350 cm Age 10: measures ≈ 500 cm Reduced number of circular folds Reduced absorptive surface area	≥ 20 years: measures at 575 cm Increased circular folds Higher absorptive surface area
Physiological	Intestinal permeability: 3-4 fold greater at birth Microbiota: Higher levels of microbes supporting developmental processes (e.g. mean bifidobacteria levels in infants: 4.4 ± 8.6×10^{10} CFU/g^{-1}) Transport system: P-gp limited during birth Intestinal transit time: ↑ in neonates and ↓during infancy Bile salt secretion and activity: ↓ in neonates and infants	Intestinal permeability: mature Microbiota: Mean levels of bifidobacteria: 1.03 ± 1.7×10^{9} CFU/g^{-1} Transport systems: mature Bile salt secretion and activity: mature

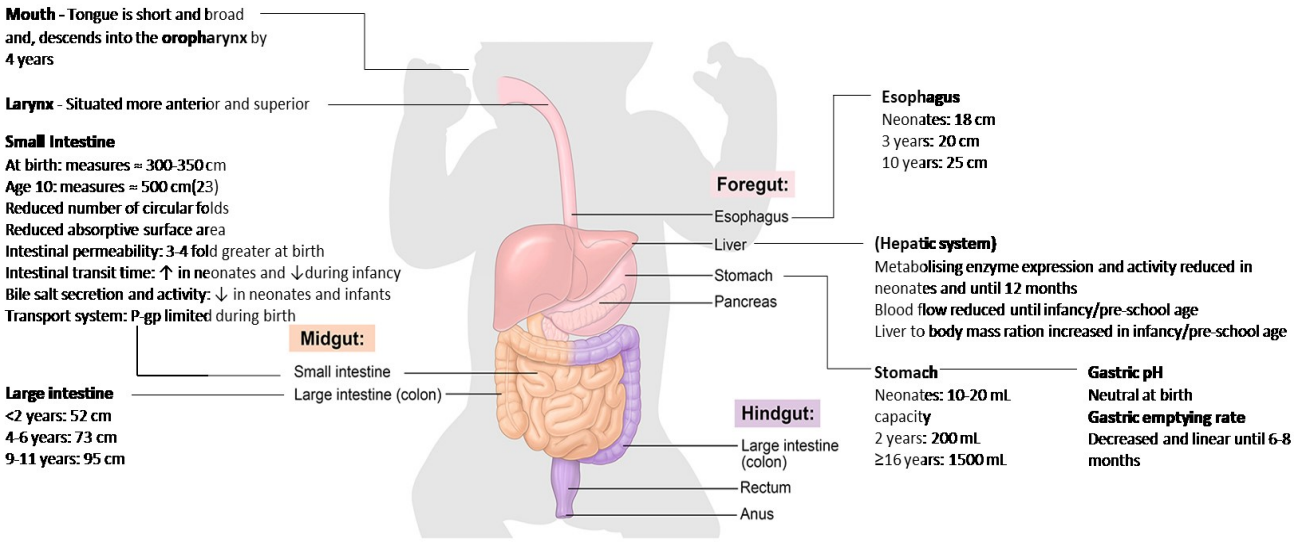

Figure 1: Diagram showing the anatomical and physiological differences of the paediatric gastrointestinal tract when compared to that of an adult. Figure adapted from Leach, J. (2020), "Fetal development: your baby's digestive system.

1.3 Paediatric pharmacokinetics

Pharmacokinetic (PK) profiles (absorption, distribution, metabolism and elimination) measure the concentration of pharmacological substances in the body and are indicative of safe and toxic therapeutic levels. The consequence of anatomical and physiological changes observed within paediatrics may significantly alter the exposure of pharmacological substances, therefore, careful dose adjustments should be considered to limit the occurrence of any adverse effects (32). This would also be true for any excipients added within the formulation, where the exposure and safety of excipients may change under influence of anatomical and physiological differences. Although excipients are considered 'inert', immature organs and lack of metabolising enzymes may lead to accumulation that may resulting in excipient toxicity (43).

As the anatomy and physiology evolves in paediatrics with age so do the pharmacokinetic considerations; pharmacokinetic profiles are non-linear, where anatomical and physiological differences in children can affect the pharmacokinetic profile (32). Pharmacokinetics (PK) studies the extent of absorption, distribution, metabolism and elimination of the pharmacological substance under review. . The variance in pharmacokinetic profiles between children and adults can have a considerable effect on the resulting concentration of the pharmacological substance under review, whether that be a drug or an excipient. Careful

dose titrations and adjustments must therefore be carried out accordingly in respect to all of the constituents involved in the formulation intended to be administered (Table 2).

Although there has been a substantial increase of data available on pharmacokinetic drug profiles, the influence of certain age specific age-related effects on PK profiles and dose requirements is not well understood and continues to exist (23). Similarly, many commonly used pharmaceutical excipients have undergone comprehensive short and long term studies for safety and toxicity in adult population, but not in paediatrics, whilst it has also been established that pharmaceutical excipients are not inert and can lead to considerable adverse effects if administered in concentrations above specified values (44). Therefore, selection of such excipients is encouraged to be based on research and guided by agencies such as the European Medicines Agency (EMA), where problematic excipients are identified and maximum daily intake values are specified. Furthermore, the dearth of authorised medicinal formulations available to paediatrics has led to the extensive use of unlicensed and off-label medicines, where excipient composition and load that is suitable and safe for paediatric patients is not guaranteed (45).

The anatomical and physiological differences observed between paediatrics and adults may significantly alter the pharmacokinetic profile of both drugs and excipients. Therefore, detailed understanding of implications of such differences is important in order to guide safe and acceptable daily intake values.

1.3.1 Absorption

Absorption – the first physiological process that governs the degree of bioavailability – can vary significantly due to differences in gastrointestinal (GI) tract development. Factors affecting extent of absorption include surface area available, intestinal permeability, gastric pH, gastric empting, GI motility and immaturity of intestinal mucosa, transport systems and secretion of bile (25). Gastric pH at birth is reported to be neutral (pH 7), where it then significantly decreases before returning back to neutral by the tenth day (46). Furthermore, gastric pH levels of 2-3, as observed in adults, are achieved in children by the age of 3 years (47). As a result of this change in gastric pH during growth and development, absorption and concentration of weakly basic, weak organic acids and/or acid labile pharmacological substances can significantly vary (48). The rate of gastric emptying in paediatrics up to the age of 6-8 months is slow and linear, where after this point proceeds to become bi-phasic, as observed in adults (25).

Intestinal transit (IT) is key for absorption and involves the travel of substances through the small intestine. In neonates, an increased IT time is observed owing to reduced GI motility and frequency of peristaltic waves, whereas during infancy, GI motility intensifies, resulting in a lower IT time (49). As a consequence of short IT times, the pharmacological substance under review may not have sufficient time to fully absorb via active and passive transporters and, therefore, may result in decreased concentrations. Bile salts are manufactured in the liver from cholesterol and serve to solubilise dietary fats within the aqueous conditions of the small intestines. Bile salts are comprised of bile acids coupled with taurine/glycine, increasing the water solubilising power of the bile salts, suggesting a positive correlation between that of bile salt concentration and pharmacological drug/substance solubility (50). Within the lower subsets of the paediatric population (neonates and infants), the secretion of bile salts is hindered, resulting in a decreased ability to solubilise and absorb fat soluble substances and lipophilic drugs (e.g. carvedilol) (51). Maturation of bile secretion and activity is achieved after 3 to 7 months post-natal. Therefore, careful dose determination and titration must be implemented (52).

1.3.2 Distribution

Following absorption, the substance then distributes relative to its physicochemical features. As a child grows and develops, total water (both intra and extracellular) concentration decreases, from around 80-90% v/w in neonates and infants, down to 55-60% v/w in adults (53). Volume of distribution of hydrophilic substances (e.g. phenobarbital) would, therefore, vary as a consequence of body water content, with neonates (having the greatest volume of distribution) requiring larger doses per weight of such compounds to achieve an equivalent therapeutic response to that of an adult (54). Additionally, the development of the blood brain barrier (BBB) in neonates is immature, thereby significantly increasing the risk of toxicity of substances (drugs and/or excipients) due to high levels entering the central nervous system(CNS) (55). This is particularly common with several commonly used functional excipients, such as ethanol and propylene glycol (56). Furthermore, the fraction of unbound drug found in neonates is higher compared to adults, due to the decreased plasma protein binding capacity, suggesting an increase in amount of pharmacological substance available for activity (57). Doses should, therefore, be determined with caution and in respect to the target age group to avoid toxicity.

1.3.3 Metabolism

The liver is predominantly the organ responsible for metabolism, where drug is metabolised into rather non-toxic and more water-soluble compounds, reducing toxicity and assisting excretion via urine and bile. In neonates, metabolising enzymes are immature, leading to

supressed enzyme expression and activity, thereby increasing potential for substance accumulation (toxicity) (58). Cytochromes P450 (CYPs) are a class of enzymes that serve to metabolise toxic substances and drugs through oxidation, so they may safely be excreted from the body. However, the amount of CYP metabolising enzymes in children aged between 6-12 months is around 50% to that of adults (59). Another factor influencing hepatic clearance is the amount of blood flow through the liver; the hepatic blood flow in a neonate is reduced, where it then reaches a rate comparable to adults by infancy/pre-school age. Nonetheless, the hepatic clearance of pharmacological substances during infancy/pre-school age is significantly increased due to a greater liver size to body weight ratio (60). Pharmacological substances undergoing high degree of metabolism are especially affected (e.g. allopurinol and benzyl alcohol) (61, 62).

1.3.4 Elimination

Elimination is the process in which the drug/substance and their metabolites are excreted from the body, predominately through the kidneys. Determinants affecting the rate and extent of elimination include glomerular filtration rate (GFR), tubular secretion and tubular reabsorption, which vary as a consequence of renal blood and plasma flow (57, 63). In newborns, the renal excretion is at its lowest, gradually increasing as the renal system matures. Renal blood flow also increases with age, where values comparable to those of adults are achieved by the age of 2 years (64). These changes in blood flow can alter the rate of excretion; therefore, the dose of substance being administered needs to be adjusted. GFR describes how well the kidneys are working by measuring the rate of blood flowing through the glomeruli; at birth, the GFR is at its lowest, increasing rapidly during the first two weeks and reaching adult levels by the age of 1 years as a result of maturation (65). It is also important to mention that the rate of increase in GFR during early days of life also varies depending on whether the baby is born before or after the 37 weeks gestation (pre-term/term). Tubular reabsorption is an occurrence in which the filtrate is absorbed back into the systemic bloodstream; tubular reabsorption increases with age, with peak maturation taking place between the age of 1 to 3 years (66). Similarly, active tubular secretion is also immature in newborns, with values comparable to adults achieved by the age of 7 to 12 months (67). Furthermore, the reabsorption of weak acids (e.g. citric acid) is under the influence of urinary pH, with a higher reabsorption of weak organic acids taking place at lower pH values, as in the case of neonates and infants (68). The most common route of elimination of substances is through the kidneys; however, some are actively secreted into the bile by the liver before being excreted in faeces. Many factors determine the extent and route of excretion and include water solubility, molecular weight and plasma protein binding (69). As discussed, paediatrics and adults can have significantly varying PK profiles as a consequence of differences in hepatic and renal system anatomy and

physiology; as a result, pharmacological substances intended for adult use cannot be assumed to be labelled as safe in paediatrics.

Table 2: Differences in hepatic and renal system physiology between children and adults and subsequent pharmacokinetic effect on commonly used pharmaceutical excipients.

Differences in hepatic and renal system anatomy and physiology	**Neonates (1 day to 1 month)**	**Infant (1 month to 2 years)**	**Adults**	**Effect on Pharmacokinetic profile**	**Types of Excipients affected**	**Example Excipient**
Hepatic system *(metabolism)*						
Metabolising enzymes (CYPs) expression and activity	Immature	Reduced (until 12 months)	Increased	Reduced metabolism	Those metabolised through CYP enzyme family	Ethanol *(solvent)*
Blood flow through liver	Lowest	Adult levels	Increased	Reduced hepatic clearance until infancy/pre-school age	Those undergoing high degree of metabolism	Propylene Glycol *(solvent)*
Liver to body mass ratio	Smaller	Larger (in infants and pre-school children)	Smaller	Increased hepatic clearance in children (infants and pre-school) ↓ AUC (plasma drug concentration over time)	Those undergoing high degree of metabolism	Benzyl alcohol *(preservative)*
First pass metabolism	Decreased	Increased (due to liver to body mass ratio)	Increased	↑ Bioavailability	Those undergoing significant first pass metabolism	Fructose *(sweetener)*
Renal system *(elimination)*						
GFR	Reduced (up to the age of 12 months)	Adult levels reached by 12 months	Increased but decreases in the elderly	Slower elimination up to the age of 12 months ↑ Levels in blood	Those renal excreted	Cyclodextrins *(solubility enhancer)*
Maturation of tubular transport (reabsorption/secretion) system	Reabsorption – Immature Secretion - Immature	Reabsorption - Adult levels reached by 1-3 year Secretion – Adult levels reached by 12 months	Increased	↑ Tubular reabsorption with age ↑ Tubular secretion with age	Disposed to tubular reabsorption/ secretion	Glucose *(sweetener)*, Sodium bicarbonate *(alkalizing agent)*, Propylene glycol
Urinary pH value	Decreased	Decreased	Increased	↑ Reabsorption at lower pH values	Weak acids/bases	Citric acid *(antioxidant)*

1.4 Pharmaceutical excipients and commonly used functional excipients in paediatric oral dosage forms

Pharmaceutical excipients are substances, other than the active pharmaceutical ingredient(s), added to achieve an intended functionality within a drug delivery system (70). The use of pharmaceutical excipients in formulations is common and have many applications (Table 3). A wide variety of sweeteners and flavours are available and incorporated to improve palatability, while colouring agents are added to differentiate between different strengths (product identification). Other applications include, aiding manufacturing processes and enhancing drug solubility and stability (71). However, unregulated excipient use in children is not recommended as it may lead to potential adverse effects. Within the paediatric population, the ability to metabolise and eliminate pharmacological substances (including excipients) varies, owing to differences in anatomy, physiology and development (32). The USP national formulary <1059> Excipient Performance, have identified functional excipient categories that are commonly used in both oral solid and liquid dosage forms (72) (Table 3).

1.4.1 Solid dosage forms (including tablets, mini-tablets, orally disintegrating tablets and capsules)

Diluents, also referred to as fillers, are constituents that simplify dose measurements and improve dose handling by increasing dosage form volume or weight. Many diluents are available and include starch (including modified starches), calcium salts such as calcium phosphate and sugars such as mannitol, sorbitol and lactose. Choice of diluent will vary depending on the manufacturing methods, compatibility with other dosage form constituents and its physical and chemical properties (73). Other than providing bulk to the dosage form, diluent are exploited for their inherent physical material attributes (e.g. particle shape, size and distribution, solubility and flow) to impart desirable manufacturing properties (e.g. powder flow and mechanical strength) and enhance performance (e.g. disintegration, dissolution and content uniformity) (74). Since diluents may comprise a substantial portion of the formulation, it is essential to measure and control critical material attributes as they will significantly affect the dosage form manufacture and performance (75).

Binders are often included in solid dosage forms for their cohesive and adhesive properties that enable for constituents to bond with one another and be held within a compact. Commonly used examples include natural and synthetic polymer such as native starches, sugars and cellulose derivatives.

Disintegrants/Superdisintegrants, a readily employed class of excipient, are responsible for effecting the dissolution profile of tablets (70). They promote and ensure the dispersion of the

tablet compact upon ingestion; through either capillary absorption, deformation or swelling mechanisms (76). Due to different physical and chemical structures, disintegrants work through different mechanisms of action however, a coalition of several mechanisms are usually responsible (77). Mechanism of action of starch-based disintegrants is believed to be through swelling, while cellulose derivatives, due to their fibrous nature exert their function through wicking, followed by swelling (78). Contrarily, wicking followed by secondary swelling and some strain recovery has been proposed as the mechanism of action of cross-linked polyvinylpyrrolidone (79). Disintegrants differ to superdisintegrants in their rate of hydration and swelling capabilities, where usually the latter is used when a faster rate of dissolution is required. Examples of disintegrants include starch, pregelatinized starch and microcrystalline cellulose (MCC), whilst superdisntrgants include modified starches (sodium starch glycolate) and cellulose (croscarmellose sodium) and cross-linked polyvinylpyrrolidone (crospovidone).

Lubricants are incorporated into formulations to reduce friction between the compact and die surface during ejection. Reducing ejection force, ensures the tablet is smoothly ejected, without any breakage or cracks (80). Depending on the functional mechanism, lubricants may be classified into one of the following categories: boundary lubricants, fluid film lubricants, or liquid lubricants. Magnesium stearate, a metallic salt boundary lubricant is one of the most frequently used lubricant in solid dosage forms as it is relatively inexpensive, chemically stable and has excellent lubrication properties (80).

Glidant and/or anticaking agent are excipients, regularly employed to improve the flow of pharmaceutical powder blends and prevent caking and clustering during bulk storage. Glidants work by firmly adsorbing at the surface of larger particles, thereby reducing interparticle adhesive and cohesive forces (81). Customarily used glidants include colloidal silicon dioxide (fumed silica) and talc.

Colourants are regularly used to characterise the final appearance of the dosage form. They aid product identification, branding and prevent counterfeiting. In addition, in paediatric formulations, colouring agents are included with an intent to promote acceptability by making the product more appealing. However, their use is in paediatrics is associated with safety concerns and therefore, are subject to federal regulations (82).

Capsule Shells are used to encapsulate pharmaceutical powders, granules and liquids and provide a means of accurate dosing and easy transportation. Apart from being able to taste mask and promote swallowability, capsule shells can be modified to control or delay the release of capsule contents (83). The capsule shell is usually derived from either animal (e.g.

bovine gelatine) or non-animal sources (e.g. polysaccharides and cellulose) and can be categorised as either hard or soft capsules. Hard capsule usually contain dry solids, are comprised of a two-piece unit and are not plasticized. On the other hand, soft capsules are normally plasticized (e.g. using glycerol, sorbitol or propylene glycol), accommodate liquids and are composed of a single unit piece. Other additional additives used in capsule shells include plasticizers, colouring agents, and preservatives(84).

Pharmaceutical dosage forms are coated for many reasons, for instance, to protect tablet ingredients from the external environment (air, light and moisture), improve stability, enhance appearance, aid swallowability and modify release of the therapeutic agent (e.g. enteric coat) (85). The materials used as coating agents can be categorised into synthetic, semisynthetic and natural materials. Hydroxypropyl methylcellulose (HPMC) is a commonly used semisynthetic material that is employed for its inert and viscoelastic properties (86). Other commonly used coating materials include natural and synthetic polymers, beeswax, paraffin, lanolin, shellac and polysaccharides.

Plasticizers are low molecular weight substances that are supplemented with a polymer to film coats and capsule shells to improve workability and flexibility (87). Plasticizers are mainly organic esters (e.g. phthalates and citrates), which exercise their effect by decreasing intermolecular and intramolecular interactions, thereby enabling molecular mobility and reducing internal friction (88).

1.4.2 Oral Liquid dosage forms (including solutions, suspensions and emulsions)

A Buffering agent (pH modifier) can be defined as “a compound or a mixture of compounds (usually a weak acid or base and its salt) that, when present in a solution, resists changes in the pH of the solution when small quantities of acid or base are added to the solution” (89). In pharmaceutical preparations, buffers or pH modifying agents are used to adjust an maintain pH values and improve the chemical stability, solubility and absorption of many pharmacological compounds, since these are pH dependant (89). Additionally, they may be added to formulations to match the pH to that of the relevant body fluid to avoid discomfort. Some commonly used pH modifying agent include acetic acid, citric acid, potassium phosphate and sodium citrate.

Solvents and solubilising agents - Many pharmacological active compounds exhibit low aqueous solubilities, rendering the need of solubilising agents. Solubilising agents are utilised to dissolve insoluble molecules, resulting in a thermodynamically stable solution (90). The

extent of solubilising power is dependent on the chemical structure or class of the solubilising agent (91). For example, cyclodextrins consist of a hydrophobic interior in which hydrophobic molecules are incorporated into, encapsulating the molecule and making it water soluble. Additionally, other solubilising agents have distinctive hydrophobic structures that are able to form inclusion complexes. After water, alcohols are most commonly used as solvent-vehicles and have a generic formula of R-OH, where R is depicted as the hydrocarbon group (92). The presence of the hydroxyl group (OH) will enable the molecule to be water soluble, whereas the hydrophobic hydrocarbon chain interacts with the hydrophobic molecules by dissolving the insoluble agent into the hydrocarbon chain, thereby facilitating solubilisation (93). Glycols are dihydroxy alcohols that are also frequently used as solubilising agents. Relative to a comparable singly hydroxyl alcohol, glycols have a higher aqueous solubility since there are more than one sites for hydrogen bonding (92). Other commonly used solubilising agents include ketones and oils.

Chelating agents (also known as sequestering agents) are excipients that interact with certain metal ions (e.g., copper, iron, manganese, lead, and calcium), removing the ions from solution to form stable, water-soluble complexes (72). Such metallic ions may otherwise promote instability in some pharmaceutical liquid formulations. Additionally, chelating agents may be equipped to increase antioxidant defences and antimicrobial efficacy by creating an environment with limited metallic ions available, thereby reducing the tendency for oxidative reactions and microbial growth (94). An ideal chelator should possess high water solubility and low toxicity, have the capacity to form non-toxic complexes and rapidly eliminate toxic metals (95). Several salt and hydrated forms of edetic acid are available and are usually employed as chelating agents in oral liquid preparations.

Antimicrobial Preservatives - Many oral liquid formulations are intended to be used as multi dose preparations and therefore require the inclusion of antimicrobial preservatives to inhibit microbial growth of bacteria, moulds and yeast during use. The action of antimicrobial preservatives on microbial proliferation is based on a variety of individual influences such as physicochemical mechanisms and biochemical reactions (96). Mechanisms of action include cell lysis, cell leakage, structural damage (e.g. cell membrane), reversible enzyme inhibition and selective permeability changes (influence on transport mechanisms) (96). Depending on the mechanism of action, the antimicrobial preservative can be classified as either be microbiostatic or microbiocidal. Commonly used antimicrobial preservatives include benzoic and ascorbic acids and their salts, phenolic compounds (parabens), alcohol and quaternary ammonium salts (e.g. benzalkonium chloride) (97).

Antioxidants are included in pharmaceutical preparations to prevent deterioration against oxidative processes, thus maintaining the integrity of the dosage form. Free radicals are unstable atoms with unpaired electrons. In the existence of oxygen, such free radicals may initiate and set of chain reactions, subsequently activating undesirable reactions with the active pharmaceutical ingredient and excipients (72). Antioxidants protects the API by retarding and/or notably reducing the occurrence of oxidative reactions. True antioxidants such as tocopherols and phenolic compounds impede the chain reaction by reacting with free radicals. In contrast, reducing agents such as ascorbic acid have a relative inferior redox potential to the API or excipient requiring protection and therefore sacrificially react with oxygen, slowing the rate of oxidative reactions (72). The third group of antioxidants are known as synergists, which facilitate the action of true antioxidants. For example, by reacting with heavy metal ions, which alongside heat and light act as catalysts during the propagation stage of oxidative chain reactions (98). Commonly used synergetic antioxidants include citric acid or chelating agents that form stable complexes with free metal catalysts (e.g. iron and copper).

Sweetening agents are used to improve the palatability of oral liquid formulations by masking any unpleasant or bitter taste. Sweeteners exert their action by binding to taste receptors on the tongue, creating a sweet sensation. Sweeteners can be classified as either: sugars (e.g. fructose and glucose), sugar alcohols (e.g. maltitol and erythritol) or artificial sweeteners (e.g. aspartame, sucralose and saccharin). Usually, the selection of sweetener is formulation dependent, however, some potentially problematic sweeting agents have been identified for paediatric use and therefore careful considerations should be taken. Additionally, patient factors such as diabetes, phenylketonuria and hypersensitivities may also influence the choice of sweetener.

Suspending and/or Viscosity-Increasing Agents – Suspending agents are aqueous biological polymers, commonly used to increase the viscosity of suspensions and emulsions to delay sedimentation of dispersed particles (99). Their effect is exerted by one of three mechanisms: increasing viscosity (by disrupting laminar flow or entrapment of solvent by macromolecular chains), gel formation or steric stabilisation (72, 100). However, the rheological profiles of the dispersions of suspending agents are non-Newtonian, where viscosity or flow change under stress (101). Suspending agent can be classified into three groups, comprising of natural agents (e.g. acacia, gelatine and kaolin), semi-synthetic agents (e.g. HPMC and sodium carboxymethylcellulose) and synthetic agents (e.g. carbopol, PVP and PVC) (102). Although suspending agents stabilise disperse systems, caution must be exercised when employing suspending agents as the acidic environment of the stomach may alter the physical properties of the formulation and therefore the rate of drug release (103).

Surfactants, also called wetting agents are excipients that adsorb to surfaces or interfaces, causing a decrease in surface tension and thereby enhancing its spreading and wetting properties (104). Such properties are exploited to enhance the solubility of drug particles by assisting the dispersion of solid particles in the liquid phase (105). Other than improving solubility, depending on the surfactant type, surfactants can also be used to promote stability of drugs in solubilised systems, improve physicochemical characteristics of the formulation and modify drug release parameters including diffusion, disintegration and dissolution (105). Surfactant molecules are composed of hydrophobic tail region coupled with a hydrophilic head group that can possess ionic, polar, or hydrogen-bond-forming groups, making them water soluble (106). Depending on the nature of the hydrophilic head, surfactants can be classified as either anionic, cationic, ampholytic or non-ionic (107). Commonly used surfactants include polysorbates, sorbitan alkyl esters, and lecithin.

Table 3: Overview of commonly used excipients in solid and liquid dosage forms.

Excipient category	**Functionality**	**Frequently used examples**
Solid dosage forms		
Binder	Bind constituents together, providing form and cohesiveness	Native starches, sugars and cellulose derivatives.
Capsule shell	Encapsulate dose, taste mask, modify/control release, provide accurate dosing	Gelatine, glycerol, sorbitol, polysaccharides and cellulose
Coating agent	Protect tablet ingredients from external environment, improve stability, enhance appearance, aid swallowability and modify release of the therapeutic agent (e.g. enteric coat)	Hydroxypropyl methylcellulose (HPMC), beeswax, paraffin, lanolin, shellac and polysaccharides
Colourant	Enhance product appeal, product identification, branding and prevent counterfeiting	Organic dyes and their lakes (e.g. Tartrazine, Sunset Yellow and Allura red), Inorganic or mineral colours (e.g. Titanium dioxide and red iron oxide) and Natural colours (e.g. β-carotene)
Diluent	Simplify dose measurements and improve dose handling, impart desirable manufacturing properties and enhance dosage form performance	Starch (including modified starches), calcium salts (e.g. calcium phosphate) and sugars (e.g. mannitol, sorbitol and lactose)
Disintegrant/ Superdisintegrant	Promote and ensure the dispersion of the tablet compact, responsible for effecting the dissolution profile	Disintegrants - Starch, pregelatinized starch and MCC) Superdisintegrants - Sodium starch glycolate,

		croscarmellose sodium and crospovidone
Glidant, anticaking agent	Improve the flow and prevent caking and clustering during bulk storage	Colloidal silicon dioxide (fumed silica) and talc
Lubricant	Reduce friction between the compact and die surface during ejection	Magnesium stearate, sodium stearyl fumarate
Plasticizer	Improve workability and flexibility of polymeric materials	Organic esters (e.g. phthalates and citrates)
Liquid dosage forms		
Buffering agent (pH modifier)	Adjust and maintain pH of formulation, enhance drug stability, solubility and absorption, maintain a consistent ionization state	Acetic acid, citric acid, potassium phosphate and sodium citrate
Solvents and solubilising agents	Enhance aqueous solubility of insoluble molecules, vehicle for oral liquid preparations	Ethanol, propylene glycol, glycerin, cyclodextrins, polyethylene glycol and mineral oil
Chelating agents	Stabilise pharmaceutical liquid formulations, Increase antioxidant defences and antimicrobial efficacy	Salt and hydrated forms of edetic acid (EDTA)
Antimicrobial Preservative	Inhibit microbial growth of bacteria, moulds and yeast	Benzalkonium chloride, benzoic acid, benzyl alcohol and methyl paraben
Antioxidants	Prevent deterioration against oxidative processes, thus maintaining the integrity of the dosage form	Tocopherols, ascorbic acid and sodium metabisulfite
Sweetening agents	Improve palatability by masking any unpleasant or bitter taste	Sorbitol, xylitol, glucose, aspartame, sucralose and saccharin
Suspending and/or Viscosity-Increasing Agents	Stabilise disperse systems such as suspensions and emulsions	Acacia, xanthan gum, carboxymethylcellulose, HPMC, PVP and PVC
Surfactants (wetting agent/emulsifier)	Improve solubility and promote stability of drugs in solubilised systems	Lecithin, polysorbates, sorbitan alkyl esters and polyethylene glycols

1.4.3 Potentially problematic excipient use paediatrics

Propylene glycol (PG) is extensively used as a solvent, co-solvent and preservative in many pharmaceutical formulations and is generally regarded as a relatively safe material (108). Nonetheless, propylene glycol use in paediatrics demonstrates safety and toxicity concerns (especially in children below the age of four years), owing to physiological immaturity (insufficient metabolism and elimination) that leads to PG accumulation (108). Higher PG levels may be safely given to children above four years, however a careful approach should be taken due to insufficient paediatric clinical data (109). With regards to such safety concerns, the EMA has stipulated safety limits on the use of PG in children (Table 4).

Ethanol is a commonly used solvent, utilised as a vehicle for lipophilic compounds in oral liquid formulations. Within the adult population, ethanol is generally regarded as safe, however, in paediatrics, the use of ethanol is associated with some serious acute and chronic adverse effects (110). Children experience rapid growth and maturation, leading to change in pharmacokinetic profiles. Such changes may potentially effect the toxicity of compounds under significant influence of pharmacokinetic processes (111). Accidental overdose may lead to acute intoxication and poising resulting in CNS depression, hypoglycaemia, hypothermia and coma. The challenge to characterise the effect of short and long term use of ethanol in paediatrics still remains, whilst establishment of appropriate intake values warrants more clinical evidence (111). Until then, the EMA have proposed that, following a single dose blood ethanol levels in children under six years should not progress beyond 1 mg/dL (112). Additionally, the FDA have restricted the ethanol content of all paediatric over the counter medicines intended for children under six years to 0.5% v/v (113).

Sucrose is a disaccharide, consisting of fructose and glucose. It is naturally produced in plants and is commonly used as a sweetener in pharmaceutical formulations. Both sucrose and fructose increase blood sugar levels, therefore should be avoided in patients who are diabetic. Their use in children suffering from hereditary fructose intolerance is also contraindicated(114). Moreover, long term fructose/sucrose use may lead to obesity, tooth enamel deterioration and dental caries, hence their use in long-term therapy is discouraged. Instead, non-caloric non-nutritive sweetening agents such as aspartame, saccharin, acesulfame-potassium and sucralose are preferred (115). These compounds have been approved as an ‘additive’ by the FDA, are GRAS listed and provide the necessary palatability without promoting calorie intake (116). Nevertheless, due to adverse effects, the FDA have established maximum allowable daily intake values for all artificial sweeteners. Aspartame consist of phenylalanine, which may be harmful in children with phenylketonuria (leading to mental disorders and seizures) (117). Similarly, saccharin should be avoided in children who are allergic to sulphonamides as it may lead to hypersensitivity reactions (118). Xylitol, and sorbitol are two alternative sweetening agents (polyols) that are not readily absorbed from the gut and therefore are suitable in children with diabetes, though they may cause gastrointestinal disorders and diarrhoea (119).

Benzyl alcohol is commonly used as a preservative in solutions and suspension for injections. In addition, it is often used as an active ingredient in many antiseptic and local anaesthetic preparations (120). However, benzyl alcohol must not be given to neonates due to the immaturity of the benzoic acid detoxification process (reduced capacity to metabolise) in newborns (121). Furthermore, the use of benzyl alcohol use is contraindicated in children up

to three years as it may cause toxic and allergic reactions (122). Despite the contraindication, benzyl alcohol may be used in children above four week old, however, caution must be exercised and preparations must be evaluated (123). Although, the EMA have proposed for more relaxed benzyl alcohol threshold values, at current, exposure values must not exceed 90mg kg^{-1} per day (123).

Lactose, a disaccharide of glucose and galactose, is an excipient widely employed as a dry powder inhaler carrier and tablet/capsule diluent. For complete digestion of lactose, the enzyme lactase is required and if deficient, either due to a rare congenital disorder or an acquired lack of intestinal lactase production leads to lactose intolerance (124). In children, lactose intolerance may be associated with acute dehydration, metabolic acidosis and prolonged diarrhoea (similar for galactose-intolerance patients) (125). Whilst, levels of lactose present in most pharmaceutical preparations is insignificant to bring about symptoms (flatulence, cramping and diarrhoea), sensitivity to lactose is highly variable in severity, and undesirable symptoms have been reported after lactose ingestion of as little as 3 g or less (126). However, the EMA have suggested a threshold of 5 g of lactose per dose (127).

Although, it is assumed that children prefer brightly coloured formulations, the use of colouring agents should be restricted, as they have been identified with adverse reactions including hypersensitivity, negative behavioural effects and ADHD (128). Consequently, both the European Commission (EU) and FDA (US) have proposed allowable daily intake values(82). Commonly used colouring agents can be divided into three categories and comprise of organic dyes and their lakes (e.g. Tartrazine, Sunset Yellow and Allura red), inorganic or mineral colours (e.g. Titanium dioxide and red iron oxide) and natural colours (e.g. β-carotene) (129).

In children, association of fibrosing colonopathy with high intake of the pancreatin products coated with methacrylic acid copolymer (Eudragit L30D-55) have been reported by Smyth et al. (1994) (130). However, other reports and data suggest the potential of gastrointestinal toxicity with all enteric-coated pancreatin products, including the HP-55 phthalate enteric-coated pancreatin preparation, Creon (131). Furthermore, paediatric exposure to plasticizers, such as di(2-ethylhexyl) phthalate (DEHP) generates concern as extrapolation data from animal studies has demonstrated varying toxicities (132). Based on the no observed effect level, the European Food Safety Authority (EFSA) have limited the allowable daily intake value of DEHP to 50 µg kg-1 per day (133).

Parabens (methyl-, ethyl- and propyl-hydroxybenzoates) are often used in pharmaceutical formulations as antimicrobial preservatives, where they are effective against a broad spectrum

of bacteria, over a wide pH range (134). However, when used in humans, such antimicrobial properties are harmful to living cells and may therefore be associated with certain risks (135). The EMA have concluded that, if possible, inclusion of preservatives and antioxidant in paediatric formulations should be avoided and their use should be justified (135). Parabens demonstrate oestrogen binding affinities, suggesting oestrogenic activity with potential reproductive effects (136). Furthermore, possible endocrine-disrupting effects have also raised concerns for paraben use in paediatric formulations (137). Nonetheless, risk assessments have led to the establishment of an acceptable daily intake of 0-10 mg/kg body weight for the total sum of methylparaben, ethylparaben and propylparaben (138).

Cyclodextrins are a family of cyclic oligosaccharides, consisting of a hydrophobic inner cavity and a hydrophilic outer surface (139). They are naturally derived from starch and are often used for delivery of poorly soluble and unpalatable pharmacological compounds. Owing to low toxicity profiles, cyclodextrins, serve as a potential alternative to problematic solubilising agents such as propylene glycol and ethanol (140). Nevertheless, at high doses (> 1000 mg/kg/day), cyclodextrins may cause cecum enlargement and digestive problems such as diarrhoea (141). The lowest observed effect levels in cyclocdextrin clinical studies has led to the conclusion of a maximum cyclodextrins threshold of 200 mg/kg/day, when administered orally (141).

Polysorbates are non-ionic surfactants, widely used as solubilising agents in injectables and liquid preparations. In the 1980s, several premature infant deaths were reported after the introduction of an intravenous vitamin E formulation (E-Ferol) containing polylobate 20 and 80 (142). Ever since, the use of polysorbates in paediatric formulations has been associated with safety concerns. Owing to similar structures and metabolic fate of all polysorbates, similar toxicokinetics are expected and therefore, the EFSA has set a maximum acceptable daily threshold of all polysorbates at 25 mg kg-1 per day (143) (Table 4).

Table 4: Overview of potentially problematic excipient use in paediatrics.

Excipient	Function	Concerns/Adverse effects	Permitted daily allowance	Most commonly found in
Propylene Glycol	Solvent, Co-solvent, Preservative	CNS depression	Neonates: 1 mg/kg Children aged 1 month–4 years: 50 mg/kg Children aged 5–17 years: 500mg/kg (108)	Oral liquids, Injectables, Topicals
Ethanol	Solvent, Anti-microbial preservative	Acute intoxication, Chronic toxicity	Children aged <6 years: 6mg/kg Children aged 6–12 years: 75mg/kg (112)	Oral liquids, Transdermal preparations
Cyclodextrins	Complexing agent, Taste masking	Cecum enlargement, Diarrhoea	200 mg/kg (141)	Oral liquid formulations
Polysorbates	Solubilising agent (Surfactant)	Hypersensitivity	25 mg/kg (143)	Injectables, Liquid formulations
Di(2-ethylhexyl) phthalate (DEHP)	Plasticizer	Endocrine disruptions, Neurotoxicity, Renal toxicity, Hepatotoxicity (144)	50 µg/kg (133)	Controlled/enteric coated formulations (Capsules)
Benzyl Alcohol/Benzoic acid/Benzoate	Preservative, Solvent	Toxicity – Multiple organ failure (62)	Total - 90 mg/kg (123)	Solutions and suspension for injections
Lactose	Tablet and capsule diluent, dry powder carrier, sweetener	Lactose intolerance - Dehydration, metabolic acidosis and prolonged diarrhoea	5 g per dose (127)	Solid dosage forms, Inhalation products
Methyl Paraben Ethyl Paraben Propyl Paraben	Antimicrobial Preservative	Oestrogenic activity Developmental toxicity	Up to 10 mg/kg in total (138)	Liquid formulations
Sweeteners				
Sucrose Fructose Aspartame, Saccharin, Acesulfame-K Sucralose Xylitol Sorbitol	Sweeting agent, Fillers (e.g. Xylitol, Sorbitol)	Hyperglycaemia Obesity Erosion of teeth Hypersensitivity Gastrointestinal disorders Aspartame - harmful in	5 g (127) 10–40 mg/kg (145) 50 mg/kg (116) 15 mg/kg (116) 15 mg/kg (116) 5 mg/kg (116) 10 g (127) 140 mg/kg (145)	Oral liquid formulations, Solid dosage forms

		children with phenylketonuria		
Colourants				
Tartrazine Sunset Yellow Curcumin Allura Red Caramels Brilliant Blue Annatto Iron oxide(s) Carmines/carmine	Colouring agent, Product identification (prevent counterfeit), Branding	Hypersensitivity, Negative behavioural effects and ADHD	7.5 mg/kg (146) 4 mg/kg 3 mg/kg 7 mg/kg 100 mg/kg 6 mg/kg 6 mg/kg 40 mg iron/day 5 mg/kg	Oral liquid formulations, Solid dosage forms

1.5 Paediatric dosage forms- Benefits and limitations

Pharmacokinetic profiles within the paediatric population are not only affected by the anatomical and physiological differences but also by dosage form design which can affect adherence and compliance. This sections covers some examples of commercial dosage forms readily used in paediatric medicine, considering their benefits and limitations, especially with the perspective of safety, quality and efficacy.

1.5.1 Paediatric dosage forms

The current trend in paediatric formulation development is towards age-appropriate dosage forms, with considerations for acceptability, safety and capability of providing variable and accurate doses according to the child's specification. Furthermore, the dosage form must exhibit acceptable palatability, contain appropriate excipients and be regulatory compliant (147). Several oral dosage forms intended for paediatric oral administration exist and include both solid (tablet, capsules, orodispersible formulations, powder for reconstitution and chewable tablets) and liquid (solutions, suspensions, elixirs and syrups) dosage forms. Solid dosage forms remain as the preferred choice of formulation for pharmaceutical industry owing to its advantages of long-term stability, manufacturing flexibility (including the ability to film coat and control API release) and overall low production cost (148).

1.5.2 Traditional tablets and capsules

Standard tablets and capsules have some major drawbacks within the paediatric population; having a fixed dose content means that only a small range of the target population can be treated, as most paediatric doses are based on child weight. The other key disadvantage arises simply from the inability of children to swallow such large dosage forms, although this is more of a concern in the lower sub-set of the population, where the risks of choking and aspiration are drastically increased (149).

1.5.3 Chewable tablets

Chewable tablets are intended to be chewed before swallowing, making them a popular choice among individuals with phagophobia (fear of swallowing). Additionally, where possible, such tablets may also be swallowed whole considering, bioavailability is not affected (150). However, the minimum age for safe use of chewable tablets is recommended from 2 years and above, due to the risk of choking in younger populations (151). As for standard tablets and capsules, chewable tablets are also limited by the lack of dose flexibility. In addition, since these tablets are designed to be chewed before swallowing, coating techniques to taste mask and control API release becomes a greater challenge (152).

When developing chewable tablets, certain criteria must be met to ensure medicinal compliance and adherence is achieved. Palatability is of utmost importance in chewable tablets, as the tablets will fragment within the oral cavity and activate taste receptors upon contact with saliva (153). Furthermore, chewable tablets must exhibit mechanical properties in which the tablet can easily be chewed without compromising its friability profile (154).

1.5.4 Orally disintegrating tablets (ODTs)/mini tablets (mini ODTs)

The term orodispersible tablet, refers to tablets that are intended to quickly disintegrate in the oral cavity in the presence of saliva. However, to label a tablet as an ODT, the FDA has suggested a disintegration time of 30 seconds and a maximum tablet weight of 500 mg (155). ODTs are emerging as a popular choice amongst paediatrics and health care professionals, as they have proven to improve patient compliance (156). After liquid preparations, ODTs are the dosage form of choice, with small size and fast disintegration times being identified as the most ideal characteristics (157). Rapid disintegration times will reduce the administration process period and, therefore, encourage medicine adherence. Similar to chewable tablets, the palatability of ODTs is crucial, as children will associate the taste of the tablets each time they need to take their medication. ODTs improve swallowability and exhibit appropriate stability profiles without the use of functional excipients (e.g. preservatives), as in the case of liquid formulations. However, once again ODTs are limited due to their rigid dose content.

Since the European Medicine Authority (EMA) set up regulations for developing age-appropriate formulations, the development of orally disintegrating mini tablets (mini ODTs) has widely gained recognition (158). A mini tablet is referred to as a tablet with a diameter equal to/less than 4 mm (159). The superiority of mini ODTs stems from their flexible dosing ability, where each unit dose incorporates a small concentration of active therapeutic substance, which can be taken either as a single tablet or as multiple tablets to fulfil higher dose requirements. Furthermore, for the upper end of the paediatric population, mini-tablets can be enclosed into capsules or compressed into a larger tablet to avoid the need to take multiple tablet units (160). Mini ODTs hold advantages inherent to both liquid and solid dosage forms,

while achieving dose flexibility, resulting in a dosage form that fulfils the definition of an age-appropriate formulation that can be utilised throughout the whole paediatric population (161).

1.5.5 Innovative solid dosage forms - Multi-particulate dosage forms (MDF)

Multi-particulate drug administration systems, usually presented in a sachet or encapsulated in a capsule, are tiny distinct units of pharmacologically active compounds, each demonstrating an extent of therapeutic response. Such dosage forms are usually intended to be reconstituted with liquid or sprinkled over soft foods, such as apple sauce, yogurt and pudding. Liquid vehicles include milk, water or juice. The discrete size of MDF improves swallowability, while their multi-particulate composition allows for increased dose flexibility. Additionally, owing to their small size, multi-particulates are evenly distributed along the GI tract, thereby improving bioavailability and minimising the occurrence of local irritation and toxicity (162). Due to their solid-sate, MDF do not require stabilising agents (e.g. preservatives and antioxidant), which have shown to be problematic excipients within the paediatric population. Co-administrating with food can promote medicine adherence by masking any unpleasant tastes; however, co –administration with foods and drinks may alter the absorption and potentially the bioavailability of the drug, leading to either reduced or increased therapeutic effects (163). Moreover, reconstituting/mixing can at times lead to incomplete ingestion of the drug, if the entire quantity in which it is mixed is not administered. Lastly, while manufacturing technologies to produce such dosage forms are widely available, packaging and dosing requirements may call for more specialised equipment and accessories, significantly increasing cost (148).

1.5.6 Liquid Formulations

Liquid formulations comprise of solutions, suspensions, elixirs, syrups, drops and emulsions. Owing to their superior ability for dose flexibility and ease of swallowing, such dosage forms are most applicable and favoured in the lower subsets of the paediatric population (up to 8 years), who are incapable or find difficulty in swallowing solid dosage forms. The capability of flexible dosing in liquid formulations allows for administration throughout the whole paediatric population, from neonates up to adolescence (16-18 years) (164).

Solutions are homogenous mixtures, where the solute is completely dissolved within the solvent. In contrasts, suspensions are heterogeneous, where the composition of components is non uniform and subject to separation and, therefore, require shaking prior to administration. Suspensions are chosen over solutions when the drug under review is insoluble in water and where use of solubilising agents is not possible (165). Emulsions are similar to suspensions; however, the mixture is comprised of two immiscible liquids and usually include an emulsifier such as polysorbates, lecithin and/or mono –and diglycerides. Syrups on the other hand are

highly concentrated sugar solutions, with or without a medicinal substance that are usually directed for paediatrics and drugs with disagreeable taste. Paediatric drops are liquid preparations (either in the form of a solution or suspension) intended to be administered in minute doses using a calibrated dropper (166). Lastly, elixirs are similar to solutions, but differ due to the fact they are sweetened, clear hydro-alcoholic liquids, with varying degree of alcohol added to maintain and evenly distribute drug particles (167).

The principal challenge associated with medicinal adherence in children is palatability, which includes and is influenced by taste, smell, texture and appearance (168). It has been revealed that more than 90% of paediatricians linked non-adherence to drugs that are bitter and unpalatable (169). Compared to solid dosage forms, this is more of a concern in liquid formulations, since simple taste masking techniques are at times not sufficient, resulting in bitter tasting formulations. Additionally, in regards to liquid preparations the dosing volume is also of significant importance when determining acceptability, where target volumes for children under 5 years is ≤ 5 mL, and ≤ 10 mL for children above the age of 5 years (170). However, the EMA draft guidance suggests a maximum dose volume of 5 mL for children under 4 years of age and 10 mL in children between 4 and 12 years (164). Due to the complex nature of liquid formulations, several functional excipients are utilised to assist manufacturing processes and optimise the formulation to promote and enhance stability and palatability (150). These include preservatives, solvents, solubilising agents, sweeteners, flavourings and colourants. However, such excipients are known to be 'problematic' and have undergone very few clinical/toxicity studies in paediatrics, resulting in unknown possibilities of observing potential adverse effects, such as hypersensitivity reactions, CNS effects and jaundice (109). As a result, the inclusion of such excipients should be justified and, where possible, limited/avoided. Furthermore, in comparison to solid dosage forms, the storage and handling cost of liquids is very high due to the bulky nature of the bottles and requirements of storing conditions, such as refrigerating and using high-density polyethylene bottles (171). Additional, safety features such as child resistant caps and special amber type glass bottles further add to the cost. Lastly, very few controlled release liquid preparations are available, suggesting the need to dose several times a day. Certainly, an increase in dosing frequency leads to a decrease in adherence, as the administration process, which is found unenjoyable by many, would need to be repeated more times (172). A simpler dosing regimen (once or twice daily) would also limit the inconvenience caused to children and caregivers who have to carry their medicines to school.

Regarding stability, liquid preparations require many considerations. Other than the inclusion of antioxidants and preservatives, many liquid formulations require to be refrigerated at

temperatures of 5ºC (+/- 3ºC) (173). This may prove to be problematic in developing countries where access to refrigeration may not be possible. Moreover, such formulations may not be suitable in countries with a warmer climate as high temperatures may result in immediate product degradation when removed from storage conditions. This not only adds to medicine handling complications but leads to excess wastage where many products may need to be replaced owing to incorrect storage. Another disadvantage of liquid formulations, is their relatively short shelf life, with an even shorter in-use shelf life (e.g. Amoxicillin Oral Suspension only has a 7 day shelf life when reconstituted).

Liquid formulations have been perceived to be the most suitable dosage from type in paediatrics, since a flexible 'sweet tasting liquid' is thought to be preferred over solids in young children (174). Globally, this has been the norm, where pharmaceutical industries opt for liquid formulations when the medicine is to be given via oral cavity. However, as more studies have been carried out to assess acceptability, results show otherwise and flexible solid dosage forms show superiority.

1.5.7 Acceptability of innovative small flexible solid oral dosage forms

Small flexible solid oral dosage forms maintain the dose flexibility of liquid preparations, while exhibiting desirable characteristics of solid dosage forms, including stability and the ability to taste mask and modify drug release. Thus, such dosage forms suggest a promising alternative to the widely accepted liquid dosage forms.

A randomised cross over study carried out by van Riet-Nales et al. (2013) concluded the preference of small 4 mm tablets in domiciliary infants and preschool children over powders, suspensions and syrups (175). Kilingmann et al. (2013 and 2015) conducted a series of randomised cross over studies comparing the acceptability of a 2 mm mini–tablet against sweet syrup; the first study compared an uncoated 2 mm tablet against 3 mL of syrup in a total of 306 children aged between 6 months and 5 years. No adverse events occurred and results showed that the uncoated 2mm tablet was more accepted over the syrup (176). The second study compared the acceptability of a 2 mm uncoated mini-tablet and 0.5 mL of syrup in neonates (2 -28 days). Out of 151 neonates, all showed competency in swallowing the mini-tablet, with increased levels of swallowability compared to the syrup (177).

Studies by Spomer et al. (2012) and Thomson et al. (2009) explored and assessed the acceptability and suitability of placebo mini-tablets for children up to the age of preschool (6 years); results showed that the acceptance of mini-tablets was higher or equal to that of the syrup (178) (179) (Table 5).

These results suggest that small solid dosage forms are actually preferred in children over liquid formulations, shifting the paradigm to small flexible solid oral dosage forms, as proposed by the World Health Organization (177). In addition to preferring flexible solid paediatric formulations as mentioned earlier, a switch to such dosage forms also provides an opportunity to improve the availability of age-appropriate paediatric medicines in both first and third world countries. This would come as a result of reduced costs of manufacturing and logistics, which are usually associated with liquid formulations but not solid dosage forms.

Table 5: Studies evaluating the acceptability of innovative small flexible solid oral dosage forms.

Author	Year	Study type	Population age (years)	Population size	Tablet size	Outcome/Acceptance of mini-tablet
Thomson et al.	2009	Randomised crossover study	2-6	100	3 mm	*Acceptance:* Age 2 years: 46% Age 3 years: 53% Age 5 years: 85%
Spomer et al	2012	Random two-way cross-over exploratory pilot study	0.5-6	60	2 mm	Mini-tablets preferred over sweet liquid formulation
van Riet-Nales et al	2013	Randomised crossover study	1-4	148	4 mm	Mini-tablet Preferred over powder, suspension and syrup
Kilingmann et al	2013	Randomised crossover study	0.5-5	306	2 mm	Mini-tablets more acceptable than liquid formulation
Kilingmann et al	2015	Randomised crossover study	2-28 days	151	2 mm	Level of swallowability higher for mini-tablets

1.5.8 Future of Paediatric formulations

The World Health Organization (WHO) has considered flexible solid oral dosage forms as the most suitable dosage form for children (180). Preparations include orodispersible, chewable and soluble tablets. Such dosage forms relieve the stresses of swallowing, as the dosage form is intended to disperse in the mouth/liquid before swallowing. Flexible solid oral dosage forms hold advantages inherent to both liquid (flexible dosing capabilities and ease of swallowing) and solid (formulation stability and low production cost) dosage forms, while

minimising their respective disadvantages (148). Currently, the focus of developing age-appropriate formulations is with flexible solid dosage forms that are easy to swallow and well accepted throughout the whole paediatric population (181, 182). Progressively, paediatric oral formulations will be present as convenient and palatable single- use multi-particulate dosage forms (MDFs) including mini-tablets, orally dispersible tablets, mini-orally disintegrating tablets, granules, sprinkles and powders, with an excipient composition and load systematically elected for paediatrics (183).

To encourage the development of flexible solid oral dosage forms, the LENA (Labelling of Enalapril from Neonates up to Adolescents) project was collaboratively initiated within Europe. The aim of the LENA project was to develop and clinically evaluate a novel age-appropriate solid oral drug formulation of enalapril. Subsequently, many developments of a novel formulation of enalapril orodispersible minitablets (ODMT) have taken place with a potential eligibility for a Paediatric Use Marketing Authorisation (PUMA) (184-186).

1.6 Factors affecting paediatric adherence and acceptability

For safe and effective pharmacotherapy, it is vital for paediatrics to adhere and comply with their regimen, ensuring accurate quantities and volumes during administration. The dosage forms design is especially important since it will dictate the willingness and ability of children to take their medicines. In addition to formulation related factors, the adherence and acceptability of dosage forms is markedly under influence by patient and disease related factors including age and whether the treatment is for an acute or chronic condition.

From a formulation perspective, to enhance and maintain medicine-adherence and acceptability, it is necessary for paediatrics to have access to dosage forms that are capable of safely delivering the dose to the child in an easy and reliable fashion. Many paediatric specific drug delivery systems are present and include oral liquids, mini-tablets, chewable tablets and orodispersible technologies. Several studies have compared the acceptability of such dosage forms within the paediatric population, with small flexible solid oral dosage forms including mini-tablets proving to be most superior (175-179). In contrast to adults, where conventional dosage forms are well accepted, the extent of acceptability of dosage forms in paediatrics greatly depends on individual child characteristics such as age, competency and developmental stage.

The WHO suggests that around 50% of medication issued for long-term conditions is not taken as advised (187). Medicine adherence assumes a consensus between the patient and the health care practitioner and can be defined as the extent to which the patient follows the agreed recommendations. High levels of medicine adherence ensures maximum possible

therapeutic benefit, improves prognosis and overall condition (clinical output) and enhances quality of life. In contrast, consequences of non-adherence include treatment failure, deteriorating condition, related psychosocial effects and increased health care costs (187). Apart from patient related factors, non-adherence may occur as a result of limitations in the drug delivery system or factors associated with the disease. Therefore, to improve adherence, paediatricians and pharmaceutical formulators should understand children's perspectives of medicines and collaboratively explore and address any limiting features in the delivery of the health care. In paediatrics, acceptability is not just limited to the child's ability and willingness to use the medicine, but also the ability and extent of compliance of the parent/caregiver to administer the drug as intended (188).

Non-adherence is usually described as either intentional or unintentional non-adherence. Intentional non-adherence is more common in older children and is when the child autonomously decides not to follow their agreed treatment recommendations; this is usually a consequence of patient related factors, such as social stigma and denial (189). Unintentional non-adherence is where the patient wants to follow the agreed recommendations, but is unable to do so and is limited by factors which are beyond one's control, such as unpalatability, level of development and required dose.

1.6.1 Patient related factors

The age of the child is a key factor when determining medicine adherence and acceptability. In the lower subsets of the paediatric population, children depend upon on their parents/caregivers; therefore, contributions and success are a direct reflection of the parents' ability to administer and follow treatment recommendations. Some reasons described by parents/caregivers for not following treatment advice include stopping medicine when symptoms improve, forgetfulness, misinterpretation of instructions and non-compliance of child(190). As the child grows and matures, they progressively begin to take ownership and responsibility of their treatment. This comes as a result of greater autonomy, where the child develops the capacity to make their own decisions. In regards to formulation acceptability, differences are observed between paediatric subgroups, where younger populations are unable to swallow solid dosage forms due to their immaturity and lack of motor and cognitive skills development (18). Other extensive differences observed between children that impact both adherence and acceptability include developmental changes/stages, disease perception, competency and biological changes (18). Due to the heterogeneity observed in children, the EMA has led to the grouping of the paediatric population into five subsets (preterm neonates, term neonates (2-28 days), infants and toddler (1-23 months), children (2-11 years) and adolescents (12-16/18 years)) (18). Each subset have unique requirements and, therefore,

the age-appropriateness of a paediatric specific dosage form type should be assessed based on capability, suitability of the dosage form and incorporation of appropriate excipients in regards to the target paediatric subset (191).

1.6.2 Formulation related factors

Palatability is the overall recognition of the dosage form in relation to its aesthetic appeal. Children have a reduced tolerance for displeasing taste that leads to unwillingness and non-adherence; therefore, as pharmaceutical manufacturers, it is important to effectively minimise any unpalatable and bitter formulations. Several taste masking techniques are available that mask unpleasant taste and include complexation, microencapsulation or addition of flavourings and sweetening agents (192). However, many flavours and sweeteners have led to adverse effects, hence their inclusions within the formulation should be justified. Moreover, both the drug and the excipients can impact and influence the palatability of the final dosage form; therefore, careful consideration should be taken when selecting constituents for paediatric specific drug dosage forms. Taste and texture are regarded as the most important factors when determining medicine acceptability, due to their effects on the overall taste sensation and capacity of mastication (193). Texture usually stipulates the relative content of different sized particles that result in a smooth, rough, gritty or slimy feel. Several studies have shown the influence of taste and texture on acceptability, where certain formulations were more acceptable than others due to taste and texture differences (175, 194). Palatability, as discussed, is crucial in both short-term and long-term paediatric medicine adherence; however, other crucial characteristics associated with long-term conditions include required dose (quantity/volume), excipient safety and convenience of dosage form.

The dose frequency and quantity should be tolerable, where once- and twice-daily regimens have shown to significantly improve patient adherence as compared to three to four times daily regimens (195). For liquid formulations, acceptable dosing volumes are considered equal to the volume of a swallow, which is reported to be around 0.27 mL/kg (applicable from 15 months onwards) (196). On the other hand, for soluble tablets, a volume of up to 20 mL is regarded acceptable in children under the age of 4 years, while 50 mL is acceptable in those above 4 years old (164). In regards to tablet size acceptability, the EMA had previously proposed certain measurements that were deemed acceptable in certain age groups (e.g. a 3-5 mm tablet is not acceptable under 2 years); however, these have since been updated and instead the EMA now suggests the acceptability of the size and shape of the tablet to be justified through appropriate studies/clinical evidence(164). Furthermore, since pharmaceutical excipients are not inert, the effect on the accumulation of such compounds

should be taken into account and assessed during long-term treatment to avoid potential adverse effects.

1.6.3 Disease related factors

Within the paediatric population, important features to be considered when assessing medication acceptability and adherence relating to the disease is whether the treatment is for an acute or chronic (lasting 3 months or more) condition (182). It has been reported that children being treated for long-term conditions (such as asthma and HIV) acquire skills to swallow tablets and capsules safely, even from a relatively early age of around 3 years (197). These swallowing capabilities arise from repetitive administration, where supporting strategies and practice encourages children, while minimising any psychological fears associated with swallowing. Nevertheless, from a social point of view, peer pressure and child reluctance are factors that may negatively affect medicine adherence in school going children that are being treated for chronic illnesses (198). It has been reported that up to approximately 70% of children with chronic conditions have poor medicine adherence because of prolonged treatment durations, symptomatic remission and increased number of medicines (199). Furthermore, unsatisfactory treatment outcomes, toxicity (either due to drug or excipients) and increased frequency of medical complications further contribute to medicine non-adherence, leading to poor clinical outcomes and increased health care costs (200).

1.6.4 Overcoming barriers in paediatric medicine non-adherence

Identifying factors affecting medicine non-adherence and acceptability authorises and opportunity to tailor design and production of paediatric specific dosage forms, guarantying appropriate dosage forms, in regards to child age and development, comorbidities and acceptable palatability.

Once- or twice-daily medication regimens have shown to increase compliance rates to more than 80% relative to three-times daily schedules (201). Where possible, modified-release formulations are available and provide longer and patient friendly dosing intervals that improve adherence (198). The simplicity and convenience of such dosage forms are especially appreciated by elderly and children that take their medicines to school. However, their use is limited by their fixed dose content, since modified-release formulations cannot be crushed as the active ingredient is intended to be taken as a whole unit. Modified-release multi-particulate dosage forms that are available for flexible dosing include mini-tablets, pellets, beads, granules, microcapsules and microparticles (202). Children taking multiple tablets (polypharmacy) as in the case of HIV patients have difficulty in adhering to their medication regimens; in such cases, fixed-dose combinations prove beneficial, where several drugs are

incorporated into a single unit. However, this may increase the size of the tablet and cause swallowing difficulties (198).

Identifying the cause of non-adherence is key in improving adherence, as barriers identified can be individually tackled by physicians, parents and other health care practitioners. Medication adherence is not solely the responsibility of the child and encouraging a "blame-free" environment, improving patient education and assessing health literacy will positively impact medicine adherence (203). Behavioural tailoring strategies aim to amend the child's behaviour towards their treatment and promote positive changes (204). Interventions include cognitive–behavioural strategies (such as exposure and relaxation) and Emotion-focused therapy (EFT) (205). Although some reports of improved adherence have been noted with behavioural interventions, firm conclusions cannot be made as evidence is small and inconsistent (204, 205). Instead, a combination of educational and behavioural techniques have been proposed to improve medication adherence (206) (Table 6).

Table 6: Summary of factors affecting paediatric medication adherence and proposed actions to overcome non-adherence.

Barriers in paediatric medication adherence	**Proposed actions to improve medication adherence**
Patient related factors	
Disease perception	Education intervention
Age and development	Increase availability of age-appropriate formulations
Resistance	Behavioural tailoring strategies
Stigma and denial	Patient education/information counselling
Neurodevelopmental disorders (e.g. ADHD, autism, conduct disorders and cerebral palsy)	Cognitive adaptation training (CAT) Multisystemic therapy (MST) for conduct disorders
Formulation related factors	
Palatability (including taste, texture, appearance and smell)	Formulation based techniques (e.g. taste masking, film coating, inclusion of paediatric compliant excipients)
Dose quantity (including different strengths available for liquid formulations)	Accurate measuring device Ensure patient/parent have identified strength before administration
Dose frequency	Modified-release formulations Fixed-dose combinations Reminders/self-management plans

1.7 Previous efforts on developing toolkits for formulation in general and for paediatric products in particular.

Despite financial incentives for paediatric formulation development, the production of medicines for children is well below anticipated levels. This is owing to the lack of standardised paediatric specific development pathways. Although some efforts have been seen on developing specific protocols or guidance documents for paediatric products, there lacks a toolkit/decision making pathway that facilitates quicker, effective and focused formulation development that combines important factors for various stages of the drug development process. Generally, formulation development methodologies exists and include preformulation, process feasibility, preliminary formulation screening, design of experiment (DoE) and evaluation, however, considerations to specific population groups such as paediatrics are not included and therefore cannot be applied to paediatric products. Further, generally such development methodologies are not systematic and again are not targeted at specific pollution groups.

As we know, pharmaceutical exceptions are an integral part of formulation development and are included to provide multiple functionalities. In 2013, the European Paediatric Formulation Initiative (EuPFI) introduced the Safety and Toxicity of Excipients for Paediatrics (STEP) database. The objective of the database is to centralise relevant paediatric excipient safety and toxicity data to provide a base for quick information retrieval when selecting/screening excipients. Additionally, the database can be useful for assessing the need to generate new data related to paediatric formulation/medicines. Further, STEP database intends to highlight excipient exposure and support risk management planning and application of in-silico models for predicting toxicity. Although safe and appropriate excipient selection forms and integral part of formulation development, using the STEP database only fulfils certain objectives and requires the use of other decision making processes that considers other paediatric needs including product acceptability (including palatability), dose banding, clinical need, product feasibility, dosage form design and product quality, safety and effectiveness.

Due to the lack of uptake of paediatric formulation developments despite financial incentives, it is clear that the paediatric population would benefit from such a toolkit/decision making platform that guides and accelerates paediatric formulation developments that are of high quality, safety and efficacy. The establishment of the paediatric decision making tool requires a systematic approach that encompasses all aspects of paediatric formulation development and considers anatomical and physiological differences, dosage from design and acceptability, dosing volumes and use of paediatric compliant excipients. Alongside the STEP database, the use of EMA guidance documents including “Guideline on pharmaceutical

development of medicines for paediatric use" and "Reflection paper: formulations of choice for the paediatric population" were evaluated to inform and guide the initial stages of the screening tools establishment, whilst ensuring all resulting products are both patient and regulatory compliant. Special emphasis should also be given on the applicability of such screening tools, ensuring they are applicable to a range of active pharmaceutical ingredients (API's) exhibiting different physicochemical properties. Model drug candidates selected within this thesis were from the inventory of the needs for paediatric medicines (EMA), with the selection criteria taking account of properties such as low aqueous solubility, stability challenges and absence of paediatric friendly dosage forms. A special consideration to poorly soluble drugs were given since the development of effective pharmaceutical products from poorly water-soluble drugs continues to be an ongoing challenge (207).

1.8 Aims and Objectives of Thesis

When developing medicines for children, the norm was to simply translate principles and technologies from adult pharmacotherapy. However, this overlooked specific needs and requirements of the paediatric population resulting in medicines with several limitations including, unknown safety and toxicity profiles, palatability concerns, acceptability issues and lack of dose flexibility. Additionally, the lack of suitable medicines for children meant that preparations were often prepared either off-label or used in an unlicensed fashion, thereby further exposing this vulnerable population group to potentially unknown adverse risks. To further add to the challenges associated with paediatric drug delivery, the choice of suitable excipients for inclusion is limited and therefore at times leads to great difficulty when trying to achieve certain functionalities such as adequate solubility, taste and stability.

The impact of the paediatric regulation and global policy reforms has led to improvements in paediatric drug developments. However, the challenge to develop and provide greater accessibility to high quality medicines that are safe and effective still remains. A distinct challenge continues to exist in this area, with an unmet need for the development of more 'age appropriate formulations', particularly with an excipient composition and load that is suitable for paediatric patients. An age appropriate formulation is a dosage form which can deliver accurate and variable doses depending on age, weight and surface area, is safe, acceptable and matched to the development and ability of individual and lastly avoids medication error.

A crucial step in paediatric formulation development is the careful screening and selection of excipients as certain excipients acceptable in adult formulations, may not be appropriate for paediatric use (208). Recently, the European Paediatric Formulation Initiative (EuPFI) introduced the STEP (Safety and Toxicity of Excipients for Paediatrics) database. The

database is a user-designed database that compiles the safety and toxicity information on excipients that is fragmented across multiple sources and presents it in one place, aiming to accelerate the excipient selection process and ensuring the excipient selection is based on relevant clinical data (208).

The overarching hypothesis of the proposal is that the establishment of an excipient screening platform will accelerate paediatric formulation development. This novel excipient screening platform for Paediatric Oral formulations systematically evaluates European regulatory paediatric formulation guidance and assesses the suitability of commonly used excipients to develop paediatric specific formulations that are safe, effective, high quality and regulatory compliant.

The objectives of the project work can broadly be categorised into the following categories

1) Development of a novel excipient screening platform for Paediatric Oral formulations. This includes:

a. Development of an excipient inclusion criteria for paediatrics based on regulatory guidelines and safety and toxicity information available through the Safety and Toxicity of Excipients for Paediatrics (STEP) database

b. Develop a “working zone” of excipients for formulation of oral paediatric medicines that meet the regulatory guidelines for paediatric medicines and excipient safety

2) Development of novel paediatric specific age-appropriate formulations informed through the excipient screening platform, including carvedilol ODMT, famotidine ODMT, nifedipine extended release mini tablets and furosemide oral solution.

3) Establishment and optimisation of the screening tool to better inform paediatric dosage form design.

4) Validation and pragmatic application of the decision making screening tool to accelerate and de-risk the production of age-appropriate formulations.

Chapter 2: Development of an age-appropriate mini orally disintegrating carvedilol tablet with paediatric biopharmaceutical considerations

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*Work from this chapter has been published in MDPI Pharmaceutics - Khan, D., et al. (2021). "Development of an Age-Appropriate Mini Orally Disintegrating Carvedilol Tablet with Paediatric Biopharmaceutical Considerations." Pharmaceutics **13**(6): 831.*

2.1 CHAPTER AIMS AND OBJECTIVES

- Develop a paediatric specific excipient 'working zone' for orodispersible tablets
- Optimise blending process as carvedilol is a low dose, potent compound
- Develop two strengths (0.5 mg and 2 mg) of an age-appropriate carvedilol formulation that possess increased dose flexibility, palatability, safety, quality and efficacy
- Characterise and evaluate formulations in defined paediatric biorelevant media

Abstract: Orally disintegrating mini-tablets (ODMT) have widely been considered as an age-appropriate formulation option that possess the ability for adequate dose flexibility, avoids swallowing difficulties and exhibits superior stability due to its solid state. The selection of an ODMT was confirmed based on the physicochemical properties of carvedilol, requirement of dosing flexibility and with an aim to limit the total number of excipient within the formulation. Carvedilol is a low dose, potent compound with low aqueous solubility. It is gastric stable and does not exhibit any taste concerns. Dosing of carvedilol is based on child weight and is usually titrated when initiated. Within this study, two strengths (0.5 mg and 2 mg) of carvedilol ODMT formulations were developed using an excipient composition and load that is appropriate for paediatric use. The formulations demonstrated adequate mechanical strength (> 30 N), and fast disintegration times (< 30 s). Dissolution profiles observed were robust and comparable to the marketed conventional tablet formulation across various part of the gastrointestinal (GI) tract in both the fed and fasted state, signifying appropriate efficacy, quality and performance. As such, the formulations developed in this study show potential to address the need of an 'age-appropriate' formulation of carvedilol, as highlighted by the European Medicines Agency (EMA) Inventory of the Needs for Paediatric Medicine.

Keywords: age-appropriate; paediatrics; ODMT; heart failure; biorelevant; DSC; disintegration; dissolution; flowability; PUMA.

2.2 Introduction

Owing to considerable differences observed in anatomy and physiology between paediatric subsets, it has been well established that children respond to drugs differently as compared to adults. Furthermore, from a formulation perspective, there is a distinct challenge to develop a dosage form that is capable of safely, accurately and reliably delivering the dose across the whole paediatric population

The benefits and limitations of current paediatric formulation platforms have been evaluated, where innovative and flexible solid oral dosage forms have shown superior acceptability among paediatric subsets (176). Other than exhibiting greater chemical and microbial stability, flexible solid oral dosage forms including pellets, multiparticulates, sprinkles and mini-tablets also display desirable characteristics associated with liquid dosage forms, such as enhanced dose flexibility and ease of swallowing. The World Health Organization (WHO) has appraised flexible solid oral dosage forms including orodispersible, chewable and soluble tablets as the most reasonable and appropriate paediatric specific dosage form (180). Orally disintegrating mini-tablet (ODMT) combines features of both innovative and flexible solid dosage forms and exhibits all characteristics of an age-appropriate formulation, including enhanced dose flexibility, delivers an accurate dose, appropriate dosing volumes, stability and palatability (209).

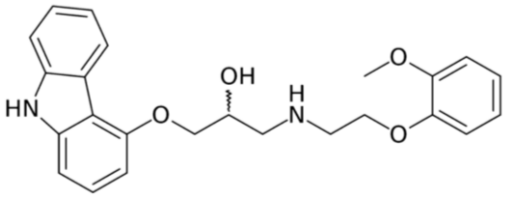
In 2007, the paediatric regulation came into force and aimed to improve the health of children by facilitating the development and availability of medicines for children (3). The paediatric use marketing authorisation (PUMA) was introduced, with an aim to stimulate research in existing compounds that are off-patent and/or to help transform known off-label and unlicensed use into authorised use. Nonetheless, the success rate of the PUMA is below anticipated levels, with only six PUMAs being granted till date (13).

Carvedilol is a weakly basic biopharmaceutical class II model drug with a pKa value of 7.8 and aqueous solubility of 4.44 mg/L (Table 7) (210). It is a third generation non selective beta blocker that also possesses alpha blocking properties. Only a small number of randomized clinical trials have taken place in paediatrics patients with heart failure, which have shown a positive impact of carvedilol on left ventricular function, clinical condition and symptoms of heart failure (211-216). Although carvedilol has been approved since 1995 and its clinical need has been established, no attempt has been made to reformulate carvedilol into an age-appropriate formulation; the only licensed medicinal form that is available is a tablet. Owing to the lack of appropriate paediatric specific formulations, specials are therefore often prescribed. The composition and excipient load, suitability and safety of such dosage forms have not been thoroughly evaluated and therefore increase the risks of potential adverse

effects. Government agencies such as the EMA have identified commonly prescribed medicines in need of age-appropriate formulations; carvedilol for treatment of hypertension and heart failure is one of the many medicines listed within the EMA Inventory of paediatric therapeutic needs (217).

The aim of the present work was to develop an age-appropriate formulation of carvedilol, with paediatric biopharmaceutical considerations for evaluation. The selection of strengths were based off of individual child weights; followed by a 'pick and mix' method ensuring applicability across the whole paediatric population whilst keeping the total number of tablets administered as low as possible. Formulation selection was based on API properties, target dose banding and suitability of delivery system for paediatric population needs. A systematic approach including excipient screening, formulation development, characterisation, dissolution studies and stability evaluation were conducted to generate a holistic preclinical data set. The application of the ODMT technology with paediatric biopharmaceutical consideration for evaluation aims to safely and accurately facilitate the development of more age-appropriate formulations for this vulnerable patient group and serves as a potential candidate for PUMA application.

Table 7: Overview of the physicochemical properties of carvedilol.

Carvedilol Monograph	
Chemical Name	1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy)ethylamino]propan-2-ol
Other Names	Carvedilol hydrochloride
Molecular Formula	$C_{24}H_{26}N_2O_4$
Molecular Weight	406.5
logP	3.8
pKa	7.8
CAS Number	72956-09-3
Carvedilol Structure	
Appearance	White to off-white powder
Melting point	114-115 °C
Solubility	4.44 mg/mL

2.3 Material and Methods

2.3.1 Material

Carvedilol was obtained by Acros Organics (New Jersey, NJ, USA). Pharmaceutical grade D-Mannitol (≥98% purity) and magnesium stearate were obtained from Sigma-Aldrich (Dorset, UK), while MCC as Pharmacel 102 was obtained from DFE Pharma (Germany). Lastly, colloidal silicon dioxide (Aerosil 200) was obtained from Evonik Industries (Essan, Germany). Pepsin from porcine gastric mucosa (≥400 units/mg protein), sodium hydroxide pellets, sodium chloride, sodium acetate and sodium monooleate were purchased from Sigma-Aldrich (UK). Sodium taurocholate hydrate, 96%, acetic acid, maleic acid and Lecithin, 60%, egg were obtained from Alfa Aesar (Lancashire, United Kingdom). Glyceryl monooleate as Monomuls 90-O 18 PH was kindly gifted by BASF (Ludwigshafen, Germany). Carvedilol tablets 3.125 mg were obtained from Bristol laboratories (Hertfordshire, United Kingdom).

For sample analysis, Acetonitrile and Methanol (HPLC-grade) were obtained from Fisher Scientific (London, UK), whereas Trifluoroacetic acid (TFA), for HPLC (≥99.0%) was purchased from Sigma-Aldrich (UK).

2.3.2 HPLC Analytical Method Development

All samples were analysed on an Agilent 1260 series (Agilent Technologies, Santa Clara, CA, USA), equipped with a reverse-phased Eclipse plus C18, 4.6 × 150 mm, 3.5µm column (Agilent Technologies, Santa Clara, CA, USA). Separation of carvedilol was achieved using an isocratic mobile phase compromising TFA: ACN (55:45 *v/v*). TFA was used at a concentration of 0.1% (*v/v*). Flow rate was set at 1 ml/min and a wavelength of 240 nm was used for detection. A 9 point calibration curve in the range 0.39 to 100 mcg/mL was prepared via serial dilution in methanol (1 in 2 dilution). All samples prepared for analysis were diluted so as to fall within the calibration range. Method validation was carried out following the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidelines (Q2(R1)).

2.3.3 Compatibility Using Differential Scanning Calorimetry (DSC)

Excipient and drug compatibility were studied using a TA DSCQ200 apparatus (TA Instruments, New Castle, DE) with TA Instruments Universal Analysis 2000 software. Data was collected under nitrogen atmosphere (flow rate: 50 mL min^{-1}) using sealed flat-bottomed TZero aluminium pans and pierced lids (sample mass 1–2 mg) at a heating rate of 10 °C min^{-1} in the range of 50 to 400 °C. For samples containing both the drug and excipient, 1 mg of each was measured in a weighing boat and gently mixed with a spatula for one minute before being transferred into the sample pan. All studies were conducted in triplicate and data is presented as mean ± standard deviation.

2.3.4 Particle Size Analysis

Particle size analysis was carried out via laser diffraction using a Sympatec Helos detector equipped with a Rodos dry disperser and vibrating feeder (Clausthal-Zellerfeld, Germany). 1 g of sample was placed on the VIBRI/I feeder and fed through the RODOS disperser. Pressure was set a 2 bars and measuring range was set between 0–175 µm. Measurements were taken in triplicates and presented as ± SD. Volume mean diameter (VMD), X10, X50 and X90 were the values obtained. Both starting materials and final blends were analysed to give an indication of flow based on particle size and to ensure acceptable flow properties are prevalent for successful direct compression.

2.3.5 Powder Flow Measurements

Angle of repose was determined using the method outline in the U.S. Pharmacopeia monograph, <1174> (218). A known amount of powder was poured through a 12mm funnel maintained at a height of 2-4 cm from the top of the powder heap. Height and diameter of the powder cone was measured and angle of repose was determined using the following equation:

Eq.1 $\tan(\Theta) = \text{Height}/0.5\ \text{Base}$

Measurements were carried out in triplicates and presented as mean ± standard deviation.

Bulk (ρbulk) and tapped densities (ρtapped) were also used to assess powder flow. A Sotax tap density tester USP II (Allschwil, Switzerland) was used as outlined in the USP monograph, <616> (219). A 25 mL measuring cylinder was tightly fastened onto the tester using a foam ring. A known amount of sample was then poured and initial volume was taken at 0 taps. Further measurement were taken after every 10, 500 and 1250 taps. Measurements were carried out in triplicates and presented as mean ± standard deviation

Carrs's index and Hausner ratios were calculated using the following equations:

Eq. 2: Carr's Index = (ρtapped - ρbulk)/ρtapped) x 100

Eq. 3: Hausner ratio = ρtapped/ρbulk

Eq. 4: ρbulk = Mass/V0 (unsettled apparent volume)

Eq. 5: ρtapped = Mass/Vf (Final tapped volume)

2.3.6 Optimising the Process of Blending for Carvedilol to Improve Content Uniformity

An Erweka AR403s Multi-Lab (Heusenstamm, Germany), equipped with a cube mixer attachment was used to mix a total of 50 g of powder that included mannitol, MCC, magnesium

stearate, colloidal silicon dioxide and carvedilol. Different speed rates, mixing times and blending orders were evaluated to analyse any consequent effects on content uniformity. In each case, magnesium stearate was added at the end and allowed to mix for a total of one minute, whilst colloidal silicon dioxide (CSD) was added second to last and allowed to mix for a total of two minutes. A Sartorius LA220S balance (Sartorius, Göttingen, Germany) was used to accurately weigh out samples.

Content uniformity was assessed using a random sampling technique, where 10 samples of 50 ± 1 mg were taken out from different parts of each powder blend. Each sample was dissolved with 50 mL of methanol in a volumetric flask and sonicated for 30 min. Post sonication, the samples were filtered through a 0.45 µm syringe filter and analysed via High Performance Liquid Chromatography (HPLC).

2.3.7 Orally Disintegrating Mini Tablet (ODMT) Production

Optimised powder blends were identified and selected, possessing acceptable tablet properties with improved content uniformity. Two strengths of carvedilol ODMTs with a target drug load of 1 and 4% *w/w* and a tablet mass of 50 mg were produced using a Specac semi-automatic hydraulic press (Slough, UK) equipped with a 4 mm multi-tip (three) with concave faced punches at a compression force of 10 KN with quick release. After step wise fine-tuning of formulation composition and tabletting parameters, two formulations for ODMT production were finalised (Table 8). The resulting ODMTs demonstrated adequate strength (> 30 N) with disintegration times of less than 30 s. The need of disintegrants and flavour enhancer was eliminated due to the careful selection of excipients that provided multiple interests including palatability, compressibility and disintegrating properties.

Table 8: Formulation composition for 500 and 2000 µg carvedilol ODMT production.

Material	**F1 (0.5 mg Tablet)**	**F2 (2 mg Tablet)**
	Concentration % w/w	Concentration % w/w
Mannitol	83.25	80.25
MCC	15	15
Carvedilol	1	4
Colloidal silica dioxide	0.25	0.25
Magnesium stearate	0.5	0.5

2.3.8 ODMT Physical Evaluation

2.3.8.1 Hardness

A Copley TBF 100 Hardness tester (Nottingham, UK) was used to measure the force required to break the tablets produced. Hardness values were measured in Newtons. Measurements were carried out in triplicates and presented as mean ± standard deviation.

2.3.8.2 Friability

Friability testing was carried out using a Sotax F2 Friabilitor (AllSchwill, Switzerland), that measures the capacity of tablets to resist mechanical stress. Six tablets were carefully de-dusted using a soft brush and an initial weight was taken. Tablets were then placed in the rotating drum and rotated for 4 min at a speed of 25 rpm (total 100 revolutions). The tablets were removed, dusted and a final weight was taken. The percentage friability was calculated using the following equation:

Eq. 6: % Friability = (initial weight- final weight)/initial weight × 100

2.3.8.3 Disintegration

Disintegration testing was carried out as stated in the official USP disintegration monograph (701). An Erweka ZT3 (Heusenstamm, Germany) disintegration tester was used. A tablet was placed in one of the six vessels and allowed to oscillate at a rate of 30 cycles per minute. In this instance, a disk was not used in order to allow for a more direct comparison of the oral cavity environment. A total of 800 mL of distilled water was used as the disintegration medium that had a constant temperature of 37 °C. Measurements were achieved for a single tablet at a time to enhance accuracy. The disintegration time was noted until there were no more residue of tablet aggregates left on the upper side of the vessel mesh. Measurements were carried out in triplicates and presented as mean ± standard deviation.

2.3.9 Adult and Paediatric Biorelevant Media Design and Development

Adult simulated gastric and intestinal media (FaSSGF, FeSSGF, FaSSIF and FeSSIF), were prepared as presented by Jantratid et al. (220) and employed as references to adjust age-specific media composition. Maharaj et al. (221) defined the composition of neonatal and infant biorelevant media after investigating and evaluating age-specific changes in intra gastric parameters including bile acid, pepsin and sodium chloride concentrations, pH and buffering capacity. A detailed summary of biorelevant media design is given in Table 9.

Table 9: Composition of adult and paediatric biorelevant media used in solubility and dissolution experiments. FaSSGF = Fasted- state Simulated Gastric Fluid, FeSSGF = Fed-state Simulated Gastric Fluid, FaSSIF = Fasted- state Simulated Intestinal Fluid, FeSSIF = Fed- state Simulated Intestinal Fluid, i = infant, n = neonate, c = cow based milk formula, s = soy based milk formula, b = breast fed.

Pre-prandial biorelevant gastric media composition			
Composition	*Adult (FaSSGF)*	*Infant (i-FaSSGF)*	*Neonate (n-FaSSGF)*
Sodium chloride (mM)	34.2	34.2	34.2
Sodium taurocholate (µM)	80	60	20
Lecithin (µM)	20	15	5
Pepsin (mg/mL)	0.1	0.025	0.015
HCl/NaOH qs	pH 1.6	pH 1.6	pH 1.6
pH	1.6	1.6	1.6
Osmolarity (mOsm/kg)	120.7 ± 2.5	120.7 ± 2.5	120.7 ± 2.5
Post-prandial biorelevant gastric media composition			
Composition	*Adult (FeSSGF)*	*Neonate-Cow (nc-FeSSGF)*	*Neonate-Soy (ns-FeSSGF)*
Sodium chloride (mM)	34.2	34.2	34.2
Acetic acid (mM)	17.12	7.25	7.25
Sodium acetate (mM)	29.75	64.65	64.65
Milk:buffer	1:1	1:1	1:1
HCl/NaOH qs	pH 5	pH 5.7	pH 5.7
pH	5	5.7	5.7
Osmolarity (mOsm/kg)	400	340	240
Buffering capacity (mmol/L/pH)	25	15	15
Pre-prandial biorelevant intestinal media composition			
Composition	*Adult (FaSSIF-V2)*	*FaSSIF-50%*	*FaSSIF-150%*
Sodium hydroxide (mM)	34.8	34.8	34.8
Sodium taurocholate (mM)	3	1.5	4.5
Lecithin (mM)	0.2	0.1	0.3
Sodium chloride (mM)	68.62	68.62	68.62
Maleic acid (mM)	19.12	19.12	19.12
HCl/NaOH qs	pH 6.5	pH 6.5	pH 6.5
Osmolarity (mOsm/kg)	180 ± 10	180 ± 10	180 ± 10
Buffering capacity (mmol/L/ph)	10	10	10

Post-prandial biorelevant intestinal media composition				
Composition	*Adult (FeSSIF-V2)*	*Neonate-breast fed (nb-FeSSIF)*	*Neonate-cow formula (nc-FeSSIF)*	*Infant-cow formula (i-FeSSIF)*
Sodium hydroxide (mM)	81.65	81.65	81.65	81.65
Sodium taurocholate (mM)	10	2.5	2.5	7.5
Lecithin (mM)	2	0.5	0.5	1.5
Sodium chloride (mM)	125.5	95	111.73	107.35
Maleic acid (mM)	55.02	55.02	55.02	55.02
Glyceryl monooleate (mM)	5	5	6.65	5
Sodium monooleate (mM)	0.8	0.8	1.06	0.8
HCl/NaOH qs	pH 5.8	pH 5.8	pH 5.8	pH 5.8
Osmolarity (mOsm/kg)	300 ± 10	330 ± 10	330 ± 10	390 ± 10
Buffering capacity (mmol/L/ph)	25	25	25	25

2.3.10 ODMT Evaluation – Biorelevant Solubility and Dissolution Studies

The saturation solubility of carvedilol in simulated dissolution media was determined according to the reported method (210). Briefly, an excess amount of carvedilol powder was added to glass vials containing 30 mL of the respective dissolution media. The vials were then screwed and placed on a hot-plate stirrer (Stuart US152, Northants, UK) maintained at 37 ± 1 °C and continuously stirred using a magnetic stirrer at a speed of 50 rpm. Samples were withdrawn after 24 h, filtered through a 0.45 µm syringe filter and analysed. The concentration of carvedilol in each sample was determined using HPLC. Solubility in each respective medium was carried out in triplicates and presented as mean ± standard deviation.

Percentage carvedilol ionisation was calculated using the following equation:

Eq. 7: $\%Ionisation = \frac{1}{1+10^{(pH-pKa)}}\ X\ 100$

where pH denotes the pH value of the dissolution medium, whilst pKa is the negative log of the acid dissociation constant of carvedilol.

Minimum dissolution volume was calculated using sink condition (Sc), determined by the ratio of solubility (S) to drug concentration (C), denoted by S/C. A value of greater than 3 is

considered as sink condition (222, 223). All dissolution test were carried out using an Erweka DT 126 with USP 2 paddle apparatus (Langen, Germany). Samples of 5 mL were drawn at appropriate time points (2, 5, 10, 15, 30, 45, 60, 75, 90,120 and 180 min) and replaced by 5 mL of fresh media to maintain sink conditions. A total of 500 mL of media per vessel, maintained at 37 °C, employed with a continuous paddle speed of 50 rpm. Drug release was determined via HPLC and adjusted for cumulative drug release (%). A total of 6 replicates were taken and data presented as mean ± standard deviation.

2.3.11 Statistical Analysis

GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA) was used to carry out one-way analysis of variance (ANOVA) with a posthoc Tukey's test to identify statistically significant differences in solubility between adult and paediatric biorelevant media. An alpha value of 0.05 was used.

2.4 Results and Discussion

2.4.1 Drug candidate selection and choice of dosage form design

The European commission have identified medicines used in cardiovascular conditions as one of the therapeutic classes in need of more age-appropriate formulations. Paediatric heart failure is well known and compromises of several conditions, including cardiomyopathy and congenital heart disease. Although disease manifestation between children and adults differ, management is based on adult data and supporting paediatric literature, meaning similar drug treatments used in adults are employed in children. Carvedilol is a BCS class II drug with low aqueous solubility (4.44 mg/L). The total frequency of prescribing and off-label carvedilol use (liquid specials) was determined and identified as 594,580 and 926 items respectively across all NHS regional teams during the last twelve months, with overall increasing trends observed since 2015 (224). Such prescribing data suggest significant off-label use and confirms the clinical need of carvedilol, therefore, presenting the opportunity to formulate carvedilol into an age-appropriate formulation that shall aim to improve safety, compliance and clinical output. Further, significant contributions of admissions due to paediatric heart failure have been reported, where the incidence of cardiomyopathies in developed countries was stated to be about 0.8–1.3 cases per 100,000 children in the 0–18 years age group and ten times higher in the 0- to 1-year old age group (225-227). This indicates the clinical need of carvedilol as an adjunct to treat paediatric heart failure among all paediatric subsets (Table 10).

Table 10: Summary of studies where carvedilol therapy has shown clinical need in neonates and infants**.**

Study	Population size	Outcome
Gachara et al. (2001)	8 Infants	Improvement in the left ventricular ejection fraction
Shaddy et al. (2007)	55 patients <24 months	These preliminary results suggest that carvedilol does not significantly improve clinical heart failure outcomes in children and adolescents with symptomatic systolic heart failure
Nishiyama et al. (2009)	15 Patients <4 years	Carvedilol may be effective in treating heart failure in children due to ischemia and myocardial disease, and complicated by tachycardia
Bruns et al. (2001)	14 patients aged 0-5 years	Carvedilol as an adjunct to standard therapy for paediatric heart failure improves symptoms and left ventricular function
Laer et al. (2002)	7 Patients < 2 years	Paediatric patients with CHF not responding to standard therapy may benefit from oral carvedilol treatment

The selection of an ODMT was confirmed based on the physicochemical properties of carvedilol, requirement of adequate dosing flexibility and with an aim to limit the total number of excipient within the formulation. Carvedilol is a low dose, potent compound with low aqueous solubility. It is gastric stable and does not exhibit any taste concerns. Dosing of carvedilol is based on child weight and is usually titrated when initiated. The availability of multiple strengths ensures to cover a wide range of therapeutic doses and, therefore, the novel ODMT formulation is aimed at children between the ages of 2 to 17 years.

2.4.2 Dose banding and proposed strengths of carvedilol mini ODTs

The dose of carvedilol according to the BNF is as follows:

For Child (2–17years) - Initially 50 micrograms/kg twice daily (max. per dose 3.125 mg) for at least 2 weeks, then increased to 100 micrograms/kg twice daily for at least 2 weeks, then increased to 200 micrograms/kg twice daily, then increased if necessary up to 350 micrograms/kg twice daily (max. per dose 25 mg) (228).

Four tablet strengths (0.25 mg, 0.5 mg, 1 mg and 2 mg) were chosen to fulfil various dose requirements of children aged between 2-12 years (Table 11). The selection of such strengths provides adequate dosing flexibility, where different strengths can be added to make up the required dose (Table 11). Furthermore, in the case of higher dose bandings, where a greater number of mini ODTs are required, older children may take multiple ODMTs or encapsulate multiple tablets into appropriate sized capsule shells to limit the frequency of administration, where the capsule can be taken as a single administration. Although four strengths are required to fulfil dose requirements, for the purpose of this study, only the 0.5 mg and 2 mg strength tablets were formulated and evaluated.

Table 11: Dose banding and number of tablets required to fulfil dose requirement.

Dose	Age and average child Weight (kg) (229)	Number of tablets required to fulfil dose requirement			
		0.25 mg	*0.5 mg*	*1 mg*	*2 mg*
50 mcg/kg	2 year: 12 kg	–	1	–	–
	3 years: 14 kg	1	1	–	–
	5 years: 18 kg	–	–	1	–
	7 years: 23 kg	1	–	1	–
	10 years: 32 kg	–	1	1	–
	12 years: 39 kg	–	–	–	1
100 mcg/kg	2 year: 12 kg	–	–	1	–
	3 years: 14 kg	–	1	1	–
	5 years: 18 kg	–	–	–	1
	7 years: 23 kg	–	1	–	1
	10 years: 32 kg	–	–	1	1
	12 years: 39 kg	–	–	–	2
200 mcg/kg	2 year: 12 kg	–	–	–	1
	3 years: 14 kg	–	–	1	1
	5 years: 18 kg	–	–	–	2
	7 years: 23 kg	–	–	1	2
	10 years: 32 kg	–	–	–	3
	12 years: 39 kg	–	–	–	4
350 mcg/kg	2 year: 12 kg	–	1	1	1
	3 years: 14 kg	–	–	1	2
	5 years: 18 kg	–	–	1	3
	7 years: 23 kg	1	1	–	4
	10 years: 32 kg	–	1	–	5
	12 years: 39 kg	–	–	–	7
Up to 25 mg (max)		Varied depending on dose. Multiple tablets may be counted and taken individually, or encapsulated and taken as a single administration, provided the child is able to swallow the respective capsule size.			

2.4.3 Paediatric excipient working zone for ODMTs.

According to the EMA, to label a tablet as an orodispersible tablet, it must completely disintegrate within 3 minutes, whilst the Food and Drug Administration (FDA) considers an ODT to rapidly disintegrate within the mouth, with an *in vitro* disintegration time of less than 30 seconds (155, 230). Particular formulation compositions and/or inclusion of certain excipients including disintegrants and superdisintegrants are used to achieve such feats. Other commonly used excipients employed in ODTs include diluents, lubricants and flow aids. Despite pharmaceutical excipients being an integral part of dosage forms, they cannot always be considered safe and acceptable for all age groups, especially in children, since most excipients intended for use are approved and have undergone comprehensive short and long

term studies for safety and toxicity solely in the adult population. On account of insufficient paediatric clinical data, it is vital that when formulating a paediatric dosage form, excipients are screened and carefully selected to avoid any potential toxicity and safety concerns (Table 12). However, appropriate excipient selection can be difficult in paediatrics, since data regarding excipient safety and toxicity is limited. In addition, the data that is available is usually dispersed and challenging to acquire. Owing to limited clinical excipient safety and toxicity data in paediatrics, it would be more appropriate and desirable to limit the total number of excipients within the formulation. Accordingly, the aim in this study was to exploit excipient characteristics that provided multiple functions, including palatability, compressibility and disintegrating properties.

Mannitol is widely used in the pharmaceutical industry as an ODT excipient due to its high water solubility, low hygroscopicity and better tolerance by patients. Additionally, it has a pleasant mouth feel, sweet taste and a cooling sensations due to its negative heat of solution (231). For such reasons, mannitol was chosen as the diluent in the proposed ODMT formulation. The choice of mannitol as a diluent in the formulation was appropriate since, although paediatric threshold data on polyols is limited, mannitol is poorly absorbed with laxative effects reported only after ingestion of quantities greater than 20 g/day (232). The maximum possible carvedilol dose of 25 mg per day contains only 20.1 mg of mannitol, which is well below the daily assumed threshold value. On the other hand, microcrystalline cellulose (MCC) was incorporated as a binder in the proposed formulation due to its safety profile, low cost and exceptional dry binding properties. MCC deforms plastically, exhibits relative low bulk density and high surface area. Such properties contribute to the unique binding properties of MCC that provide mechanical robustness and cohesiveness to tablets, even at small compression forces (233). In addition, at concentrations of 5-15%, MCC also exhibits disintegrating properties, making it a useful excipient in tabletting of ODTs via the direct compression method (234).

Magnesium stearate was included into formulation to reduce friction between the compact and die surface during ejection, whereas colloidal silicon dioxide (CSD) was incorporated to enhance flowability. Magnesium stearate is a metallic salt lubricant, commonly used as a lubricant in pharmaceutical tableting due to its high lubrication properties, chemical stability and low cost (235). CSD is a glidant used to improve the flow properties of powders. Mechanism of actions include, reducing electrostatic and attractive forces between host particles, reduction of surface roughness and reducing friction between particles (236). The safety of the aforementioned excipients were further established after reviewing the STEP database, where safe use in children was affirmed (237). Nonetheless, the proposed selection

of excipients are approved as additives by the FDA and included in the database of substances generally regards as safe (GRAS) (238).

Table 12: Functionality and rational for inclusion of selected excipients for the production of carvedilol ODMT.

Excipient	Function	Max ADI	Reason for selection
Mannitol	Diluent, Sweetener	10 g	Serves dual function- Provides bulk to the dosage form and enhances palatability. Low hygroscopicity so can be used with moisture sensitive actives
MCC	Binder, Disintegrant	Not specified	Provides strength to formulation, exhibits excellent compressibility properties and serves as a disintegrant at concentration used, thereby minimising the total number of excipients
Magnesium stearate	Lubricant	No ADI allocated	Reduce friction between the compact and die surface during ejection
Colloidal silica dioxide	Glidant (flow aid)	NA	Improve the flow properties of powder blend- ensuring consistent die fill

2.4.4 Compatibility using Differential scanning calorimetry (DSC)

In order to limit the total number of excipients within the formulation, one of the objectives of this study was to exploit excipient characteristics that provided multiple functions including palatability, compressibility and disintegrating properties.

Drug and excipient compatibility is an important part of preformulation study, where concomitant use of excipient and active pharmaceutical ingredients (API) are assessed. Commonly used excipients in ODT formulation were scanned alongside carvedilol to determine compatibility.

Carvedilol displayed a sharp endothermic peak at 117.33 °C, indicating its melting point and crystalline structure (Figure 2). The phase transition enthalpy value was 149.3 J/g. Polymorphism is a well-known phenomenon for fatty acids, and their salts (239). The broad melting range of magnesium stearate is typical of commercial samples because commercial magnesium stearate consists of either crystalline hydrates (di or trihydrate, or a mixture of), or a poorly crystallised anhydrate (234, 240). Magnesium stearate displayed two endothermic signals: a small one with a peak temperature of 87.92 °C (enthalpy: 19.70 J/g), and a larger one with a peak temperature of 115.03 °C (enthalpy: 274.7 J/g), where the former corresponds

to dehydration and the latter indicates the fusion of constituent fatty acids (241). The DSC curve of MCC shows a broad endothermic signal between 50–125 °C (peak value: 78.51 °C and enthalpy: 37.37 J/g) corresponding to the loss of humidity and an endothermic peak at 337.73 °C (enthalpy: 321.1 J/g) with an onset temperature of 314.88 °C, corresponding to the melting point of MCC. Mannitol displayed a sharp melting peak with onset at 166.03 °C and a transitional enthalpy value of 321.7 J/g. Owing to the extremely high melting point of colloidal silicon dioxide that occurs at 1600 °C, no phase transitions or peaks were observed in the range of 50–250 °C (234).

The sharp melting peak of carvedilol remained consistent when combined with magnesium stearate, suggesting compatibility between the two components (Figure 2). However, the peak corresponding to the dehydration of magnesium stearate has shifted to a peak temperature of 102.93 °C (enthalpy: 29.51 J/g) from 87.92 °C (enthalpy: 19.70 J/g). The third endothermic peak at 127.16 °C (enthalpy: 11.53 J/g), indicating the fusion of constituent fatty acids also shifted from a peak temperature of 115.03 °C (enthalpy: 274.7 J/g) with reduced enthalpy value. Such changes may be due to the different polymorphic forms of magnesium stearate in which melting points vary depending on the concentration of the specific polymorphic form present (242). It may be possible for a certain polymorphic form to be present at a higher concentration in the mixture as compared to the individually screened magnesium stearate. Additionally, due to the similar melting points of both components in the mixture, it is possible that less amount of energy was required to fulfil the fusion of constituent fatty acids in magnesium stearate since the melting of carvedilol occurred first and therefore less energy was needed to fulfil the later fusion. No substantial peak shifts, appearance of new peaks or changes in enthalpy values were observed between carvedilol and mannitol, MCC and CSD mixtures, thereby demonstrating compatibility (Figure 2).

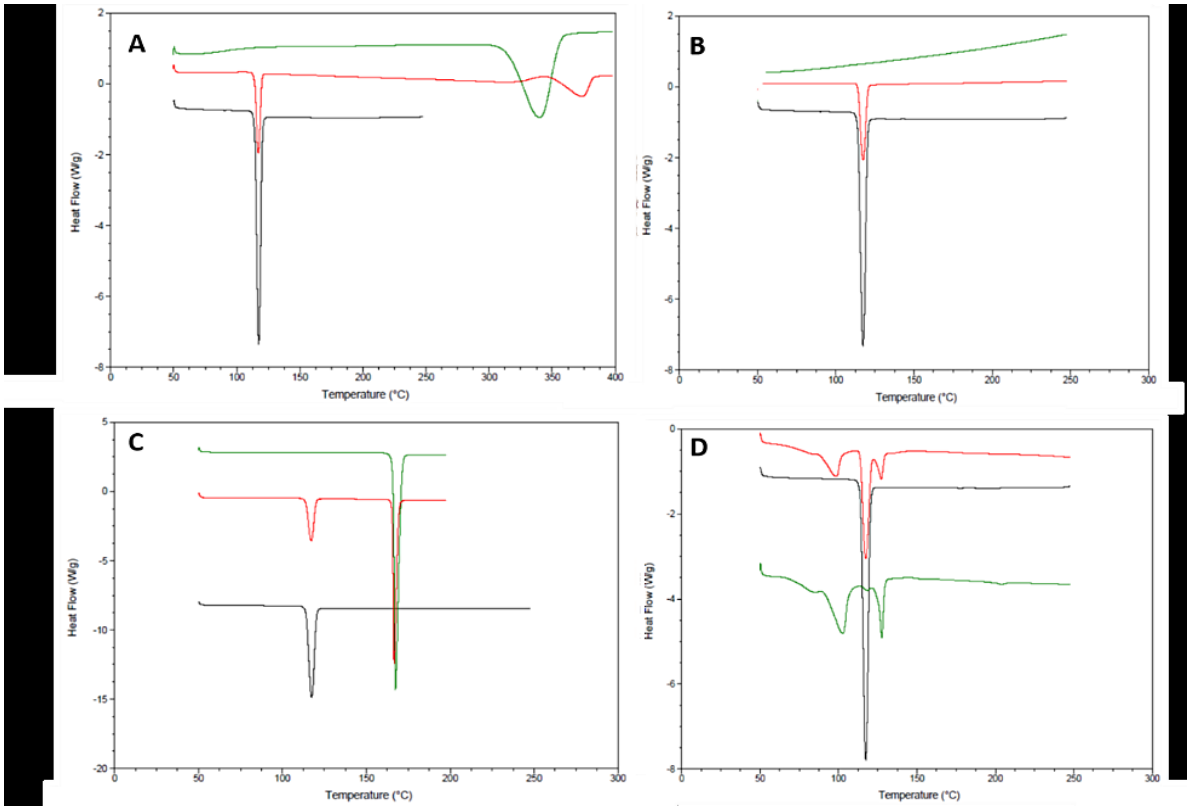


Figure 2: DSC thermograms showing compatibility between carvedilol and selected excipients. A) MCC, B) CSD, C) Mannitol, D) Magnesium stearate. In each thermogram, the black, red and green curves represent carvedilol, the excipient, and a 1:1 of carvedilol and excipient, respectively.

2.4.5 Placebo tablet production and stepwise finetuning of mini ODT properties

Concentrations of 0.5 and 1.0 %w/w were used to compare the effect of magnesium stearate on tablet hardness and disintegration when compressed with mannitol. During our preliminary work it was observed (results not shown) that an increase in lubricant concentration resulted in a decrease in hardness and increase in disintegration time. Magnesium stearate is hydrophobic in nature and forms a film at the surface of other excipients and/or API. An increased concentration of magnesium stearate will result in a stronger film being formed, whereby the disintegration medium will take a longer time to penetrate the particle surface, resulting in slower wettability and as a consequence increased disintegration time (243). Additionally, in the case of 1%w/w magnesium stearate, the stronger lubricant film formed around the mannitol particles resulted in reduced inter particle bonds to form between subsequent mannitol particles, thereby reducing the hardness of the compact. To avoid increased disintegration times and decreased hardness, lubrication concentration of 0.5% w/w was chosen.

The impressive binding properties of MCC comes from its plastically deforming nature, which when compressed, plastically deforms and thus maximises the area of inter-particle bonding (233). When MCC is deformed, it creates many slip planes leading to a large proportion of exposed hydroxyl groups (244). The presence of exposed hydrogen groups on adjacent cellulose molecules thus allows the formation of numerous hydrogen bonds, thereby increasing tablet strength (245). In addition. The shape of MCC also contributes to its increased tabletability, as irregularly shaped and elongated particles result in mechanical interlocking (246). Placebo tablet production using a concentration of 5% w/w MCC, resulted in a hardness value of 12.63 ± 2.71 N and a disintegration time of 21.33 ± 0.578 s. The disintegration time is ideal however the mechanical strength of the tablets is unacceptable. At such values, the tablets would not be able to withstand mechanical shock during handling and transportation. The friability also had failed as the tablets broke into pieces during the friability test. The low concentration of MCC was not enough to provide adequate binding properties and strength to the formulation. At 25 %w/w, the hardness was noted as 47.87 ± 2.57 N, percentage weight loss as 0.07% and disintegration time of 27.00±4.58 s. This higher concentration of MCC allowed the formation of many hydrogen bonds during compression and therefore produced a robust tablet. However, the disintegration time achieved at 25%w/w was at the upper limits of the recommended 30s disintegration time from FDA, therefore 15 %w/w MCC was chosen as it showed reduced disintegration times of 15.00 ± 1.00 s. The friability test showed a percentage loss of 0.22% and hardness values of 35.20 ± 11.72 N, suggesting a mechanically sound tablet. As discussed previously, at concentrations of 5-15 %w/w, MCC exhibits disintegrating properties that reduces to disintegration time, while maintaining adequate tablet strength. In addition to MCCs swelling ability, the disintegrating properties arise from its highly hygroscopic nature and capacity to increase liquid transport into a tablet matrix, accelerating both diffusion and capillary action (247, 248).

The effect of CSD on tablet properties were investigated using two different concentrations (0.5 and 0.25%). As expected, the higher concentration of CSD (0.5%) proved to improve flow properties; however, negative effects were observed on disintegration times. Disintegration time achieved for 0.5 %w/w CSD was 42.67 ± 8.08 s, taking it above the recommended 30 seconds (FDA recommendation). The increase in disintegration time was due to the nano-size of CSD, which coats the host particles thereby decreasing the ability of disintegration medium to be drawn into the compact. The increase in hardness is a consequence of CSD's ability to enhance hardness in compacts containing materials that deform plastically such as MCC (249). CSD decreases any negative effects on hardness by preventing lubricants such as magnesium stearate occupying the surface of plastically

deforming material such as MCC, therefore allowing increased bonding between MCC particles (233). CSD at 0.25 %w/w was therefore selected.

After a step wise fine-tuning of formulation composition, an ODT comprising of adequate strength with disintegration times of less than 30 s was successfully achieved.

2.4.6 Pre Compression Material Characterisation

For low concentration drugs, as in the case of carvedilol (1 and 4 % w/w), the success of direct compression is dictated by the excipients to ensure content uniformity, flow and compressibility (250). It is essential for the powder to flow evenly, ensuring the right amount of powder enters the tabletting die, allowing consistent tablet weight and content uniformity.

The cohesiveness of a material must be determined before blending to evaluate flow properties (Table 13). Cohesive materials have an average particle size of less than 50 µm and tend to aggregate, leading to intermitted flow (251). On the other hand, non-cohesive materials have a larger particle size (>50 µm) and are expected to have good flow properties for successful tableting via the direct compression method (252). The volume mean diameter (VMD) of carvedilol was 24.68 ± 1.97 µm, suggesting its cohesive nature and tendency to aggregate. MCC on the other hand displayed a larger VMD of 84.20 ± 0.40 µm, suggesting non-cohesive behaviour. Larger particles, as in the case of MCC and mannitol (70.92 ± 3.27 µm) exhibit better flow compared to materials with smaller particle sizes due to the reduced surface area available for inter-particulate interactions (253). Magnesium stearate showed a VMD of 17.4 ± 1.96 µm indicating its cohesive nature. However, magnesium stearate when mixed, forms a boundary layer around particles in the formulation, resulting in reduced friction between particles and optimising the flow of the powder blend (80). The hydrophobic nature of magnesium stearate also reduces inter-particulate interactions by minimising wan der Waals forces, thereby improving flow (254).

Table 13: Particle size analysis of starting materials and optimised formulation blends (F1 and F2).

Sample	VMD (µm)
Mannitol	70.92 ± 3.27
MCC	84.20 ± 0.40
Carvedilol	24.68 ± 1.97
Magnesium stearate	17.49 ± 1.96
F1	77.50 ± 0.60
F2	77.18 ± 1.17

The angle of repose (AOR) indicated carvedilol to possess poor flow (46.47 °) (Table 14); this is expected, because the small particle size of carvedilol means there is a greater surface area available for interaction between carvedilol particles. On its own, magnesium stearate showed passable flow (40.33 °); however, it is well known that lubricants improve flow properties when added to powders. MCC and mannitol showed excellent and fair flow properties (23.73 and 32.97°, respectively). It is important for MCC and mannitol to possess good flow properties as they will dictate the overall flow of the formulation blend, since they are at high concentrations and make the bulk of the powder blend. After incorporating CSD, the angle of repose for formulations 1 and 2 both displayed excellent flowability. Flow aids enhance flow by adsorbing onto the particles within the formulation, thereby increasing surface roughness of host particles, resulting in reduced van der Waals forces (255).

According to Carr's index and Hausner ratio, mannitol exhibited very poor flow (33.33% and 1.5) whereas MCC shows passable flow (23.08% and 1.3) (Table 14). These results are in disagreement with results presented by the angle of repose. This may be due to the shape of the particles, where angular particles, as in the case of MCC, may initially pack loosely but when subjected to force result in significant repacking, resulting in higher Hausner ratio and leading to an untrue representation of actual flow property (256). The fibrous and irregular particle shape of MCC may also contribute to its inter-particulate void volume, leading to a higher volume to mass ratio (219, 257). The same can be said for F1 and F2 where flow evaluation based on CI and HR indicates poor flow, where in fact, both formulation blends actually possess excellent flowability.

Table 14: Angle of repose, Carr's index (CI), Hausner ratio (HR) and evaluation based on CI and HR.

Sample	AOR (°)	CI (%)	HR	Evaluation based on CI and HR
Mannitol	32.97	33.33	1.50	Very, very poor
MCC	23.73	23.08	1.30	Passable
Carvedilol	46.47	57.89	2.38	Very, very poor
Magnesium stearate	40.33	44.41	1.80	Very, very poor
F1	15.97	27.78	1.38	Poor
F2	16.36	27.78	1.38	Poor

2.4.7 Optimising the Process of Blending for Carvedilol to Improve Content Uniformity

Mixing of particulate solids is an important process step in achieving homogenous blends and key to assuring that each resulting unit dose possesses the specified amount of drug to achieve effective therapeutic levels. For low concentration drugs, as in the case of carvedilol, the success of direct compression is dictated by the excipients which are incorporated into the powder blend, with an aim to optimise flow and compressibility. It is essential for the powder to flow evenly, ensuring the right amount of homogeneous powder enters the tableting die, ensuring consistent tablet weight and content uniformity (258).

The three major mechanisms of mixing include, convective, shear and diffusive mixing. Convective mixing is referred to as macromixing and involves bulk movement or gross displacement of particles from one part of the powder bed to another (259). During shear mixing, the use of an agitator arm or blast of air is used to create shear forces, ensuring large cohesive particle clumps are de-agglomerated and dispersed throughout the bulk powder (260). Lastly, diffusive mixing, also referred to as micromixing, is caused by the random movement of particles; it involves the materials to be tilted where gravitational forces cause the upper layers to slip and diffusion of individual particles to take place over newly developed surfaces (261). The main mechanism of mixing using a cube mixer is through diffusion. The powders are added into the mixer and allowed to move by tilting the powdered materials past the angle of repose using gravity to drive flow. This allows for a gentle mixing process with the potential to achieve a true random mix (234).

Blending optimisation requires careful consideration of order of addition of excipients together with fine tuning of process parameters. It was expected that longer mixing time would provide better content uniformity, since the API particles would have more contact time with other particles, increasing the chances of collision and resulting in improved homogeneity (262, 263). However, this was not the case as an increase in mixing time to fifteen minutes increased the percentage recovery of carvedilol. The increase in mixing time may have led to the segregation of carvedilol particles, owing to its particle size, density, and cohesive nature (264). Denser particles, as in the case of carvedilol, promote de-mixing and non-homogeneity in the mix, since dense particles consistently move downwards and settle at the base of the powder blend. Additionally, longer blending times impose greater particle-particle collisions, resulting in increased particle mobility and dilation of the powder bed. Blending optimisation requires careful consideration of order of addition of excipients together with fine tuning of process parameters. A total mixing time of 5 minutes at a speed of 250 rotations per minutes and with MCC added first provided optimised conditions for successful tableting and content uniformity (Table 15). MCC exhibits high surface roughness, enabling it to improve content uniformity of fine materials by promoting particle interlocking and acting as a host with multiple cavities for API entrapment (265). Additionally, high surface could potentially result in fewer adhesive interactions between particles, thereby improving flow and increasing the chances of achieving a homogenous blend (266).

Table 15: Effect of processing parameters and blending order on percentage recovery of carvedilol. F1) 250 rpm for 5 min, F2) 250 rpm for 10 min, F3) 100 rpm for 5 min, F4) 100 rpm for 10 min, F5) 100 rpm for 15 min, F6) 250 rpm for 15 min, F7) 250 rpm for 5 min (MCC added first), F8) 100 rpm for 10 min (MCC added first). Data presented as mean ± standard deviation (n=10).

Blend	% carvedilol recovery	RSD
1	111.8 ± 7.4	6.61
2	102.4 ± 7.4	7.27
3	104.9 ± 8.2	7.85
4	93.7 ± 5.9	6.31
5	148.6 ± 12.3	8.31
6	140.6 ± 9.2	6.56
7	103.5 ± 3.2	3.06
8	104.4 ± 5.3	5.05

2.4.8 ODMT Evaluation

Both formulations produced a balance between mechanical strength and fast disintegration times (Table 16). Friability was below the 1% European pharmacopeia limit for both formulations, suggesting minimal residual mass loss during transportation and handling. Formulations disintegrated within the recommended US Food and Drug Administration (FDA) disintegration time limit of 30 s (and well within the European Pharmacopoeia limit of 3 minutes) (267, 268). Fast disintegration times allows tablets to be safely administered to children either directly into the mouth or suspended on a spoon prior to administration. Regarding uniformity of dosage units, the United States Pharmacopeia (USP) states that the uniformity of content (CU) rather than weight variation (WV) be evaluated for uncoated tablets that have a dose of <25mg or drug ratio <25% w/w (269). Since the dose of carvedilol is less than 25mg in both ODMT strengths, CU was evaluated. In order to meet the acceptance criteria, the first ten dosage units must be within 85-115% of the stated label claim. All 10 units were within the acceptance values of 85-115% of the stated label claim. The percentage recovery of carvedilol tablets (F1 and F2) were within the specified values, suggesting appropriate therapeutic concentrations and safety.

Table 16: Tablet properties. Hardness value and disintegration time presented as mean ± standard deviation (n = 3). Content uniformity presented as mean ± standard deviation (n = 10).

Formulation	Hardness (N)	Friability (% loss)	Disintegration (s)	Content Uniformity (%)
F1 (0.5 mg ODMT)	35.20 ± 11.72 N	0.22	11.2 ± 3.2	102.2 ± 2.9
F1 (2 mg ODMT)	33.62 ± 7.39 N	0.23	10.6 ± 2.4	101.7 ± 3.6

2.4.9 ODMT Evaluation – Biorelevant Saturation solubility and Dissolution Studies

2.4.9.1 Solubility

Carvedilol is a weakly basic compound with a pKa value of 7.8, exhibiting the distinctive pH-dependent solubility profile of a weak base, with low solubility in dissolution media of high pH values and high solubility in media of low pH values (210). The pH of adult and paediatric FaSSGF is 1.6, with 100% ionisation of carvedilol. In contrast, biorelevant FaSSIF exhibited a pH of 6.5 and 95.23% of ionised carvedilol (Table 17). An increase in percentage of ionisation suggests the availability of more protonated free base, capable of forming salts with the anion of the buffer species, which may influence the solubility of carvedilol (210, 270).

Additionally, the type of buffer system involved may also affect the solubility of carvedilol, where buffers with an increased salt concentration may lead to a decrease in carvedilol solubility, due to their higher ionic strength (210). Adult, neonate and infant biorelevant FaSSGF showed higher solubility values in comparison to adult and paediatric FeSSGF, FaSSIF and FeSSIF (Figure 3), suggesting that carvedilol is more soluble in acidic media and therefore should demonstrate a higher dissolution rate, especially within the fasted state of the gastric media. In contrast, adult and paediatric FaSSIF displayed lower solubility values due to the basic pH value of 6.5. Differences in solubility owing to varying bile salt and lipid micelles additive components between adult and paediatric FaSSGF were insignificant, as an increase in pepsin, sodium taurocholate and lecithin did not necessarily improve solubility as demonstrated by infant FaSSGF, exhibiting a significantly (p value< 0.0001) larger solubility value (319.1 µg/mL) in comparison to adult FaSSGF (237.0 µg/mL), despite adult FaSSGF possessing higher concentrations of pepsin, sodium taurocholate and lecithin (Table 9). This affirms the effect of pH on carvedilol solubility. However, within the small intestine, the extent of solubility owing to varying bile salt and lipid micelles concentrations were more noticeable with increased solubility values reflecting higher concentrations of bile salt and lecithin, FaSSIF-V2 (18.7 µg/mL), FaSSIF-50% (17.9 µg/mL) and FaSSIF-150% (44.4 µg/mL). A similar observation was made between adult and paediatric FeSSIF, where the solubility of adult FeSSIF-V2 was greater than all paediatric biorelevant FeSSIF (Table 18).

Despite ns-FeSSGF and nc-FeSSGF media having similar pH values of 5.7, solubility values of ns-FeSSGF were half (p value< 0.0001) of that of nc-FeSSGF (Table 18). This may be due to the increased osmolarity of nc-FeSSGF (Table 9). An increase in osmolarity suggests the presence of more base of the main salt that is able to form salts with the anion of the buffer species, thereby increasing the solubility of carvedilol (210).

During dissolution, sink condition is a mandatory prerequisite, otherwise dissolution data may not be accurate (271). Without sink conditions, the total dissolution may considerably decrease; once the saturation point has been achieved, concentrations above this value will not be reflected within the dissolution data. All adult and paediatric biorelevant media within this study provided sink conditions (sink values >3) (Table 18).

Since carvedilol is rapidly absorbed with peak plasma concentrations attained within 1-1.5 hours after oral administration, there is some gastric absorption taking place (272). Carvedilol being a weak base suggests that the solubility profiles of carvedilol in FaSSGF would be the most credible values due to the acidic pH, with all FaSSGF variations exhibiting high solubility values. In contrast, owing to variability in intestinal fluid compositions, the solubility of carvedilol amongst the small intestine is variable across the population group (Table 18).

Carvedilol exhibits the distinctive pH-dependent solubility profile of a weak base, with low solubility in dissolution media of high pH values and high solubility in media of low pH values. This may pose a risk of precipitation as the drug travels along the GIT from a low pH in the stomach to a high pH in the intestine. Although not within the scope of the current work, formulations containing carvedilol could be optimised to enhance the solubility in intestinal media to mitigate precipitation risk by either including precipitation inhibitors within the formulation composition or by reducing the particle size of carvedilol (273). Nonetheless, intestinal media is comprised of bile salts and lecithin that combine to form micelles and increase the solubility of hydrophobic molecules and therefore minimises the risk of drug precipitation (274).

Table 17: Percentage ionisation of carvedilol (pKa 7.8) at various pH values, calculated using Henderson-Hasselbach equation (*Eq. 7*).

pH	Ionised carvedilol (%)	Unionised carvedilol (%)
1.6	100	0
5.0	99.84	0.16
5.7	99.21	0.79
5.8	99.01	0.99
6.5	95.23	4.77

Table 18: pH, saturation solubility (S), sink value (S/C) and sink condition of carvedilol in various adult and paediatric biorelevant media. **C: intrinsic solubility of carvedilol.*

Dissolution media	pH	Solubility (S, µg/mL)	Sink value (S/C)	Sink condition
FaSSGF Adult	1.6	237.0±3.1	59.3	Yes
n-FaSSGF	1.6	212.2±8.9	53.1	Yes
i-FaSSGF	1.6	319.1±8.0	79.8	Yes
FeSSGF Adult	5.0	123.0±2.0	30.8	Yes
nc-FeSSGF	5.7	100.2±1.8	25.1	Yes
ns-FeSSGF	5.7	49.0±6.7	12.2	Yes
FaSSIF-V2	6.5	18.7±0.4	4.7	Yes
FaSSIF 50%	6.5	17.9±0.8	4.5	Yes
FaSSIF 150%	6.5	44.4±2.0	11.1	Yes
FeSSIF-V2	5.8	265.3±2.3	66.3	Yes
nb-FeSSIF	5.8	82.6±12.4	20.7	Yes
nc-FeSSIF	5.8	87.9±3.6	22.0	Yes
ic-Fessif	5.8	147.3±9.9	36.8	Yes
**C = 4 µg/mL*				

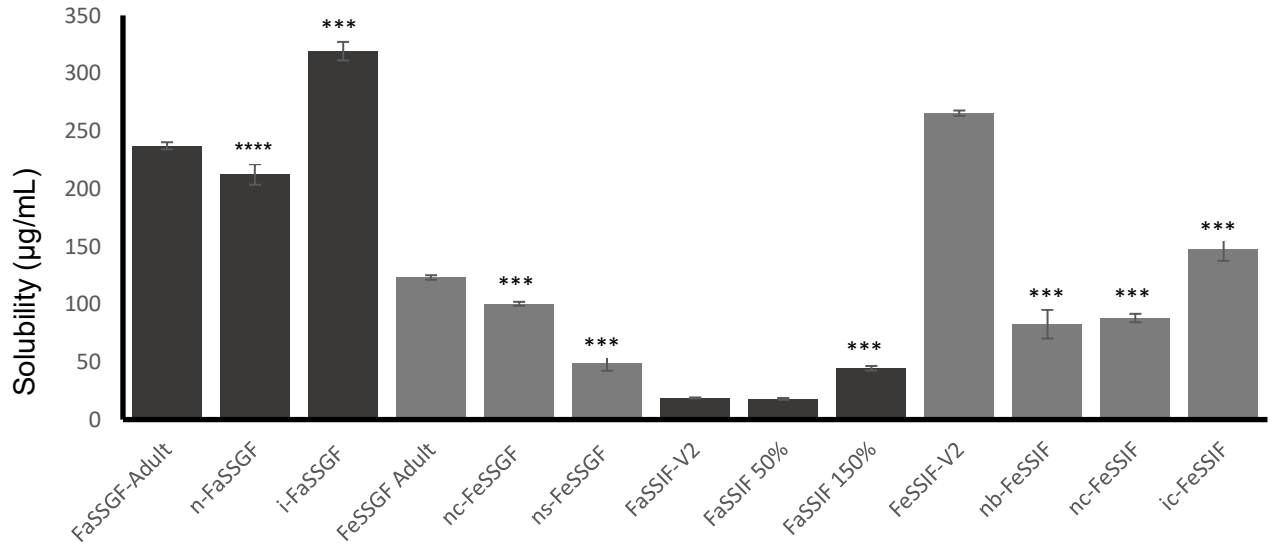


Figure 3: 24 h carvedilol saturation solubility (mean ± standard deviation, n = 3) in adult and paediatric biorelevant gastrointestinal media. Statistically significant solubility differences compared to the adult media are denoted with **** (P ≤ 0.0001). FaSSGF = Fasted- state Simulated Gastric Fluid, FeSSGF = Fed- state Simulated Gastric Fluid, FaSSIF = Fasted-state Simulated Intestinal Fluid, FeSSIF= Fed- state Simulated Intestinal Fluid, i = infant, n = neonate, c = cow based milk formula, s = soy based milk formula, b = breast fed.

2.4.9.2 Dissolution

All adult and paediatric biorelevant media prepared for dissolution testing are categorised as level II media (biorelevant) that mimic various parts of the GI tract in regards to adjusted osmolality, pH, buffer capacity, bile components and phospholipid concentration, and therefore, are superior to conventional USP/BP dissolution methods as they better reflect the solubilisation capacity of adult and paediatric GI fluids both during the fed and fasted state (207).

The initial rate of release of carvedilol from the novel ODMTs (0.5 mg and 2 mg strengths) in pre-prandial state was immediate, with carvedilol percentage release exceeding 80% after the first 10 minutes (Figure 4A, Figure 5A). The absorption of carvedilol is rapid, where maximum serum concentration is achieved between 1 to 2 hours post administration (275). Both strengths of the ODMT began to approach plateau levels approximately after 45 minutes within all fasted state simulated gastric media, with no significant differences observed between the adult and age-specific biorelevant media. However, relative to FaSSGF and i-FaSSGF the 0.5 mg ODMT in n-FaSSGF depicted a slower rate of percentage carvedilol release between 10 to 90 minutes (Figure 4A). The reduced percentage carvedilol release may be due to decreased carvedilol solubility owing from limited bile salt (sodium taurocholate) concentration

in n-FaSSGF as compared to FaSSGF and i-FaSSGF (276). Nonetheless, a similar extent of dissolution values were observed towards the end of the experiment.

Moving onto the fed state gastric media, it was expected for the dissolution rate to decrease in adult and paediatric FeSSGF due to the higher pH values of 5 and 5.7. This was true as the percentage release of carvedilol was only about 30-40% after the first 10 minutes. In addition, relative to adult and paediatric FaSSGF, the total percentage release of carvedilol 0.5 mg ODMT was lower in both adult and paediatric FeSSGF, with values of 91.5, 90.5 and 88.5% respectively. Further, percentage release in nc-FeSSGF (pH 5.7) was considerably lower compared to adult FeSSGF (pH 5) for the 2 mg ODMT, 89.4 and 96.2% respectively (Figure 4B). In adult FeSSGF the percentage of ionised carvedilol is 99.84% as compared to 99.21% in nc-FeSSGF. Other than a slight increase in percentage carvedilol ionisation, the total extent of dissolution may have also been enhanced owing to the buffer composition where the inclusion of increased acetate buffer allows the formation of water-soluble acetate salt, thereby increasing solubility of carvedilol (210). Nonetheless, similar values overall were observed after 120 minutes within adult and paediatric FeSSGF for both the 0.5 and 2mg ODMTs.

Bile salt concentrations within the fasted proximal small intestine depicted high degree of variability between paediatric and adult studies (221). On such account, age-specific FaSSIF was not developed, instead two alternative media were developed to simulate bile salt and lipid concentrations of 50% and 150% to that of FaSSIF-V2. Lecithin enhances the solubility of drugs through complexation, whilst sodium taurocholate holds the ability to produce micelles. Other than micellar solubilisation, bile salts may alter drug polarity and therefore solubility through ion pairing, when interacting with drug molecules (276). In line with the solubility data, the dissolution data also reflected the solubilising effect of bile salts and lipids within the fasted state intestinal media, where the extent of dissolution within FaSSIF-150% (0.5 mg: 84.8%, 2 mg: 86.4%) was greater in comparison to FaSSIF 50%, where the total percentage release of carvedilol 0.5 mg and 2 mg ODMT was limited to 80.0 and 79.0% respectively. Nonetheless, the total carvedilol percentage release was much lower (p value$<$ 0.0001) compared to adult and paediatric FaSSGF, FeSSGF and FeSSIF due to the higher pH (6.5), with an average total percentage release of 84.6% (0.5 mg) and 84.0% (2mg) after 180 minutes across FaSSIF-V2, FaSSIF 50% and FaSSIF 150%.

Adult and paediatric FeSSIF and FeSSGF both exhibited similar pH values (5.8 and 5.7). Hence, it was expected to observe similar dissolution trends. Although, the rate of dissolution was similar between paediatric biorelevant media, the rate and total percentage carvedilol release in FeSSIF-V2 was greater relative to adult FeSSGF. This may be due to the

composition of FeSSIF-V2, where the addition of glyceryl monooleate and sodium oleate may have enhanced the solubility and dissolution of carvedilol (277). Similar differences were observed between adult and paediatric FeSSIF, where both the 0.5 mg and 2 mg ODMT displayed faster dissolution rate and overall a greater extent of percentage carvedilol release in FeSSIF-V2. Glyceryl monooleate (GMO) is a glyceryl ester of fatty acids, typically used as an emulsifying agent within the pharmaceutical industry. In addition to its emulsifying properties, GMO when administered alongside bile salts may increase the effect of bile salt function by forming a mixed micellar form (278).

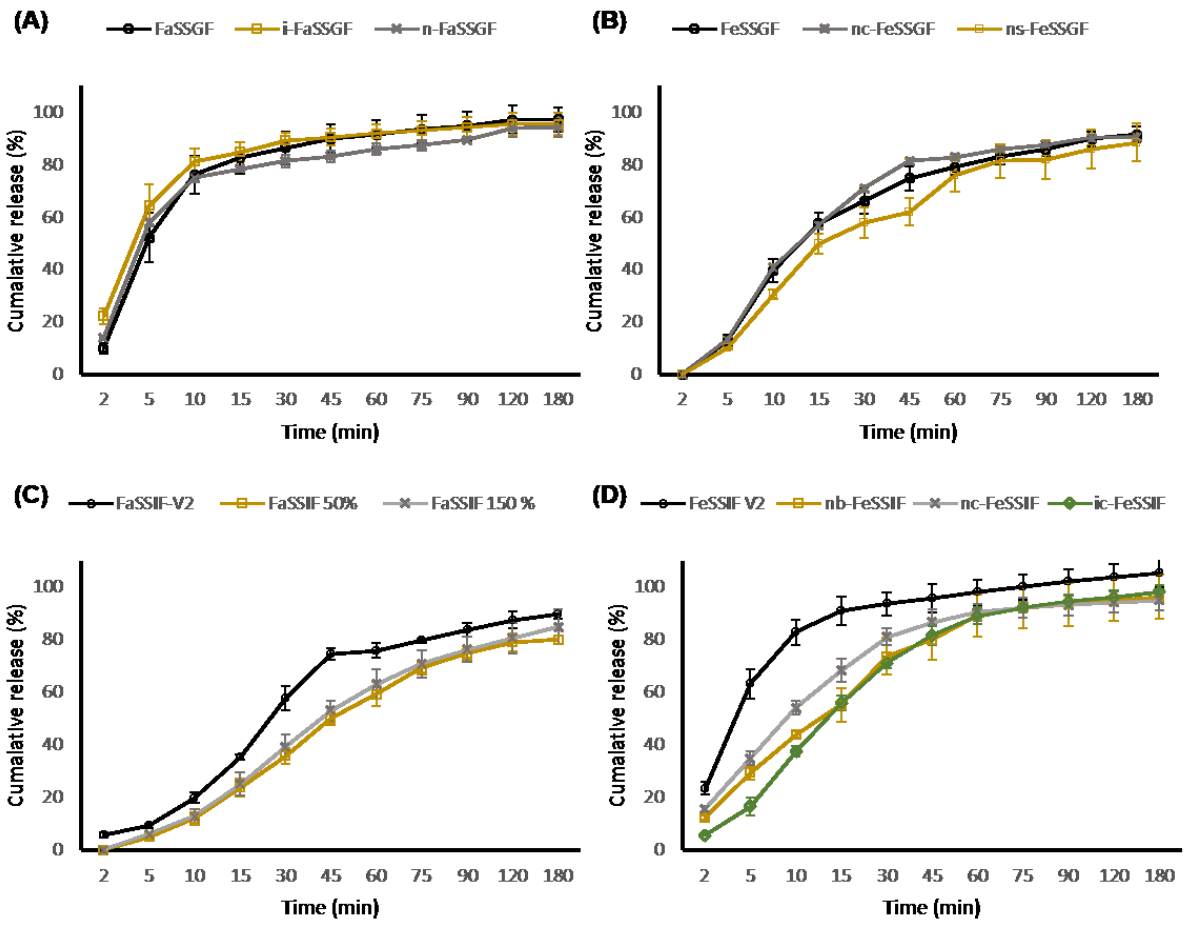


Figure 4: Dissolution profile of carvedilol 0.5 mg ODMT in adult and paediatric biorelevant media. Data presented as mean ± standard deviation (n=6). FaSSGF = Adult Fasted- state Simulated Gastric Fluid, FeSSGF = Adult Fed- state Simulated Gastric Fluid, FaSSIF-V2 = Adult Fasted- state Simulated Intestinal Fluid, FeSSIF= Fed- state Simulated Intestinal Fluid, i = infant, n = neonate, c = cow based milk formula, s = soy based milk formula, b = breast fed.

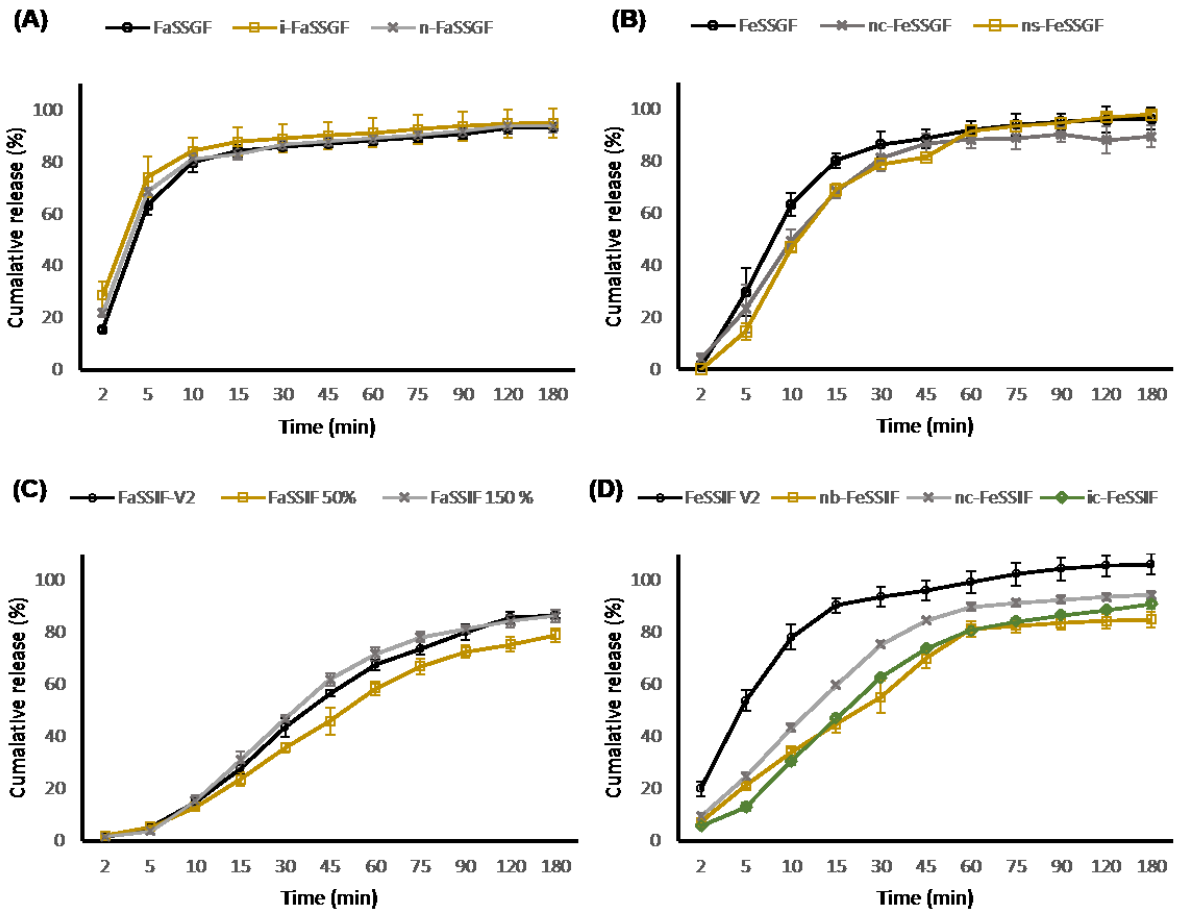


Figure 5: Dissolution profile of carvedilol 2 mg ODMT in adult and paediatric biorelevant media. Data presented as mean ± standard deviation (n=6). FaSSGF = Adult Fasted- state Simulated Gastric Fluid, FeSSGF = Adult Fed- state Simulated Gastric Fluid, FaSSIF-V2 = Adult Fasted- state Simulated Intestinal Fluid, FeSSIF= Fed- state Simulated Intestinal Fluid, i = infant, n = neonate, c = cow based milk formula, s = soy based milk formula, b = breast fed.

Figure 6 compares the *in vitro* drug performance of both the 0.5 mg and 2 mg ODMT formulations against a marketed carvedilol tablet (MAT). Since the dose of carvedilol in paediatrics is variable, the control (marketed carvedilol tablet) was selected as 3.125 mg (lowest available strength). Although not labelled as an ODT, the marketed tablet constitutes of povidone and crospovidone type A and B, all of which display disintegrating and dissolution and solubility enhancing properties. Crospovidone is a water insoluble superdisintegrant exhibiting high capillary action and marked hydration capacity (234). ODMT formulations displayed similar rates and extent of carvedilol release in all adult biorelevant media. The choice of excipients and concentrations utilised, alongside fine-tuned production parameters for the manufactured carvedilol ODMT ensured a balance between adequate mechanical strength and dissolution profile, comparable to marketed formulation without including

dedicated functional excipients such as superdisintegrants. Exclusion of such functional excipients decreases overall production costs and limits the total number of excipients within the formulation, thereby limiting the number of potential excipient related safety and toxicity concerns. Since data regarding excipient toxicity in paediatrics is limited, excipients must be kept to a minimum and avoided where possible. Tablet disintegration is a prerequisite for dissolution, where orodispersible formulations must disintegrate rapidly. However, the use of superdisintegrants in and as a model formulation constituent is not preferred since many are incompatible with various drugs compounds, are moisture sensitive (leading to instability) and lack toxicity data in both adults and paediatrics (234).

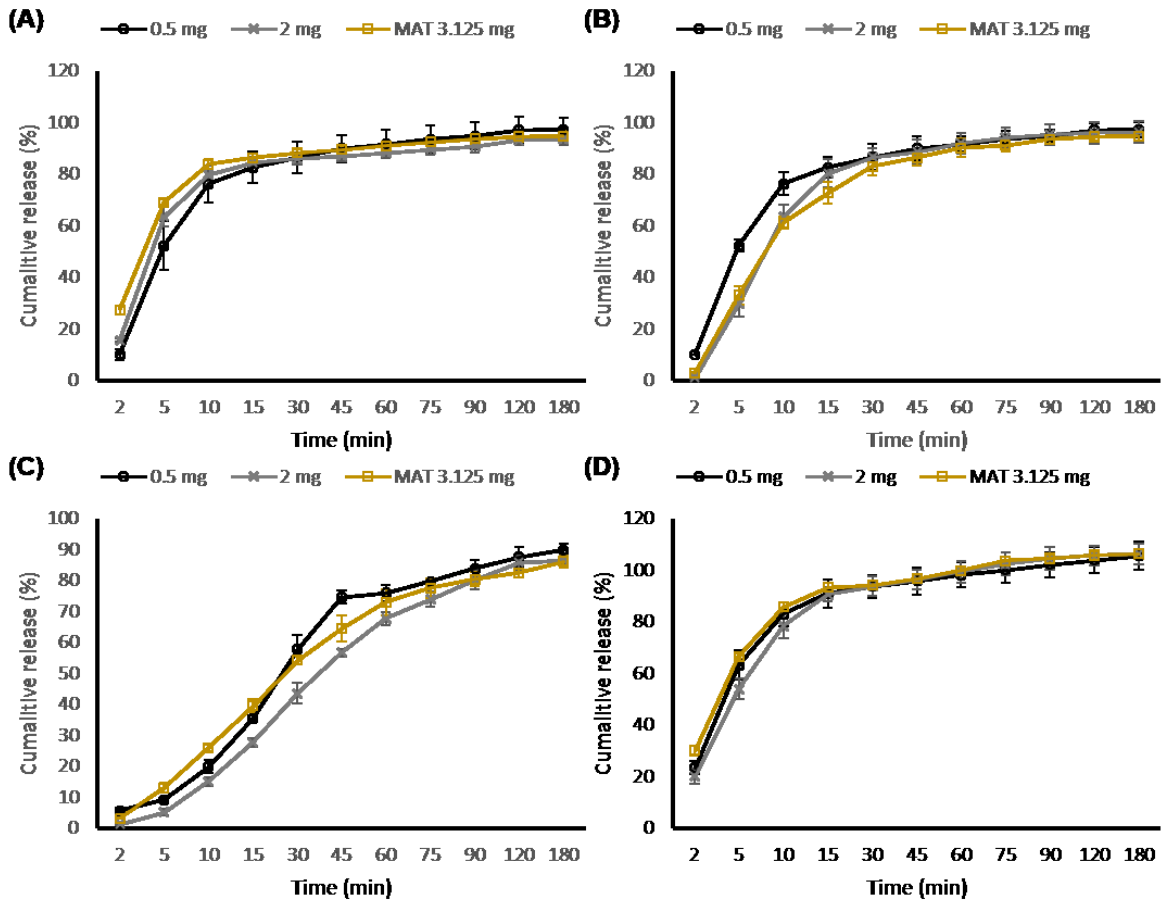


Figure 6: Dissolution profile comparison between carvedilol 0.5 mg ODMT, 2 mg ODMT and marketed 3.125 mg tablet in adult and paediatric biorelevant media. Data presented as mean ± standard deviation (n=6). MAT = Marketed adult tablet. A: FaSSGF, B: FeSSGF, C: FaSSIF-V2, D: FeSSIF-V2.

2.4.10 General discussion

Due to the chemical properties of carvedilol, solubility is dependent upon the specific biorelevant media characteristics. The range of physiological pH of different parts of the gastrointestinal (GI) tract may influence solubility and consequently affect dissolution rate and total extent of carvedilol release. Although the fasted state of the gastric media is ideal for rapid and complete dissolution, it has been advised that heart failure patients take their medication with food to avoid a sharp increase in plasma concentration and limit the risk of orthostatic hypotension (279). Total percentage release between the fasted and fed state gastric media in adult and paediatric were comparable; however, our data confirmed the impact of food on dissolution rate in fed state adult and paediatric biorelevant media. Due to regular feeding intervals, neonates and young infants are generally always in a continuous state of post prandial, thus, most likely would follow the dissolution trend as depicted for FeSSGF (Figure 4B, Figure 5B), where drug release is gradual, thereby restricting sudden increase in drug plasma concentrations and limiting the risk of observing adverse effects.

Other than co-administration of food, anatomical and physiological differences observed in the GI tract between adults and paediatrics may also affect drug solubility and dissolution. Key variations include gastric and intestinal capacity, pH, gastric emptying rate, intestinal transit time and bile salt secretion. As mentioned earlier, the Biopharmaceutics Classification System (BCS) classifies drugs into one of four categories depending on its solubility and permeability. However, the adult BCS must be used with caution when applied to paediatrics. This is because factors including upper GI lumen fluid volume, dose and total volume being administered alongside differ between adult and paediatric populations and may alter the defined solubility within the BCS (280).

Paediatric biorelevant media provided a better reflection of paediatric GI fluid composition in comparison to conventional USP/BP recommended dissolution media and displayed some significant dissolution differences against adult biorelevant media *in vitro*. However, to better predict paediatric *in vivo* drug performance, *in vitro* data should preferably be amalgamated with physiological based pharmacokinetic (PBPK) modelling and simulation that take into account factors influencing absorption and permeability such as gastric emptying rate, metabolising enzymes, active transport process and intestinal microflora composition (281, 282). However, paediatric GI physiology and factors affecting paediatric permeability (microbiota, transport systems and metabolising enzymes) are still not fully understood (280, 283). Bridging such gaps would certainly provide improved paediatric *in vivo* prediction tools, compiled with superior biorelevant media reflecting specific paediatric subset characteristics, which would be of great interest for the paediatric pharmaceutical industry, regulatory authorities and most importantly child safety.

2.4.11 Stability analysis

The stability and shelf life of drug products can be impacted by chemical degradation caused by moisture, air, and light. Carvedilol is relatively both thermally and photolytically stable. A forced degradation study carried out by Rizwan, M., et al. (2009) showed that exposure to moist heat or temperatures of 80 °C did not result in any decomposition of carvedilol (284). Similarly, the photochemical stability of carvedilol was confirmed by Stojanović, J., et al. (2007) who monitored the percentage of the degradation product 4-Hydroxycarbazole for up to 100 days (285). Although, carvedilol undergoes degradation under acidic and basic stress, this would not affect the stability of ODMTs in packaged conditions (284).

Over the 12 month course of the stability testing (long term) at 25°C and 60% RH, no significant changes were observed. The assay of both formulations remained within acceptable limits and within 5% of their initial values (Figure 7). Additionally, the formulations did not show any signs of physical tablet defects and no significant changes in hardness values were observed (results not shown), thereby showing adequate stability according to the ICH guidelines (286).

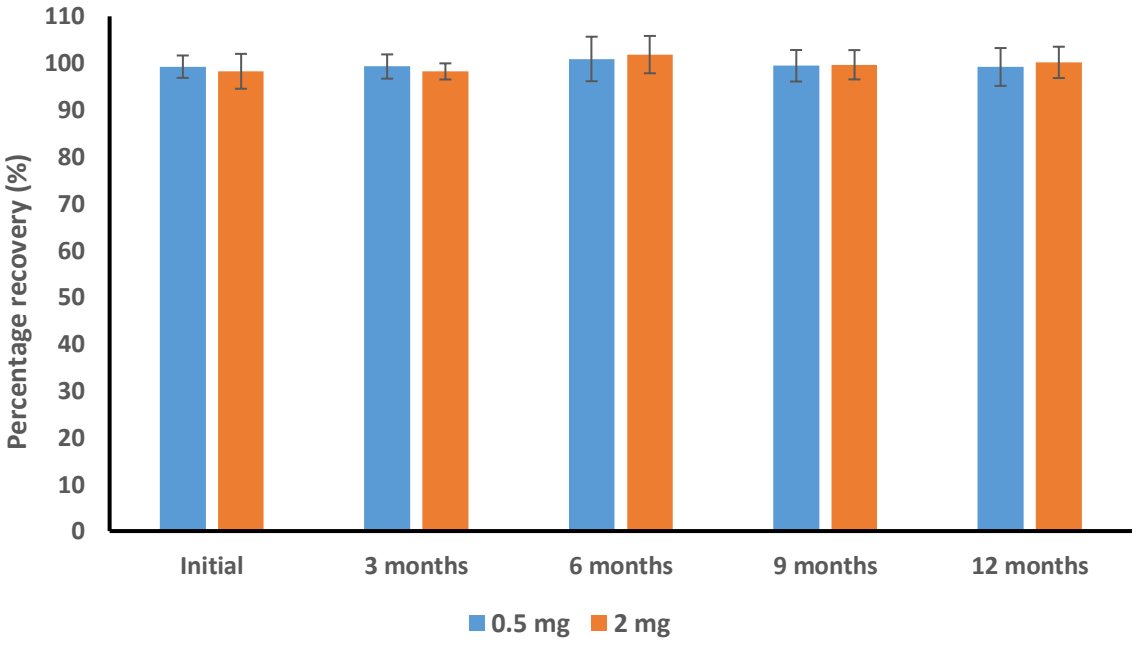


Figure 7: Percentage carvedilol recovery for long term stability. Results are generated from triplicate repeats (n=3) and error bars indicate standard deviation.

The criteria to determine any 'significant changes' are outlined by the ICH stability testing of new drug substances and products Q1A(R2) and are as follows:

1) A 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency when using biological or immunological procedures;

2) Any degradation product's exceeding its acceptance criterion;
3) Failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e.g., color, phase separation, resuspendibility, caking, hardness, dose delivery per actuation); however, some changes in physical attributes (e.g., softening of suppositories, melting of creams) may be expected under accelerated conditions;

and, as appropriate for the dosage form:

4) Failure to meet the acceptance criterion for pH; or
5) Failure to meet the acceptance criteria for dissolution for 12 dosage units.

2.5 Conclusion

Two strengths of carvedilol ODMT formulations were developed using an excipient composition and load that is appropriate for paediatric use. The novel ODMTs displayed all relevant features of an age-appropriate formulation including enhanced dose flexibility, palatability, excipient safety and concordance to development and ability for safe administration. Dissolution profiles observed were robust and comparable to the marketed formulation across various part of the GI tract in both the fed and fasted state. The systematic selection of drug candidates listed within the EMA Inventory of paediatric therapeutic needs, alongside paediatric specific formulation approach provides an encouraging starting point that ultimately aims to increase the availability of more age-appropriate formulations for children through PUMA applications. Lastly, further updated and more clinically relevant *in vitro* methodologies reflecting impact of age, paediatric hydrodynamics and method of administration between various paediatric subsets would prove to be beneficial for a more accurate in-vivo prediction.

Chapter 3: Development of an age-appropriate ethanol free furosemide oral solution

3.1 CHAPTER AIMS AND OBJECTIVES

- Develop a paediatric specific excipient 'working zone' for oral solutions
- Enhance furosemide solubility via cyclodextrins, pH adjustment and salt formation
- Develop two strengths (4 mg/mL and 8 mg/mL) of an age-appropriate ethanol free furosemide formulation that possess increased dose flexibility, palatability, safety, quality and efficacy
- Exploit excipient properties to limit the number of excipients within the formulations and omit potentially harmful excipients
- Develop a preservative free version of the formulation

3.2 Introduction

The National institute for health and care excellence (NICE) guidelines are evidence based recommendations that help manage disease and conditions. For the management of heart failure, NICE guidelines states the routine use of diuretics as the first line of treatment for the relief of congestive symptoms and fluid retention in people with heart failure (287). Furosemide is one of the most effective, least toxic and commonly used diuretic employed within the paediatric population (288, 289), primarily used to treat hypertension and oedema associated with heart failure. Furosemide inhibits the apical sodium/potassium/chloride transporter in the thick ascending limb of the loop of Henle, thereby enhancing water and electrolyte excretion from the body (290). Typical furosemide dose for neonates is 0.5 – 2 mg/kg every 12 – 24 hours, the same dose is given 2 – 3 times a day for children up to age of 11, and for children between 12 and 17 years of age a daily dose of 20 – 40 mg is specified (291).

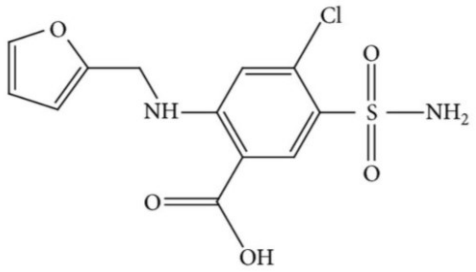
Ethanol is a commonly used solvent with some preservative action that is utilised as a vehicle for lipophilic compounds in oral liquid formulations. Within the adult population, ethanol is generally regarded as safe; however, in paediatrics, the use of ethanol is associated with some serious acute and chronic adverse effects (110). Children experience rapid growth and maturation, leading to changes in pharmacokinetic profiles; such changes may potentially affect the toxicity of compounds under significant influence of pharmacokinetic processes (111). Accidental overdose may lead to acute intoxication and poisoning resulting in CNS depression, hypoglycaemia, hypothermia and coma.

The challenge to characterise the effect of short and long term use of ethanol in paediatrics still remains, whilst establishment of appropriate intake values warrants more clinical evidence (111). Until then, the EMA have proposed that, following a single dose, blood ethanol levels

in children under six years should not exceed 1mg/100mL and 12.5mg/100mL in children aged between 6 – 12 years (112). The US Food and Drug Administration (FDA) have also restricted the ethanol content of all oral products intended for paediatrics to ≤0.5%, 5% and 10% for children <6 years, children 6-12 and children >12 years, respectively (292). At present, available medicinal forms of furosemide include traditional tablet and oral solution. However, the concentrations of ethanol in all authorised marketed furosemide solutions do not act in accordance with paediatric regulatory bodies and are well above the paediatric threshold values and therefore cannot be recommended. The ethanol concentration in the following marketed formulations is as follows, 7.9 %, 9.8 % and 11.9 % w/v Frusol 20mg/5ml (Rosemont Pharmaceuticals Ltd; registered in the UK), Impugan 10 mg/mL (Actavis Group hf.; registered in Sweden) and LasixR liquid 10 mg/mL (Sanofi-Aventis Deutschland GmbH, Germany), respectively (293-295).

Furosemide is a biopharmaceutical class (BCS) IV drug with a pKa value of 3.9 and an aqueous solubility of 18.25 µg/mL (Table 19) (296). In order to solubilise the desired concentration of drug, and with an aim to avoid toxic or 'problematic' excipient, alternative solubilising methods are called for. Such solubilising techniques include particle size reduction, solid dispersions, pH adjustment, salt formation and/or inclusion of cyclodextrins, surfactants and/or co-solvents. The undesirable and bitter taste of furosemide also warrants for taste masking techniques in order to enhance palatability (297). Taste masking techniques include functional (sweeteners, bitter blockers), physical (polymer coating) and biochemical (chemical modifiers, ion-exchange resins) masking (192). Cyclodextrins are a family of cyclic oligosaccharides, consisting of a hydrophobic inner cavity and a hydrophilic outer surface (Figure 8) (139). They are naturally derived from starch and are often used for delivery of poorly soluble and unpalatable pharmacological compounds. Owing to low toxicity profiles, cyclodextrins, serve as a potential alternative to problematic solubilising agents such as propylene glycol and ethanol (140). Nevertheless, at high doses (> 1000 mg/kg/day), cyclodextrins may cause cecum enlargement and digestive problems such as diarrhoea (141). The lowest observed effect levels in cyclodextrins clinical studies has led to the conclusion of a maximum cyclodextrins threshold of 200 mg/kg/day, when administered orally (141).

Table 19: Overview of the physicochemical properties of furosemide.

Furosemide Monograph	
Chemical Name	sodium;4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoate
Other Names	Sodium furosemide
Molecular Formula	$C_{12}H_{10}ClN_2NaO_5S$
Molecular Weight	352.7
logP	2.3
pKa	3.9
CAS Number	41733-55-5
Furosemide Structure	
Appearance	White or slightly yellow, solid powder or crystals
Melting point	220 °C
Solubility	Slightly soluble in chloroform, ether. Soluble in acetone, methanol, DMF, aqueous solutions above pH 8.0. Less soluble in ethanol
Sensitivities	Oxidation, solutions of acidic pH and light

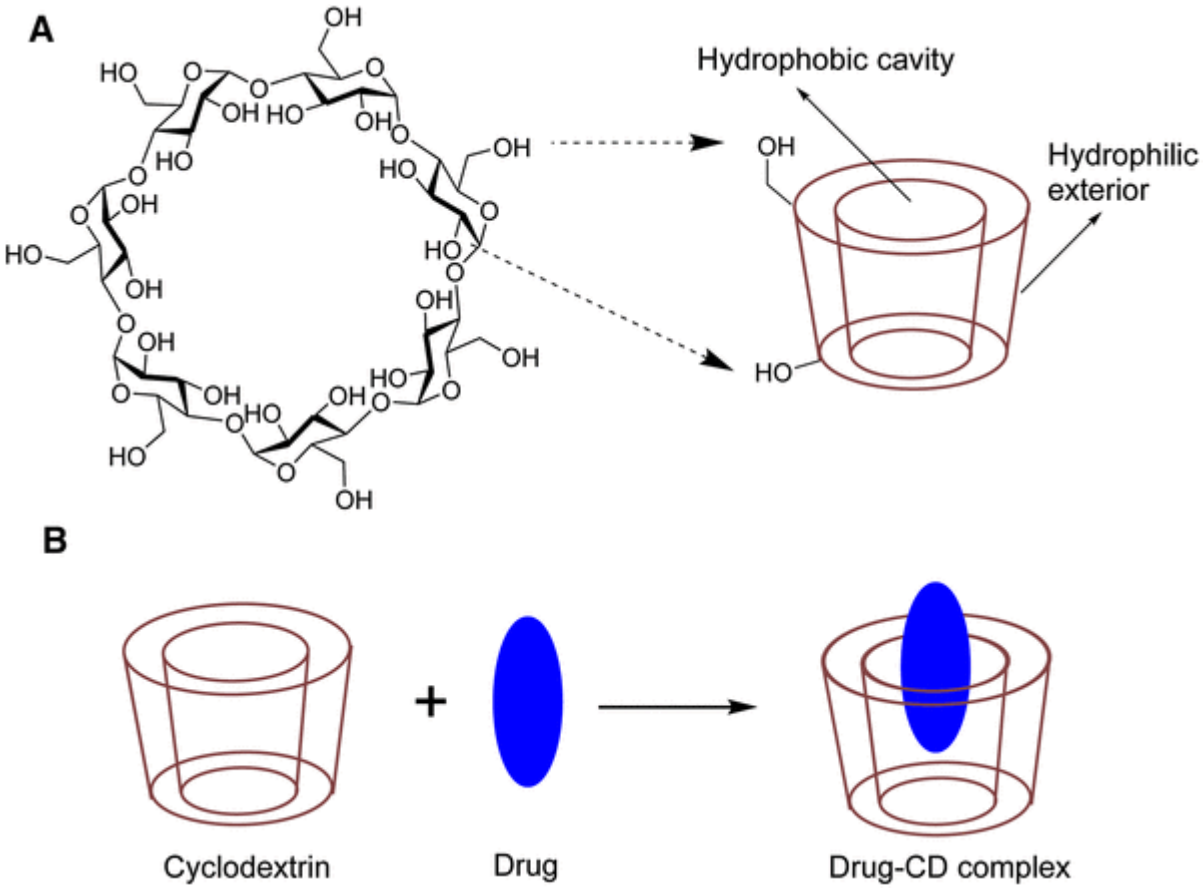


Figure 8: Figure from Qiu, N., et al. (2017) – diagram of cyclodextrins structure and their ability to form inclusion complexes(298).

The pH of an aqueous system can affect the solubility of the drug compound. As pH scales are logarithmic, their inverse indicates the concentration of hydrogen ions in a solution. As the pH of a solution is decreased, ionic compounds containing basic anions become more soluble as protons present will associate with the basic anion thus effectively lowering their concentration (299). In liquid formulations, the pH can readily be adjusted to a certain pH level at which the solute is ionised to a degree that affords solvation of the drug compound (300). Regarding furosemide, a weak acid with a carboxylic acid functional group, its aqueous solubility increases as a function of pH, where solubility increases as pH increases (301).

This study aims to develop two strengths of an ethanol free furosemide solution using alternative methods for enhancing solubilisation, eliminating the need for 'problematic' and potentially toxic excipients. A systematic bottom up approach with careful considerations given to paediatrics in regards to excipient safety, palatability and acceptability was ensured.

3.3 Material and Methods

3.3.1 Materials

Pharmaceutical standard Furosemide ≥98%, (2-Hydroxypropyl)-β-cyclodextrin (HP-β-CD), Sodium hydroxide, Xylitol, Methyl paraben, and Disodium hydrogen phosphate were purchased from Sigma-Aldrich (Dorset, UK). Citric acid was obtained from Alfa Aesar (Lancashire, UK), whilst strawberry flavour was gifted by Azelis (Antwerp, Belgium).

Methanol (HPLC-grade) and acetonitrile (ACN) for sample dilution and analysis was obtained from Fisher Scientific (London, UK).

3.3.2 HPLC Analytical Method Development

All samples were analysed on an Agilent 1260 series (Agilent Technologies, Santa Clara, CA, USA), equipped with a reverse-phased Kinetex 5 µm C8 100 A LC Column, 150 x 4.6mm (Phenomenex, Macclesfield, UK). Separation of furosemide was achieved using an isocratic mobile phase compromising of ACN and water, adjusted to pH 3.3 (70:30 v/v). Flow rate was set at 1.0 mL/min, injection volume was 10 µL and a wavelength of 233 nm was used for detection. An 8-point calibration curve ranging from 0.78 – 100 µg/mL was exercised and all samples prepared for analysis were diluted so as to fall within the calibration range. Method validation was carried out following The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidelines (Q2 (R1)) (302).

3.3.3 Dose banding and proposed strengths

The British national formulary for children (BNFc) was referred to determine paediatric furosemide dose (291). For each age, the dose range was determined based on average child weights and strengths were proposed accordingly so that strengths provide adequate flexibility whilst using acceptable dosing volumes.

3.3.4 Child friendly excipient working zone development and selection of excipients

A child friendly excipient working zone was developed. Firstly, an excipient inclusion criteria was developed to meet the regulatory guidelines for paediatric medicines and excipient safety. Inclusion criteria included excipients to be affirmed as GRAS (generally recognized as safe) or approved as an “additive” by the European Commission and/or FDA. Potentially problematic excipients were identified through literature and the Safety and Toxicity of Excipients for Paediatrics (STEP) database was employed to identify maximum excipient daily allowance of such excipients. Further, published guidance provided by the EMA in regards to excipient use in paediatric pharmaceutical development were followed to ensure safety and regularity compliance(164). Only excipients included within the ‘working zone’ were utilised;

excipient selection was based on safety profiles and functionalities to produce a stable and effective oral solution.

3.3.5 Drug – Excipient compatibility using differential scanning calorimetry (DSC)

Drug and excipient compatibilities were studied using a TA DSCQ200 apparatus (TA Instruments, New Castle, DE, USA) with TA Instruments Universal Analysis 2000 software. Data was collected under nitrogen atmosphere (flow rate: 50 mL min^{-1}) using sealed flat-bottomed TZero aluminium pans and pierced lids (sample mass 1–2 mg). A heating rate of 10 °C min^{-1} in the range of 50 to 400 °C was employed. For samples containing both the drug and excipient, 1 mg of each was measured in a weighing boat and gently mixed with a spatula for one minute before being transferred into the sample pan. All studies were conducted in triplicate and data are presented as mean ± standard deviation.

3.3.6 Furosemide solubility enhancement using cyclodextrins

3.3.6.1 Cyclodextrins Phase Solubility Studies

Phase solubility studies were carried out according to the method described by Higuchi and Connors (303). Briefly, solutions of increasing concentration of HP-β-CD (0-30 mM) were prepared in distilled water. Excess furosemide was then added to each solution and allowed to stir for a total of 48 hours at a temperature of 25°C, after which equilibrium was reached. Samples were filtered through a 45 µm filter, appropriately diluted and analysed via HPLC.

3.3.6.2 Effect of pH on furosemide and HP-β-CD complexation

The effect of pH on drug complexation was evaluated. As described above, the phase solubility was determine according to the method reported by Higuchi and Connors (303); however, the pH was adjusted once excess drug was added to the increasing concentrations of HP-β-CD solutions. The pH values explored were 4, 6.5 and 7.5 respectively. The pH range was selected based on the target pH of the final formulation, as well as to compare the effect of both acidic and alkaline environments on complexation.

3.3.6.3 Determination of Furosemide – HP-β-CD apparent stability constant (Kc) and complexation efficiency (CE)

The binding strength of furosemide/ HP-β-CD was calculated in terms of apparent stability constant (Kc) using the following equation (Equation (8)):

$$Kc = \text{slope} / S_o \times (1 - \text{slope})$$

Where S_o is the intrinsic aqueous solubility of furosemide and slope is the slope of phase solubility diagram.

The ratio of complexation to free HP-β-CD concentration, also known and complexation efficiency (CE) describes the solubilising effect of HP-β-CD. The CE was calculated using the following equation (Equation (9)):

CE=slope / (1−slope)

3.3.7 Furosemide solubility enhancement via pH adjustment

3.3.7.1 Saturation solubility and percentage ionisation

The saturation solubility of furosemide at various pH values was determined. Here, an excess amount of furosemide was added to a set volume of distilled water in a screw cap glass vial. The pH was then adjusted using either 0.1 M hydrochloric acid (HCl) or 0.1 M sodium hydroxide (NaOH) to pH values ranging from 5-9 and allowed to stir until equilibrium was established (24 h). Samples were withdrawn after 24 h, filtered through a 0.45-µm syringe filter, and analysed via HPLC. Saturation solubility at each respective pH level was carried out in triplicates and presented as mean ± standard deviation. Percentage ionisation was calculated using the following equation:

Eq. 7: $\%Ionisation = \frac{1}{1+10^{(pH-pKa)}}\ X\ 100$

Where pH denotes the pH value of the solution, whilst pKa is the negative log of the acid dissociation constant of furosemide.

3.3.8 Formulation development

Formulations were prepared using ultra-pure water and volumetric glassware. Excipients were weighed out and dissolved in a defined amount of water using a magnetic stirrer. A controlled temperature of 25°C was used to aid solubilisation. Once dissolved, furosemide was then added gradually while adjusting the pH simultaneously to ensure adequate ionisation to fully solubilise furosemide. Upon dissolving furosemide into the excipient solution, formulations were then made up to volume and transferred into amber glass bottles.

3.3.9 Preservative efficacy (PET) test

Shortlisted formulations were put through PET to assess the ability of the preserving system, and is required by pharmacopeia standards. The challenge test procedure was similar to that described in United States Pharmacopeia (USP) <51> (304). In summary, the formulation was challenged by simulating contamination through a series of inoculations containing microorganisms.

3.3.9.1 Preparation of inoculum

Specified microorganisms (Escherichia coli, Pseudomonas aeruginosa, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus niger* and *Zygosaccharomyces rouxii*) were inoculated at the surface of a suitable volume of solid agar medium. The inoculum culture conditions for each microorganism are described in table 2 of (USP) <51> (304). Cultures were harvested using sterile saline to obtain a microbial count of about 1×10^8 colony-forming units (cfu) per mL.

3.3.9.2 Procedure

Shortlisted formulations (category 3 product) was separated out into 6 different containers, where each one was challenged with one of the six challenge microorganism such that the final concentration of the test preparation after inoculation was between 1×10^5 and 1×10^6 cfu per mL of the product. The plate count method was employed to determine the concentration of each standard inoculum. The standard concentrations were used to estimate the initial concentration of each microorganism in the test preparation. Inoculated test containers were incubated at 22.5 ± 2.5°C for an interval of no less than 28 days.

During the 28 day incubation period, the product was evaluated at specific time intervals (14 days and 28 days for category 3 products). At day 14 and 28, the number of cfu present in each test preparation was determined using the plate-count technique and any changes observed in appearance during this period were noted. At each sampling interval, each inoculated preparation was neutralised and plated using appropriate dilution and plating methods.

Following the respective microbial recovery incubation periods (Table 2 of (USP) <51>), surviving microorganism were counted and the change was reported as log reduction. The log reduction of microorganisms at prescribed time intervals (14 and 28 days) were determined to quantitatively evaluate the antimicrobial effectiveness of the formulation to inhibit microbial proliferation. Passing criteria for category 3 products can be found in Table 3 of (USP) <51> (304).

3.3.10 Stability testing

Formulations were evaluated for stability at 25°C and 60% RH using a Binder KBF 115 stability chamber (Tuttlingen, Germany) for a period of six months. Stability studies included physical appearance (including colour and precipitation), pH and percentage recovery.

3.4 Results and Discussion

The challenges identified during formulation development included solubility enhancement, sufficient antimicrobial preservative efficacy, palatability, age-appropriateness and stability. A bottom up paediatric specific formulation development approach coupled with application of principal pharmaceutical design resolved inherent drug incompatibles and formulation challenges using alternative and/or 'safer' formulation techniques and procedures. This included the use of a polyol/citric acid preserving system to produce a preservative free oral solutions and enhancing solubility and replacing ethanol through pH adjustment and/or inclusion of cyclodextrins. This ensured that appropriate excipient compositions and loads were present within the final dosage form. Further, the excipient screening tool (mentioned above in carvedilol chapter) and a bottom-up paediatric specific formulation development approach also aims to exploit excipients properties to provide multiple functionalities thereby limiting the total number of excipients and removing toxic excipients along the way.

3.4.1 Dose banding and proposed strengths

The paediatric dose of furosemide for neonates is 0.5–2 mg/kg every 12–24 hours, 0.5–2 mg/kg 2–3 times a day for child between 1 month-11 years and 20–40 mg daily for child 12-17 years (291). Owing to the high variability and range of potential doses, two strengths (4 mg/mL and 8 mg/mL) were elected to deliver across the paediatric population whilst maintaining acceptable dosing volumes (Table 20). The deduced strengths are also in agreement with strengths of current market furosemide oral liquid preparations.

Table 20: Proposed strengths based on child weights and dosing requirements. 2 strengths have been provided to provide greater flexibility and keep dosing volumes as low as possible.

Age	Weight (Kg)	Dose range (mg)	Required volume (mL)	Proposed strength
Neonate (up to 28 days)	3.5	1.8 - 7	0.5 - 2	4 mg/mL
1 mth	4.3	2.2 - 8.6	0.5 - 2	
2 mths	5.4	2.7 - 10.8	0.7 - 2.5	
3 mths	6.1	3.1 - 12.2	0.8 - 3	
4 mths	6.7	3.4 - 13.4	0.9 - 3.5	
6 mths	7.6	3.8 - 15.2	1 - 4	
1 yr	9	4.5 - 18	1 - 4.5	
3 yrs	14	7 - 28	1 – 3.5	8 mg/mL
5 yrs	18	9 - 36	1 – 4.5	
7 yrs	23	11.5-46	1.5 - 6	
10 yrs	32	16-64	2 - 8	

3.4.2 Child friendly excipient working zone development and selection of excipients

Although oral liquid formulations of furosemide are available, they are not particularly child-friendly. Current marketed oral liquid products of furosemide (aimed at paediatric patients) contain potentially harmful excipients (namely alcohol and parabens) (Table 21). Parabens (methyl-, ethyl- and propyl-hydroxybenzoates) are often used in pharmaceutical formulations as antimicrobial preservatives, where they are effective against a broad spectrum of bacteria, over a wide pH range (134). However, when used in humans, such antimicrobial properties are harmful to living cells and may therefore be associated with certain risks (135). The EMA have concluded that, if possible, inclusion of preservatives and antioxidant in paediatric formulations should be avoided and their use should be justified (135). Parabens also demonstrate oestrogen binding affinities, suggesting oestrogenic activity with potential reproductive effects (136). Furthermore, possible endocrine-disrupting effects have also raised concerns for paraben use in paediatric formulations (137). Nonetheless, risk assessments have led to the establishment of an acceptable daily intake of 0-10 mg/kg body weight for the total sum of methylparaben, ethylparaben and propylparaben (138).

Table 21: Overview of current marketed oral liquid preparations of furosemide including excipient composition and ethanol content.

Marketed solution	List of excipients	Ethanol content (%v/v)
Frusol 20mg/5ml, 40mg/5ml, and 50mg/5ml oral solution (Rosemont Pharmaceuticals Ltd) Marketed: UK	Ethanol, sodium hydroxide, cherry flavour (containing ethanol and propylene glycol (E1520)), liquid maltitol (E965), disodium hydrogen phosphate (E339), citric acid monohydrate (E330) and purified water	7.9% (397.28 mg/5ml) - Above thresholds (293)
Furosemide 4mg/ml, 8mg/ml and 10mg/ml Oral Solution (Thame Laboratories) Marketed: UK	Citric acid monohydrate (E330), Ethanol, Sodium hydroxide (E524), Liquid maltitol (E965), Cherry flavour [containing propylene glycol (E1520)], Disodium phosphate, anhydrous (E339), Purified water	8.3% (416 mg/5ml) - Above thresholds (305)
Impugan 10 mg/mL oral drops Marketed: Sweden	Saccharin sodium (sweetener), sodium hydroxide, ethanol (alcohol content 9.8%) and water	9.8 % (500 mg/5ml) - Above thresholds (294)
LasixR liquid 10 mg/mL Marketed: Germany	Purified water, ethanol, sorbitol solution 70% (non-crystallizing) (Ph.Eur.), Glycerol 85%, sodium hydroxide, quinoline yellow (E 104), yellow orange S (E 110), orange flavor, methyl (4-hydroxybenzoate) and propyl (4-hydroxybenzoate) (parabens) as preservatives.	11.9% (595 mg/5ml) - Above thresholds (295)

The development of oral liquid preparations require several excipients including antimicrobial preservatives, antioxidants, buffering systems, solvents and flavours that provide specific functionalities. However, careful consideration should be given when electing excipients, ensuring a selection/inclusion of paediatric compliant excipients. Where identified, potentially harmful excipients may be used if the benefits outweigh the risk; however, such excipient use must be within established allowable daily allowances (ADI). An excipient working zone for liquid dosage forms (in particular, furosemide oral solution) is compiled in Table 22.

Table 22: Excipient 'working zone' for liquid dosage forms. The excipient working zone is not exhaustive but provides an overview of functional category, advantage of excipient use and max ADI of commonly used excipients in oral solutions (in particular, for the successful development of an ethanol free furosemide oral solution).

<table>
<tr><th>Excipient</th><th>Functional category</th><th>Comments</th><th>Max ADI</th></tr>
<tr><td colspan="4">
<table>
<tr><th>Preservative</th><th>Concentration (% w/w)</th><th>pH working range</th><th>Max ADI</th></tr>
<tr><td>Methyl-4-hydroxybenzoate</td><td>0.05 – 0.1</td><td>1 – 8.5</td><td>5 mg/kg/day - 0-10 mg/kg body weight for the total sum of methylparaben, ethylparaben and propylparaben</td></tr>
<tr><td>Propyl-4-hydroxybenzoate</td><td>0.05 – 0.1</td><td>1 – 8.5</td><td>5 mg/kg/day</td></tr>
<tr><td>Sodium benzoate</td><td>0.01 – 0.2</td><td><5</td><td>benzoic acid and its salts has been established to 0–5 mg/kg bw/day</td></tr>
<tr><td>Potassium sorbate</td><td>0.14</td><td>3.5-5.5</td><td>Sorbic acid and its salts are 3 mg/kg bw/day</td></tr>
<tr><td>Propylene glycol</td><td>15–30</td><td>1-14</td><td>50 mg/kg/day in children less than 5 years old, and 1 mg/kg/day</td></tr>
</table>
</td></tr>
<tr><td>Cyclodextrins</td><td>Solubilizing agent; stabilizing agent.</td><td>Aqueous solubility of unsubstituted natural cyclodextrins including αCD, βCD, and γCD, and their complexes is limited, hence the more soluble derivatives such as 2-hydroxypropyl-βCD (HPβCD) are favoured</td><td>children under the age of 2 years treated with up to 200 mg HP-β-CD/kg/day for 2 weeks were well tolerated and considered safe</td></tr>
<tr><td>Xylitol (dual function)</td><td>Non-cariogenic sweetening agent; preservative</td><td>Lower water activity and a higher osmotic pressure than sucrose, therefore enhancing product stability and freshness. Demonstrated to exert certain specific bacteriostatic and bactericidal effects</td><td>20 g/day</td></tr>
<tr><td>Sucralose</td><td>Sweetening agent.</td><td>Sweetening power approximately 300–1000 times that of sucrose and has no aftertaste. It has no nutritional value, is non-cariogenic, does not promote dental caries, and produces no glycaemic response. Used in concentrations of 0.03–0.24% w/w.</td><td>0 to 15 mg/kg bw</td></tr>
<tr><td>Citric Acid (dual function)</td><td>Acidifying agent; antioxidant; buffering agent; chelating agent;</td><td>
<table>
<tr><th>Function</th><th>Concentration (% w/w)</th></tr>
<tr><td>Buffer solutions</td><td>0.1–2.0</td></tr>
</table>
</td><td>No recommended daily allowance</td></tr>
</table>

	flavour enhancer; preservative.	Flavour enhancer	0.3–2.0	has been specified
		Sequestering agent	0.3–2.0	
		Antioxidant/Preservative	0.2-1.0	
Sodium Phosphate	Buffering agent; sequestering agent.	level of dibasic sodium phosphate used as an excipient in a pharmaceutical formulation is not usually associated with adverse effects		40 mg/kg bw per day
Propylene glycol	Antimicrobial preservative; disinfectant; humectant; plasticizer; solvent; stabilizing agent; water-miscible co-solvent.	Oral solutions – 10-25% w/w		Neonates: 1 mg/kg Children aged 1 month–4 years: 50 mg/kg Children aged 5–17 years: 500mg/kg
Xanthan gum	Gelling agent; stabilizing agent; suspending agent; sustained-release agent; viscosity increasing agent.	Although primarily used as a suspending agent, xanthan gum has also been used to prepare sustained-release matrix tablets.		0 to 10 mg/kg bw
Polysorbates	Dispersing agent; emulsifying agent; non-ionic surfactant; solubilizing agent; suspending agent; wetting agent.	They have been found to be useful in improving the oral bioavailability of drug molecules that are substrates for P-glycoprotein		Group ADI of polysorbates to 10 mg/kg body weight/day

3.4.3 Solubility enhancement via cyclodextrins complexation and pH adjustment

3.4.3.1 Cyclodextrins Phase Solubility Studies

Cyclodextrins are mainly employed as complexing agents to enhance bioavailability by increasing drug solubility. Besides enhancing drug solubility, inclusion complexes can mask bitter tastes caused by active pharmaceutical ingredients and prevent drug degradation. The aqueous solubility of unsubstituted natural cyclodextrins including αCD, βCD, and γCD, and their complexes is limited, hence the more soluble derivatives such as 2-hydroxypropyl-βCD (HPβCD) are favoured (306). As with many excipients, cyclodextrins safety and toxicity data within the paediatric population is deficient. Nonetheless, the oral availability of cyclodextrins is extremely low, and although few, paediatric data has shown children under the age of 2 years treated with up to 200 mg HP-β-CD/kg/day for 2 weeks were well tolerated and considered safe (141).

The amount of cyclodextrins required to solubilise the drug dose within the aqueous vehicle was determined using phase solubility studies. It is important to note that an excess is usually accounted for in order to prevent drug precipitation upon storage and formulation usage (306). Phase-solubility analysis is a conventional approach to determine the stability constant value and also provide useful information of the stoichiometry of the equilibrium.

At pH 4, the solubilisation of furosemide using HP-β-CD increased with increasing concentrations of HP-β-CD (Figure 9). Although an overall positive trend was observed, the data was not linear and in practical terms the amount of furosemide being solubilised with increasing concentrations of HP-β-CD was negligible. This suggests that inclusion complexes were not being formed. A physical visual observation after 48 hours also displayed a cloudy solution suggesting the lack of complexation. Similarly, at pH 6.5 and 7.5, the data does not present a linear trend and the increase in solubility was rather due to the increase in pH values (Figure 10, Figure 11). As the pH value increases, there is a progressive increase in drug ionised form (Table 23) and therefore the affinity of furosemide for the hydrophobic HP-β-CD decreases.

The equilibrium constant is often calculated and provides an indication of strength of the interaction between drug and cyclodextrins, whilst it is also useful for the assessment of changes in physicochemical and biopharmaceutical properties of the guest drug molecule included within the CD cavity (307). However, the equilibrium constant ($K_{1:1}$) is extremely sensitive to the intrinsic solubility of the drug and can therefore lead to incorrect values, especially for low solubility drugs, as in the case of furosemide. Consequently, determination of complex efficiency (CE) usually provides a more accurate representation. The complex efficiency of furosemide and HP-β-CD at pH 4, 6.5 and 7.5 were determined to be 0.102, 0.0058 and 0.0026 respectively. These values are extremely low and suggest the lack of complexation. Additionally, the progressive decrease in complexation efficiency as the pH increases confirms the effect of drug ionisation on binding affinity, where ionised drug binding affinity for the HP-β-CD decreases as the drug becomes more ionised. It has been reported that an increase in intrinsic solubility results in enhanced complexation; however, although this may be true, we decided to discontinue the use of cyclodextrins since pH adjustment alone proved enough to solubilise our intended dose (8 mg/mL), whilst no positive effect of HP-β-CD on furosemide solubility was observed (306). As with any paediatric formulation, a minimum excipient load and composition is preferred, hence cyclodextrins use was discontinued and solubility was enhanced using a buffered pH system.

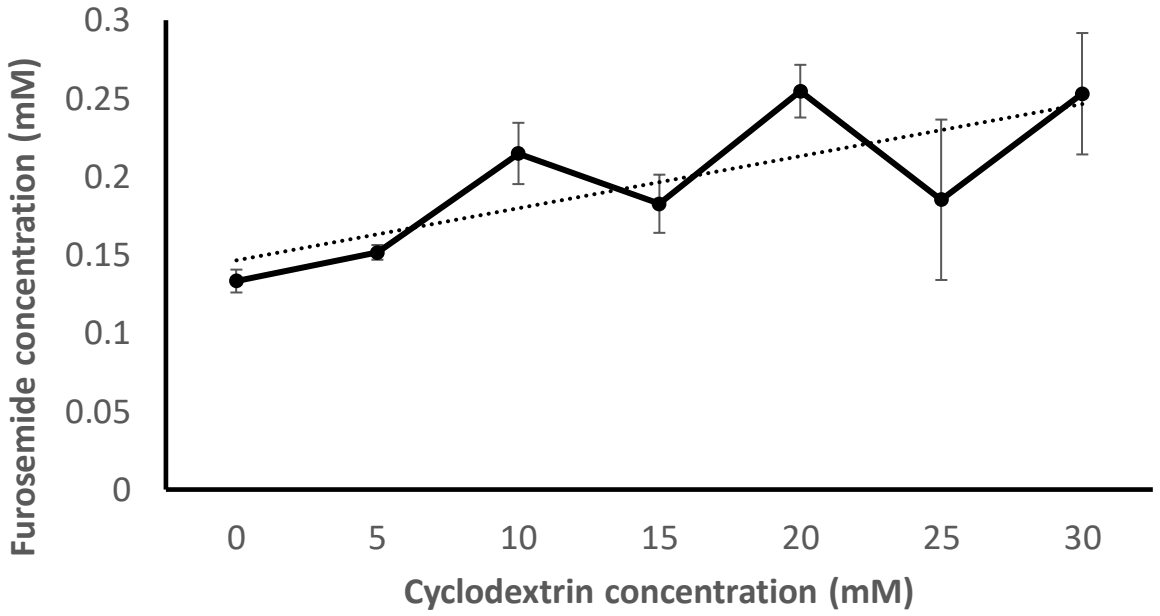


Figure 9: Phase solubility profile for Furosemide complexed with HP-β-CD at pH 4. Increasing concentrations of HP-β-CD were prepared from 0-30 mM with excess furosemide added to each solution to assess the effect of complexation and solubility as function of HP-β-CD concentration. Results are presented as the average (n=3) and error bars indicate standard deviation. Samples were filtered, diluted and assayed using HPLC.

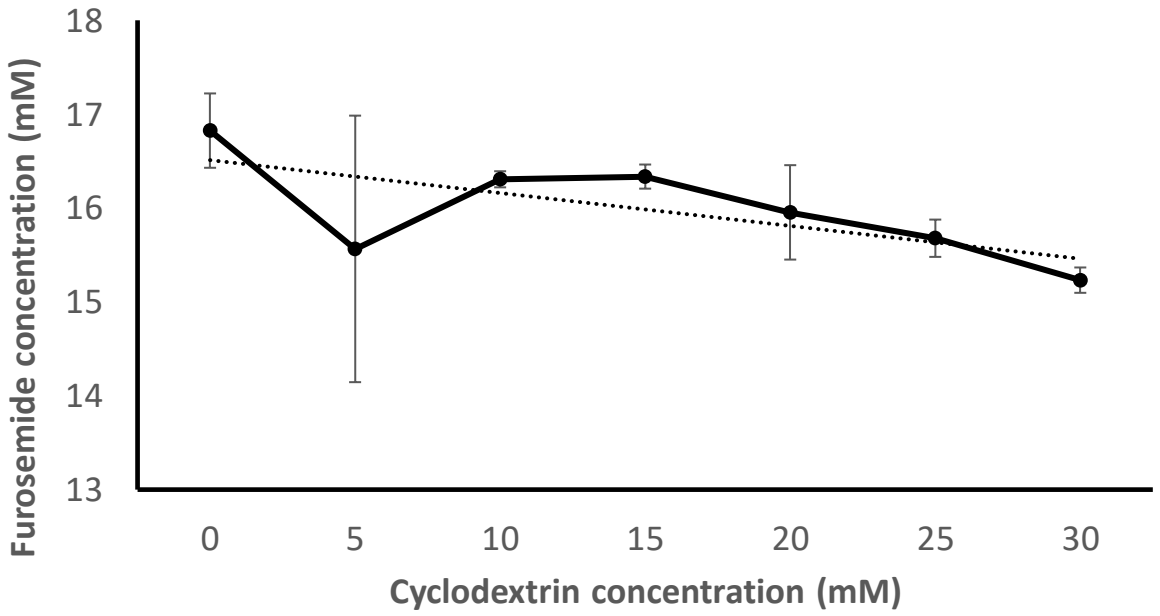


Figure 10: Phase solubility profile for Furosemide complexed with HP-β-CD at pH 6.5. Increasing concentrations of HP-β-CD were prepared from 0-30 mM with excess furosemide added to each solution to assess the effect of complexation and solubility as function of HP-

β-CD concentration. Results are presented as the average (n=3) and error bars indicate standard deviation. Samples were filtered, diluted and assayed using HPLC.

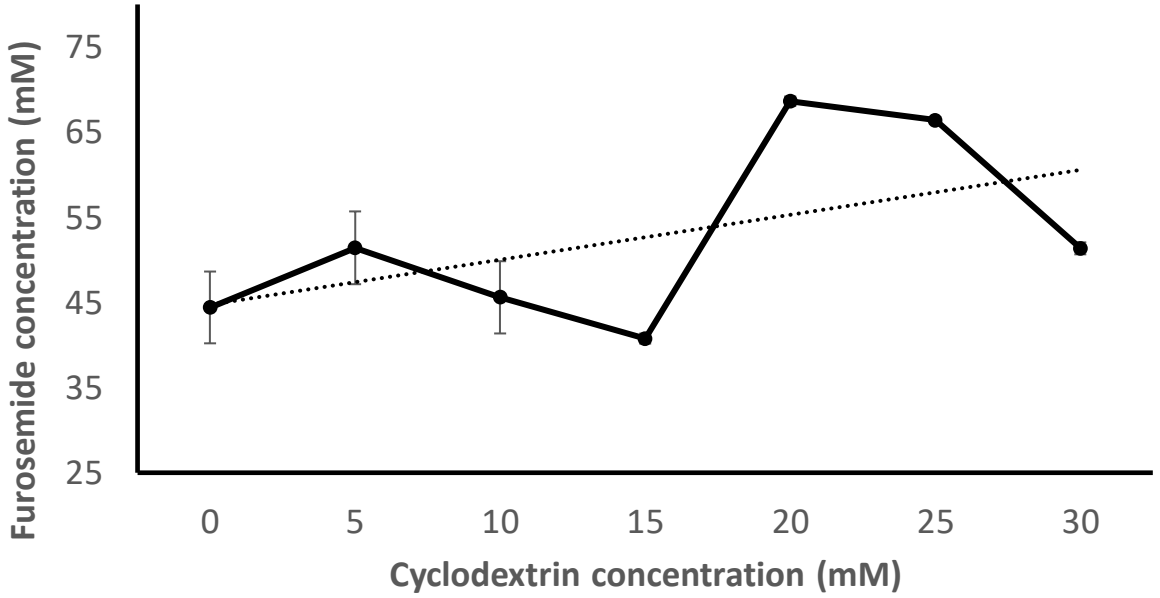


Figure 11: Phase solubility profile for Furosemide complexed with HP-β-CD at pH 7.5. Increasing concentrations of HP-β-CD were prepared from 0-30 mM with excess furosemide added to each solution to assess the effect of complexation and solubility as function of HP-β-CD concentration. Results are presented as the average (n=3) and error bars indicate standard deviation. Samples were filtered, diluted and assayed using HPLC.

The effect of pH variation on solubilisation of furosemide (pK_a 3.9) was investigated. Furosemide is a weak acid with a carboxylic acid functional group, where solubility increases as a function of pH. At pH 5, the solubility of furosemide was 0.15 ± 0.007 mg/mL and increasing up to 40.46 ± 0.70 mg/mL at pH 9 (Figure 12). At pH 7, the saturation solubility was determined to be 9.57 ± 0.1 mg/mL. At this neutral pH range, 99.92 % (Table 23) of the drug molecule is ionised thereby existing in a more polar form and subsequently enhancing solubility by increasing drug molecule attraction with polar water molecules. An optimum pH of around 7 was used in order to efficiently solubilise furosemide and limit the amount of sodium hydroxide added, since sodium hydroxide presents a soapy burning taste when used in large quantities (308).

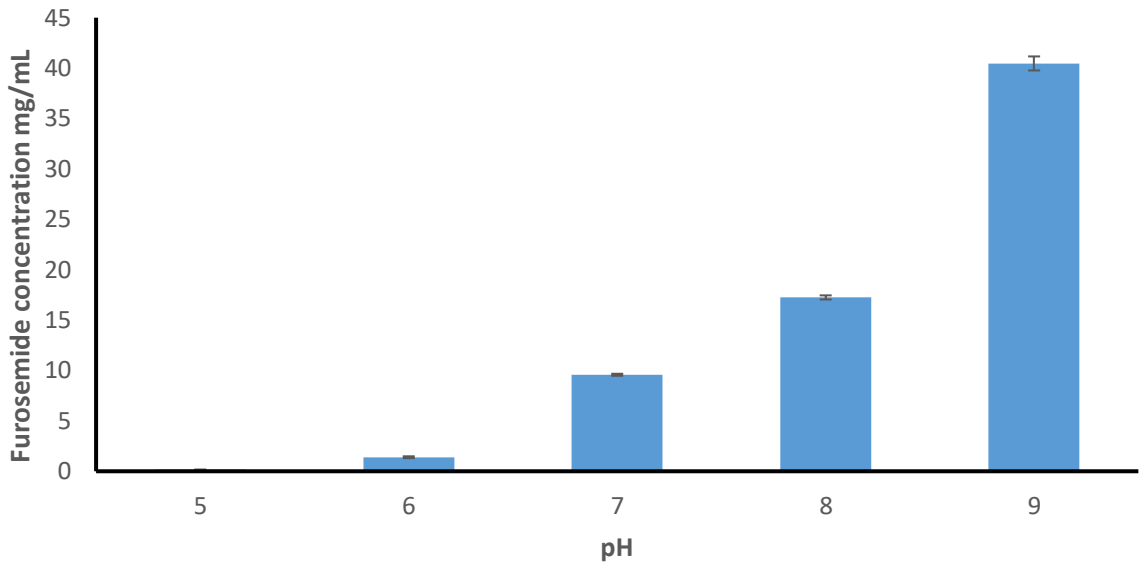


Figure 12: Solubility of furosemide at various pH values. Results are presented as the average (n=3) and error bars indicate standard deviation.

Table 23: Percentage ionisation of furosemide (pKa 3.9) at various pH values. Percentage ionisation calculated using the Henderson–Hasselbalch equation.

pH	% ionised	% unionised
5	92.64	7.36
6	99.21	0.79
7	99.92	0.08
8	99.99	0.01
9	100.00	0.00

3.4.4 Excipient selection and exploiting multiple excipient functionalities

With an aim to limit the total number of excipients, sodium sulphite was explored as a functional excipient that provides antioxidant, antimicrobial and pH adjusting properties. Initial studies involved investigating the power of sodium sulphite to adjust the solution to our chosen pH of 7, since this pH value presented an adequate percentage of ionised drug form, required to achieve the desirable solubility. Sodium sulphite concentrations ranging from 0.2 to 10 % v/v were explored. At 0.2 %, adequate solubilisation was not achieved, the percentage recovery was 4.5 ± 0.2 mg/mL (theoretical concentrations: 8 mg/mL) and the final pH of the solution was 6.14. The lack of solubility can be explained by the low pH value where solubility findings suggested a maximum saturation solubility of furosemide of 1.39 ± 0.1 mg/mL at pH 6. A gradual in increase in sodium sulphite concentration on furosemide solubility was

investigated, where a minimum of 3 % v/v of sodium sulphite was required to effectively soluble the higher strength of our formulation (8 mg/mL). Although the intended solubility was achieved, we decided to discontinue with sodium sulphite since 3 % v/v sodium sulphite would result in a delivery of sodium sulphite which is above the acceptable daily intake (ADI) value of 0.7 mg/kg bw (309). Additionally, the resulting solution exhibited a sulphuric smell which can present a problem in regards to paediatric acceptability.

A particular concern regarding paediatric formulation is the excipient composition and load. As with any oral liquid formulation, the use of an antimicrobial system is warranted in order to prevent microbial proliferation and keep the product sterile and fit for purpose. Due to our working pH, the choice of applicable preservatives were limited, with only parabens and propylene glycol as potential candidates (Table 24). However, such excipients have been identified as problematic in medicines for children (109). As described above (section 1.4.3), parabens are associated with potential adverse effects including oestrogenic activity with potential reproductive effects, hypersensitivity reactions and hyperbilirubinemia in neonates (109). In regards to propylene glycol, adverse effects include central nervous system (CNS) depression and intoxication, especially in new-borns and infants (< 4years). This is due to the reduced capability of metabolism and renal clearance that leads to accumulation of propylene glycol. The EMA have concluded the following daily threshold values of 1 mg/kg, 5 mg/kg and 500 mg/kg for neonates, children aged 1 month to 4 years and children aged 5 to 17 years respectively (108).

Although the use of parabens within allowable concentrations (up to 10 mg/kg/day) was an option, we also decided to opt for a preservative free formulation on the recommendation from the EMA. To overcome this challenge and mask the bitter taste of furosemide, the properties of sugar alcohols (such as xylitol) were explored to provide multiple functionalities. Xylitol is highly effective at enhancing flavour and masking unpleasant taste, while at certain concentrations exerts certain bacteriostatic and bactericidal effects (310, 311). It has been reported that xylitol exerts its bacteriostatic effect by entering the bacteria phospho-transferase system where it competes with phosphofructokinase and, therefore, results in the inhibition of glycolysis (312). Upon inhibition of glycolysis, the microorganism is unable to metabolise glucose and therefore unable to produce adenosine triphosphate (the molecule responsible for carrying energy into the cells) through this pathway (313). Thus, xylitol acts in a bacteriostatic manner by reducing the amount of energy required by bacteria for normal cellular metabolism. Another way in which xylitol exerts its bacteriostatic properties is by lowering the water activity (Aw) within the preparation. Micro-organisms require water to maintain their turgor pressure and carry out metabolic activity; when the Aw outside of the microorganism is lower than inside, water leaves the cell through via diffusion and results in

reduction of turgor pressure and metabolic activity (314). Lastly, xylitol can also enhance product stability by potentiating the antimicrobial effects of other preservatives within the formulation. The inclusion of citric acid within our formulation possesses some antimicrobial effects (315). The combination of both xylitol and citric acid therefore was envisioned to synergistically produce adequate preservative effects, thereby limiting the need of a dedicated preservative.

The concentration of xylitol used within formulations (40 %w/v), coupled with the inclusion of citric acid and strawberry flavour would also ensure palatability and mask the bitter taste of furosemide. Although taste assessments were not carried out, when comparing the compositions of current marketed furosemide oral solutions (that are widely accepted), levels of sweeteners and flavours were found to be similar. Additionally, xylitol when compared to other sugar alcohols included in marketed products results in a less viscous solution (316). This would be beneficial for palatability as less viscous products are favoured as they are less adhesive and easier to manipulate within the mouth, resulting in improved swallowability (317).

Table 24: Overview of commonly used preservatives in oral liquid formulations, optimum pH working range, potential risks and recommendations.

Preservative used in oral formulations	Concentration (%)	pH range	Risk	Recommendation
Sodium benzoate (most commonly used in paediatric formulations)	0.01 – 0.2	<5	May increase the risk of jaundice in new born babies	There are adequate data to establish an overall no-observed-adverse-effect level (NOAEL) of 500 mg/kg bw/day. Therefore the acceptable daily intake (ADI) for benzoic acid and its salts has been established to 0–5 mg/kg bw/day (EMA, 2014)
Potassium sorbate (shows the best risk-benefit-relationship)	0.14	3.5-5.5	There are no data showing any risk by using recommended concentrations	Recommended acceptable daily intake for sorbic acid and its salts are 3 mg/kg bw/day (EFSA, 2015)
Methyl-4-hydroxybenzoate (MHB)	0.05 – 0.1	1 – 8.5	May cause allergic reactions (possibly delayed)	The use of MHP in oral formulations up to 0.2 % of the product (as within the recommended effective concentrations as a preservative) is not a concern for humans including the paediatric population (EMA, 2013)
Propyl-4-hydroxybenzoate (PHB)	0.05 – 0.1	1 – 8.5	Propyl-4-hydroxybezoate binds to oestrogen receptors but with a much weaker affinity than the natural ligand	A permitted daily exposure (PDE) value of 5 mg/kg/day can be calculated for the use of PHB in adults and children older than 2 years with mature metabolic capacity
Propylene glycol	15–30	1-14	May cause alcohol-like symptoms	In the absence of compelling data the maximum daily intake is 50 mg/kg/day in children less than 5 years old, and 1 mg/kg/day in pre-term and term neonates due to known immaturity of both metabolic and renal clearances of propylene glycol in these populations (EMA, 2018)

Citric acid (CA) was chosen as the antioxidant where it delays/inhibits the oxidation of molecules, preventing degradation and improving stability and shelf life of pharmaceutical preparations (318). The selection of citric acid was again based on exploiting multiple functionalities including antioxidant, antimicrobial and taste enhancing properties. Other than its antioxidant properties, citric acid is widely employed as a microbial preservative. Citric acid is a weak organic acid, capable of crossing the cell membrane and influencing intracellular pH, leading to acidification of the intracellular media. A low intracellular pH within cells leads to damage of protein, extracellular membrane and suppression of NADH oxidation, resulting in bacteria cell death (319). Citric acid is especially effective against gram negative bacteria; however, studies indicate the antimicrobial effectiveness of citric acid against Staphylococcus aureus, E. coli and Pseudomonas (320, 321). Although previous studies have reported the antimicrobial effect of CA is due to the combined effect of the molecule and acidic environment, a recent study has demonstrated that an ionised version of CA is in fact more effective in destroying bacteria (322). Since citric acid is a weak acid, as the pH is increased the percentage ionisation of citric acid also increases. Our formulations exhibit a final pH of around 7.4, suggesting that more than 50 % of citric acid is in its tribasic form CA^{3-}, therefore demonstrating a higher antimicrobial efficacy in comparison to CA in its un-charged, un-dissociated state (CAH3) (322).

3.4.5 Allowable daily intake values for selected excipients

Other than exploiting excipient functionalities, the excipient selection process was based on the fact that included excipients satisfy the inclusion criteria of the screening tool (section 3.3.4 and section 6.3.1). We can confirm that the excipients are GRAS listed and within acceptable threshold values (where applicable).

Parabens - Risk assessments have led to the establishment of an acceptable daily intake of 0-10 mg/kg body weight for the total sum of methylparaben, ethylparaben and propylparaben (138). The maximum dose (40 mg per day) will deliver a total of 0.5 mg of methyl paraben. This is well below the daily threshold values.

Citric Acid – No recommended daily allowance has been specified. However, very small amounts of this excipient has been used and do not anticipate doses to be significant enough to warrant any safety concerns

Sodium Phosphate - The European Food Safety Authority (EFSA) panel has re-assessed the safety of phosphates and derived, for the first time, a group acceptable daily intake [ADI] of 40 milligrams per kilogram of body weight [mg/kg bw] per day (323). The maximum amount of sodium phosphate in a maximum 40 mg dose is 15 mg. This is well below the daily threshold values.

Sodium hydroxide - No recommended daily allowance has been specified. However, very small amounts of this excipient has been used and do not anticipate doses to be significant enough to warrant any safety concerns

Xylitol – Xylitol has been allocated an ADI “not specified” following review of the available safety information in both animals and humans. In children doses below 30g/day are unlikely to cause gastrointestinal discomfort (324). The maximum amount of xylitol in a maximum 40 mg dose is 2 g.

The compositions of optimised formulations is tabulated below (Table 25).

Table 25: Composition of all final formulated preparations. Four different versions of a 4 mg/mL and 8 mg/mL strength solution were developed. These include preservative free, sweetened and flavoured preparations.

Formulation	Type	List of excipients
4 mg/mL		
1	Flavoured Sweetened Preservative free	Xylitol (40 % w/v), Sodium hydroxide (1.3 % v/v), Sodium phosphate (0.3 % w/v), Strawberry flavour (0.2 % w/v), Citric acid (0.2 % w/v), Purified water
2	Flavoured, Sweetened Preservative	Xylitol (40 % w/v), Sodium hydroxide (1.3 % v/v), Sodium phosphate (0.3 % w/v), Strawberry flavour (0.2 % w/v), Citric acid (0.2 % w/v), Methyl-paraben (0.1 %), Purified water
3	Unflavoured Sweetened Preservative free	Xylitol (40 % w/v), Sodium hydroxide (1.3 % v/v), Sodium phosphate (0.3 % w/v), Citric acid (0.2 % w/v), Purified water
4	Unflavoured Sweetened Preservative	Xylitol (40 % w/v), Sodium hydroxide (1.3 % v/v), Sodium phosphate (0.3 % w/v), Citric acid (0.2 % w/v), Methyl-paraben (0.1 %), Purified water
8 mg/mL		
1	Flavoured Sweetened Preservative free	Xylitol (40 % w/v), Sodium hydroxide (1.3 % v/v), Sodium phosphate (0.3 % w/v), Strawberry flavour (0.2 % w/v), Citric acid (0.2 % w/v), Purified water
2	Flavoured, Sweetened Preservative	Xylitol (40 % w/v), Sodium hydroxide (1.3 % v/v), Sodium phosphate (0.3 % w/v), Strawberry flavour (0.2 % w/v), Citric acid (0.2 % w/v), Methyl-paraben (0.1 %), Purified water
3	Unflavoured Sweetened Preservative free	Xylitol (40 % w/v), Sodium hydroxide (1.3 % v/v), Sodium phosphate (0.3 % w/v), Citric acid (0.2 % w/v), Purified water
4	Unflavoured Sweetened Preservative	Xylitol (40 % w/v), Sodium hydroxide (1.3 % v/v), Sodium phosphate (0.3 % w/v), Citric acid (0.2 % w/v), Methyl-paraben (0.1 %), Purified water

3.4.6 Preservative Efficacy

In order to satisfy the BP/EP criteria for preservative efficacy in oral preparations, there should be at least a 3 log reduction for bacteria from the initial count after 14 days, with no increase thereafter, whilst a minimum of 1 log reduction is required for yeasts/moulds (325). F2 and F4 includes a preservative (Methyl-paraben (0.1 %)), where preservative efficacy criteria has been met for each tested microorganism, suggesting adequate antimicrobial efficacy (Figure 14 and Figure 16). In contrast, the preservative free (preservative free system: xylitol-citric acid) formulations (F1 and F3) demonstrated adequate preservative efficacy against *Pseudomonas aeruginosa*, *Aspergillus* and *Zygosaccharomyces rouxii*. However, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) narrowly failed, where a log reduction of 2.77 and 2.85 for F1 and 2.01 and 2.02 for F3 was achieved, respectively (Figure 13 and Figure 15). This may be due to the minimum inhibitory concentration (MIC) of citric acid, where studies have reported a MIC (wt %) of 0.25-0.5 against *S. aureus,* and up to 1 for *E.coli* (326, 327). The citric acid concentration within the preservative free formations was 0.2 % w/v respectively. Although, more than 99% reduction of both *S. aureus* and *E.coli* was achieved, the BP/EP preservative efficacy requirements were not met and therefore, in future preservative free formulation developments, we would suggest a citric acid concentration of at least 0.25 % w/v. However, the PET result indicate that the xylitol-citric acid preservative system if highly effective, since a log reduction of more than 2 was shown for *S. aureus* and *E.coli*, despite using a MIC below reported values. This is owing to the synergistic antimicrobial effect of xylitol and citric acid when used simultaneously. When comparing the log reduction of *S. aureus* and *E.coli* for F1 and F3, the higher strength (8 mg/mL) furosemide perseverative free solution displayed a greater log reduction. This would be due to the lower Aw of F1 where the addition of more solute results in more water being in the bound state, reducing the amount of water available to microorganism for microbial growth and proliferation (328).

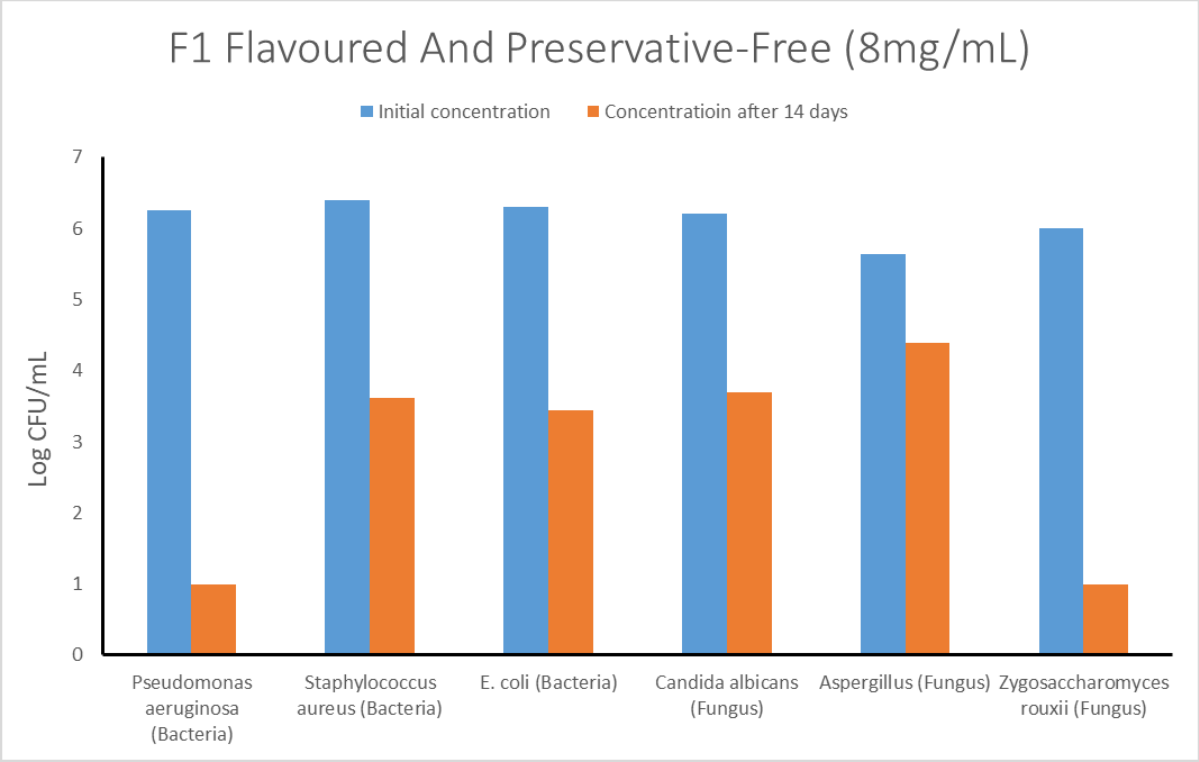


Figure 13: Preservative efficacy data displaying the degree of log reduction of various microorganisms. F1 (Flavoured and Preservative free version of 8 mg/mL).

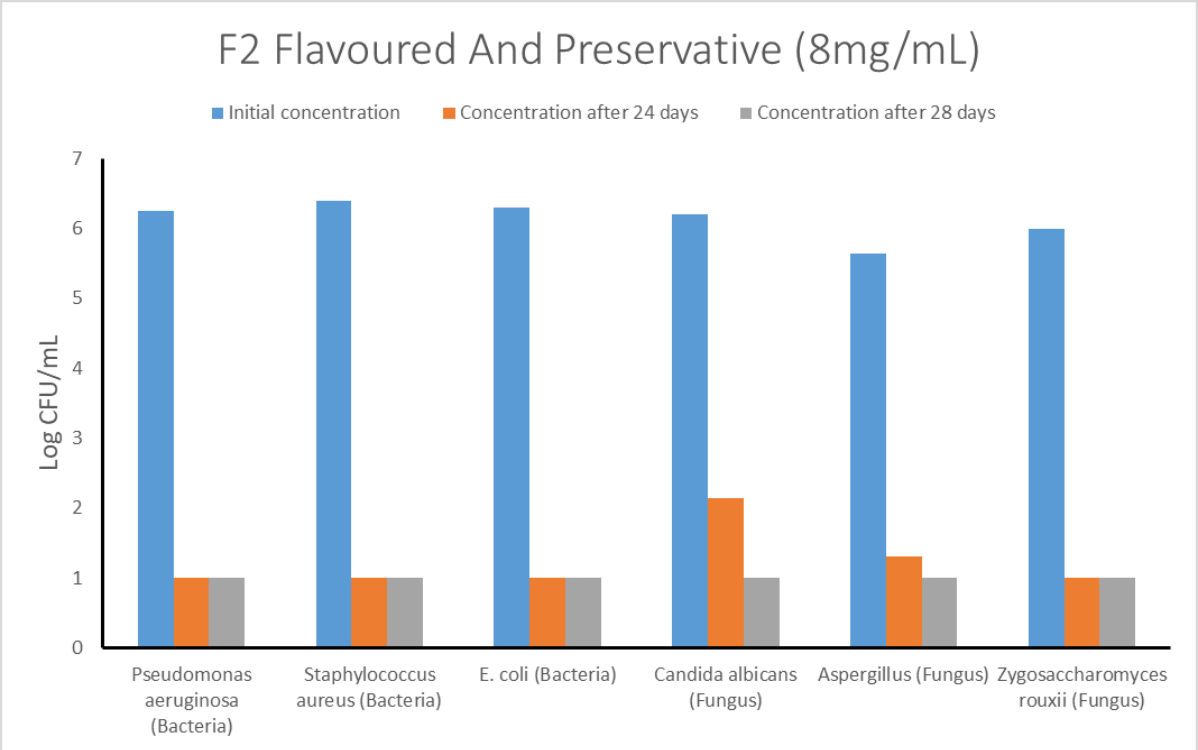


Figure 14: Preservative efficacy data displaying the degree of log reduction of various microorganisms. F2 (Flavoured and Preservative version of 8 mg/mL).

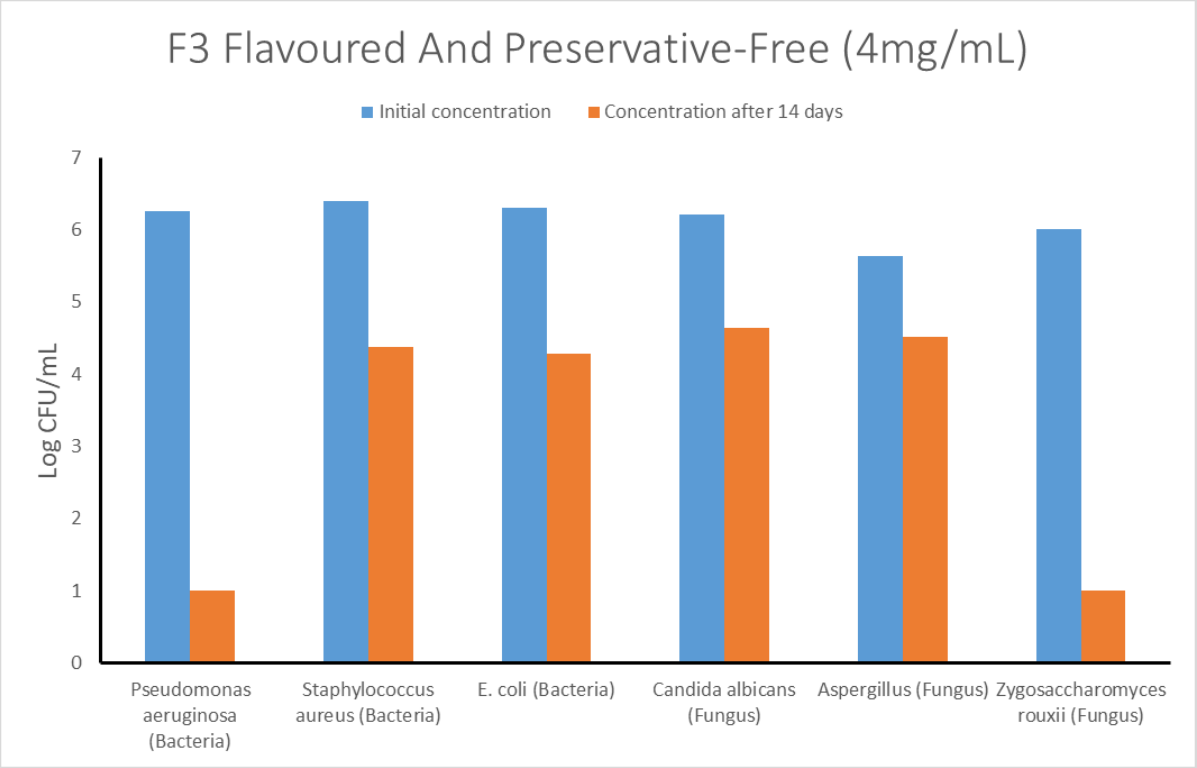


Figure 15: Preservative efficacy data displaying the degree of log reduction of various microorganisms. F3 (Flavoured and Preservative free version of 4 mg/mL).

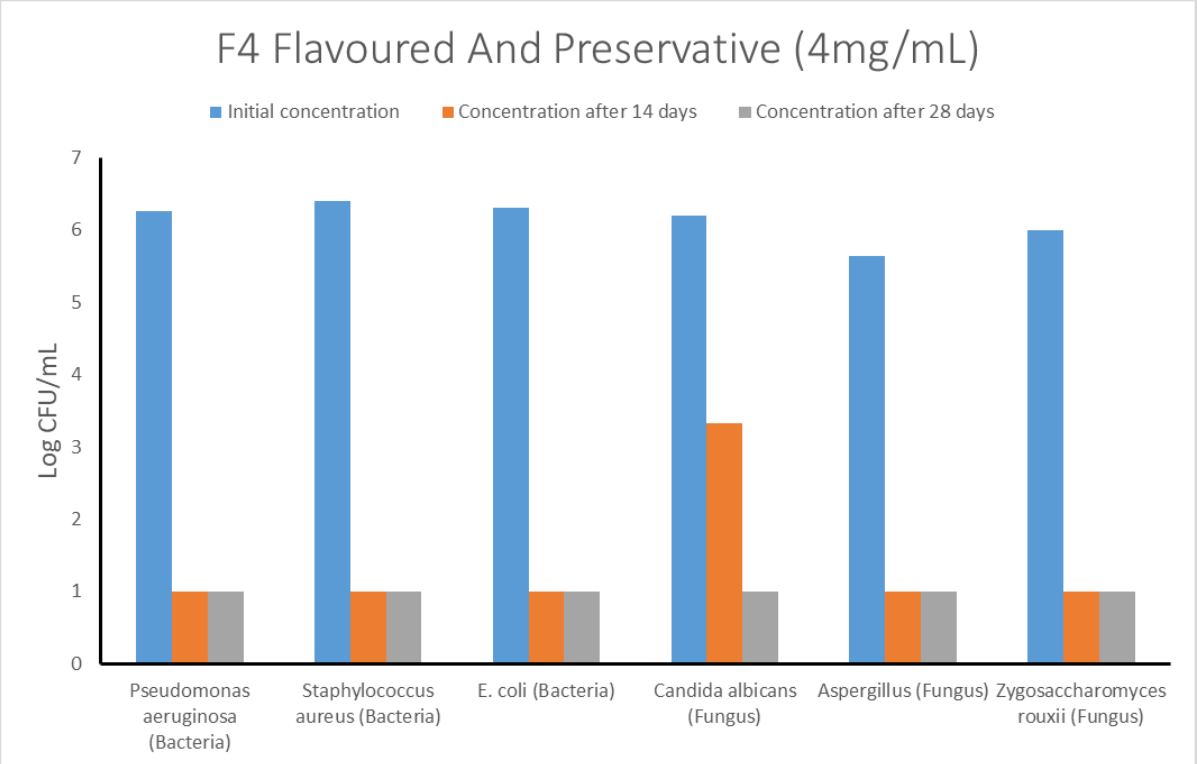


Figure 16: Preservative efficacy data displaying the degree of log reduction of various microorganisms. F4 (Flavoured and Preservative version of 4 mg/mL).

3.4.7 Stability

Stability testing is a procedure that aims to determine shelf-life, recommended storage conditions and influence of external environmental factors including light, humidity and temperature are tested on drug product quality. All furosemide products were subjected to a long term stability, lasting 12 months at 25°C and 60% relative humidity (RH). These conditions were chosen to closely simulate the environment the formulations would experience during manufacture, storage and handling.

In order to satisfy the pharmacopeia requirements, the percentage of furosemide in an oral solution should not be less than 90.0 percent and not more than 110.0 percent of the labelled amount (329). Whereas, the stability testing guidelines set out by the ICH (Q1A (R2)) have defined a 'significant' change as: A 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency when using biological or immunological procedures (330).

Furosemide in acidic solutions is unstable and undergoes acid catalysed hydrolysis, yielding to 4-chloro-5- sulfamoylanthranilic acid and furfuryl alcohol that converts to levulinic acid (331). In order to prevent drug degradation a buffered alkaline pH (>7) solution should first be prepared to which the drug is then added gradually and mixed to solubilise. By increasing the pH of the solution we are not only improving solubility but also the stability, since furosemide exhibits good stability in alkaline solutions (332). Additionally, furosemide is also photosensitive, however in alkaline solutions furosemide is relatively stable to photodegradation (333). Therefore, furosemide should always be prepared in alkaline pH that is protected from light. All solutions were packaged and stored in amber glass bottles to minimise risk of photodegradation and comply with storage recommendations from pharmacopeia.

All 8 mg/mL versions we stable throughout the 12 month stability period with no significant changes observed. All formulations were within 90.0 to 110.0% of the stated amount with no more than 5% change in assay from their initial values (Figure 18). Regarding the 4 mg/mL varieties, F1, F2 and F3 were within 90.0 to 110.0% of the stated amount, however, F1 and F3 displayed a change in assay of more than 5% at the 9 month time point (Figure 17). Surprisingly, F4 displayed an initial concentration of 109.5% of the theoretical 4 mg/mL and a total percentage change of 7.75% (Table 26). This may be due to furosemide instability, however the data observed for the same version of the 8 mg/mL would suggest otherwise. Nonetheless, it would be important to mention that other than the diuretic property o furosemide, furosemide also possess antibacterial properties since it is formally a sulphonamide (334). Higher strength formulations may have benefited from this and can be a reason for possessing better stability overall when compared to the lower strengths. The

discrepancy for this high initial concentration as well as some formulations (F1,F2,F3,F6 and F7) displaying an increase in concentration at 3 and 6 months of the stability study would suggest experimental and/or analytical error. The pH of all preparations remained consistent (suitable pH for maintaining FUR solubility) and there was no observed change in appearance.

Table 26: Percentage change (%) from initial values.

Formulation	Description	Percentage change (%)	Pass/Fail
F1	4 mg Preservative free with Flavour	6.28	FAIL
F2	4 mg Preservative free & Unflavoured	0.83	PASS
F3	4 mg Preservative with Flavour	5.86	FAIL
F4	4 mg Preservative and Unflavoured	7.75	FAIL
F5	8 mg Preservative free with Flavour	3.11	PASS
F6	8 mg Preservative free & Unflavoured	4.52	PASS
F7	8 mg Preservative with Flavour	1.51	PASS
F8	8 mg Preservative & Unflavoured	1.64	PASS

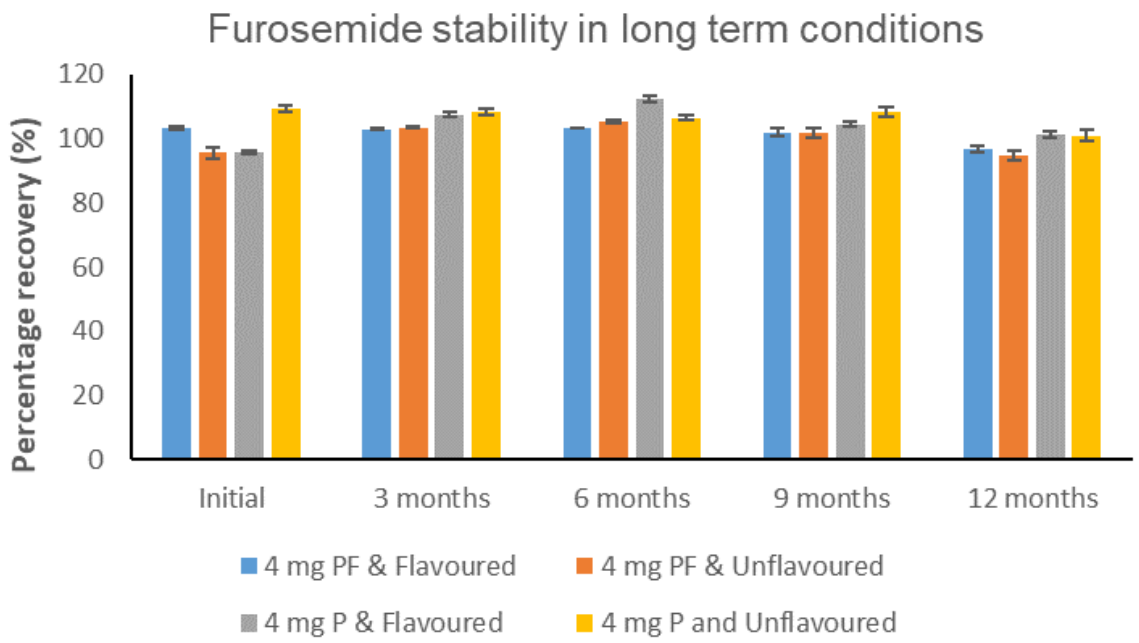


Figure 17: Long term stability data for all variations of the 4 mg/mL formulations at 25°C and 60% relative humidity (RH). Data presented as mean (n=3) and error bars indicate standard deviation.

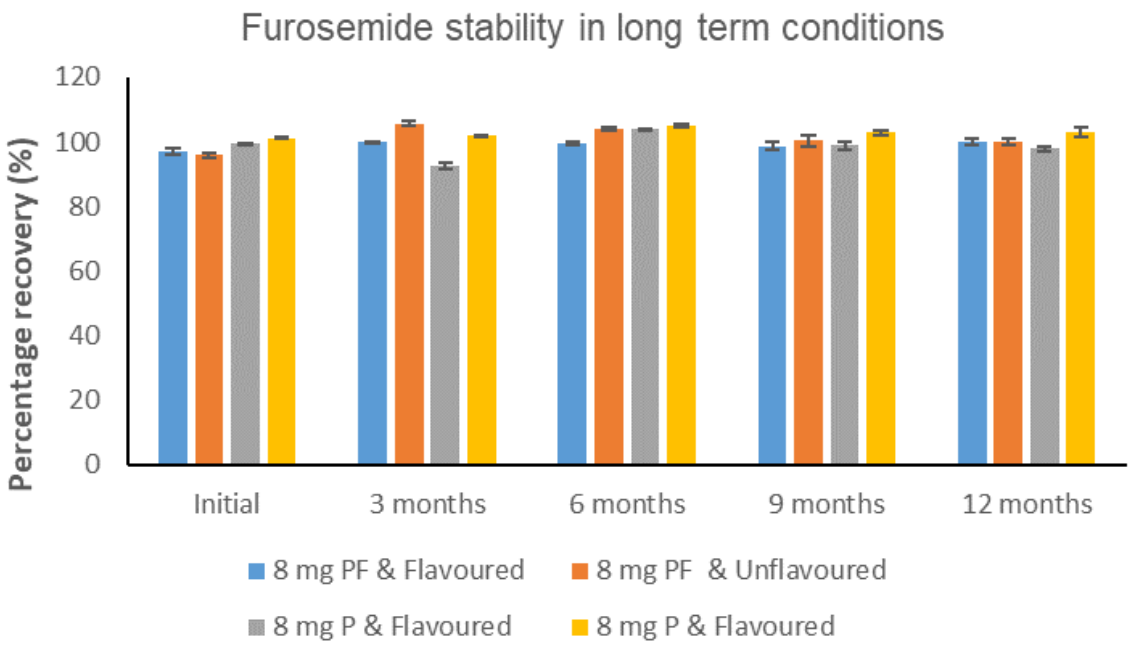


Figure 18: Long term stability data for all variations of the 8 mg/mL formulations at 25°C and 60% relative humidity (RH). Data presented as mean (n=3) and error bars indicate standard deviation.

The criteria to determine any 'significant changes' are outlined by the ICH stability testing of new drug substances and products Q1A(R2) and are as follows:

6) A 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency when using biological or immunological procedures;
7) Any degradation product's exceeding its acceptance criterion;
8) Failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e.g., color, phase separation, resuspendibility, caking, hardness, dose delivery per actuation); however, some changes in physical attributes (e.g., softening of suppositories, melting of creams) may be expected under accelerated conditions;

and, as appropriate for the dosage form:

9) Failure to meet the acceptance criterion for pH; or
10) Failure to meet the acceptance criteria for dissolution for 12 dosage units.

3.5 Conclusion

Four versions of two strengths (4 mg/mL and 8 mg/mL) of furosemide ethanol free oral solutions were developed for the paediatric population. From an excipient composition regard, the formulations developed display superiority as they only included paediatric appropriate excipients. The paediatric specific bottom up development approach ensured formulations were comprised of suitable excipients that provide multiple functionalities including palatability, stability, antimicrobial stability and solubility. A 12-month stability study at 25°C and 60% relative humidity (RH) indicated that all, with an exception of F4 displayed an acceptable content of furosemide and were stable throughout the 12 month period with no significant observed changes in pH and appearance. Although our preservative free formulations displayed good preservative efficacy, it was not enough to satisfy the recommended criteria for preservative efficacy. In future, we anticipate that an increase in citric acid concentration from 0.2 % w/v to 0.5 % w/v would provide that extra preserving effect required to meet the BP/EP preserving efficacy test criteria. Nonetheless, the ethanol free formulations with preservative (0.1 % w/v methyl paraben) displayed sufficient antimicrobial properties.

Chapter 4: Systematic development strategy of an age-appropriate formulation of famotidine for the paediatric population

4.1 CHAPTER AIMS AND OBJECTIVES

- Review existing famotidine products
- Build on the paediatric specific excipient 'working zone' for orodispersible tablets
- Review dosing requirements to determine applicable dosage form strengths
- Develop two strengths (2 mg and 10 mg) of an age-appropriate famotidine formulation that possess increased dose flexibility, palatability, safety, quality and efficacy
- Characterise and evaluate formulation performance in biorelevant conditions

Abstract

Among children, famotidine is commonly used to treat gastroesophageal reflux disease (GORD) and peptic ulcers. However, he lack of suitable dosage forms available results in routine unlicensed and off-label use. All current marketed famotidine preparations either consist of potentially problematic excipients that may not be suitable for the paediatric population or have a composition comprised of several excipients. A systematic paediatric specific formulation development strategy is employed, taking into consideration dosage form feasibility and acceptability, followed by evaluation of dosage form quality and performance in biorelevant media. Mannitol and MCC are excellent direct compression excipients that provide multiple functionalities, and when coupled with principal pharmaceutical design eliminate the need of dedicated excipients and result in ODTs with good mechanical strengths (≈ 40 N) and fast disintegration times (< 15 seconds). The resulting formulations displayed robust release profiles in FaSSGF, FeSSGF, FaSSIF and FeSSIF with complete dissolution obtained within the first 2 minutes. Over the 6 month course of the stability testing (medium term) at 25°C and 60% RH, no 'significant' change was observed.

Keywords: age-appropriate; paediatrics; ODMT; GORD; biorelevant; mini-tablets; disintegration; dissolution; flowability; excipients.

4.2 Introduction

Famotidine is a histamine type-2 (H_2) receptor antagonist that is commonly used in children for the management of gastroesophageal reflux disease (GORD) and peptic ulcers (Gastric or duodenal ulceration). Famotidine exerts it effects by competitively binding to H_2 receptors located on the surface of gastric parietal cells, thereby disrupting the stimulation of gastric acid secretion (335). Famotidine is present as a white to yellow crystalline powder, which is soluble in water and possess a logP and pKa of -0.64 and 6.7 respectively (336).

The first Famotidine tablet (Pepcid®) was introduced on the market by Merck, Sharp and Dohme in 1986. The safety and effectiveness of famotidine (Pepcid®) has been well established in paediatric patients for the treatment of peptic ulcer disease and GORD. Evidence from controlled and well-reported studies of Pepcid® in adults as well as published pharmacokinetic and pharmacodynamics information in paediatric patients supports the use of PEPCID in children (337). However, paediatric patients weighing under 40 kg are not recommended to use PEPCID 20 and 40 mg tablets, since these strengths exceed the dose prescribed for these patients.

Although H_2 receptor antagonists including famotidine have an established role in the management of paediatric GORD and peptic ulcers; the lack of suitable dosage forms, however, result in ongoing routine off-label and unlicensed medicine use. Currently, in the UK, the only marketed (for adults) available preparation of famotidine is a 20 mg and 40 mg film coated tablet. The United States do have alternative preparations available including powder for suspension, chewable tablet and oral disintegrating tablet (ODT); however, the strengths (20 mg and 40 mg) available are targeted at the adult population and would require manipulation if given to children. Pepcid® (famotidine) for oral suspension when reconstituted delivers 40 mg per 5 mL and serves as an appropriate dosage form option for paediatrics, however, contains potentially harmful excipients (namely sodium benzoate and parabens). Furthermore, liquid dosage forms require special storage conditions (refrigeration) and are more susceptible to degradation compared to solid dosage forms. The uncertainty of excipient safety in current market formulations, coupled with inadequate dosing flexibility opportunities and palatability concerns suggests the need of an age- appropriate formulation of famotidine that is specifically designed and developed for the paediatric sector. An age-appropriate formulation ensures the formulation is safe, effective, palatable, high quality, concordant to development and ability, inclusion of appropriate excipients and uses acceptable dosing volumes (338).

Despite the recent advances in paediatric formulation development and the increase in availability of suitable and approved medicines, there yet remains a standard strategy that encompasses paediatric formulation needs with principle pharmaceutical design.

The work in this chapter aims to build on the systematic paediatric specific formulation development strategy that was introduced in the last two chapters (carvedilol and furosemide). At the same time, successful development of an age appropriate formulation using the screening tool (as mentioned in previous chapters) coupled with a systematic paediatric specific formulation development strategy would suggest validation of the screening tool and/or provide opportunities to further refine and improve the screening tool's effectiveness.

4.3 Materials and Methods

4.3.1 Materials

Famotidine was purchased from Alfa Aesar (Lancashire, United Kingdom). Pharmaceutical grade D-Mannitol (≥98% purity) and magnesium stearate were obtained from Sigma-Aldrich (Dorset, UK), while MCC as Pharmacel 102 was obtained from DFE Pharma (Germany). Lastly, colloidal silicon dioxide (Aerosil 200) was obtained from Evonik Industries (Essan, Germany). For the preparation of biorelevant media for dissolution, FaSSIF, FeSSIF, FaSSGF and FeSSGF powder and buffer concentrates were purchased from Biorelevant (London, United Kingdom)

For sample analysis, Acetonitrile and Methanol (HPLC-grade) were obtained from Fisher Scientific (London, UK), whereas Trifluoroacetic acid (TFA), for HPLC (≥99.0%) was purchased from Sigma-Aldrich (UK).

4.3.2 HPLC Analytical Method Development

All samples were analysed on an Agilent 1260 series (Agilent Technologies, Santa Clara, CA, USA), equipped with a reverse-phased Eclipse plus C18, 4.6 × 150 mm, 3.5µm column (Agilent Technologies, Santa Clara, CA, USA). Separation of famotidine was achieved using an isocratic mobile phase compromising of Methanol: Water, adjusted to pH 3.3 (55:45 *v/v*). Flow rate was set at 0.5 mL/min, injection volume was 5 µL and a wavelength of 265 nm was used for detection. A 9 point calibration curve in the range 0.39 to 100 mcg/mL was prepared via serial dilution in methanol (1 in 2 dilution). All samples prepared for analysis were diluted so as to fall within the calibration range. Method validation was carried out following the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidelines (Q2(R1)).

4.3.3 Dose banding and proposed strengths

Determining the dose of famotidine in paediatrics was based on available pharmacokinetic/dynamic studies and the variety of doses used in clinical trials. For each age,

the dose range was determined based on average child weights and strengths were proposed accordingly so that strengths provide adequate flexibility whilst using acceptable dosing volumes.

4.3.4 ODT development strategy

The exploration of current patents and prior art for famotidine ODT was undertaken to provide key characteristics of a suitable ODT, and technologies used to achieve a palatable formulation with adequate mechanical strength and fast disintegrating times.

4.3.5 Development of paediatric excipient 'working zone' for ODTs and pre-screening

The development of ODTs requires several functional excipients including fillers/binders, disintegrants, tabletting excipients and taste enhancers. To begin with, excipients considered safe for use in children were determined by referring to literature, regulatory guidance documents and utilising the STEP database (44, 164, 339, 340). It was especially important to use excipients that would ensure reasonable hardness of the tablets, but also quick dissolution so that the tablets could either be taken whole or dispersed in a teaspoon of liquid prior to ingestion.

4.3.6 Pre-compression analysis

4.3.6.1 Particle Size Analysis

Particle size analysis was carried out via laser diffraction using a Sympatec Helos detector equipped with a Rodos dry disperser and vibrating feeder (Clausthal-Zellerfeld, Germany). 1 g of sample was placed on the VIBRI/I feeder and fed through the RODOS disperser. Pressure was set a 2 bars and measuring range was set between 0–175 µm. Measurements were taken in triplicates and presented as ± SD. Volume mean diameter (VMD), X10, X50 and X90 were the values obtained. Both starting materials and final blends were analysed to give an indication of flow based on particle size and to ensure acceptable flow properties are prevalent for successful direct compression.

4.3.6.2 Powder Flow Measurements

Angle of repose was determined using the method outline in the U.S. Pharmacopeia monograph, <1174> (218). A known amount of powder was poured through a 12mm funnel maintained at a height of 2-4 cm from the top of the powder heap. Height and diameter of the powder cone was measured and angle of repose was determined using the following equation:

Eq.1: $\tan(\Theta) = \text{Height}/0.5\ \text{Base}$

Measurements were carried out in triplicates and presented as mean ± standard deviation.

Bulk (ρbulk) and tapped densities (ρtapped) were also used to assess powder flow. A Sotax tap density tester USP II (Allschwil, Switzerland) was used as outlined in the USP monograph, <616> (219). A 25 ml measuring cylinder was tightly fastened onto the tester using a foam ring. A known amount of sample was then poured and initial volume was taken at 0 taps. Further measurement were taken after every 10, 500 and 1250 taps. Measurements were carried out in triplicates and presented as mean ± standard deviation

Carrs's index and hausner ratios were calculated using the following equations:

Eq. 2: Carr's Index = (ρtapped - ρbulk)/ρtapped) x 100

Eq. 3: Hausner ratio = ρtapped/ρbulk

Eq. 4: ρbulk = Mass/V0 (unsettled apparent volume)

Eq. 5: ρtapped = Mass/Vf (Final tapped volume)

4.3.7 Orally Disintegrating Mini Tablet (ODMT) Production

Optimised powder blends were identified and selected, possessing acceptable tablet properties with improved content uniformity. Two strengths of famotidine ODMTs with a target drug load of 4 and 20 % *w/w* and a tablet mass of 50 mg were produced using a Specac semi-automatic hydraulic press (Slough, UK) equipped with a 4 mm multi-tip (three) with concave faced punches at a compression force of 10 KN with quick release.

4.3.8 ODMT Physical Evaluation

4.3.8.1 Hardness

A Copley TBF 100 Hardness tester (Nottingham, UK) was used to measure the force required to break the tablets produced. Hardness values were measured in Newtons. Measurements were carried out in triplicates and presented as mean ± standard deviation.

4.3.8.2 Friability

Friability testing was carried out using a Sotax F2 Friabilitor (AllSchwill, Switzerland), that measures the capacity of tablets to resist mechanical stress. Six tablets were carefully de-dusted using a soft brush and an initial weight was taken. Tablets were then placed in the rotating drum and rotated for 4 min at a speed of 25 rpm (total 100 revolutions). The tablets were removed, dusted and a final weight was taken. The percentage friability was calculated using the following equation:

Eq. 6: % Friability = (initial weight- final weight)/initial weight × 100

4.3.8.3 Disintegration

Disintegration testing was carried out as stated in the official USP disintegration monograph (701). An Erweka ZT3 (Heusenstamm, Germany) disintegration tester was used. A tablet was

placed in one of the six vessels and allowed to oscillate at a rate of 30 cycles per minute. A total of 800 mL of distilled water was used as the disintegration medium that had a constant temperature of 37 °C. Measurements were achieved for a single tablet at a time to enhance accuracy. The disintegration time was noted until there were no more residue of tablet aggregates left on the upper side of the vessel mesh. Measurements were carried out in triplicates and presented as mean ± standard deviation.

4.3.9 Dissolution Studies

All dissolution tests were carried out using an Erweka DT 126 with USP 2 paddle apparatus (Langen, Germany). Samples of 5 mL were drawn at appropriate time points (2, 5, 10, 15, 30, 45, and 60 minutes) and replaced by 5 mL of fresh media to maintain sink conditions. A total of 500 mL of media per vessel, maintained at 37 °C was employed with a continuous paddle speed of 50 rpm. Drug release was determined via HPLC and adjusted for cumulative drug release (%). A total of 6 replicates were taken and data presented as mean ± standard deviation.

Simulated gastric and intestinal (biorelevant) media (FaSSGF, FeSSGF, FaSSIF and FeSSIF), was prepared as presented by Jantratid et al. (220). The composition of biorelevant media is presented in Table 27.

Table 27: Composition of biorelevant media used for dissolution studies. FaSSGF = Fasted-state Simulated Gastric Fluid, FeSSGF = Fed- state Simulated Gastric Fluid, FaSSIF = Fasted- state Simulated Intestinal Fluid, FeSSIF = Fed- state Simulated Intestinal Fluid.

Pre-prandial biorelevant gastric media composition	
Composition	*FaSSGF*
Sodium chloride (mM)	34.2
Sodium taurocholate (µM)	80
Lecithin (µM)	20
Pepsin (mg/mL)	0.1
HCl/NaOH qs	pH 1.6
pH	1.6
Osmolarity (mOsm/kg)	120.7 ± 2.5
Post-prandial biorelevant gastric media composition	
Composition	*FeSSGF*
Sodium chloride (mM)	34.2
Acetic acid (mM)	17.12
Sodium acetate (mM)	29.75
Milk:buffer	1:1
HCl/NaOH qs	pH 5
pH	5
Osmolarity (mOsm/kg)	400
Buffering capacity (mmol/L/pH)	25
Pre-prandial biorelevant intestinal media composition	
Composition	*FaSSIF-V2*
Sodium hydroxide (mM)	34.8
Sodium taurocholate (mM)	3
Lecithin (mM)	0.2
Sodium chloride (mM)	68.62
Maleic acid (mM)	19.12
HCl/NaOH qs	pH 6.5
Osmolarity (mOsm/kg)	180 ± 10
Buffering capacity (mmol/L/ph)	10
Post-prandial biorelevant intestinal media composition	
Composition	*FeSSIF-V2*
Sodium hydroxide (mM)	81.65
Sodium taurocholate (mM)	10
Lecithin (mM)	2
Sodium chloride (mM)	125.5
Maleic acid (mM)	55.02
Glyceryl monooleate (mM)	5
Sodium monooleate (mM)	0.8
HCl/NaOH qs	pH 5.8
Osmolarity (mOsm/kg)	300 ± 10
Buffering capacity (mmol/L/ph)	25

4.3.10 Statistical Analysis

GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA) was used to carry out one-way analysis of variance (ANOVA) with a posthoc Tukey's test to identify statistically significant differences in solubility between adult and paediatric biorelevant media. An alpha value of 0.05 was used.

4.4 Results and Discussion

4.4.1 Summary of famotidine physiochemical properties and influence on dosage form feasibility – use of screening tool to inform dosage form design

Preformulation study intends to establish physicochemical parameters of drug substance under review and evaluate compatibility with other constituents to develop a stable, safe and effective dosage form (341). Several properties can be characterised and involve solubility, stability, compatibility and compressibility. Bulk characteristics include particle shape, size and distribution, flow and organoleptic properties (342). Since physicochemical properties are inherent to each drug, characterisation provides a means to analyse and determine properties that may influence selection of excipients, manufacturing processes, rational development of dosage form type and formulation composition (343, 344). Additionally, preformulation studies are useful in providing regulatory relief as characterisation ensures the suitability of individual formulation components for the intended population and establishes any safety and toxicological concerns of the medicinal product under review (345).

Famotidine is a BCS class III drug, exhibiting high solubility-low permeability (346). It is a weak base with a pKa of 6.7, a molecular weight of 337.44 g/mol and logP of -0.64 (336) (Table 28). Famotidine is rapidly absorbed and acts within one hour of oral administration, with peak plasma concentrations being reached within 1-3 hours (336). Famotidine is freely soluble in glacial acetic acid (500 mg/mL), slightly soluble in methanol (3 mg/mL), very slightly soluble in water (1 mg/mL) and practically insoluble in ethanol (<0.1 mg/mL) (336).

Famotidine is unstable within the acidic environment and is thought to be one of the reasons for its variable absorption, with its total bioavailability ranging between 40-50% (347). A study carried out by Islam and Narurkar determined the effect of pH on famotidine stability. It was reported that famotidine is most stable at pH 6.3 (348). The decomposition of famotidine in aqueous solution was also studied by Junnarkar and Stavchansky, which was confirmed both in acidic and alkaline media and that maximum stability occurred at pH 6.52, 6.61 and 6.92 when solutions were heated to 85, 70 and 55 ˚C respectively (349). Famotidine concentration is reported to reduce by 34 % after 1 hour in simulated gastric media and by approximately 88 % after 3 hours (350). This acidic mediated degradation is said to be more significant in the elderly population due to increased gastric emptying time (123 minutes), whilst the

decomposition of famotidine is only around 36% in young children due to a mean gastric emptying time of 50 minutes (351).

It has been reported that the presence of food does not affect the bioavailability of famotidine; however, co-administration of potent antacids may significantly reduce the oral absorption by 20-30 % (347). Although clinically insignificant, it is worth mentioning that contrasting statements have been made where studies report that the bioavailability of famotidine slightly increases with food and decreases with co-administration of antacids (352, 353). This may affect the younger subsets of the paediatric population since they are always in a continuous fed state. According to pharmacokinetic data from clinical trials and a published study in paediatric patients, children aged 0-3 months exhibit decreased plasma clearance and a longer elimination half-life than older children, whilst pharmacokinetic parameters calculated for paediatric patients are comparable to those determined for adults between the ages of 3 months and 15 years (354). Nonetheless, it is worth noting that total bioavailability of the drug may vary among paediatric subsets owing to varied gastric emptying times and pH values.

Famotidine exhibits solid-state polymorphism, with the marketed form being the metastable polymorph B and possessing a greater solubility to that of form A (355). However, Hassan *et al.* (1997) states that among the commercially available forms of famotidine, the B form is probably the most stable and therefore has the lowest aqueous solubility (0.55 mg/ml)(356). Compared to the conformation of form B which is folded, form A presents as an extended conformation. The polymorphic form B of famotidine can be obtained when famotidine is crystallised from an aqueous solution at a high initial concentration, whereas form A can be obtained through manual grinding (355, 356). Since the commercial form is metastable, there is always a possibility for it to convert to form A (stable) during transport and storage. This may significantly impact and influence drug properties including solubility, stability, efficacy and toxicity (357). In an attempt to solve the polymorphism problem in famotidine, a new stable salt formation was prepared by Russo *et al.* (2014) that exhibits enhanced solubility and stability profiles compared to form A and B (355). The new conformer was obtained by either solvent evaporation or co-milling of famotidine and maleic acid.

Further, in an effort to enhance the solubility and stability profiles of famotidine, Ain *et al*. (2013) investigated the potential of drug-cyclodextrins complexation. The study concluded that the kneaded complex of famotidine with β-cyclodextrin significantly increases the solubility and dissolution rate of famotidine by a 2.34, 1.83 and 2.01 fold increase in water, HCl and phosphate buffer, respectively (358). Similar approaches that use cyclodextrins have also been reported by other authors which claim an improvement of famotidine chemical stability, oral bioavailability and bitter taste (348, 359, 360).

Compared to BCS class II drugs, famotidine exhibits good solubility and will present fewer challenges when it comes to the preparation of liquid dosage forms. However, other than being able to provide flexible dosing and easy swallowability, liquid dosage forms come with many disadvantages. These include stability concerns, special storage requirements, palatability concerns for bitter tasting drugs and the need of several functional excipients including preservatives, antioxidants, sweeteners and solvents and/or solubility enhancing excipients. Based on the physicochemical properties of famotidine, various dosage form types are possible (Figure 19). However, for the successful development of age-appropriate medicines one must consider other factors including dose banding, drug excipient compatibilities and guidance and recommendations from regulatory bodies and/or health agencies such as the European Medicines Agency (EMA) or World Health Organization (WHO).

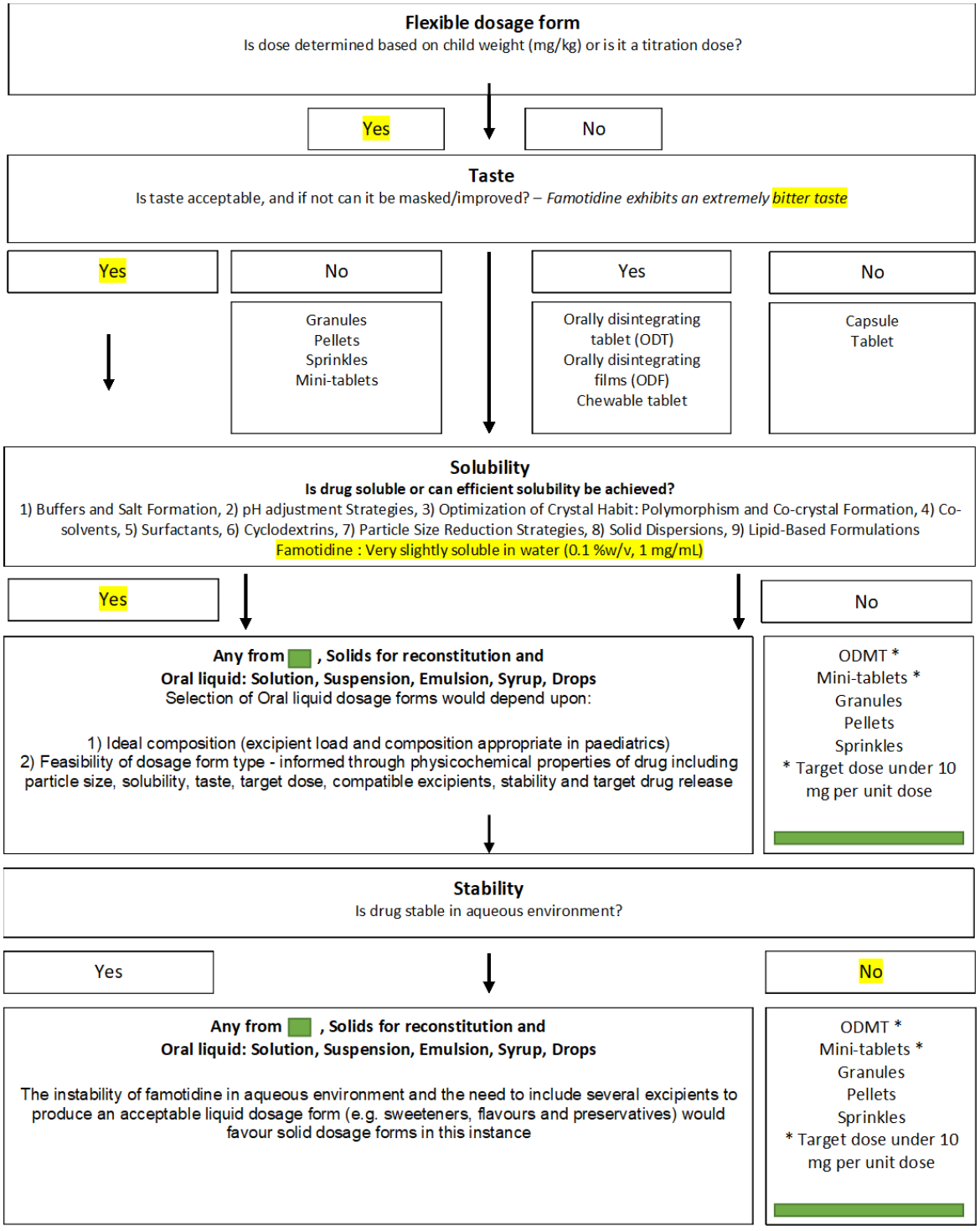
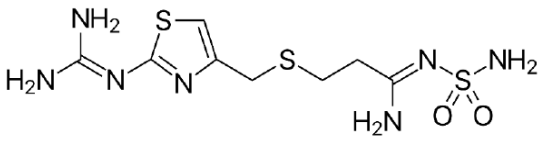


Figure 19: Systematic flow chart that identifies appropriate and feasible dosage form types. Dose flexibility and inherent drug physicochemical properties are considered.

Table 28: Overview of the physicochemical properties of famotidine(336).

Famotidine Monograph	
Chemical Name	3-[2-[(aminoiminomethyl) amino]-4-thiazolyl-methylthio]-N-(aminosulfonyl)-propanimidoamide
Other Names	Famotidine HCl
Molecular Formula	$C_8H_{16}ClN_7O_2S_3$
Molecular Weight	373.9
logP	-0.64
pKa	6.7
CAS Number	125193-62-6
Famotidine Structure	
Appearance	White to light yellow crystalline powder
Melting point	163–164° C
Solubility	Freely soluble in glacial acetic acid (500 mg/mL), slightly soluble in methanol (3 mg/mL), very slightly soluble in water (1 mg/mL) and practically insoluble in ethanol (<0.1 mg/mL)(336).
Sensitivities	Light and acidic conditions

4.4.2 Dose banding and proposed strengths of famotidine mini ODTs

Famotidine dosing recommendations for paediatric patients are not well established and have not been listed within the British National Formulary for Children (BNFc). In light of the available pharmacokinetic/dynamic studies and the variety of doses used in clinical trials, famotidine is usually administered at 0.5 to 1.0 mg/kg once or twice daily within the paediatric population (361-364). Two tablet strengths (2 mg and 10 mg) were chosen to fulfil various dose requirements of children aged between 1-12 years. The selection of the proposed strengths aim to provide enhanced dosing flexibility, where different strengths can be combined to make up the required dose, whilst keeping administration quantities as low as possible. In order to reduce any potential risk of confusion when handling/administering different strengths, perhaps the 7 year olds would be better just starting off with one 10 mg tablet, and 10 year olds with perhaps two (Table 29). Either way, it would be essential to have some way of distinguishing between the two with markings and/or colour, possibly.

Table 29: Dose banding and number of tablets required to fulfil dose respective requirements.

Dose	Age and average child Weight (kg) (229)	Number of tablets required to fulfil dose requirement		
		Dose (mg)	*2 mg*	*10 mg*
0.5 mg/kg	1 year: 9 kg ≈ 9 kg	4.5 ≈ 4	2	–
	3 years: 14 kg ≈ 15 kg	7.5 ≈ 8	4	–
	5 years: 18 kg ≈ 20 kg	10	–	1
	7 years: 23 kg ≈ 25 kg	12.5 ≈ 10	–	1
	10 years: 32 kg ≈ 30 kg	16 ≈ 20	–	2
	12 years: 39 kg ≈ 40 kg	20	–	2
1 mg/kg	1 year: 9 kg ≈ 10 kg	10	–	1
	3 years: 14 kg ≈ 14 kg	14	2	1
	5 years: 18 kg ≈ 20 kg	20	–	2
	7 years: 23 kg ≈ 24 kg	24	2	2
	10 years: 32 kg ≈ 30 kg	30	–	3
	12 years: 39 kg ≈ 40 kg	40	–	4

4.4.3 Existing famotidine products and rationale for selection of ODMT

A compilation of current marketed famotidine products including available strengths and inactive ingredients was prepared. This allows us to compare the current formulations and identify any gaps/improvements that can be applied when selecting and developing our version of an age-appropriate formulation of famotidine.

All current marketed liquid preparations contain three preservatives and sucrose, neither of which are ideal for a paediatric formulation (Table 30). The preservatives included are sodium benzoate and parabens. The EMA have concluded that, if possible, inclusion of preservatives and antioxidant in paediatric formulations should be avoided and their use should be justified (135). Parabens demonstrate oestrogen binding affinities, suggesting oestrogenic activity with potential reproductive effects (136). Furthermore, possible endocrine-disrupting effects have also raised concerns for paraben use in paediatric formulations (137). With benzoic acid (and its Na or K salts), an important safety concern is its ability to displace bilirubin from albumin. This is more of a concern in preterm and full-term neonates, since immaturity of metabolic enzymes may result in accumulation of benzoic acid (365).

PEPCID RPD™ contains two potentially problematic excipients including aspartame and red ferric oxide (colouring agent). Aspartame consist of phenylalanine, which may be harmful in children with phenylketonuria (leading to mental disorders and seizures) (117). Colouring agents including iron oxides are associated with concerns of hypersensitivity, negative behavioural effects and ADHD. The allowable daily intake allowance of aspartame is set at 50 mg/kg, whilst a total daily allowance of iron (including iron oxides) has been set of 40 mg

(145, 146). The composition of Fluxid™ consists of methacrylic acid copolymer and sucrose. In children, the use of methacrylic acid copolymer has been associated with fibrosing colonopathy and gastrointestinal toxicity (130). Sucrose is a disaccharide, consisting of fructose and glucose. Although, sucrose is GRAS listed, long term sucrose use may lead to obesity, tooth enamel deterioration and dental caries (366).

All other tablet preparations (generic famotidine, Pepcid® and Zantac-360) either consist of potentially problematic excipients that may not be suitable for the paediatric population or have a composition comprised of too many excipients including methacrylic acid copolymer, sodium benzoate, parabens and colouring agents (Table 28). Nonetheless, all marketed products have a strength aimed for adults and would require some sort of manipulation if given to children.

The World Health Organization (WHO) has considered flexible solid oral dosage forms as the most suitable dosage form for children (180). Preparations include orodispersible, chewable and soluble tablets. Such dosage forms relieve the stresses of swallowing, as the dosage form is intended to disperse in the mouth/liquid before swallowing. Flexible solid oral dosage forms hold advantages inherent to both liquid (flexible dosing capabilities and ease of swallowing) and solid (formulation stability and low production cost) dosage forms, while minimising their respective disadvantages (148). Currently, the focus of developing age-appropriate formulations is with flexible solid dosage forms that are easy to swallow and well accepted throughout the whole paediatric population (181, 182). Given the current recommendations from WHO for innovative small dosage forms for paediatric use and the lack of suitable dosage forms with age-appropriate design and dose flexibility has led to the recommendation to develop an age-appropriate formulation as an ODMT.

Considering the extensive variability between the paediatric populations, a systematic approach to paediatric formulation development should be employed to ensure safe and effective treatment, where paediatric specific drug delivery systems are available which contain non-toxic excipients, are palatable, grant minimal dosage and frequency, are applicable to all ages and exhibit easy and reliable administration. Although many age-appropriate formulation types were possible, the decision to develop an ODMT would benefit from improved chemical stability as famotidine is in solid form. Additionally, it is anticipated that there would be improved bioavailability due to some buccal absorption (367). Multiple strengths allow for enhanced dosing flexibility, ensuring accurate dosing and applicability across the whole paediatric population. Lastly, compared to liquid formulation options that require increased production and storage challenges, the choice of an ODMT would possibly be the simplest and/or cheapest option to formulate using either a dry granulation or direct

compression approach. The choice to manufacture a famotidine ODMT aims to provide an age-appropriate formulation option for the treatment of paediatric GORD and peptic ulcers; however, there are some limitations that are worth mentioning. Because of their small size, mini-tablets present several challenges from a formulation perspective including blend homogeneity, flowability, and conent uniformity (368). Additionally, several strengths would need to be manufactured in order to provide adequate dose flexibility. Also, other than requiring moisture-resistant packaging to prevent any moisture related negative impacts on tablet properties, it would also be advisable to consider packaging that simplifies the selection of different strengths and avoids accidental dosing errors.

Table 30: Overview of current market formulations including dosage form type, marketing authorisation (MA) holder and list of inactive ingredients.

Product	Medicinal form	MA holder	Inactive ingredients
Famotidine 20mg & 40mg	Tablet	Tillomed Laboratories Limited	Microcrystalline cellulose, Lactose monohydrate, Macrogol 4000, Magnesium stearate, Hypromellose, Sodium starch glycolate (type A), Silica, colloidal anhydrous, Pregelatinized starch, Talc, Titanium dioxide
Zantac-360 20 mg	Tablet	Sanofi	Carnauba wax, Corn starch, Hydroxypropyl cellulose, Hypromellose, Magnesium stearate, Microcrystalline cellulose, Red iron oxide, Sodium starch glycolate, Talc, Titanium dioxide, Yellow iron oxide
Pepcid® 20mg & 40mg	Tablet	Merck & Co.	Hydroxypropyl cellulose, Hypromellose, Iron oxides, Magnesium stearate, Microcrystalline cellulose, Corn starch, Talc, Titanium dioxide, and Carnauba wax
Pepcid® 40mg/5mL	Oral suspension	Merck & Co./Salix Pharmaceuticals Inc.	Citric acid, Flavours, Microcrystalline cellulose, Carboxymethylcellulose sodium, Sucrose, Xanthan gum, Sodium benzoate 0.1%, Sodium methylparaben 0.1%, and Sodium propylparaben 0.02%
PEPCID RPD™ 20mg & 40mg	ODT	Merck & Co.	Aspartame, Mint flavour, Gelatine, Mannitol, Red Ferric oxide, and Xanthan gum
Fluxid™ 20 mg & 40 mg	ODT	Schwarz Pharmaceuticals	Citric acid, Colloidal silicon dioxide, Corn starch, Crospovidone, Hypromellose, Magnesium stearate, Mannitol, Methacrylic acid copolymer, Microcrystalline cellulose, Natural and artificial cherry flavour, Sodium bicarbonate, Sucralose and sucrose

Famotidine 40mg/5mL (Generic)	Powder for Suspension	Lupin Pharmaceuticals Inc.	Monohydrate citric acid, Powdered cellulose, Sucrose, Xanthan gum and banana, cherry and peppermint wash flavour, Methylparaben sodium, Propylparaben sodium and Sodium benzoate
Famotidine 40mg/5mL (Generic)	Powder for Suspension	Hi-Tech Pharmacal Co. Inc.	Citric acid, Confectioner's sugar, Natural and artificial cherry flavour, Natural and artificial peppermint flavour, Natural banana flavour, Sodium hydroxide, and xanthan gum, Methylparaben, Propylparaben, and Sodium hydroxide

4.4.4 Prior famotidine ODT development strategy

Lura *et al* compared isomalt with a mannitol based co-processed excipient in orodispersible mini-tablets for paediatric use. Both, high and low drug loaded ODMTs were successfully produced by direct compression of formulations consisting of commercialised tabletting aids: isomalt (galenIQ™721) and Ludiflash®. Compared to Ludiflash®, isomalt based formulations complied to USP dissolution requirements, even without a disintegrant, and also provided a greater degree of freedom to further optimise dosage form properties (369).

The patent filed by Pilgaonkar *et al.* (370) details a composite that is prepared by spray drying a water-soluble excipient with calcium silicate, the composite is then tableted via direct compression to provide an ODT with fast dispersing times. However, the multi-step process may prove to be uneconomical and complicated.

Ahuja *et al.* (371) protected a famotidine ODT formulation that incorporates inulin and the drug, followed by sublimation of the solvent and forming the ODT. The patent claims that the ODT formulation exhibits fast dispersing times. Building on this, a new patent was submitted by Sonavane *et al*. in 2018 (372) that provides for an improved third generation fast disintegrating dosage form based on a matrix comprising a combination of maltodextrin and hyaluronic acid (HA) or a pharmaceutically acceptable salt thereof. The invention claims to provide fast disintegrating dosage forms with relatively high tensile strength.

A study carried out by Furtado *et al.* (2008) evaluated the effect of camphor as a subliming agent on the mouth dissolving property of famotidine tablets (373). Famotidine disintegrating tablets were prepared with camphor as the subliming agent and sodium starch glycolate in combination with croscarmellose sodium as superdisintegrants. The formulations were tested for weight variation, hardness, friability, drug content, wetting time, *in vitro* and *in vivo* dispersion, mouth feel, and *in vitro* dissolution. All the formulations showed low weight variation with dispersion time less than 30 seconds and rapid *in vitro* dissolution. Formulations including camphor displayed significantly reduced wetting time and *in-vitro* dispersion time.

However, the harmful effects of exposure to small amounts of camphor-containing products on infants and children have long been recognised and therefore should be used with caution (374).

A patent filed by Yutaka *et al* in 2022 (375), protected a famotidine containing ODT. The key to this invention was matching the particle size distribution of famotidine with that of the diluent (lactose). Pharmatose 125M (size distribution, particle shape & morphology of sieved lactose provide good flowability and mixing characteristics) was employed as the diluent, polyvinylpyrrolidone as a binder, whilst menthol and sweetener were used as taste enhancers. Famotidine and excipients were granulated using ethanol.

Reviewing the current market/patent landscape has helped to inform and conceptualise the formulation approach that has been perused within (Table 31). These included identifying key excipients and techniques that provide good flowability, fast disintegration times and content uniformity. However, as in line with the lack of available information on paediatric dosage form technologies, the current market/patent landscape for famotidine ODT did not consider the paediatric population.

Table 31: Prior and/or current reported methods for the development of famotidine ODTs.

Author	Formulation approach	Key characteristics
P.S. Pilgaonkar *et al*, WO2007/113856, Filed 31 Mar 2006.	Spray-drying a water-soluble excipient with calcium silicate	Fast dispersing times
V. Ahuja *et al*, WO2011/120903, Filed 28 Mar 2011. Followed by Sonavane *et al*, WO2018 / 130603 Filed Jan. 11 , 2018	Lyophilisation - a drug is physically trapped in a matrix composed of inulin Matrix comprising a combination of maltodextrin and hyaluronic acid (HA) or a pharmaceutically acceptable salt thereof	Relatively high tensile strength Fast dispersing times
S. Furtado., *et al* (373)	Use of camphor as a subliming agent in combination with super-disintegrants	Low weight variation with dispersion time less than 30 seconds and rapid in vitro dissolution
Lura, A., *et al* (369)	Mini-tablets were successfully produced by direct compression of formulations based on commercialized tabletting aids: isomalt (galenIQ™721) and Ludiflash®	Fast and complete drug release Simplified formulation
M. Yutaka *et al*, JP2002087958, Filed 08 Sep 2000	Match the particle size distribution of famotidine with that of the lactose diluent.	Fast disintegration times

	The components (Pharmatose 125M (diluent) polyvinylpyrrolidone (binder), sweetener and menthol (flavour)) of the formulation were granulated using ethanol	

4.4.5 Development of paediatric excipient 'working zone' for ODTs and pre-screening

This section adds to the excipient working zone of solid dosage forms as introduced above (section 2.4.3) and list potential excipients that can be exploited to provide the desired characteristics, at the same time being appropriate for paediatric use.

Commonly used fillers/binders used in the production of ODTs include polyols, cellulose derivatives, starch (including modified starches), calcium salts and sugars. Disintegrants can be divided as traditional disintegrant or superdisintegrant and include starch, sodium starch glycolate (SSG), croscarmellose sodium (CCS) and crospovidone. Tabletting excipients include magnesium stearate and fumed silica while taste enhancers included either sweeteners or flavouring agents. An inclusion criteria for excipient candidates for safe paediatric use was defined. Only excipients that are listed in the FDA GRAS database alongside being considered safe for paediatric use according to the STEP database and relevant literature were included within the excipient working zone. Although many excipients were included within the excipient working zone, excipients that were able to provide multiple functionalities were given preferences in order to limit the total number of excipients within the model formulation. The following excipients were included within the excipient working zone for the development of ODTs (in no specific order):

Microcrystalline cellulose (MCC) is commonly used as a binding agent due to its exceptional dry binding properties, safety profile and low cost. MCC deforms plastically, exhibits relative low bulk density and high surface area. Such properties contribute to the unique binding properties of MCC that provide mechanical robustness and cohesiveness to tablets, even at small compression forces (233). In addition, at concentrations of 5-15%, MCC also exhibits disintegrating properties, making it a useful excipient in tabletting of ODTs via the direct compression method (234). Cellulose, in its original form is tightly bound together by several intermolecular hydrogen bonds and van der Waals forces, resulting in a water insoluble fibrous substance that is inert and with no significant absorption (376). Upon evaluation of chronic toxicity studies performed with cellulose and cellulose derivatives, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that it is not required to determine a numerical ADI and therefore allocated an acceptable daily intake (ADI) of 'not specified' (377).

Since milk, including breast milk and formula milk, contain lactose, lactose monohydrate is considered safe for paediatric consumption. Other than possessing excellent compressibility profiles, lactose is commonly employed as a diluent/binder to produce tablets with sufficient mechanical strength while maintaining good disintegration properties (378). Other significant factors contributing to the popularity of lactose as an excipient include low cost, palatability (bland taste), compatibility with other constituents, stability and very good aqueous solubility (378). Despite the desired properties of lactose, there are some disadvantages associated with lactose that are worth mentioning. These include, the cariogenic potential of lactose and inappropriateness in individuals with lactose intolerance or galactosaemia. Nonetheless, the cariogenic potential of lactose does not affect its safety profile since the residence time of the formulation is brief and the quantities ingested as an excipient is no more than a couple of millilitres of milk (379). Furthermore, lactose is well tolerated by most children. Whilst levels of lactose present in most pharmaceutical preparations is insignificant to bring about symptoms (flatulence, cramping and diarrhoea), sensitivity to lactose is highly variable in severity, and undesirable symptoms have been reported after lactose ingestion of as little as 3 g or less (126). However, the EMA have suggested a threshold of 5 g of lactose per dose (127).

Mannitol is a widely used excipient that features good mechanical properties, rapid dissolution, and a pleasant taste. Due to its properties, it is often used as a diluent or binder in orally disintegrating formulations. However, one should be aware of its laxative effect when consumed in large quantities (≥20 g) (380). During preformulation experiments, model formulations with desirable disintegrating times required at least 40% of mannitol and up to around 80% without a specified functional disintegrant/superdisintegrant within the formulation composition (data not shown). Even with around 80% w/w mannitol, a typical amount of mannitol ingested in a mini tablet would be around 20-40mg. This would be well below the allowable daily threshold of 10 g (381).

Starch and its derivatives are multifunctional excipients that are widely employed in orally disintegrating tablet formulations. As an excipient, starch's versatility lies largely in its derivatisation, where derivatives offer advantages including improved flowability, compressibility and disintegrant and binding properties (79). Examples of some commonly used modified starches include maltodextrin, pregelatinised starches and cyclodextrins. Following safety re-evaluation in 2017, based on the short and long-term toxicity, carcinogenicity and reproduction toxicity, the EFSA concluded that "no safety concern exists for the use of modified starches as food additives at the reported use levels and applications for the general population" and that a numerical ADI is not necessary (382).

The European Medicines Agency (EMA) requires a tablet to completely dissolve within 3 minutes to be designated as an orodispersible tablet, while the Food and Drug Administration (FDA) believes an ODT should dissolve within 30 seconds *in vitro* (155, 230). A disintegrant is an additive that helps tablets break down when they are in contact with liquids, which results in the fragmenting of the tablets into smaller pieces. Traditional disintegrants include starch, whilst superdisintegrants include sodium starch glycolate (SSG), croscarmellose sodium and crospovidone. In the preparation of dispersible tablets and fast-dissolving tablets, superdisintegrants are often used. Generally speaking, superdisintegrants are products of natural or synthetic pharmaceuticals that are modified so that their swelling properties are enhanced, which facilitate significant water uptake and the subsequent disintegration when delivered to their intended target sites (383).

Crospovidone is a cross-linked, water-insoluble superdisintegrant that exerts its disintegrating effects through capillary action and swelling. Upon evaluating the safety of crospovidone (E 1202) and owing to the lack of adverse effects in the available repeated dose toxicity studies, the JECFA Panel concluded that there is no need for numerical acceptable daily intake (ADI) value (384). SSG is the sodium salt of cross-linked carboxymethyl starch, which acts as a superdisintegrant by rapidly expanding due to high water adsorption, resulting in faster disintegration. Among children, the acceptable daily intake (ADI) is at 80 mg/kg body weight (385).

Sucrose is a disaccharide, consisting of fructose and glucose. It is naturally produced in plants and is commonly used as a sweetener in pharmaceutical formulations. Both sucrose and fructose increase blood sugar levels, therefore should be avoided in patients who are diabetic. Their use in children suffering from hereditary fructose intolerance is also contraindicated (114). Moreover, long term fructose/sucrose use may lead to obesity, tooth enamel deterioration and dental caries, hence their use in long-term therapy is discouraged. Instead, non-caloric non-nutritive sweetening agents such as aspartame, saccharin, acesulfame-potassium and sucralose are preferred (115). These compounds have been approved as an ‘additive’ by the FDA, are GRAS listed and provide the necessary palatability without promoting calorie intake (116). Nevertheless, due to adverse effects, the FDA have established maximum allowable daily intake values for all artificial sweeteners (refer to table in introduction chapter). Aspartame consist of phenylalanine, which may be harmful in children with phenylketonuria (leading to mental disorders and seizures) (117). Similarly, saccharin should be avoided in children who are allergic to sulphonamides as it may lead to hypersensitivity reactions (118). Xylitol, and sorbitol are two alternative sweetening agents (polyols) that are not readily absorbed from the gut and therefore are suitable in children with diabetes, though they may cause gastrointestinal disorders and diarrhoea (119). Comparable

to sweeteners, artificial flavours too have a questionable reputation and are suspected of activating sensitivities.

Tabletting agents including magnesium stearate and Colloidal silica dioxide are usually incorporated in small amounts (that do not warrant any concern) to help with the tableting process. Magnesium stearate is a lubricant that helps reduce friction between the tablets and die surface during ejection and is usually incorporated at concentration of 0.25–5.0% in tablet and capsule manufacture, whilst silica is employed as a glidant at concentrations of 0.1–1.0% to improve the flow of powders during tablet manufacturing by reducing friction and adhesion between particles (234).

To minimise potential excipient related risks and to limit the number of exceptions within the formulation, it was decided to not consider the use of sweeteners and flavouring agents within this study. Instead, mannitol was selected as a dual function excipient that was intended to be used as a sweetening agent and diluent, whilst MCC was employed as a binder and disintegrating agent. From all pre-formulations screened, two formulations were selected for ODMT production. The two strengths formulated had a famotidine concentration of 4% and 20% respectively (Table 32).

An important consideration that should be addressed with regards to ODMTs is the taste and palatability since the product is intended to quickly disintegrate within the oral cavity before swallowing. For the focus of the current work, sweeteners and flavours were not incorporated, rather the composition aimed to provide the following; 1) a fine-tuned ODT composition that fulfils the disintegration and mechanical strength criteria, whilst using minimal excipients. 2) Validation of the OMDT composition success as introduced in chapter 2 (section 2.4.5). Once a base formula has been generated and validated in regards to fulfilling ODT requirements, different sweeteners and flavours can always be added if needed. Nonetheless, the concentration of mannitol used (>60 % w/w) may suggest effective taste masking.

Table 32: Optimised composition of famotidine ODMTs. F1: 2 mg ODMT, F2: 10 mg ODMT.

Composition	Formulation 1 (F1) % w/w	Formulation 2 (F2) % w/w
Mannitol	80.25	64.25
MCC	15	15
Famotidine	4	20
Colloidal silica dioxide	0.25	0.25
Magnesium stearate	0.5	0.5

4.4.6 Challenges faced during pre-formulations and process parameters optimisation

The main challenges faced during pre-formulations was ensuring dose accuracy (content uniformity) and appropriate flowability, since experiments during preliminary screening displayed impaired flowability with increasing famotidine concentrations. This is because famotidine has a volume median diameter (VMD) of 15.71 ± 0.025 µm and has poor flowability. Blending parameters investigated included mixing time, speed and order of addition of excipients. A mixing time of five minutes at a speed of 250 rpm with MCC added first proved to be the optimised parameters that resulted in improved content uniformity (optimised parameters were identified, applied and validated from the carvedilol ODMT manufacture study). According to the angle of repose (19.6 and 18.4), both formulations (4% and 20% drug load) displayed excellent flowability. Since a dedicated disintegrant/superdisintegrants was excluded within our formulations, the effect of processing parameters including compression force and concentration of excipients greatly impacted the physical characteristics of the ODMTs. A compression force of 9.81 kN with quick release with the following concentrations of excipients (Table 32) resulted in ODMTs with desired characteristics including adequate mechanical robustness, low friability and rapid disintegrations times.

4.4.7 Pre Compression Material Characterisation

Application of conventional tableting equipment and with the availability of tableting excipients that provide improved flow, compressibility, and disintegration properties, direct compression is the easiest and most cost-effective method for manufacturing orally disintegrating tablets (ODT) (386). However, successful tabletting through direct compression may be challenging when using high or low doses of API. Many APIs possess poor compressibility profiles which may negatively affect tablet production if required in large amounts, whilst low dose APIs may present a challenge in achieving good content uniformity and blend homogeneity (387).

The cohesiveness of a material may significantly influence flow properties. Cohesive materials have an average particle size of less than 50 µm and tend to aggregate, leading to intermitted flow (251). On the other hand, non-cohesive materials have a larger particle size (>50 µm) and are expected to have good flow properties for successful tableting via the direct compression method (252). The volume mean diameter (VMD) of famotidine was 15.71 ± 0.03 µm, suggesting its cohesive nature and tendency to aggregate (Table 33). MCC and mannitol on the other hand displayed a larger VMD of 84.20 ± 0.40 µm and 70.92 ± 3.27 µm, suggesting non-cohesive behaviour. Larger particles, as in the case of MCC and mannitol exhibit better flow compared to materials with smaller particle sizes due to the reduced surface area available for inter-particulate interactions (253). Magnesium stearate showed a VMD of 17.4 ± 1.96 µm indicating its cohesive nature. However, magnesium stearate when used in small

quantities, forms a boundary layer around particles in the formulation, resulting in reduced friction between particles and optimising the flow of the powder blend (80). The hydrophobic nature of magnesium stearate also reduces inter-particulate interactions by minimising van der Waals forces, thereby improving flow (254).

Other than inherent material characteristics, mixing of particulate solids is an important process step in achieving homogenous blends and key to assuring that each resulting unit dose possesses the specified amount of drug. Blending optimisation requires careful consideration of order of addition of excipients together with fine tuning of process parameters. Optimised mixing parameters identified in the work above (section 2.4.7) was referred to when preparing famotidine powder blends. A total mixing time of 5 minutes at a speed of 250 rotations per minutes and with MCC added first provided optimised conditions for successful tableting and content uniformity.

Initially, it was expected that longer mixing time would provide better content uniformity, since the API particles would have more contact time with other particles, increasing the chances of collision and resulting in improved homogeneity (262, 263). However, similar to the findings observed in section 2.4.7, an increase in mixing time to fifteen minutes increased the percentage recovery of famotidine (data not shown). The increase in mixing time may have led to the segregation of famotidine particles, owing from its particle size, density, and cohesive nature (264). Denser particles, as in the case of famotidine, promote de-mixing and non-homogeneity in the mix, since dense particles consistently move downwards and settle at the base of the powder blend. Additionally, longer blending times impose greater particle-particle collisions, resulting in increased particle mobility and dilation of the powder bed.

Table 33: Particle size analysis of starting materials and formulation blends. Results are presented as the mean (n=3) and standard deviation.

Sample	VMD (µm)
Mannitol	70.92 ± 3.27
MCC	84.20 ± 0.40
Famotidine	15.71 ± 0.03
Magnesium stearate	17.49 ± 1.96
F1	72.93 ± 2.45
F2	59.61 ± 1.94

The angle of repose (AOR) indicated famotidine to possess poor flow (46.20 °) (Table 34); this is expected, because the small particle size of famotidine means there is a greater surface area available for interaction between particles. Magnesium stearate showed passable flow (40.33 °), however, it is well known that lubricants improve flow properties when added to powders. MCC and mannitol showed excellent and fair flow properties (23.73 and 32.97°). It is important for MCC and mannitol to possess good flow properties as they will dictate the overall flow of the formulation blend, since they are at high concentrations and make the bulk of the powder blend. After incorporating CSD, the angle of repose for formulations 1 and 2 both displayed excellent flowability. Flow aids enhance flow by adsorbing onto the particles within the formulation, thereby increasing surface roughness of host particles, resulting in reduced van der Waals forces (255).

According to Carr's index and Hausner ratio, mannitol exhibited very poor flow (33.33% and 1.5) whereas MCC shows passable flow (23.08% and 1.3) (Table 34). These results are in disagreement with results presented by the angle of repose. This may be due to the shape of the particles, where angular particles, as in the case of MCC, may initially pack loosely but when subjected to force result in significant repacking, resulting in higher Hausner ratio and leading to an untrue representation of actual flow property (256). The fibrous and irregular particle shape of MCC may also contribute to its inter-particulate void volume, leading in a higher volume to mass ratio (219, 257). The same can be said for F1 and F2 where flow evaluation based on CI and HR indicates poor flow, where in fact, both formulation blends actually possess good flowability.

Table 34: Angle of repose, Carr's index (CI), Hausner ratio (HR) and evaluation based on CI and HR.

Sample	AOR (°)	CI (%)	HR	Flow property based on CI and HR
Mannitol	32.97	33.33	1.50	Very, very poor
MCC	23.73	23.08	1.30	Passable
Famotidine	46.20	57.89	2.38	Very, very poor
Magnesium stearate	40.33	46.78	1.82	Very, very poor
F1	19.60	35.00	1.54	Poor
F2	18.40	25.00	1.33	Poor

4.4.8 ODMT Evaluation

Both formulations produced a balance between mechanical strength and fast disintegration F1 and F2 both displayed a balance between mechanical strength and fast disintegration times (Table 35). Friability was below the 1% European pharmacopeia limit for both formulations, suggesting adequate mechanical strength required for during transportation and handling. Formulations disintegrated within the recommended US Food and Drug Administration (FDA) disintegration time limit of 30 s (and well within the European Pharmacopoeia limit of 3 minutes) (267, 268). Fast disintegration times allows tablets to be safely administered to children either directly into the mouth or suspended on a spoon prior to administration. The British Pharmacopeia (BP) states that in order for the dosage form to fulfil the requirement, the content of famotidine in each unit dose must be between 95-105% of the stated amount (388). The percentage recovery of both strengths were within the specified values, suggesting good content uniformity of the powder blends.

Table 35: Tablet properties. Hardness value and disintegration time presented as mean ± standard deviation (n=3). Content uniformity presented as mean ± standard deviation (n=10).

Formulation	Hardness (N)	Friability (% loss)	Disintegration (s)	Content Uniformity (%)
F1 (2 mg ODMT)	39.50±4.35 N	0.34	14.61±0.55	101.87±3.19
F2 (10 mg ODMT)	38.20±4.31 N	0.79	13.37±2.31	100.6±1.08

4.4.9 Dissolution Studies

Previously (as for carvedilol ODMT), for the evaluation of dissolution profiles of tablets used in paediatrics, paediatric biorelevant media was developed that mimicked various parts of the GI tract in regards to adjusted osmolality, pH, buffer capacity, bile components and phospholipid concentration. However, no significant difference was observed between adult and paediatric biorelevant media. This was also true for another study that was carried out by Van der Vossen *et al*. (2019) that concluded that the dissolution of nifedipine formulations was not affected by age-related changes, rather differences in dissolution profile were owing to the fed and fasted states (280). Therefore, for the purpose of this study, biorelevant media was not further modified. The intrinsic solubility of famotidine is 1 mg/mL (Table 28), which is adequate to maintain sink conditions in all tested biorelevant media. Adult simulated gastric and intestinal media (FaSSGF, FeSSGF, FaSSIF and FeSSIF), were prepared as presented by Jantratid et al. (220). Robust release profiles were observed for both the 2 mg and 10 mg famotidine ODMTs in FaSSGF, FeSSGF, FaSSIF and FeSSIF with complete dissolution

obtained within 2 minutes (Figure 20 and Figure 21). This can be related to the extremely fast disintegration times of famotidine ODMTs, where tablets disintegrate in under 15 seconds. Interestingly, although literature displays robust famotidine dissolution profiles, in our study the rate of dissolution was much quicker. This may be a result of the micronised size (VMD 15.71 ± 0.025 micron) of famotidine particles used within the formulation blends. Other than an increase in surface area with decreasing particle size, a small particle has a higher disjoining pressure than a large particle, so it has a higher interfacial solubility, and therefore results in an increase in dissolution rate (389). Nonetheless, the dissolution profiles suggest that the bioavailability of famotidine is not limited by dissolution and that incomplete absorption is a result of limited permeability and degradation in acidic environments.

Other than co-administration of food, anatomical and physiological differences observed in the GI tract between adults and paediatrics may also affect drug solubility and dissolution. Key variations include gastric and intestinal capacity, pH, gastric emptying rate, intestinal transit time and bile salt secretion. As mentioned earlier, the Biopharmaceutics Classification System (BCS) classifies drugs into one of four categories depending on its solubility and permeability. However, the adult BCS must be used with caution when applied to paediatrics. This is because factors including upper GI lumen fluid volume, dose and total volume being administered alongside differ between adult and paediatric populations and may alter the defined solubility within the BCS (280). To better predict paediatric *in vivo* drug performance, *in vitro* data should preferably be amalgamated with PBPK modelling and simulation that take into account factors influencing absorption and permeability such as gastric emptying rate, metabolising enzymes, active transport process and intestinal microflora composition (281, 282). However, paediatric GI physiology and factors affecting paediatric permeability (microbiota, transport systems and metabolising enzymes) are still not fully understood (280, 283). Bridging such gaps would certainly provide improved paediatric *in-vivo* prediction tools, compiled with superior biorelevant media reflecting specific paediatric subset characteristics, which would be of great interest for the paediatric pharmaceutical industry, regulatory authorities and most importantly child safety.

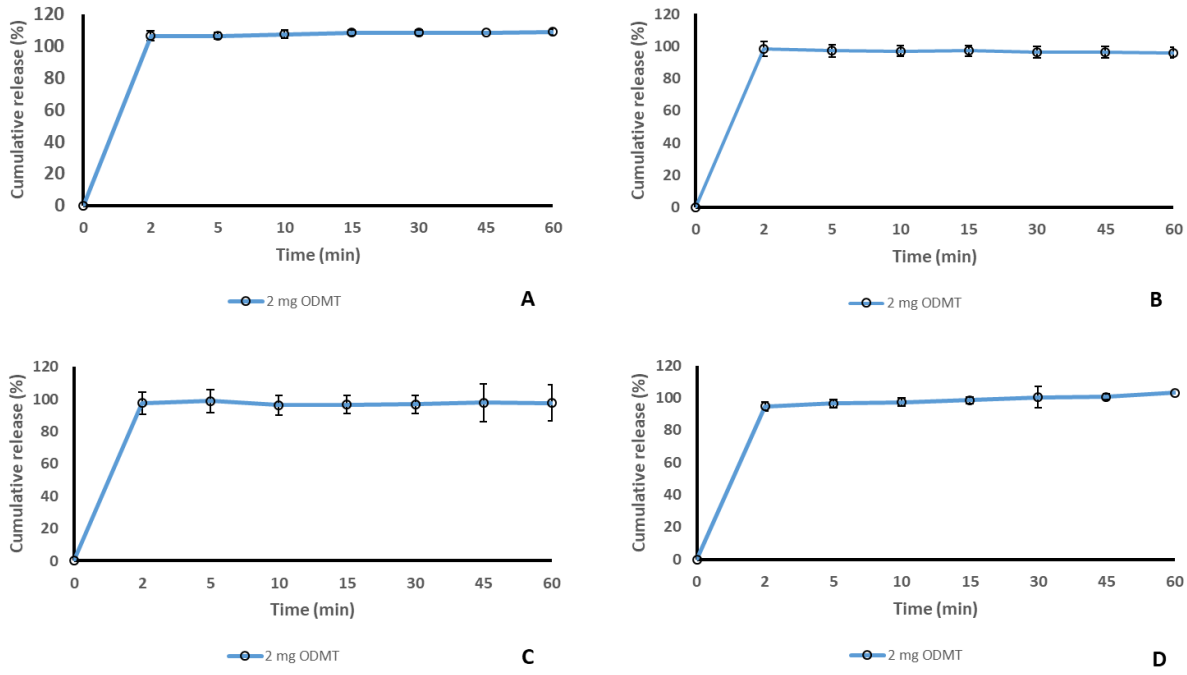


Figure 20: Dissolution profiles for famotidine 2 mg ODMT. A: FaSSGF, B: FeSSGF, C: FaSSIF, D: FeSSIF. Data presented as mean ± standard deviation (n=6).

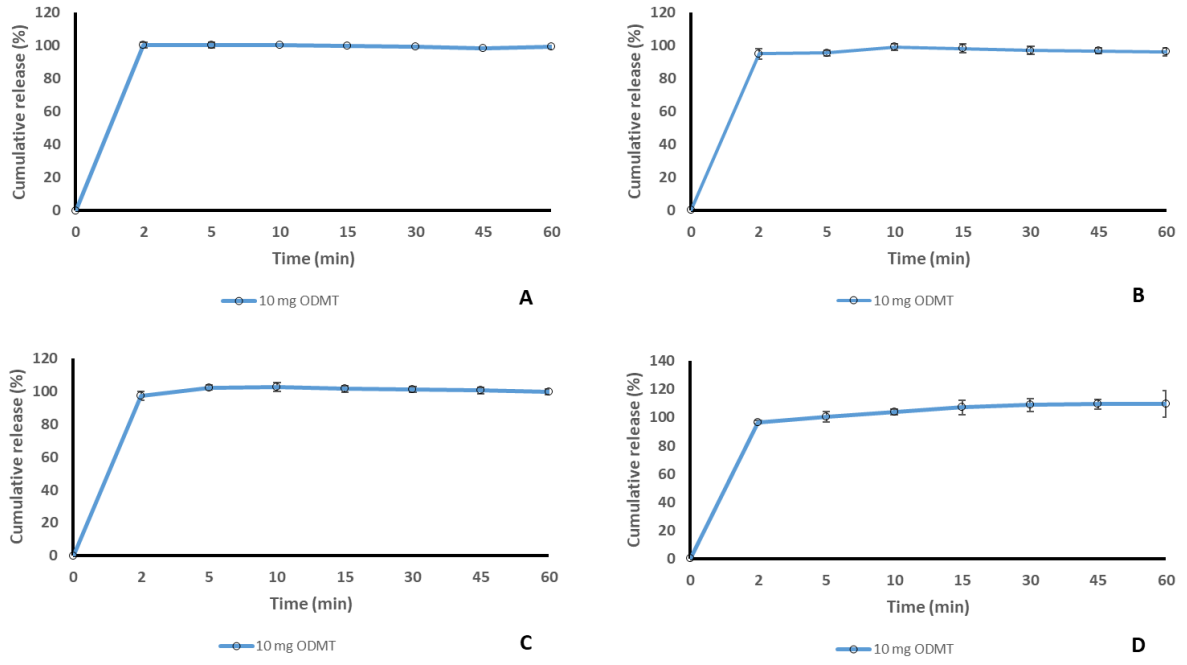


Figure 21: Dissolution profiles for famotidine 10 mg ODMT. A: FaSSGF, B: FeSSGF, C: FaSSIF, D: FeSSIF. Data presented as mean ± standard deviation (n=6).

4.4.10 Stability analysis

Other than analysing the percentage recovery of famotidine over a 6 month period (Figure 22), the mini-tablets were also tested for changes in their mechanical strength and disintegration times (Figure 23). Over the 6 month course of the stability testing (medium term) at 25°C and 60% RH, no 'significant' change was observed. The assay of both formulations remained within 5% of their initial values. Additionally, the formulations did not show any signs of physical tablet defects and no significant changes in hardness values or disintegration times were observed, thereby showing adequate stability according to the ICH guidelines (286). Famotidine displays extensive degradation under oxidative, acidic and basic conditions (390). As a solid dosage form, it would be appropriate to package the products in foil blisters to avoid permeation of oxygen, whereas instability in acidic and basic environments would require further optimisation if a liquid dosage form was to be chosen.

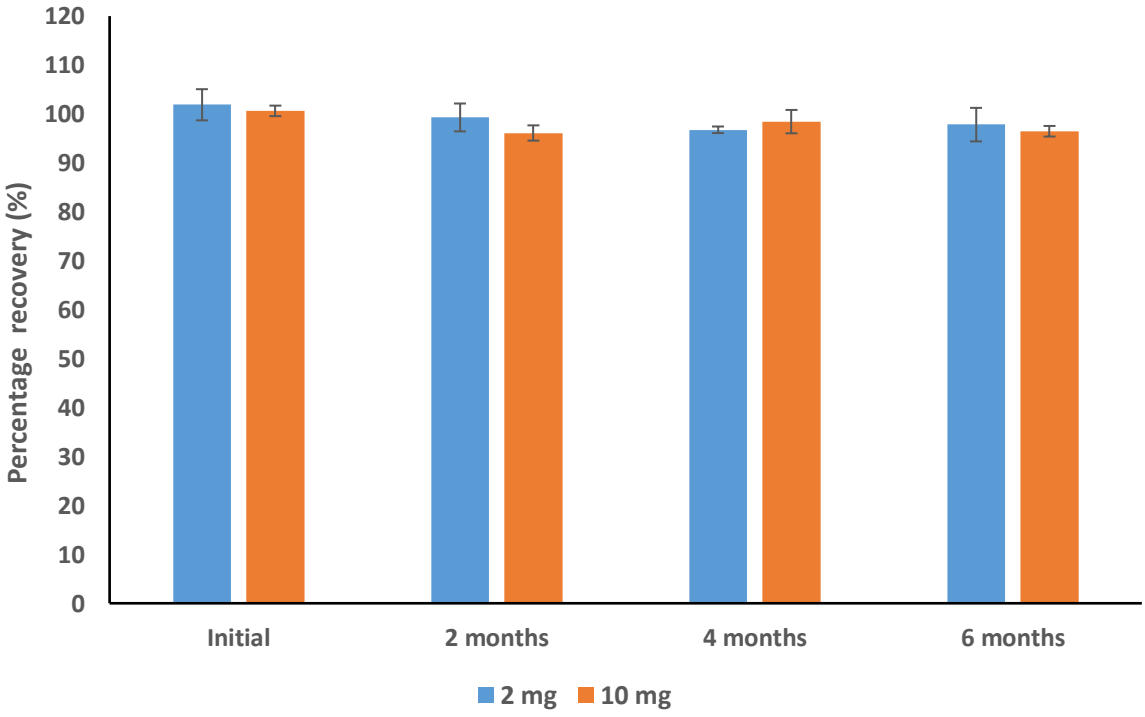


Figure 22: Percentage famotidine recovery for medium term stability. Data presented as mean ± standard deviation (n=3).

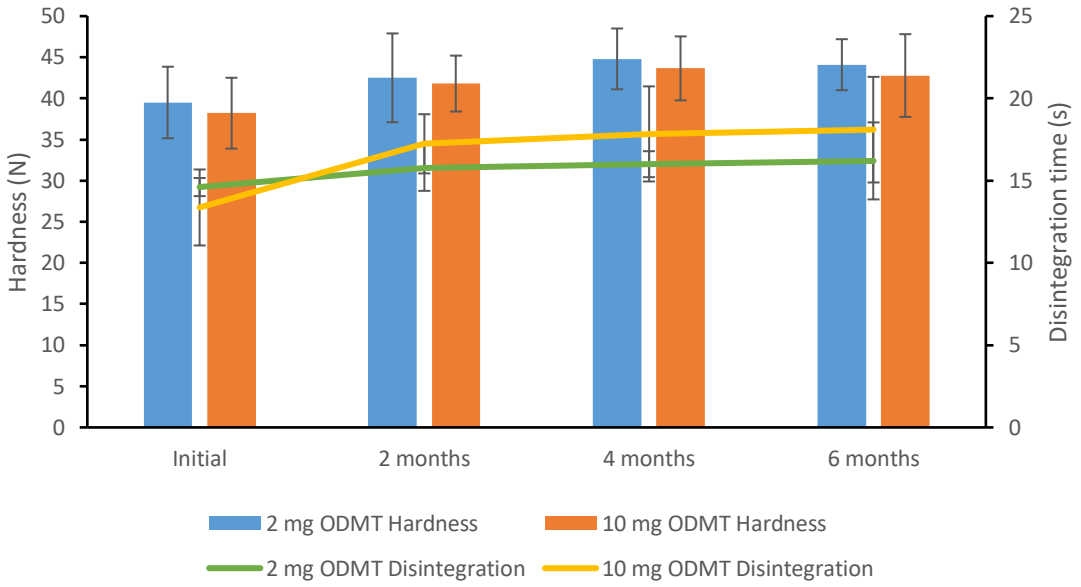


Figure 23: Change in hardness and disintegration times over the 6 month course of the stability testing (medium term) at 25°C and 60% RH. Data presented as mean ± standard deviation (n=3).

The criteria to determine any 'significant changes' are outlined by the ICH stability testing of new drug substances and products Q1A(R2) and are as follows:

11) A 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency when using biological or immunological procedures;
12) Any degradation product's exceeding its acceptance criterion;
13) Failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e.g., colour, phase separation, resuspendibility, caking, hardness, dose delivery per actuation); however, some changes in physical attributes (e.g., softening of suppositories, melting of creams) may be expected under accelerated conditions;

and, as appropriate for the dosage form:

14) Failure to meet the acceptance criterion for pH; or
15) Failure to meet the acceptance criteria for dissolution for 12 dosage units

4.4.11 Conclusion

An age-appropriate formulation of famotidine was successfully developed using the screening tool, as has been developed and applied in previous chapters. The effectiveness of the screening tool was validated and where needed optimised to better inform paediatric specific formulations that are appropriate, safe, effective and of high quality. Prior to formulation development, a detailed understanding of inherent physicochemical properties of the drug indicates potential challenges, feasibility and appropriateness of the intended dosage form. Moving forward, a comprehensive summary of current marketed formulated products help identify gaps within the market and opportunities to fulfil unmet needs. As a starting point, it is beneficial to explore current technologies and methods currently employed to develop the selected dosage form type as this allows to determine the most effective techniques that result in desired dosage form characteristics. These methods can then be further refined and modified to produce a superior product with great simplicity. Regarding excipient use in children, an inclusion criteria ensures all potentially problematic excipients are accounted for and that the resulting product is safe from any excipient related adverse effects.

Chapter 5: Clinically informed development of a novel extended release nifedipine mini-tablet for paediatrics using physiologically based pharmacokinetics (PBPK) simulation

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Work from this chapter has been published in MDPI Pharmaceutics - Khan, D., et al. (2023). "Virtual Clinical Trials Guided Design of an Age-Appropriate Formulation and Dosing Strategy of Nifedipine for Paediatric Use" Pharmaceutics 2023, 15, 556. https://doi.org/10.3390/pharmaceutics15020556.

5.1 CHAPTER AIMS AND OBJECTIVES

- Integrate PBPK modelling to a paediatric specific formulation development approach to clinically inform an appropriate dosage from design
- Identify and optimise dosing strategy
- Evaluate different grades and concentrations of HPMC for their effect on release rate
- Determine the mechanism of release using kinetic modelling
- Development of an age-appropriate extended release nifedipine formulation
- Characterise and evaluate formulation performance

Abstract

Nifedipine is a rapid acting antihypertensive agent with maximum peak concentrations being achieved within 30 to 60 minutes after oral administration. This rapid onset of action causes a precipitous reduction in blood pressure leading to adverse effects associated with reflex sympathetic nervous system (SNS) activation, including tachycardia and worsening myocardial and cerebrovascular ischemia. As a result, short acting nifedipine preparations are not recommended. Furthermore, current marketed dosage forms are often inappropriate for use in paediatric prescribing, leading to a dependence on unlicensed medicine use, increasing the risk of potential unknown adverse effects. Here we report a paradigm shift in the process of paediatric formulation development; PBPK modelling to inform innovative dosage form design of a patient centric sustained release mini tablet that will meet the needs of the paediatric population. In order to identify a possible dosing approach for use in children, dose adjustments were considered through 100 µg/kg increments to achieve the majority of subjects with trough plasma concentrations within the therapeutic window. A 5 mg sustained release mini-tablet was successfully developed with duration of release extending over 24 h and an informed optimised dosing strategy of 450 µg/kg twice daily. The resulting formulation provides flexible dosing opportunities, improves patient adherence by reducing frequent administration burden and enhances patient safety profiles by maintaining efficacious levels of consistent drug plasma levels over a sustained period of time.

Keywords: Paediatrics; PBPK; pharmacokinetics; age-appropriate; mini-tablets; modified release; adherence

5.2 Introduction

Nifedipine is a dihydropyridine calcium channel blocker that exerts its effect directly on vascular smooth muscle and myocardial cells and inhibits the influx of calcium ions by blocking voltage dependant L-type $Ca2^{+}$ channels. This results in reduced intracellular calcium, thereby reducing peripheral arterial vascular resistance and dilatation of coronary arteries, leading to improved myocardial oxygen delivery and reduced blood pressure (391).

The use of nifedipine was once well established, being one of the most widely prescribed medicines to treat hypertension. However, owing to safety and tolerability concerns and with the introduction of newer agents, the use of nifedipine has become less desirable (392). In 1995, concerns regarding the safety of nifedipine surfaced after a meta-analysis of clinical trials of nifedipine was published. The report concluded that the use of short-acting (SA) nifedipine in moderate to high doses results in an increase in total mortality in patients with coronary heart disease (393). Other reports have also mentioned safety concerns with SA nifedipine preparations, associating its use with cerebral ischemia and myocardial infarction (394, 395). As opposed to concerns regarding the safety of SA nifedipine in adults, studies evaluating the safety of SA nifedipine in children suggest otherwise, with several studies concluding SA nifedipine to be an important, safe, and effective oral antihypertensive agent (396-398).

The advantages of SA nifedipine use is the rapid onset of action and lack of central nervous system (CNS) depression; however, the precipitous reduction in blood pressure means nifedipine is associated with adverse effects, including reflex tachycardia, retinal ischemia and myocardial ischemia and infarction (399). Consequently, long-acting formulations have become available, addressing the drawbacks of SA nifedipine preparations. However, there are no modified release preparations of nifedipine authorised for paediatric use. Consequently, children are given unlicensed preparations where current marketed dosage forms are manipulated prior to administration, increasing the risk of potential unknown adverse effects (400-402). The challenge to develop such sophisticated dosage forms is owing to limited formulation development strategies for such formulations alongside little to no work reported on modified/sustained release paediatric formulations. Challenges are further compounded by the distinct differences seen in the way in which drugs perform in the paediatric population. Further, performing clinical studies in children is a challenge as children are an exceptional population with specific ethical and clinical concerns.

As a consequence of anatomical and physiological variances observed between children and adults, pharmacokinetic parameters can significantly differ and often lead to sub therapeutic and/or toxic plasma drug concentrations (403). The Simcyp™ (physiologically based

pharmacokinetics) PBPK simulator is one of the most sophisticated programs employed within the pharmaceutical industry to predict drug performance from virtual populations. Simcyp™ is especially useful in modelling drug performance, evaluating PK variability and determining dosing in paediatrics, since it contains extensive information on demographics, developmental physiology, and the ontogeny of drug elimination pathways. The considerations of such characteristics is important as they can greatly impact the pharmacokinetics of the drug under review.

For the first time, an attempt to integrate PBPK modelling to a paediatric specific formulation development approach to clinically inform an appropriate dosage from design and strategy is made. An integrated approach such as this not only aims to inform dosage from design to improve formulation safety profiles but also provide the necessary paradigm shift in paediatric formulation development that is vital for avoiding unnecessary paediatric studies, ensuring clinical trial dose selection is based on science, and minimising clinical trial enrolment.

In the present study, several grades and concentrations of Hydroxypropyl methylcellulose (HPMC) were compared for their effect on release rate. Resulting dissolution profiles were then evaluated to determine the mechanism of release using kinetic modelling. In order to pragmatically assess the translation of resultant dissolution profiles to the paediatric populations, virtual clinical trials simulations were conducted using physiologically-based pharmacokinetic (PBPK) modelling. In the context of formulation development, PBPK simulation was employed to determine and inform optimised formulations that achieve plasma concentrations that remain within the therapeutic window throughout the dosing strategy.

5.3 Methods and Materials

5.3.1 Materials

Nifedipine was purchased from Alfa Aesar (Lancashire, UK). Lactose monohydrate and magnesium stearate were obtained from Sigma-Aldrich (Dorset, UK), whilst microcrystalline cellulose (MCC) as Pharmacel 102 was obtained from DFE Pharma (Germany). Colloidal silica dioxide (Aerosil 200) was obtained from Evonik Industries (Essan, Germany) and grades of hydroxypropyl methylcellulose (HPMC) as METHOCEL™ were obtained from Colorcon (Dartford Kent, UK). Market extended release nifedipine preparations including Nifedipress MR 10 and Adalat LA 30 were obtained from Dexcel Pharma (Daventry, UK) and Bayer (Reading, UK).

Dissolution media comprised of a phosphate/citrate buffer with Sodium lauryl sulphate (SLS). Dibasic sodium phosphate and citric acid were purchased from Acros Organics (Morris Plains, NJ, USA), whereas phosphoric acid and SLS were obtained from Sigma-Aldrich (Dorset, UK).

For sample analysis, Acetonitrile and Methanol (HPLC-grade) were obtained from Fisher Scientific (London, UK), whereas Trifluoroacetic acid (TFA), for HPLC (≥99.0%) was purchased from Sigma-Aldrich (Dorset, UK).

5.3.2 HPLC Analytical Method Development

Samples were analysed using an Agilent 1260 series (Agilent Technologies, Santa Clara, CA, USA), equipped with a reverse-phased Eclipse plus C18, 4.6 × 150 mm, 3.5 µm column (Agilent Technologies, Santa Clara, CA, USA).

Nifedipine separation was achieved using an isocratic mobile phase compromising TFA: ACN (25:75 v/v). TFA was used at a concentration of 0.1% (v/v). Flow rate was set at 0.8 mL/min and a wavelength of 235 nm was used for detection. A 9-point calibration curve in the range 0.39 to 100 mcg/mL was prepared via serial dilution in methanol (1 in 2 dilution). All samples prepared for analysis were diluted so as to fall within the calibration range. Method validation was carried out following The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidelines (Q2 (R1)).

5.3.3 Dose banding and selection of strengths

The dose of nifedipine indicated for treatment of hypertension in children between the ages of 1 month and 11 years is 200–300 micrograms/kg 3 times a day. The frequency of dosing depends on the preparation used, however, there is no standardised modified preparation nifedipine available for paediatric use. The selection of strengths was based on dosing requirements according to the BNF and reported optimised dose strategy post PBPK modelling.

5.3.4 Dissolution

Paediatric specific biorelevant media was not employed owing to previous findings where nifedipine did not show any significant age-related difference in dissolution of nifedipine when compared to adult biorelevant media (280). Similarly, evaluation of the effect of age-related difference on dissolution on another BCS class II drug (carvedilol) did not show any significant effect of varying physiological differences on drug release profiles (338). Therefore, dissolution media was prepared as per the USP monograph for nifedipine extended release tablets. In brief, 330.9 g of dibasic sodium phosphate and 38 g of citric acid were dissolved in water in a 1-L volumetric flask. 10 mL of phosphoric acid was then added, and the resulting concentrate was diluted with water to volume. 125.0 mL of concentrate (buffer) and 1 L of 10% sodium lauryl sulphate solution were mixed and diluted to 10 L. The medium was adjusted to a pH of 6.8.

All dissolution tests were carried out using an Erweka DT 126 with USP 2 paddle apparatus (Langen, Germany). Each vessel contained 900 mL of media, maintained at a temperature of

37 °C with a continuous paddle speed of 50 rpm. Samples of 5 mL were drawn at appropriate time points (2, 3, 4, 5, 6, 8, 12, 20 and 24 hrs) and replaced by 5 mL of fresh media to maintain sink conditions. Drug release was determined via HPLC and adjusted for cumulative drug release (%). A total of 6 replicates were taken and data presented as mean ± standard deviation.

5.3.5 Kinetic modelling

In an effort to further understand and compare the mechanism of nifedipine release from formulations, dissolution data was fitted within the following four kinetic models: zero order, first order, Higuchi and Korsmeyer-Peppas models. Method for data fitting was followed as described by Costa and Lobo (2001), Higuchi, T. (1963) and Korsmeyer, R. W., et al. (1983) (404-406) and summarised in Table 36.

Where *Q* is the amount of drug released or dissolved, Q_0 is the initial amount of drug release or dissolved (usually *zero*), $Q_t/Q\infty$ is the fraction of drug released at time t, K is the rate constant, and *n* indicates the release mechanism. In situations where the release mechanism is not well known or more than one type of release could be involved, the Peppas model is commonly used where an *n* value < 0.5 = Quasi-Fickian diffusion, *n* equals 0.5 = Fickian diffusion, 0.5 < *n* < 1. 0 = anomalous (Non - fickian transport), *n* equals 1.0 = Case II transport and *n* greater than 1 indicates Super case II transport drug transport mechanism (404).

Table 36: Kinetic drug release models.

Model Equation	Equation
Zero order	$Q_1=Q_0+K_0t$ *(Eq. 10)*
First order	$\text{Log } C_t = \text{Log } C_0 - k\,t / 2.303$ *(Eq. 11)*
Higuchi	$Q = K_H\, t^{1/2}$ *(Eq. 12)*
Korsmeyer-Peppas	$Q_t/Q\infty = Kt^n$ *(Eq. 13)*

5.3.6 Extended-release Mini Tablet Production

Mini-tablets were produced with a target drug load of 10% w/w and a tablet mass of 50 mg using a Specac semi-automatic hydraulic press (Slough, UK), equipped with a 4-mm multi-tip (three) with concave faced punches at a compression force of 10 KN with quick release. Optimised blending and tabletting parameters for low dose mini-tablets were applied as described by Khan, D., et al. (2021) (338).

5.3.7 Hardness

Tablet hardness was assessed using a Copley TBF 100 Hardness tester (Nottingham, UK) that measured the force required to break the tablets. Hardness values were measured in Newton's and carried out in triplicates and presented as mean ± standard deviation.

5.3.8 Friability

Friability testing was carried out using a Sotax F2 Friabilator (Allschwill, Switzerland) that measured the capacity of mini tablets to resist mechanical stress. 10 tablets were carefully de-dusted using a soft brush and an initial weight was taken. Tablets were then placed in the rotating drum and rotated for 4 min at a speed of 25 rpm (total 100 revolutions). The tablets were removed, dusted, and a final weight was taken. The percentage friability was calculated using the following equation:

Eq. 6: % Friability = (initial weight − final weight)/initial weight × 100

5.3.9 Physiologically-based pharmacokinetic (PBPK) modelling

In order to pragmatically assess the translation of resultant dissolution profiles to the paediatric populations, virtual clinical trials simulations were conducted using the physiologically-based pharmacokinetic (PBPK) modelling tool Simcyp in both adults and children (Simcyp Ltd, a Certara company, Sheffield, UK, Version 21). Unless otherwise stated, mixed genders (50:50) were incorporated into all simulations.

5.3.9.1 Validation of the nifedipine model in adults

We utilised the previously validated and published nifedipine PBPK model incorporated into the Simcyp Simulator and demonstrated confirmatory validation of model development by considering 4 studies within healthy populations: (i) 18 healthy male volunteers (23-29 years old) received a single oral dose of nifedipine 20 mg (immediate release) in 3 studies (407); (ii) 6 healthy male volunteers (aged 20-25 years old) receiving a single oral dose of nifedipine 20 mg (immediate release) (408); (iii) 6 healthy male volunteers (aged 20-30 years old) receiving a single oral dose of nifedipine 20 mg (immediate release) (409); (iv) 6 healthy male volunteers (aged 22-34 years old) receiving nifedipine 10 mg (immediate release) as a single dose or three times a day for 5 days (410).

In order to simulate a MR formulation system a Weibull function was fit to a 30 mg modified release reference in-vitro release profile (Adalat OROS®) (411).

5.3.9.2 Simulation in virtual adults

For adult studies, the dissolution profiles for F1 (No HPMC), F2 (30 % HPMC) and F3 (50 % HPMC) were incorporated into a 10x10 trial design (n=100) with Healthy Adults aged 20-50 years of age with dosing at either 30 mg twice daily or 60 mg once daily for 6 days.

5.3.9.3 Simulation in virtual children

For paediatric studies, the dissolution profiles for F1 (No HPMC), F2 (30 % HPMC) and F3 (50 % HPMC) were incorporated into a 10x10 trial design (n=100) with children aged 5-11 years of age with dosing at 250 µg/kg once, twice or three times daily. Results were demarked for 5-7 year olds and 7-11 year olds.

5.3.9.4 Developing a dosing approach in children

In order to identify a possible dosing approach for use in children, dose adjustments were considered through 100 µg/kg increments to achieve the majority of subjects with trough plasma concentrations within the therapeutic window. The formulation attaining plasma concentration throughout the therapeutic window was selected and identical trial design was utilised as described in Section 5.3.9.4.

5.3.9.5 Predictive performance

To confirm the predictive performance during validation, prediction of pharmacokinetic metrics to within two-fold (0.5-2.0 fold ratio) of that published in clinical data was accepted (412-414). Further, a visual predictive checking (VPC) strategy was utilised to visually compare predicted concentration–time profiles with retrospective observed data, with predictions valid when the predicted data points overlapped with the observed data sets (415-417). Observed data acquired from retrospective published studies, extracted using WebPlotDigitizer v.3.10 (http://arohatgi.info/WebPlotDigitizer/).

5.3.10 Statistical analysis

Statistical analysis of the drug release profiles was carried out by one-way analysis of variance (ANOVA) with post-hoc Tukey test using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA).

5.4 Results and discussion

5.4.1 Acceptability of mini-tablets

Children are not small adults, nor are all children alike. Similarly, paediatric drug development is not a one-size-fits-all process. It relies on factors such as disease type, inherent physicochemical characteristics, age and maturity of the patient, and formulations/dosing options. Developing an age-appropriate formulation, especially for children is an immense challenge from a scientific, ethical and logistical standpoint. This is owing to a lack of translatable dosage form development technologies and the absence of paediatric clinical data. The use of PBPK aims to minimise drug related adverse effects and inform formulation design and development, alongside contributing to the need of availability of more pharmacokinetic data in paediatrics.

The target age range for the novel 4 mm extended release mini tablets was specified from the age of 3 years and onwards. In a recently published review article, an attempt to survey currently available evidence on acceptability of oral medicine in paediatrics to guide the selection of appropriate dosage form types in future paediatric formulation development was made, resulting with several key findings, including the superior acceptability of small innovative dosage forms over other conventional dosage form types (418). A particular study carried out by van Riet-Nales, D. A., et al. (2013) evaluated the acceptability of a 4 mm mini tablet in 183 children aged 1-4 years. The study concluded that a 4 mm tablet is well accepted for children from the age of 1 year (175). Further, appraisal of Paediatric Investigation Plans (PIPs) by regulators including the European Medicines Agency (EMA) and its Paediatric Committee (PDCO) demonstrate concurrence with the acceptability of small (0–4 mm) tablets in children aged 2-5 years of age (419). Older children can either take multiple mini tablets or a conventional adult marketed modified release preparation.

5.4.2 Paediatric formulation development approach for extended release mini-tablets and formulation composition optimisation

When developing a paediatric specific formulation, the inclusion of excipients must be justified. There are several techniques to achieve modified release (MR) including matrix, membrane controlled and osmotic pump systems. In order to guide the selection of safe and age appropriate excipients to achieve an extended release preparation, the compositions of current market nifedipine preparations were initially explored. Reference to the STEP database was made to ensure excipient selection and loads were paediatric compliant. However, upon evaluating we found that marketed formulations were composed of numerous excipients (10 to 15). This not only adds to cost but increase the potential of unknown excipient related toxicity and safety concerns.

Moving forward, current modified release mini tablet preparations with a paediatric license were evaluated. As of now, there are only three modified release mini tablet preparations (Orfiril Long®, Slenyto and Pancrease MT®) licensed for paediatric use. However, the MR technique within these formulations use methacrylic acid copolymer, which has been associated with fibrosing colonopathy (420). For such reasons and to limit the total number of excipients within the formulation, it was decided to explore hydrophilic monolithic systems where HPMC is used as the gold standard. A monolithic system exposes all of its surface area for potential solubilisation and is a simple and effective technique where different grades of HPMC can be compared to ascertain a required baseline behaviour of disintegration and drug release. HPMC is hydrophilic, non-ionic, enzyme resistant, GRAS listed and appropriate for paediatric use at levels below 660–900 mg/kg per day (421). Several grades of HMPC

were explored to optimise drug release in order to achieve either ONCE daily or TWICE daily administration.

Since low-viscosity HPMC is advised for low aqueous soluble substances such as nifedipine, we decided to firstly explore various concentrations of HPMC E3. In order to determine the baseline behaviour of disintegration and drug release with HPMC E3, three very different concentrations were used (0, 50 and 89.5 % w/w). It was envisioned that since nifedipine is poorly soluble, HPMC E3 would provide erosion based extended release. However, all three formulations presented a similar drug release profile, suggesting that the effect of HPMC E3 on providing extended release was nominal. This is owing to the low viscosity of this particular HPMC grade where the viscosity is 3 cP (of a 2% solution at 25°C) (422).

Another observation made here was that total nifedipine release was limited to around 60%. This was due to the degradation of nifedipine where nifedipine is known to be highly sensitive to light. Upon exposure to light, nifedipine degrades to nitro- and nitroso-pyridine analogues by intramolecular processes, as well as a few minor secondary products generated from inter-molecular interactions between primary degradation products and their intermediates (423). Moving forward, all dissolution experiments were conducted after the apparatus was fully covered to prevent any light entering the dissolution vessels.

For an effective controlled-release matrix, rapid hydration is required to form a protective gelatinous layer, as it prevents the drug and excipients in the matrix from dissolving prematurely (424). HPMC E3 not only displayed a slow rate of hydration but also formed a relatively weak gel layer. This was observed during the 6 hour dissolution mark, where the tablet fully fragmented. The low viscosity of HPMC E3 suggests reduced ether groups available for hydrogen bonding with water molecules, while the presence of poorly water soluble nifedipine resulted in further disruption of hydrogen bonding, thereby decreasing the amount of water bound to the polymer (425). For these reasons HPMC E3 was discounted and various concentrations of medium viscosity HPMC K4M were explored (Figure 24).

In general, as polymer viscosity increases, drug diffusion and release rates decrease. This is because high viscosity hypromellose results in a turbid gel that resists erosion and dilution, since hypromellose chains swell more quickly and prevent further liquid from getting into the pores (424). It follows that if a good gel layer is formed, the rate of drug release will be reduced and will be dependent on the rate at which drug molecules diffuse through it, as well as the rate of mechanical destruction of the gel layer by attrition and unravelling of the matrix (424). The effect of increasing concentrations of HPMC K4M on nifedipine release rate is significant (p-value<0.05) as after 6 hours the total drug released for F1 is 96.18 ± 1.76 % and 77.43 ± 0.77 %, 44.83 ± 0.33 % and 41.11 ± 5.18 % for F2, F3 and F4 respectively.

After comparing different concentrations of HPMC K4M, F3 was chosen as the optimum formulation that provided a release profile extending over 24 h (Figure 24). Other formulation constituents include lactose as the diluent and magnesium stearate and AEROSIL® as tabletting excipients (Table 37). Lactose is considered to be safe because of its presence in various forms of milk, including breast milk and formula milk. Lactose is approved as an 'additive' by the FDA and listed as GRAS and has a recommended threshold of 5 g per dose (127).

Table 37: Composition of extended release nifedipine mini-tablets.

Composition	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)	F4(% w/w)
Nifedipine	10	10	10	10
Lactose monohydrate	89.25	59.25	39.25	19.25
HPMC (K4M)	-	30	50	70
Mg stearate	0.5	0.5	0.5	0.5
AEROSIL®	0.25	0.25	0.25	0.25

The mixing and tabletting strategy employed was as described by Khan, D., et al. (2021), where optimised blending and processing parameters (mixing for 5 minutes at a speed of 250 rpm and compression force of 10 KN with quick release) resulted in mini-tablets that displayed good mechanical strength and content uniformity (Table 38) that meets pharmacopeia requirements (338).

Table 38: Mini-tablet characterisation.

Formulation	Hardness (N)	Friability (%)	Content Uniformity (%)
F3 (50 % w/w HPMC)	62.10 ± 1.90 N	0.25	98.57± 4.26

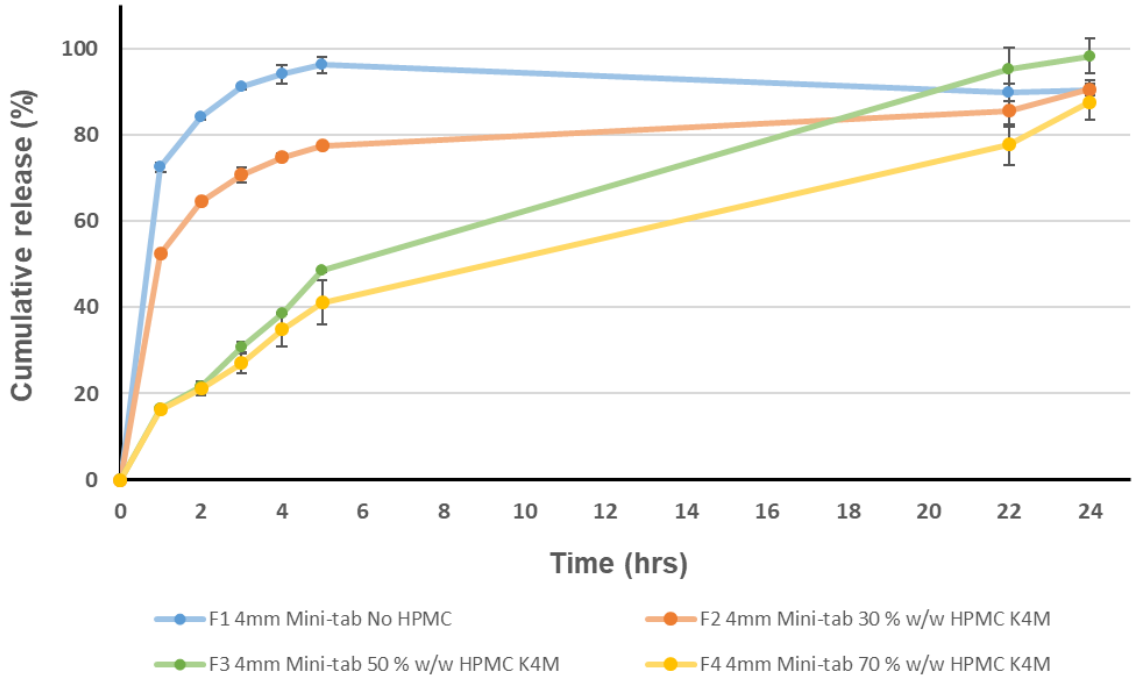

Figure 24: Dissolution profiles of mini-tablets composed of various grades of HPMC K4M. Data presented as mean ± standard deviation.

5.4.3 Kinetic modelling

In 1963, Higuchi described a mathematical model that describes drug release from a matrix system, with the assumption that the amount of drug released is proportional to the square root of time, representing a linear function (405). Additionally, Higuchi also proposed that for low concertation drugs, solubility and release occurs through porosity of the dosage form (426). The Korsmeyer-Peppas model describes drug release from a polymeric system that considers Non - Fickian transport and is useful when the release mechanism is uncertain or where more than one type of release phenomenon is involved (406, 427).

Fitting the release data to the following models was used to study the kinetics of nifedipine release: zero order, first order, Higuchi and Korsmeyer-Peppas models (Table 39). Based on correlation coefficient (R^2) values, formulations containing HPMC most closely fit the Higuchi and Korsmeyer-Peppas kinetic model. This shows that the extended release formulations describe drug release from a matrix polymeric system through either Fickian or non-Fickian diffusion. When a diffusion process is governed by Fick's law and the gradient of the penetrant concentration, it is said to be fickian diffusion (428). On the other hand, non-Fickian diffusion does not follow Fick's law and may occur when diffusion is through porous media or when there is swelling of polymers due to penetration the diffusing species (429). HPMC K4M is a water soluble cellulose based polymer that when in contact with water hydrates and forms a

gel layer (430). As water permeates into the tablet, the tablet matrix further swells and the gel layer continues to expand. The closely fitted Higuchi and Korsmeyer-Peppas kinetic model suggests that the HPMC swells, creating a gel layer and porous matrix from which the drug diffuses from through non-Fickian diffusion.

The release exponent (n) was found to be 0.15 for F2 (suggesting a drug release mechanism by Quasi-Fickian diffusion) and between $0.5 < n < 1$ for F3 and F4 (anomalous (Non - Fickian transport) drug transport mechanism) (431). Nifedipine release in F1 (immediate release) followed first order kinetics; this was expected as the formulation did not possess any extended release properties. It is also important to mention that the release of nifedipine formulations containing HPMC displayed an initial burst release, followed by a decrease in release rate over time (Figure 24). This may be due to drug close to the matrix surface being released before the surrounding polymer reached the polymer disentanglement concentration, since the diffusion coefficients for drug molecules were higher than the polymer at early stages (432). Lastly, it is evident that the release rate of nifedipine from formulations containing HPMC K4M decreases as a function of increasing polymer concentration. This can be seen as a result of increasing linearity when release data is fitted to the Higuchi and Korsmeyer-Peppas kinetic model (Table 39).

Table 39: Kinetic modelling of F1-F4.

Formulation	Zero order	First order	Higuchi	Korsmeyer-Peppas	(n)
F1 (immediate)	0.645	0.987	0.348	0.331	0.05
F2 (30% HPMC)	0.387	0.717	0.638	0.895	0.15
F3 (50 % HPMC)	0.936	0.985	0.993	0.984	0.58
F4 (70 % HPMC)	0.932	0.971	0.994	0.990	0.53

5.4.4 Virtual clinical trials analysis of nifedipine model in adults and children

Having identified optimal formulations and resulting dissolution profiles, we next attempted pragmatically to assess the in-vivo translation using virtual clinical trials analysis.

These approaches utilised physiologically-based pharmacokinetic modelling and knowledge of population variability in physiological and biochemical properties governing a drugs absorption, distribution, metabolism, and elimination, to develop virtual populations containing the matching inherent physiological and biochemical variability identified within clinical trials populations.

In order to validate the applicability of the model, we assessed the ability of the model to recapitulate retrospective nifedipine plasma concentration-time profiles in adults from 4 studies. Pharmacokinetic parameters were consistent with observed data and ranges (Figure 25) and, within 2-fold for the geometric mean of the reported pharmacokinetic parameters (Table 40), confirming successful validation.

Table 40: Validation results. AUC: area under the curve; Cmax: maximum plasma concentration; tmax: time to maximum plasma concentration; nr: not reported; - :not calculated. Mean Cmax, tmax and AUC ratio represent the ratio of predicted:observed pharmacokinetic parameter. G1,G2 and G3: represents gestational trimester 1,2 and 3.Data reported as geometric mean ± (standard deviation).

Study	Dose		Cmax	tmax	AUC	Mean Cmax	Mean tmax	Mean AUC
			(ng/mL)	*(h)*	*(ng/mL.h)*			
Ohashi et al (1993)	Single (20 mg)	Predicted	245 ± 152	0.6 ± 0.2	501 ± 124	-	-	-
		Observed G1	nr	nr	680 ± 135	-	-	0.74
		Observed G1	nr	nr	809 ± 318	-	-	0.62
		Observed G1	nr	nr	579 ± 191	-	-	0.87
Tateishi et al (1989)	Single (20 mg)	Predicted	245 ± 152	0.6 ± 0.2	501 ± 124	-	-	-
		Observed	236 ± 70	1 ± 0.9	623 ± 139	1.04	0.6	0.8
Ohashi et al (1990)	Single (20 mg)	Predicted	238 ± 142	1.1 ± 0.8	357.1 ± 124.9	-	-	-
		Observed	421 ± 177	nr	453.6 ± 176.2	0.57	-	0.79
Smith et al (1987)	Single (20 mg)	Predicted	101 (62-225)	1 ± 0.5	279 (307-435)	-	-	-
		Observed	131 (97-179)	0.5	266 (322-415)	0.77	2	1.04
	Multiple (10 mg)	Predicted	82 (62-225)	1 ± 0.5	201 (142-235)	-	-	-
		Observed	56 (42-74)	0.5	134 (113-158)	1.46	2	1.5
Wonnerman et al (2008)	Single OROS (30 mg)	Predicted	21.8 (15.9-42)	5 (3-17)	319 (214-517)	-	-	-
		Observed	17.6 (11.6-30.8)	6 (5-24)	358 (160-906)	1.23	0.83	0.89

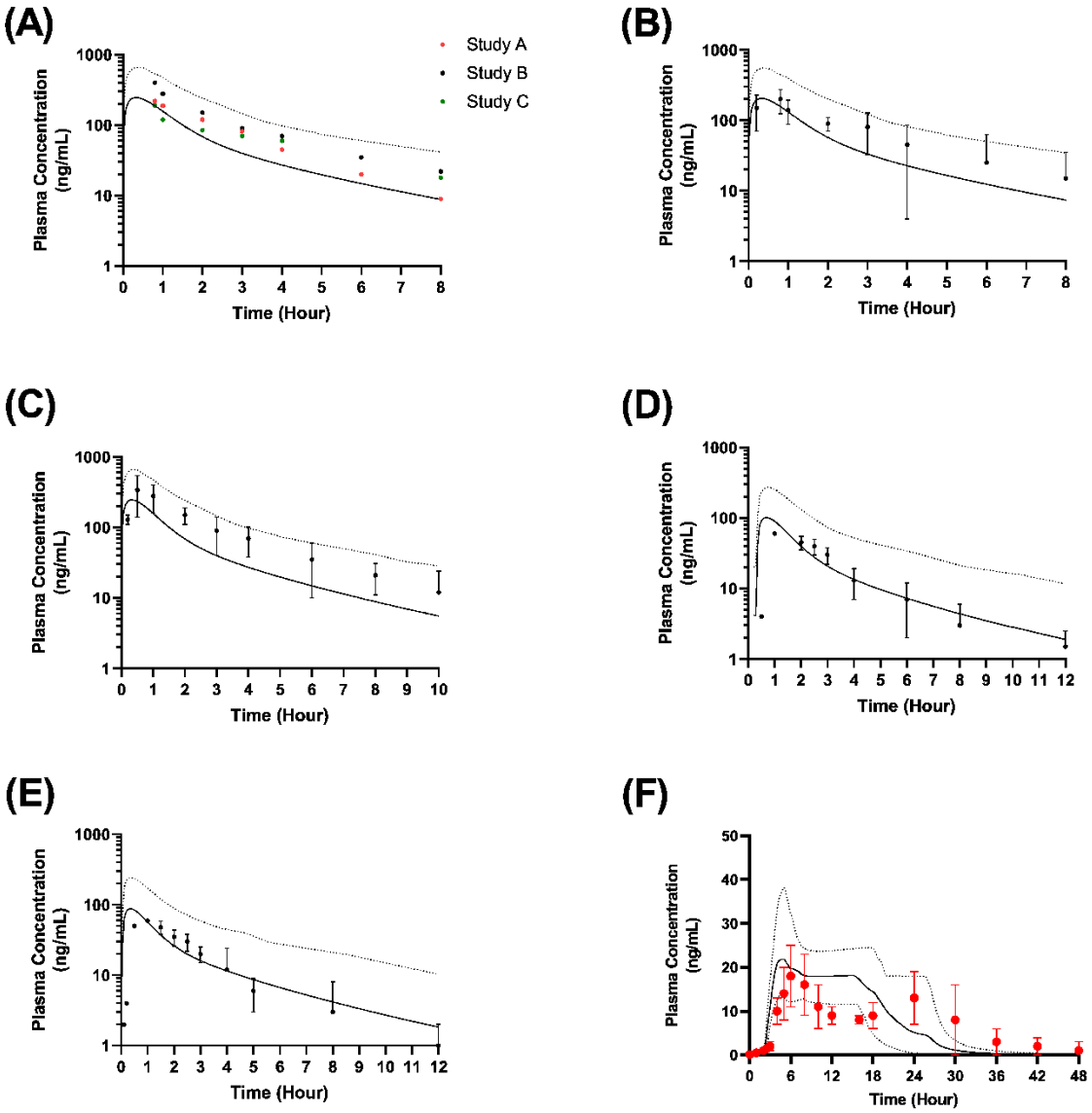

Figure 25: Simulated plasma concentration-time profiles of nifedipine in Healthy Adults. (A) Single oral dose of nifedipine 20 mg (immediate release) in 3 studies (407); (B) Single oral dose of nifedipine 20 mg (immediate release) (408); (C) Single oral dose of nifedipine 20 mg (immediate release) (409); Nifedipine 10 mg (immediate release) as a single dose (D) (410) or three times a day for 5 days (E) (410); (F) Single or dose of 30 mg modified release nifedipine (411). Filled circles indicate the observed clinical data, with dotted lines indicating the corresponding 5th and 95th percentile range of the predicted mean (solid lines). Vertical lines indicate standard deviation.

5.4.5 Simulations in adults

In order to pragmatically assess the impact of HPMC content on pharmacokinetics, dissolution profiles from formulations F1 (No HPMC), F2 (30 % HPMC) and F3 (50 % HPMC) mini-tablets were used to drive predictions of oral pharmacokinetics using the validated adult model. 60 mg once daily and 30 mg twice daily dosing was examined to identify an appropriate dosing approach to target the therapeutic range of 25-100 ng/mL (433) (Figure 26).

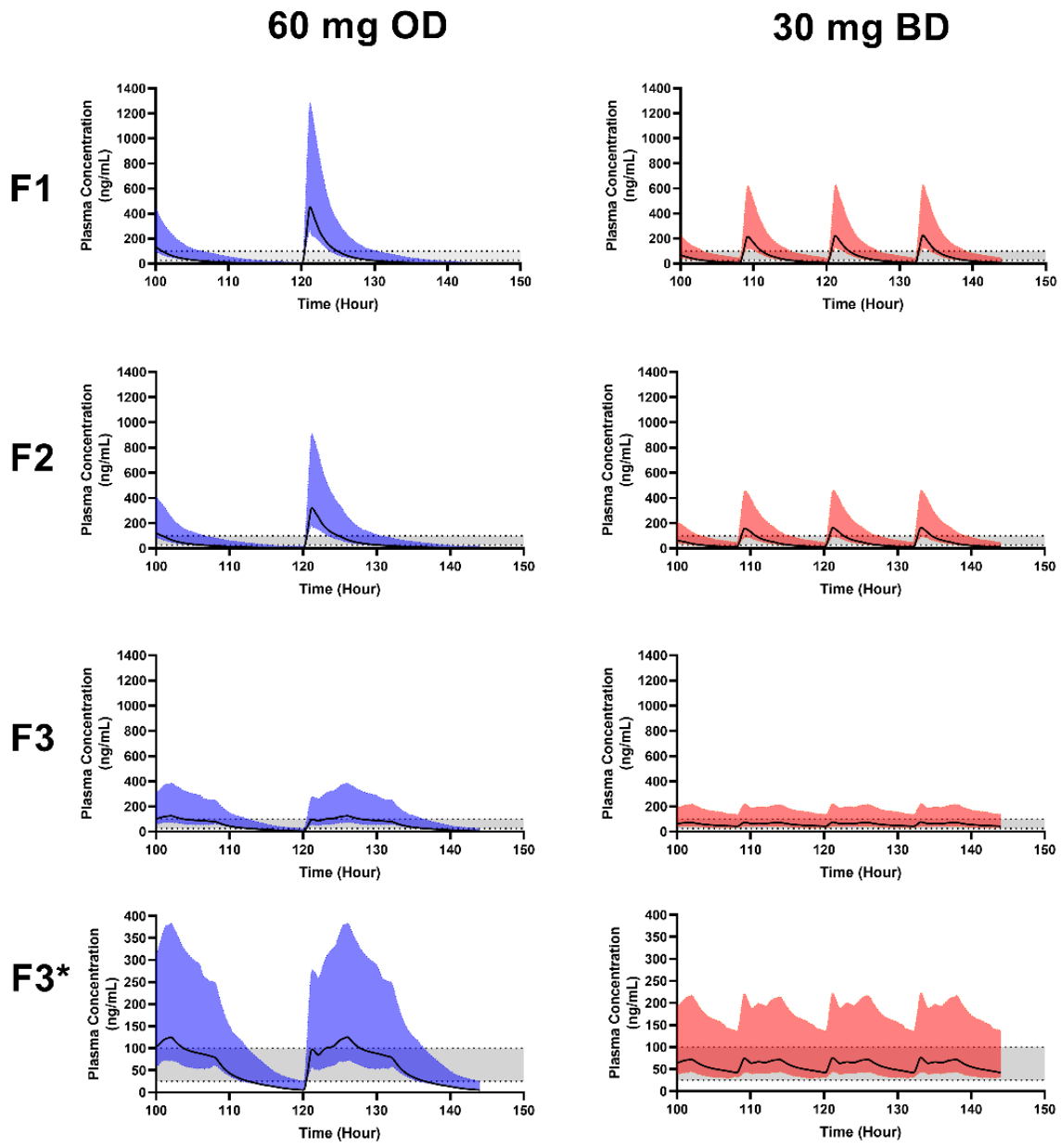

Figure 26: Simulated plasma concentration-time profiles of formulated nifedipine mini-tablets in healthy adults. The predicted plasma concentrations of nifedipine when formulated into F1 (No HPMC), F2 (30 % HPMC) and F3 (50 % HPMC) mini-tablets, following dosing at 60 mg once daily (left panels) or 30 mg twice daily (right panels). F3*: Represents F3 with an axis

range from 0-400 ng/mL. Solid lines indicate predicted plasma concentrations, shaded regions indicate the 5^{th}-95^{th} percentile range around the predicted mean. The suggested therapeutic window (25-100 ng/mL) is indicated by the shaded horizontal region.

Table 41: Simulated mini-tablet pharmacokinetics in healthy adults. F1: No HPMC mini-tablet; F2: 30 % HPMC mini-tablet; F3: 50 % HPMC mini-tablet; AUC: area under the curve; Cmax: maximum plasma concentration; tmax: time to maximum plasma concentration; Data reported as geometric mean ± (standard deviation).

Formulation	Dose	Cmax (ng/mL)	tmax (h)	AUC (ng/mL.h)
F1	30 mg BD	229 ± 94.2	1.2 ± 0.15	703 ± 425.6
	60 mg OD	466 ± 187	1.2 ± 0.24	1459 ± 876.8
F2	30 mg BD	167.1 ± 70.16	1.23 ± 0.18	617.9 ± 366.8
	60 mg OD	331 ± 133	1.25 ± 0.26	1270.7 ± 751
F3	30 mg BD	76.68 ± 39.5	2.25 ± 1.99	711.6 ± 407
	60 mg OD	126.8 ± 62	5.5 ± 1.34	1432.8 ± 817

Formulation F3 provided a broadly lower Cmax for both twice and once daily dosing, 76.68 ± 39.5 ng/mL and 126.8 ± 62 ng/mL respectively, compared to F1 and F2 (Table 41), with twice daily dosing mean plasma concentrations more appropriately targeting the therapeutic window throughout the dosing approach (Figure 26). The results from adults demonstrate the ability of the F3 mini-tablet to provide the required plasma concentration within the therapeutic window for a longer duration than that from F1 and F2, which provides a more rapid dissolution (as a result of the reduced HPMC content) and hence rapid absorption into the systemic circulation (434).

5.4.6 Simulations in children

In order to pragmatically assess the impact of HPMC content on pharmacokinetics in children, F1-F3 dissolution profiles were used to drive predictions of oral pharmacokinetics in children aged 5-7 and 7-11 years at a fixed dose of 250 µg/kg. A dose of 250 µg/kg was chosen as a median dose to the indicated dose of 200-300 micrograms/kg 3 times a day for children 1 month to 11 years (435).

Three dosing strategies were assessed, OD, BD and TDS. At all three dosing approaches, predicted mean plasma concentrations were broadly within the therapeutic widow for some of the dosing period (Table 42) (Figure 27); however, for F3 a BD or TDS dosing approach resulted in predicted mean plasma concentrations within the therapeutic widow for the entire dosing period, for both age groups. With increasing dosing frequency, Cmax increased by 60 % for TDS vs OD dosing for both age groups with consistent AUCs for each age groups (Table 42) (Figure 27).

F1 (immediate release) displayed large fluctuations in peak-to-trough concentrations and a Cmax above the therapeutic range, indicating a negative impact on clinical response and tolerability. Subsequently, the need for an extended release preparation is advised. F3 given BD was chosen as the optimum dosing approach as it was able to provide consistent drug plasma concentrations, a reduction in the peak-to-trough fluctuations and potential for improved patient compliance.

Table 42: Simulated mean mini-tablet pharmacokinetics in children dosed at 250 µg/kg. F1: No HPMC mini-tablet; F2: 30 % HPMC mini-tablet; F3: 50 % HPMC mini-tablet; AUC: area under the curve; Cmax: maximum plasma concentration; tmax: time to maximum plasma concentration; OD: once daily; BD: twice daily; TDS: three times daily. Data reported as geometric mean (SD omitted for clarity).

Formulation	Dosing	Age range (years)	Cmax (ng/mL)	Tmax (h)	AUC (ng/mL.h)
F1	OD	5-7	113	1.2	398.2
		7-11	101.6	1.2	300
	BD	5-7	113	1.6	397.7
		7-11	105.8	1.36	300
	TDS	5-7	113	1.43	398.1
		7-11	107.1	1.38	301
F2	OD	5-7	113	2.2	364
		7-11	101	2.16	274
	BD	5-7	111	2.2	363
		7-11	102	2.16	275
	TDS	5-7	123	2.15	362
		7-11	106	2.13	274
F3	OD	5-7	39	5.3	418
		7-11	31	4.7	314
	BD	5-7	46	3	417
		7-11	37	2.45	314
	TDS	5-7	63	2.4	418
		7-11	49	2.16	313

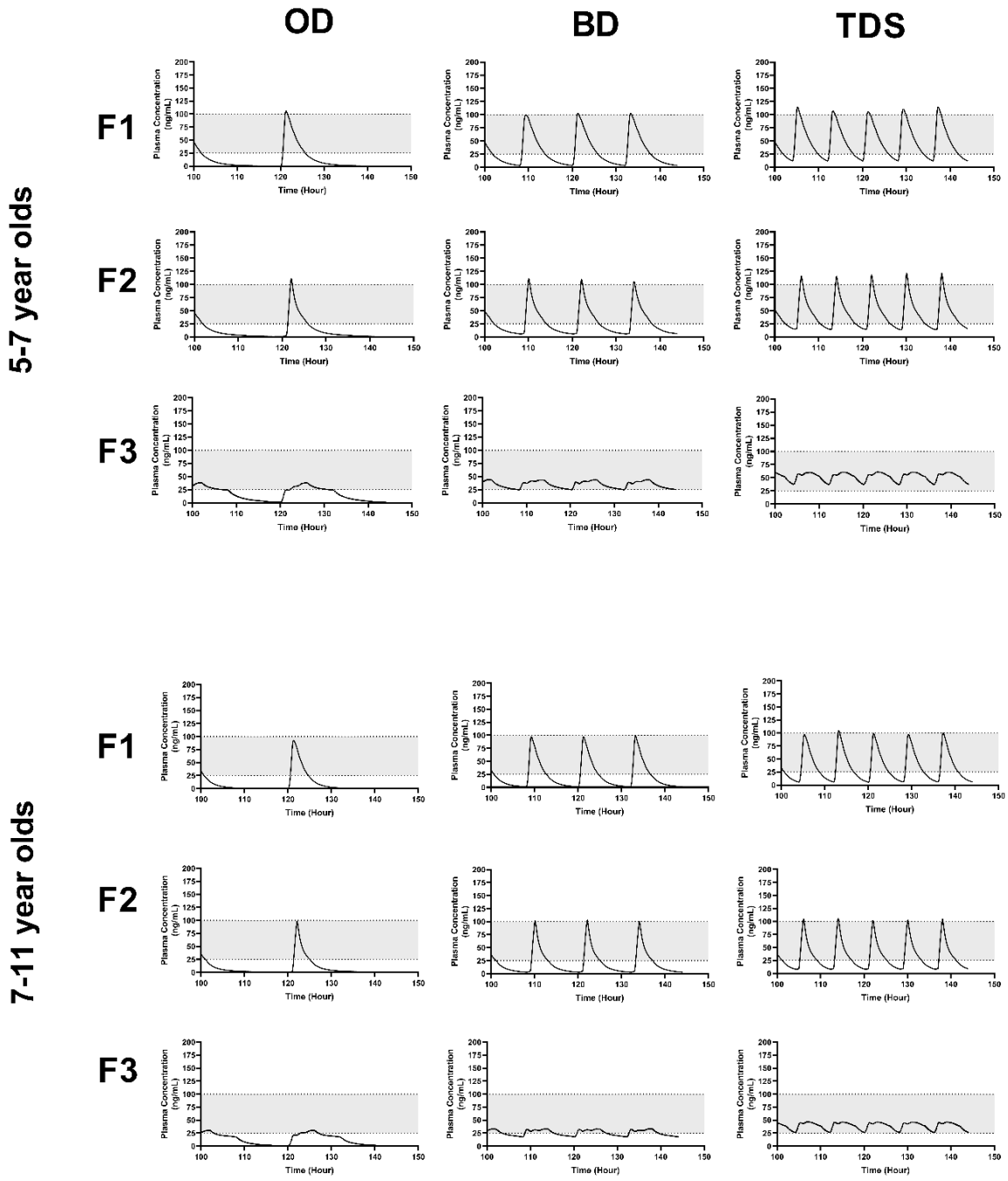

Figure 27: Simulated plasma concentration-time profiles of formulated nifedipine mini-tablets in children. The predicted plasma concentrations of nifedipine when formulated into F1 (No HPMC), F2 (30 % HPMC) and F3 (50 % HPMC) mini-tablets, following dosing at 250 µg/kg in children aged 5-7 years (top panels) or 7-11 years (bottom panels), following a once (OD), twice (BD) or three (TDS) times daily dosing (left, middle and right panels respectively). Solid lines indicate predicted plasma concentrations, shaded regions indicate the 5^{th}-95^{th} percentile range around the predicted mean. The suggested therapeutic window (25-100 ng/mL) is indicated by the shaded horizontal region.

5.4.7 Developing a dosing approach in children

Based upon our initial predictions, a BD dosing approach was selected, reflecting a more realistic dosing approach for children compared to TDS dosing utilising F3 (Figure 27). Given the maximum dose incorporated limitations within each mini-tablet (~ 10 mg), dosing at age groups above 7 years of age would require more than one mini-tablet BD (Figure 27).

We therefore considered an increase in the overall dose from 250 µg/kg, by 100 µg/kg increments, to a maximum dose limit of 10 mg within each mini-tablet dose, with age groups of 3-5- and 5–7-year-olds. A key driver for an optimal dosing approach was to identify a dosing regimen resulting the fewest subjects with trough plasma concentrations below the lower limit of the therapeutic window, 25 ng/mL, but maintaining the drug loading limits within the formulation.

A dose of 350 µg/kg resulted in 36 % (3–5-year-olds) and 46 % (5-7 year olds) of subjects with trough concentrations below the lower end of the therapeutic window (Table 43) (Figure 28), with peak concentrations broadly within the therapeutic window (Figure 28B) and mean trough concentrations close to the lower limit (3-5 year olds: 31.76 ng/mL ± 25.9 ng/mL; 5-7 year olds: 25.6 ng/mL ± 15.6 ng/mL) (Figure 28C).

However, a dose of 450 µg/kg resulted in the lowest percentage of subjects with trough concentrations below the lower end of the therapeutic window, 23 % (3–5-year-olds) and 20 % (5-7 year olds) (Table 43) (Figure 29). Under this dosing strategy, trough concentrations were approximately 30 % higher than those for the 350 µg/kg, with the final mean dose being below the 10 mg limit, 7.4 mg ± 1.1 mg (3–5-year-olds) and 9.04 ± 1.7 (5–7-year-olds).

After an optimised dosing strategy of 450 µg/kg was confirmed, dose banding was used to ensure a maximum of no more than two mini-tablets are dosed at any one time. A 5 mg strength was selected on the basis of being able to provide enhanced dosing flexibility options (Table 44).

Table 43: Simulated optimised F3 mini-tablet pharmacokinetics in children. a Percentage of subjects with trough concentration below the lower limited of the therapeutic window. AUC: area under the curve; Cmax: maximum plasma concentration; Cmin: minimum plasma concentration; tmax: time to maximum plasma concentration; Data reported as geometric mean ± (standard deviation).

Dose	Age range (years)	Cmax (ng/mL)	Cmin (ng/mL)	tmax (h)	AUC (ng/mL.h)	< 20 ng/mL [a] (%)	Dose (mg)
350 µg/kg	3-5	62.48 ± 35	31.76 ± 25.9	3.35 ± 2	563.3 ± 376	36	5.75 ± 0.87
	5-7	53.97 ± 25.3	25.6 ± 15.6	2.59 ± 2	458.9 ± 248.3	46	7.03 ± 1.37
450 µg/kg	3-5	80.45 ± 45.8	40.85 ± 33	3.35 ± 2	726 ± 485.2	23	7.4 ± 1.1
	5-7	69.5 ± 32.6	32.8 ± 20.1	2.59 ± 2	590.8 ± 319	20	9.04 ± 1.7

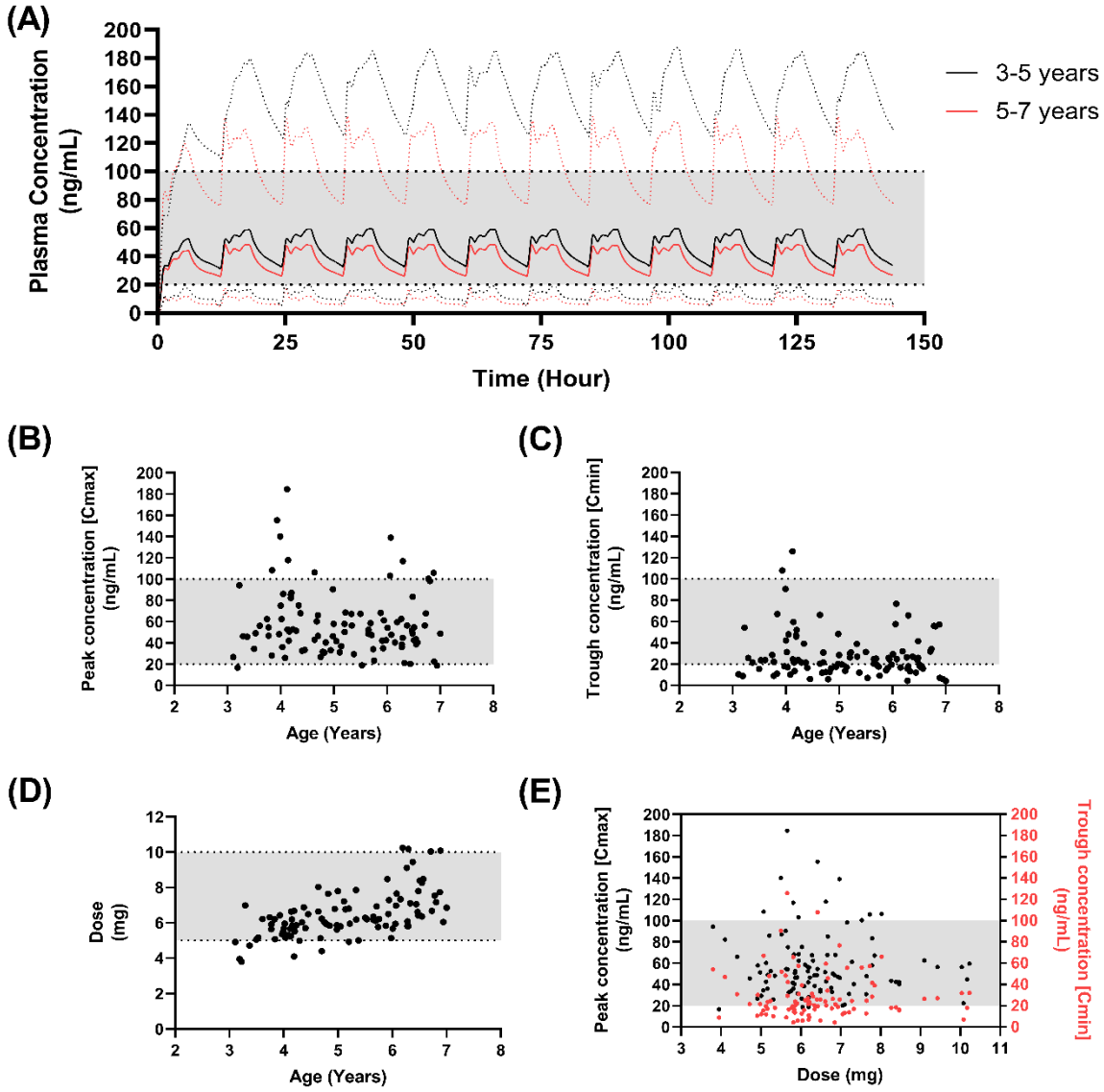

Figure 28: Simulated pharmacokinetics of 350 µg/kg F3 nifedipine mini-tablets in children. (A) The predicted mean plasma concentrations of F3 nifedipine, following dosing at 350 µg/kg twice daily in children aged 5-7 years (black line) or 7-11 years (red line) with dashed lines indicating the 5th-95th percentile range around the predicted mean; (B) The predicted peak plasma concentrations of F3 nifedipine with age; (C) The predicted trough plasma concentrations of F3 nifedipine with age; (D) Dose (mg) administered per dosing interval (12 hours); (E) Peak and trough plasma concentration related to dose (mg) administered per dosing interval (12 hours). Shaded horizontal regions indicated the suggested therapeutic window (25-100 ng/mL) (A,B,C and E) or ideal dose range to be incorporated into the mini-tablet (D).

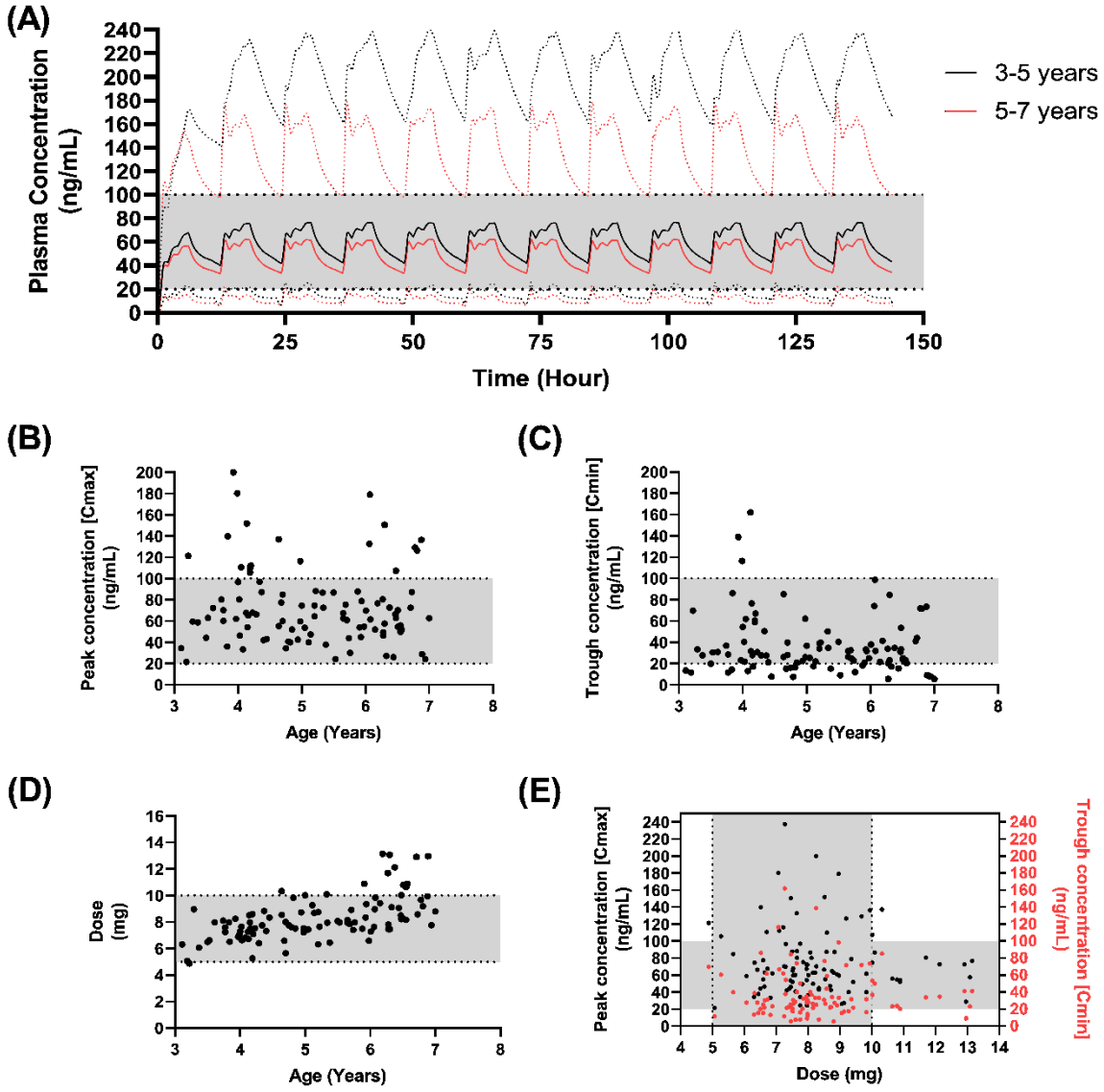

Figure 29: Simulated pharmacokinetics of 450 µg/kg F3 nifedipine mini-tablets in children. (A) The predicted mean plasma concentrations of F3 nifedipine, following dosing at 450 µg/kg twice daily in children aged 5-7 years (black line) or 7-11 years (red line) with dashed lines indicating the 5^{th}-95^{th} percentile range around the predicted mean; (B) The predicted peak plasma concentrations of F3 nifedipine with age; (C) The predicted trough plasma concentrations of F3 nifedipine with age; (D) Dose (mg) administered per dosing interval (12 hours); (E) Peak and trough plasma concentration related to dose (mg) administered per dosing interval (12 hours). Shaded horizontal regions indicated the suggested therapeutic window (25-100 ng/mL) (A, B, C and E) or ideal dose range to be incorporated into the mini-tablet (D).

Table 44: Dose banding.

Dosing strategy	Average weight (kg)	Dose (mg)	No of 5mg tablet required
250 micrograms/kg	3 years: 14 kg 5 years: 18 kg 7 years: 23 kg	3.5 ≈ 5 4.5 ≈ 5 5.8 ≈ 5	1 1 1
350 micrograms/kg	3 years: 14 kg 5 years: 18 kg 7 years: 23 kg	4.9 ≈ 5 6.3 ≈ 5 8.1 ≈ 10	1 1 2
450 micrograms/kg	3 years: 14 kg 5 years: 18 kg 7 years: 23 kg	6.3 ≈ 5 8.1 ≈ 10 10.4 ≈10	1 2 2

5.4.8 Process of paediatric formulation development using PBPK modelling

The PBPK informed formulation developmental approach mentioned within is able to clinically inform and optimise age-appropriate formulations. An appropriate prototype formulation was initially developed after reviewing market needs, manufacturing feasibility and applying a paediatric specific formulation development approach. This was done through the screening process as described in previous chapters where feasible dosage form designs are identified, followed by dose banding and selection of appropriate paediatric compliant excipients. Formulations containing nifedipine require extended release properties to avoid sudden drops in blood pressure. Upon review of the current landscape of nifedipine formulations available, there is lack of age-appropriate formulations that provides adequate dosing flexibilities and safe therapeutic profiles. Consequently, extended release mini-tablets were identified to fulfil this gap. Multiple mini-tabs can be counted and taken to fulfil dose requirements while PBPK modelling allows for pragmatic determination of paediatric plasma concentrations to support drug licensing and clinical dosing.

PBPK modelling was applied to translate in-vitro profiles and predict drug plasma concentration in-vivo. Based upon findings, the choice of dosage form design is then reconsidered/optimised to be able to facilitate any adjustments, for example optimising the formulations composition to further provide extended release properties to achieve consistent drug plasma level. In this study PBPK modelling successfully informed the unacceptability of nifedipine as an immediate release preparations and suggested the need of an extended release preparation that can provide consistent drug plasma concentrations, a reduction in the peak-to-trough fluctuations and improved patient compliance. In this case, formulation 3 (compromised of 50 % w/w HPMC K4M) was chosen as the optimised formulation as the release profiles translated in providing consistent nifedipine plasma concentrations and the ability to reduce dosing frequency.

This holistic formulation development approach integrates PBPK modelling within the novel excipient screening platform (discussed in chapter 6) to provide an additional layer to formulation screening that ensures resulting developments are effective and safe (figure 30). Further, this formulation development process can assist in avoiding unnecessary and potentially harmful drug exposure in sensitive population groups such as paediatrics.

Figure 30: Process overview of informed formulation optimisation using PBPK.

5.5 Conclusion

A key driver for this study was to identify both a clinically informed age-appropriate formulation and a potential dosing strategy within the remits of compliance within paediatrics. The final suggested dosing strategy at 0.45 mg/kg was applicable across age group 3–7-year-old whilst maintaining the limitation on drug loading (< 10 mg) to ensure a maximum of no more than two mini-tablets are dosed. Further, this dosing strategy is within recommended guidelines (435), and has been used in previous studies: (i) children 11.6 ± 5.3 years across a dose of 0.04-0.69 mg/kg (397); in a study by (396) who dosed across 0.1-1.2 mg/kg in paediatrics; (iii) in a study by (398) with 182 patients aged 0.2-17.9 years with a dosing range of 0.04–0.67 mg/kg per dose. Further, plasma concentration levels observed at a dosing strategy of 0.45 µg/kg are broadly within the range reported by (436).

However, there is a paucity in nifedipine pharmacokinetics in paediatric age groups, and further studies are needed to demonstrate plasma concentrations in paediatrics would support further validation of our findings. Despite these drawbacks, this study has, for the first time, provided a pragmatic estimation of a possible dosing approach that could be applied to the use of a novel nifedipine mini-tablet for use in children.

Chapter 6: Development of novel excipient screening platform for paediatric oral formulations (thesis summary)

6.1 CHAPTER AIMS AND OBJECTIVES

- Review regulatory guidelines for paediatric medicines
- Develop an excipient inclusion criteria and 'working zone' for paediatric oral formulations
- Review and identify paediatric formulation considerations
- Develop, Optimise and Validate the novel excipient screening platform

6.2 Introduction

Despite recent advances in paediatric drug delivery, the lack of appropriate medicines available for the paediatric population means that medicines are often subject to unlicensed and off-label use. This not only leads to medicine non-compliance and non-adherence but may significantly put this vulnerable group at risk in terms of clinical safety and efficacy. The safety aspect applies to both the API and other inactive ingredients within the formulation, since many excipients intended for adult use are approved and have undergone comprehensive short and long term studies for safety and toxicity in adult population but not in paediatrics. Further, compared to a licensed paediatric formulation, medicines manipulated prior to administration remove regulatory safeguards that would otherwise ensure the formulation has thoroughly been evaluated in all aspects of safety, stability and efficacy and is appropriate for paediatric use.

The insufficiency of suitable medicines for children can be owed to several reasons including the lack of translatable dosage form development technologies that provide palatability, dosing flexibility and an excipient composition and load suitable for paediatrics, and by applying theories and practises that are considered standard in adult pharmacotherapy, without considering the particular needs and requirements of children.

In order to assist paediatric formulation development, several guidance documents have been published (164, 437). However, for formulated products to be effective, safe and of high quality, a more comprehensive form of guidance is required where formulations are systematically formulated with considerations of end user acceptability, clinical efficacy, age-appropriateness and patient centricity. Further, a focus on assessing excipient appropriateness in paediatrics is essential as different dosage from types require the use of different functional excipients. The work presented within aims to develop a novel screening platform that accelerates and de-risks the development of paediatric oral formulations.

6.3 Methods

6.3.1 Excipient inclusion criteria

The initial development steps of the screening tool involved using the information available through the Safety and Toxicity of Excipients for Paediatrics (STEP) database and reviewing the regulatory guidelines for paediatric medicines and excipient safety to develop an excipient inclusion criteria and help identify potentially problematic excipients.

6.3.2 Excipient working zone

The next step included the development of the "excipient working zone", which was constructed by compiling a list of commonly used excipients in oral preparations, such as liquid (solutions and suspensions) and solid (Orally disintegrating tablets, mini tablets) dosage forms. The excipient inclusion criteria was then applied to ensure the resulting "working zone" of excipients only includes excipients that comply with the regulatory guidelines for paediatric medicines and excipient safety.

Before starting the development process of the screening platform, four model APIs were selected, that would be employed to both develop and validate the screening tool. The model drugs were chosen after reviewing existing European Regulatory prioritised paediatric medicines and input from Proveca Ltd, our industrial sponsor.

6.3.3 Differential scanning calorimetry (DSC)

A key process involved in all age-appropriate formulations development was assessing drug-excipient compatibility for selected excipients in order to verify that there is no reciprocal interaction among the components. Any incompatibilities were confirmed using supplementary techniques including FT-IR spectroscopy and X-ray powder diffraction.

6.3.4 Development of excipient screening platform through development of carvedilol as an age-appropriate formulation

The development of an age-appropriate formulation of Carvedilol ODMT (medicine used in paediatric heart failure) was responsible for shaping and giving direction to the screening platform. Inherent physicochemical properties and dosing needs helped identify feasible dosage form types whilst a patient centric formulation approach influenced appropriate excipient selection and further informed appropriate dosage form type that would provide therapeutic efficacy and patient acceptability and compliance. Within the early stages of the development of the screening platform, a comprehensive review of the current landscape of paediatric medicines and differences in physiology and anatomy was undertaken to help identify key challenges and provide adequate knowledge to consider such differences and further guide the selection of appropriate dosage form types. These include variability among children, lack of translatable dosage form development technologies and insufficient paediatric

clinical data. This evidence based approach aims to ensure the resulting products are of high quality, safe and clinically efficacious for the entire paediatric population range. At this point, the 'ICH guideline Q8 (R2) on pharmaceutical development' was revised to implement Quality-by-Design (QbD), Critical quality attributes (CQA) and quality target product profile QTPP within the formulation development process.

6.3.5 Development of excipient screening platform through development of furosemide as an age-appropriate formulation

The development of an ethanol free solution of furosemide was responsible for further developing, adding to and refining the screening platform. Although, several appropriate dosage form types were possible with furosemide, we opted for an oral liquid as a response to market drivers and with a desire for the screening tool to be applied to both solid and liquid dosage forms.

6.3.6 Validation and optimisation of excipient screening platform through development of famotidine and nifedipine as age-appropriate formulations

The development of famotidine ODMT was exercised as a validation opportunity where the draft screening tool was employed to assure whether we can accelerate and develop an age-appropriate formulation with desired characteristics. Lastly, extended release nifedipine mini-tablets were developed to broaden the aspects of use and further widen the ability of the screening tool to inform, optimise and accelerate the development of age-appropriate formulations that not only are age-appropriate but superior to current market formulations in regards to therapeutic safety profiles and reduced administration burden.

6.4 Results and Discussion

6.4.1 Excipient inclusion criteria and development of excipient working zone

The safety and toxicity of excipients for paediatrics were assessed using the STEP database which the European (Eu) and United States (US) Paediatric Formulation Initiatives (PFIs) have collaboratively created. The STEP database is a user-designed free resource that compiles the safety and toxicity information of excipients that is constructed from selected information/database sources. Here, excipient safety and toxicity has been established through either clinical, non-clinical or in-vitro data. This educates and helps formulators decide which excipients are appropriate and suitable for paediatrics and at which concentrations. The database is also supplemented with information regarding maximum excipient daily allowance, regulatory status and excipient functionality.

The “reflection paper: formulations of choice for the paediatric population” was extensively used to inform development of paediatric formulations (18). The intention of the document was to assist in the development of paediatric formulations which allow paediatrics to have access to a range of authorised dosage forms that are safely and effectively adapted to the needs of paediatric patients and a comprised of excipients known to be safe and effective for the age of the paediatric patient. Similarly, the “Guideline on pharmaceutical development of medicines for paediatric use” document was used to provide additional guidance for the pharmaceutical development of medicinal products for children between birth and 18 years of age (164). However, as mentioned in both documents, these guidelines are not intended as regulatory guidance document which define requirements to be fulfilled and therefore should be read in conjunction with relevant EU legislative and guidance documents.

These resources helped identify potentially problematic excipients. These include ethanol, propylene glycol, Benzalkonium chloride and colouring and sweetening agents (Table 45).

Table 45: Overview of potentially problematic excipients including functionality, concerns and permitted daily allowance.

Excipient	Function	Concerns/Adverse effects	Permitted daily allowance	Most commonly found in
Propylene Glycol	Solvent, Co-solvent, Preservative	CNS depression	Neonates: 1 mg/kg Children aged 1 month–4 years: 50 mg/kg Children aged 5–17 years: 500mg/kg (108)	Oral liquids, Injectables, Topicals
Ethanol	Solvent, Anti-microbial preservative	Acute intoxication, Chronic toxicity	Children aged <6 years: 6mg/kg Children aged 6–12 years: 75mg/kg (112)	Oral liquids, Transdermal preparations
Cyclodextrins	Complexing agent, Taste masking	Cecum enlargement, Diarrhoea	200 mg/kg (141)	Oral liquid formulations
Polysorbates	Solubilising agent (Surfactant)	Hypersensitivity	25 mg/kg (143)	Injectables, Liquid formulations
Di(2-ethylhexyl) phthalate (DEHP)	Plasticizer	Endocrine disruptions, Neurotoxicity, Renal toxicity, Hepatotoxicity (144)	50 µg/kg (133)	Controlled/enteric coated formulations (Capsules)

Benzyl Alcohol/Benzoic acid/Benzoate	Preservative, Solvent	Toxicity – Multiple organ failure (62)	Total - 90 mg/kg (123)	Solutions and suspension for injections
Benzalkonium chloride	Preservative	Bronchospasm from anti-asthmatic drugs	0.1 mg/kg	Liquid formulations
Lactose	Tablet and capsule diluent, dry powder carrier, sweetener	Lactose intolerance - Dehydration, metabolic acidosis and prolonged diarrhoea	5 g per dose (127)	Solid dosage forms, Inhalation products
Methyl Paraben Ethyl Paraben Propyl Paraben	Antimicrobial Preservative	Oestrogenic activity Developmental toxicity	Up to 10 mg/kg in total (138)	Liquid formulations
Sweeteners				
Sucrose Fructose Aspartame, Saccharin, Acesulfame-K Sucralose Xylitol Sorbitol	Sweeting agent, Fillers (e.g. Xylitol, Sorbitol)	Hyperglycaemia Obesity Erosion of teeth Hypersensitivity Gastrointestinal disorders Aspartame - harmful in children with phenylketonuria	5 g (127) 10–40 mg/kg (145) 50 mg/kg (116) 15 mg/kg (116) 15 mg/kg (116) 5 mg/kg (116) 10 g (127) 140 mg/kg (145)	Oral liquid formulations, Solid dosage forms
Colouring agents				
Tartrazine Sunset Yellow Curcumin Allura Red Caramels Brilliant Blue Annatto Iron oxide(s) Carmines/carmine	Colouring agent, Product identification (prevent counterfeit), Branding	Hypersensitivity, Negative behavioural effects and ADHD	7.5 mg/kg (146) 4 mg/kg 3 mg/kg 7 mg/kg 100 mg/kg 6 mg/kg 6 mg/kg 40 mg iron/day 5 mg/kg	Oral liquid formulations, Solid dosage forms

As per the guidance of the EMA, if it is not possible to avoid the use of excipients that have a known risk in the formulation of a particular pharmaceutical dosage form, the use could be considered based on a benefit to risk ratio (164). However, a comprehensive rationale should be provided where an argument of the added value of the chosen dosage form type and inclusion of certain excipients takes into consideration the relative benefits and the risks of possible alternative pharmaceutical dosage forms and routes of administration that are not dependent on the use of such excipients.

Several functional excipients are required to achieve a certain dosage form type, these may include tableting excipients such as magnesium stearate and AEROSIL® for solid dosage forms and preservative, pH modifiers, buffering agents and solubility enhancers in liquid dosage forms. A compilation of commonly used excipients in oral preparations can be found in Table 46. The inclusion criteria defined was that excipients must be either approved as an "additive" by the European Commission and/or FDA or Affirmed as (generally recognized as safe) GRAS. Additionally, the concentration of excipients should be under the allowable daily intake value. After applying the inclusion criteria, a summarised excipient working zone was formed (Table 47, Table 48).

Table 46: Commonly used excipients in oral preparations, such as liquid (solutions and suspensions) and solid (Orally disintegrating tablets, mini tablets) dosage forms.

Excipient category	Functionality	Commonly used examples
Solid dosage forms		
Binder	Bind constituents together, providing form and cohesiveness	Native starches, sugars and cellulose derivatives.
Capsule shell	Encapsulate dose, taste mask, modify/control release, provide accurate dosing	Gelatine, glycerol, sorbitol, polysaccharides and cellulose
Coating agent	Protect tablet ingredients from external environment, improve stability, enhance appearance, aid swallowability and modify release of the therapeutic agent (e.g. enteric coat)	Hydroxypropyl methylcellulose (HPMC), beeswax, paraffin, lanolin, shellac and polysaccharides
Colourant	Enhance product appeal, product identification, branding and prevent counterfeiting	Organic dyes and their lakes (e.g. Tartrazine, Sunset Yellow and Allura red), Inorganic or mineral colours (e.g. Titanium dioxide and red iron oxide) and Natural colours (e.g. β-carotene)
Diluent	Simplify dose measurements and improve dose handling, impart desirable manufacturing properties and enhance dosage form performance	Starch (including modified starches), calcium salts (e.g. calcium phosphate) and sugars (e.g. mannitol, sorbitol and lactose)
Disintegrant/ Superdisintegrant	Promote and ensure the dispersion of the tablet compact, responsible for effecting the dissolution profile	Disintegrants - Starch, pregelatinized starch and MCC) Superdisintegrants - Sodium starch glycolate, croscarmellose sodium and crospovidone
Glidant, anticaking agent	Improve the flow and prevent caking and clustering during bulk storage	Colloidal silicon dioxide (fumed silica) and talc
Lubricant	Reduce friction between the compact and die surface during ejection	Magnesium stearate, sodium stearyl fumarate
Plasticizer	Improve workability and flexibility of polymeric materials	Organic esters (e.g. phthalates and citrates)
Liquid dosage forms		
Buffering agent (pH modifier)	Adjust and maintain pH of formulation, enhance drug stability, solubility and absorption, maintain a consistent ionization state	Acetic acid, citric acid, potassium phosphate and sodium citrate
Solvents and solubilising agents	Enhance aqueous solubility of insoluble molecules, vehicle for oral liquid preparations	Ethanol, propylene glycol, glycerin, cyclodextrins, polyethylene glycol and mineral oil
Chelating agents	Stabilise pharmaceutical liquid formulations, Increase	Salt and hydrated forms of edetic acid (EDTA)

	antioxidant defences and antimicrobial efficacy	
Antimicrobial Preservative	Inhibit microbial growth of bacteria, moulds and yeast	Benzalkonium chloride, benzoic acid, benzyl alcohol and methyl paraben
Antioxidants	Prevent deterioration against oxidative processes, thus maintaining the integrity of the dosage form	Tocopherols, ascorbic acid and sodium metabisulfite
Sweetening agents	Improve palatability by masking any unpleasant or bitter taste	Sorbitol, xylitol, glucose, aspartame, sucralose and saccharin
Suspending and/or Viscosity-Increasing Agents	Stabilise disperse systems such as suspensions and emulsions	Acacia, xanthan gum, carboxymethylcellulose, HPMC, PVP and PVC
Surfactants (wetting agent/emulsifier)	Improve solubility and promote stability of drugs in solubilised systems	Lecithin, polysorbates, sorbitan alkyl esters and polyethylene glycols

Table 47: Excipient 'working zone' for solid dosage forms.

Excipient	Functional category	Comments	Max ADI
SOLID DOSGAE FORMS (Tablets, ODT, Minitablets, ODMT)			
Magnesium stearate	Lubricant	Tabletting excipient. Reduce friction between the tablets and die surface during ejection	No ADI allocated
AEROSIL®	Flow aid	Tabletting excipient. Improve the flow of powders during tablet manufacturing by reducing friction and adhesion between particles	NA
Ludipress®	Co-processed excipients	Formulation for fast disintegrating solid oral dosage forms. Mixture of Lactose monohydrate (93%), Kollidon® 30 (3.5%) and Kollidon® CL (3.5%).	NA
Ludiflash®	Co-processed ingredients	Formulation for fast disintegrating solid oral dosage forms. Mixture of Crospovidone (5%), Mannitol (90%) and Polyvinyl acetate (5%).	NA
*Microcrystalline cellulose (MCC) (**dual function**)*	Binder, disintegrant	Tablet disintegrant 5–15 Tablet binder/diluent 20–90	not required to determine a numerical ADI – Not specified
Lactose	Diluent	10–90% w/w	5 g
*Mannitol (**dual function**)*	Diluent	10–90% w/w	10 g
*Starch (**dual function**)*	Tablet and capsule diluent; tablet and capsule disintegrant; tablet binder; thickening agent.	Tablet disintegrants at concentrations of 3–25% w/w	numerical ADI is not necessary
Crospovidone (Kollidon® CL grades) *SSG* *Croscarmellose sodium*	Disintegrant (Water-insoluble) Disintegrant Disintegrant	2–5% w/w 2–8 % w/w 0.5–5.0 % w/w	Not specified 80 mg/kg 30 g/day
Sucrose	Confectionery base; coating agent; granulation aid; suspending agent; sweetening agent; tablet binder; tablet and capsule diluent; viscosity-increasing agent	Function / Concentration (% w/w) Syrup for oral liquid formulations: 67 Sweetening agent: 67 Tablet binder (dry granulation): 2–20 Tablet binder (wet granulation): 50–67	NA
HPMC	Binder; film-coating; extended-release agent; thickening and viscosity increasing agent	Concentrations between 2% and 5% w/w may be used as a binder. High-viscosity grades may be used to retard the release of drugs from a matrix at levels of 10–80% w/w. Depending upon the viscosity grade, concentrations of 2–20% w/w are used for film-forming solutions to film-coat tablets.	0 to 25 mg/kg bw (As sum of total modified celluloses

Table 48: Excipient 'working zone' for liquid dosage forms.

<table>
<tr><th>Excipient</th><th>Functional category</th><th>Comments</th><th>Max ADI</th></tr>
<tr><td colspan="4">LIQUID DOSAGE FORMS (Solutions, suspensions, elixir, syrup)</td></tr>
<tr><td colspan="4">
<table>
<tr><th>Preservative</th><th>Concentration (% w/w)</th><th>pH working range</th><th>Max ADI</th></tr>
<tr><td>Methyl-4-hydroxybenzoate</td><td>0.05 – 0.1</td><td>1 – 8.5</td><td>5 mg/kg/day - 0-10 mg/kg body weight for the total sum of methylparaben, ethylparaben and propylparaben</td></tr>
<tr><td>Propyl-4-hydroxybenzoate</td><td>0.05 – 0.1</td><td>1 – 8.5</td><td>5 mg/kg/day</td></tr>
<tr><td>Sodium benzoate</td><td>0.01 – 0.2</td><td><5</td><td>benzoic acid and its salts has been established to 0–5 mg/kg bw/day</td></tr>
<tr><td>Potassium sorbate</td><td>0.14</td><td>3.5-5.5</td><td>Sorbic acid and its salts are 3 mg/kg bw/day</td></tr>
<tr><td>Propylene glycol</td><td>15–30</td><td>1-14</td><td>50 mg/kg/day in children less than 5 years old, and 1 mg/kg/day</td></tr>
</table>
</td></tr>
<tr><td>Cyclodextrins</td><td>Solubilizing agent; stabilizing agent.</td><td>Aqueous solubility of unsubstituted natural cyclodextrins including αCD, βCD, and γCD, and their complexes is limited, hence the more soluble derivatives such as 2-hydroxypropyl-βCD (HPβCD) are favoured</td><td>children under the age of 2 years treated with up to 200 mg HP-β-CD/kg/day for 2 weeks were well tolerated and considered safe</td></tr>
<tr><td>Xylitol (dual function)</td><td>Non-cariogenic sweetening agent; preservative</td><td>Lower water activity and a higher osmotic pressure than sucrose, therefore enhancing product stability and freshness. Demonstrated to exert certain specific bacteriostatic and bactericidal effects</td><td>20 g/day</td></tr>
<tr><td>Sucralose</td><td>Sweetening agent.</td><td>Sweetening power approximately 300–1000 times that of sucrose and has no aftertaste. It has no nutritional value, is non-cariogenic, does not promote dental caries, and produces no glycaemic response. Used in concentrations of 0.03–0.24% w/w.</td><td>0 to 15 mg/kg bw</td></tr>
<tr><td>Citric Acid (dual function)</td><td>Acidifying agent; antioxidant; buffering agent; chelating agent; flavour enhancer; preservative.</td><td>
<table>
<tr><th>Function</th><th>Concentration (% w/w)</th></tr>
<tr><td>Buffer solutions</td><td>0.1–2.0</td></tr>
<tr><td>Flavour enhancer</td><td>0.3–2.0</td></tr>
<tr><td>Sequestering agent</td><td>0.3–2.0</td></tr>
<tr><td>Antioxidant/Preservative</td><td>0.2-1.0</td></tr>
</table>
</td><td>No recommended daily allowance has been specified</td></tr>
<tr><td>Sodium Phosphate</td><td>Buffering agent; sequestering agent.</td><td>level of dibasic sodium phosphate used as an excipient in a pharmaceutical formulation is not usually associated with adverse effects</td><td>40 mg/kg bw per day</td></tr>
<tr><td>Propylene glycol</td><td>Antimicrobial preservative; disinfectant;</td><td>Oral solutions – 10-25% w/w</td><td>50 mg/kg/day in children less than 5</td></tr>
</table>

	humectant; plasticizer; solvent; stabilizing agent; water-miscible co-solvent.		years old, and 1 mg/kg/day
Xanthan gum	Gelling agent; stabilizing agent; suspending agent; sustained-release agent; viscosity increasing agent.	Although primarily used as a suspending agent, xanthan gum has also been used to prepare sustained-release matrix tablets.	0 to 10 mg/kg bw
Polysorbates	Dispersing agent; emulsifying agent; non-ionic surfactant; solubilizing agent; suspending agent; wetting agent.	They have been found to be useful in improving the oral bioavailability of drug molecules that are substrates for P-glycoprotein	Group ADI of polysorbates to 10 mg/kg body weight/day

6.4.2 Development of excipient screening platform through development of carvedilol as an age-appropriate formulation

To begin with, inherent physicochemical properties of carvedilol were identified. Carvedilol is a weakly basic biopharmaceutical class II model drug with a pKa value of 7.8 and aqueous solubility of 4.44 mg/L (210). It is stable within the gastric environment and does not possess any palatability concerns. For a child (2–17years), carvedilol is initially prescribed at a dose of 50 micrograms/kg twice daily (max. per dose 3.125 mg) for at least 2 weeks, then increased to 100 micrograms/kg twice daily for at least 2 weeks, then increased to 200 micrograms/kg twice daily, then increased if necessary up to 350 micrograms/kg twice daily (max. per dose 25 mg)(228). From the inherent physicochemical properties and dosing requirements, several dosage form types were possible, however, the selected dosage form type must be able to provide dosing flexibility. A dose banding strategy was developed to inform the selection of strengths and confirm whether a solid dosage form is able to achieve the dosing requirements without increasing pill burden (Table 49). Further, as with all formulation developments throughout the project work, we intended to limit the number of excipients within the formulation where and when possible. To achieve this, the favour was with solid dosage forms as it removed the need of including several functional excipients that would otherwise be required in a liquid dosage form (preservative, buffering agent and solubilising agents).

Besides opting for a solid dosage form type to minimise excipient load, the World Health Organization (WHO) has also considered flexible solid oral dosage forms as the most suitable dosage form for children (180). Preparations include chewable tablets, mini-tablets, orally disintegrating tablets and mini-orally disintegrating mini tablets. Such dosage forms are easy to swallow and well accepted throughout the paediatric population (181, 182).

Due to the wide range of dosing needs and low dosing concentrations, an ODMT was considered a feasible choice as different strengths could be counted to provide adequate dosing flexibility whilst the low dosing concentrations could easily be incorporated within the small size of the mini-tablets. After the dosage form type was provisionally selected, the 'excipient working zone' was then referred to do select excipients required to develop an ODMT. Although we had an option to formulate an ODMT using dedicated functional excipients, we decided to further supplement and refine the screening tool.

During formulation development, several different excipient combinations and/or compositions and process parameters were explored to provide desired characteristic. Fundamental pharmaceutics knowledge allowed exploitation of excipients that provide multiple functionalities, thereby limiting the total number of excipients required within a particular formulation. Subsequent excipients are noted as 'dual function' excipients within the excipient working zone (Table 47 and 48).

Table 49: Dose banding and number of tablets required to fulfil dose requirement.

Dose	Age and average child Weight (kg) (229)	Number of tablets required to fulfil dose requirement			
		0.25 mg	*0.5 mg*	*1 mg*	*2 mg*
50 mcg/kg	1 year: 9 kg ≈ 10 kg	–	1	–	–
	3 years: 14 kg ≈ 15 kg	1	1	–	–
	5 years: 18 kg ≈ 20 kg	–	–	1	–
	7 years: 23 kg ≈ 25 kg	1	–	1	–
	10 years: 32 kg ≈ 30 kg	–	1	1	–
	12 years: 39 kg ≈ 40 kg	–	–	–	1
100 mcg/kg	1 year: 9 kg ≈ 10 kg	–	–	1	–
	3 years: 14 kg ≈ 15 kg	–	1	1	–
	5 years: 18 kg ≈ 20 kg	–	–	–	1
	7 years: 23 kg ≈ 25 kg	–	1	–	1
	10 years: 32 kg ≈ 30 kg	–	–	1	1
	12 years: 39 kg ≈ 40 kg	–	–	–	2
200 mcg/kg	1 year: 9 kg ≈ 10 kg	–	–	–	1
	3 years: 14 kg ≈ 15 kg	–	–	1	1
	5 years: 18 kg ≈ 20 k	–	–	–	2
	7 years: 23 kg ≈ 25 kg	–	–	1	2
	10 years: 32 kg ≈ 30 kg	–	–	–	3
	12 years: 39 kg ≈ 40 kg	–	–	–	4
350 mcg/kg	1 year: 9 kg ≈ 10 kg	–	1	1	1
	3 years: 14 kg ≈ 15 kg	–	–	1	2
	5 years: 18 kg ≈ 20 kg	–	–	1	3
	7 years: 23 kg ≈ 25 kg	1	1	–	4
	10 years: 32 kg ≈ 30 kg	–	1	–	5
	12 years: 39 kg ≈ 40 kg	–	–	–	7
Up to 25 mg (max)		Varied depending on dose. Multiple tablets may be counted and taken individually, or encapsulated and taken as a single administration, provided the child is able to swallow the respective capsule size.			

6.4.3 Development of excipient screening platform through development of furosemide as an age-appropriate formulation

In contrast to the preference of solid innovative small flexible solid oral dosage forms as mentioned above, furosemide was developed as an ethanol free oral solution. This was as a response to market drivers, where oral liquid preparations of furosemide are extensively prescribed and widely accepted. However, the amount of ethanol in all current marketed formulations are well above the allowable daily intake threshold values (Table 50). Other than market drivers, the decision to opt for a liquid preparation and its feasibility was further informed and supported through the flow chart (Figure 31). The requirement of dosing flexibility and solubility and stability profiles are key factors that influence dosage form type. The dosing for furosemide in paediatrics is based on child weights and therefore requires a dosage form type that can provide dosing flexibility. Although furosemide exhibits low aqueous solubility and is unstable in acidic conditions, the choice of an oral solution was still preferred due to the market need. In parallel to identifying feasible dosage form types, formulation challenges and requirements from a paediatric perspective are also identified. These may include dosage form acceptability, solubility and stability concerns, palatability and manufacture feasibility. During the formulation development of furosemide, a bottom up paediatric specific formulation developmental approach was employed where inherent physicochemical properties and potential formulation challenges were identified.

Furosemide exhibits extremely low aqueous solubility (0.01825 mg/mL) and sensitivities to light, air and acidic conditions. Initial studies focused on evaluation of pH solubility profile that improved the solubility to 9.57 mg/mL at pH of 7. The need of ethanol or other 'toxic' solubilising agents was, therefore, removed. Owing to the safety concerns of many commonly used antimicrobial preservatives and pH stability profiles of furosemide solutions (most stable at pH 5.5 and above), the choice of preservative was limited. The low water activity and specific bacteriostatic and bactericidal effects of xylitol were exploited to remove the need of dedicated functional preservatives. Additionally, xylitol imparts a cooling effect and is highly effective at enhancing flavour and masking unpleasant taste. Citric acid was employed as an antioxidant, stability enhancer and taste masking agent; exploitation of excipient functionalities to serve multiple functions limits the total number of excipients required within the formulation. Exploiting paediatric applicable excipients to offer multiple functionalities, alongside careful formulation design tailored to paediatric specificities, ensured the development of an age-appropriate furosemide liquid formulation with an excipient composition and load that is suitable across the entire paediatric population. It is important to mention that during preformulation studies, excipient and drug compatibility was also assed using differential scanning calorimetry (DSC). DSC is a thermal analysis apparatus determining temperature

and heat flow corresponding to material transitions as a function of time and temperature (438). DSC is extensively used and usually serves as a first choice of compatibility testing due to its ability to rapidly screen for incompatibility in pharmaceutical mixtures (439). However, DSC results should be interpreted with caution, as the conclusions based on DSC results alone can be often misleading and any incompatibilities should be supported with complementary techniques such as Fourier transform infrared (FTIR) or X-ray powder diffraction (XRPD) (440, 441).

Table 50: Overview of current marketed oral liquid preparations of furosemide including excipient composition and ethanol content.

Marketed solution	List of excipients	Ethanol content (%v/v)
Frusol 20mg/5ml, 40mg/5ml, and 50mg/5ml oral solution (Rosemont Pharmaceuticals Ltd) Marketed: UK	Ethanol, sodium hydroxide, cherry flavour (containing ethanol and propylene glycol (E1520)), liquid maltitol (E965), disodium hydrogen phosphate (E339), citric acid monohydrate (E330) and purified water	7.9% (397.28 mg/5ml) - Above thresholds (293)
Furosemide 4mg/ml, 8mg/ml and 10mg/ml Oral Solution (Thame Laboratories) Marketed: UK	Citric acid monohydrate (E330), Ethanol, Sodium hydroxide (E524), Liquid maltitol (E965), Cherry flavour [containing propylene glycol (E1520)], Disodium phosphate, anhydrous (E339), Purified water	8.3% (416 mg/5ml) - Above thresholds (305)
Impugan 10 mg/mL oral drops Marketed: Sweden	Saccharin sodium (sweetener), sodium hydroxide, ethanol (alcohol content 9.8%) and water	9.8 % (500 mg/5ml) - Above thresholds (294)
LasixR liquid 10 mg/mL Marketed: Germany	Purified water, ethanol, sorbitol solution 70% (non-crystallizing) (Ph.Eur.), Glycerol 85%, sodium hydroxide, quinoline yellow (E 104), yellow orange S (E 110), orange flavour, methyl (4-hydroxybenzoate) and propyl (4-hydroxybenzoate) (parabens) as preservatives	11.9% (595 mg/5ml) - Above thresholds (295)

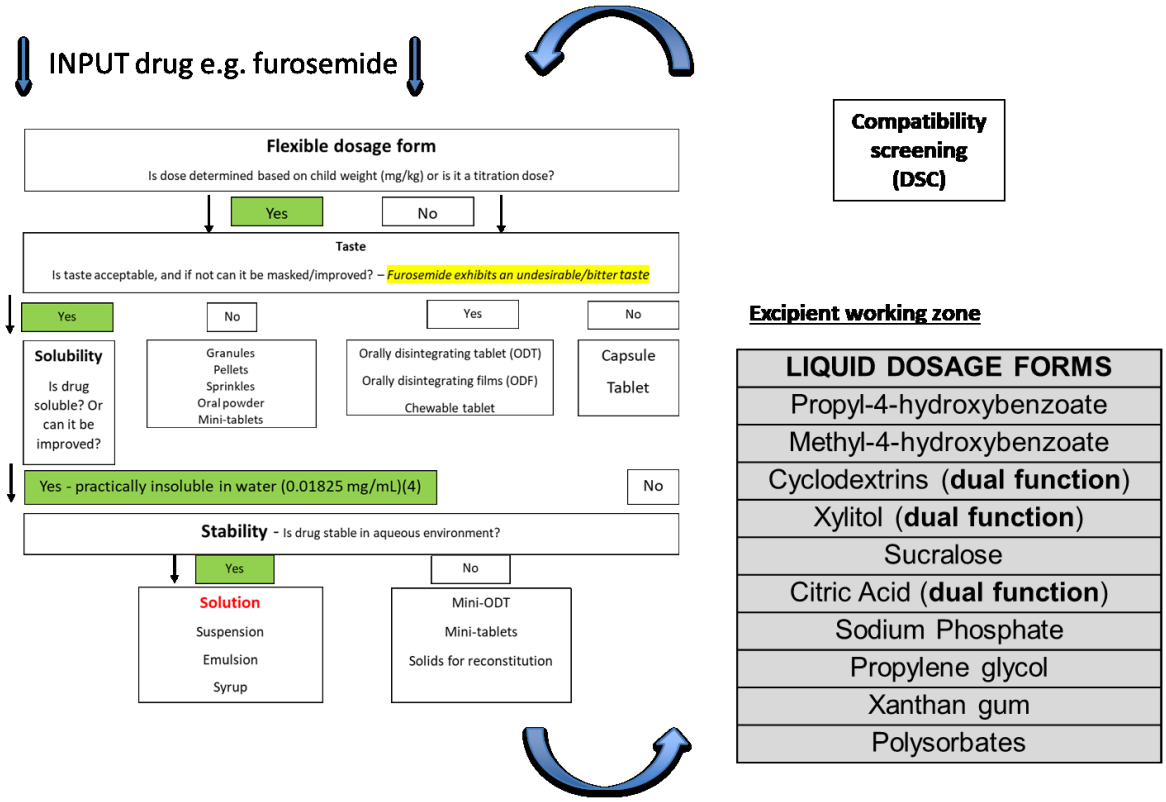

Figure 31: A depiction of the process that identifies feasible dosage form types, followed by excipient selection though the excipient working zone and compatibility studies.

6.4.4 Validation of the excipient screening platform through development of famotidine as an age-appropriate formulation

Formulating carvedilol and furosemide as an age appropriate formulation resulted in a decision making pathway that considers specific paediatric needs, manufacturing feasibility and utilises principle pharmaceutics knowledge that's ensures the development of age-appropriate formulations that are clinically efficacious, safe and of high quality. The development of famotidine as an age appropriate formulation was completed as a validation opportunity to evaluate the effectiveness of the excipient screening tool/ decision making pathway. Similarly, as above, famotidine was processed through the flow chart and several feasible dosage form options were provided (Figure 19). In parallel, manufacturing feasibility, patent landscape, prior art and market drivers were identified and explored to further support the choice of drug dosage form. Once an ODMT was confirmed as the choice of dosage form type, the excipient working zone was referred to, assisting the formulation development process and ensuring the resultant product is safe and regulatory compliant from a excipient composition and load standpoint. During formulation of carvedilol ODMT, several excipient combinations and process parameters were explored to achieve desired characteristics. The conclusions within this work were carried forward and applied to the development of famotidine ODMT (338). This included optimised blending parameters for low dose concentration drugs, optimised excipient concentrations and combinations as well as tablet manufacture parameters.

6.4.5 The integration of the screening tool in PBPK informed formulation development

The development of nifedipine as extended release mini-tablets further contributed to the screening tool ability to inform and optimise age-appropriate formulations. This was through modelling drug performance, evaluating pharmacokinetic (PK) variability and informing dose adjustments in paediatrics using physiologically based pharmacokinetics (PBPK) simulation. PBPK based paediatric modelling was employed to inform innovative dosage form design of a truly patient centric sustained release mini tablet that will meet the needs of paediatric population.

Since short acting preparations cause precipitous reduction in blood pressure, nifedipine is often given as a sustained-release formulation. Currently, the challenge to develop such sophisticated dosage forms is owing to limited formulation development strategies for such formulations alongside little to no work reported on modified/sustained release paediatric formulations.

Initially, PBPK simulation informed that an immediate release preparation of nifedipine warrants safety and toxicity concerns as mean values of systemic concentration in plasma were well above the therapeutic window of 25-100 ng/mL. The unacceptability of an

immediate release preparation suggested the need for a modified release preparation. Using the dosing range provided by the BNFc, intermediate prototype formulations were developed that provided extended release properties. The release data generated from these formulations were then processed into the PBPK simulation.

The choice of technology used to achieve modified release was informed after reviewing manufacturing feasibility, patent landscape and prior art of nifedipine and applying the process knowledge embedded within the screening tool. After reviewing current marketed nifedipine formulations, it was noted that the composition of all marketed nifedipine sustained release preparations are excipient heavy and involve technologies which are complicated and add to overall cost. As with all previous formulation development approaches described above, the formulation of nifedipine extended release mini-tablets aimed to limit the total number of excipients used within the formulation. Consequently, hydrophilic monolithic systems were explored, with several grades of HPMC at various concentrations assessed to provide the desired release rate.

Based upon initial predictions, a twice daily (BD) dosing approach was suggested, reflecting a more realistic dosing approach for children compared to three times a day (TDS). After assessing several dosing regimens, a final dosing strategy of 0.45 mg/kg was suggested that would be applicable across age group 3–7-year-old whilst maintaining the limitation on drug loading (< 10 mg) to ensure a maximum of two mini-tablets are dosed. Owing to the adjusted dose, the dose banding and drug load requirement per unit dose was adjusted accordingly. The integration of the screening tool in PBPK informed formulation development permitted the development of nifedipine as an age-appropriate formulation that exhibits improved safety and consistent therapeutic response profiles and reduced administration burden.

6.4.6 General discussion and future work

In order to meet patient needs and consistently achieve the intended product performance, a systematic approach to pharmaceutical development as mentioned within the ICH Q8 may prove to be beneficial. The approach mentioned within establishes the quality target product profile (QTPP), and takes into consideration the specific needs of the paediatric population (164). The quality target product profile can be defined as an overview of the quality characteristics of a drug product that can be achieved as a goal for the product to achieve the desired quality, taking into account the product's safety and efficacy [ICH Q8 (R2)]. On the basis of the QTPP, critical quality attributes (CQAs) and formulation/process parameters that may affect them should then be identified. CQA is defined as ‘a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range,

or distribution to ensure the desired product quality' and include aspects affecting product purity, strength, drug release and stability (437).

The unique systematic formulation development approach developed functions in a similar method where quality characteristics of the drug product are defined with a patient centric perspective, followed by identifying formulation challenges that may prevent achieving the desired product attributes.

Moving forwards, the concept/methodology of the screening tool is similar to that of Quality-by-Design (QbD). The pharmaceutical Quality-by-Design (QbD) concept is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [ICH Q8 (R2)]. QbD aims to maintain product consistency and enable continuous refinement and innovation throughout the product lifecycle.

The consideration of QbD, QTPP and CQA not only leads to the successful development of an intended dosage form but also acts as a regulatory safeguard where formulations are developed in accordance to regulatory guidelines.

It would be important to mention that this specific formulation development tool is thought to be an evolving tool where more number of formulations developed through the tool would add to its effectiveness. This is because each API comes with its own challenges owing to inherent physicochemical properties. Challenges include stability and palatability concerns, drug sensitivities and limited solubility profiles. Until now, it would be fair to say that formulation developments of drugs possessing similar physical chemical properties to the ones already put through the screening tool would be relatively straightforward as specific challenges encountered for those APIs have already been addressed and the knowledge gained could be used for drugs possessing similar physicochemical properties. An example would be optimising blending for low dose APIs (section 2.4.7). Blending was initially optimised to improve content uniformity for carvedilol ODMTs, the process optimisation was then applied to famotidine ODMTs and proved successful. The screening tool presented within is applicable to most pharmaceutical APIs intended for oral delivery. Although physicochemical properties of certain drugs may present challenges, the bottom-up paediatric specific development approach described above ensures safe and effective paediatric formulation developments. Although formulation developments through the screening tool follows a systematic approach, biologics or medicines intended for delivery routes other than oral (parenteral, inhalation and topical) may not work with this system. This is because these would present with specific formulation challenges that are yet to be explored (biologics are very sensitive and parenterals require significant considerations such as enhanced stability

profiles, sterility, isotonicity and compatibility between drug and excipients). These challenges not only need to be addressed from a principle pharmaceutics point of view but from a patient specific (paediatrics) point of view. Future work would involve subjecting the screening tool to molecules such as biologics or drugs intended for parenteral routes.

Additionally, since palatability is a major factor for medicine compliance in paediatrics, features for palatability testing (including taste, mouth feel, smell, swallowability and aftertaste) could be added to ensure developed products are palatable. From a dosage form perspective, other considerations that can be integrated within the screening tool could include product packaging, product usability and need for delivery devices. Other considerations may include process robustness and quality from a manufacturing point of view and supply chain considerations from a distribution point of view.

Another key element that would better de-risk and accelerate formulation developments is formulation evaluation. Products developed through the screening tool are intended for paediatrics and therefore evaluation of the product should be in relevant conditions. An effort was made where carvedilol ODMTs were evaluated in paediatric biorelevant media (section 2.4.9.2). However, currently accepted in-vitro analytical tools (including dissolution and disintegration) do not mimick relevant paediatric environments and therefore the evaluation may be questionable. As with development of paediatric specific formulations, an effort in developing and validating appropriate analytical methods for evaluation of such formulations should also be made.

6.5 Conclusion

A novel excipient screening platform was developed which aids the acceleration of paediatric formulation development. The process of developing the tool included a holistic and pragmatic approach and utilised fundamental pharmaceutics knowledge whilst considering product scale up and manufacturing feasibility. It was ensured that the tool employed the principles of QbD and QTPP whilst directing the formulation scientist to develop a product which is clinically efficacious and patient centric. Lastly, it is worth mentioning that this unique systematic approach is not considered as conclusive but evolving, as the more age-appropriate formulations developed through the use of this systematic approach, the more contribution it has to the screening tool's effectiveness.

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