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Development of thin film oral drug delivery devices for use in paediatric and palliative care patient populations

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Abstract

There is a lack of “age-appropriate” medicines available for children which has led to the routine use of unlicensed or off-label medicines in children. Oral liquids and suspensions often contain preservatives and solvents which may be harmful to a developing child, and also rely on accurate measurement of small volumes to administer the correct dose. In order to determine the scale of the problem locally, an audit of current prescribing and excipient exposure was conducted in a sample of neonates at the Princess Royal Maternity, Glasgow.

Oral thin films (OTFs), composed principally of water soluble polymers, dissolve quickly in saliva without the need to chew or drink water. They are ideally suited to patients who struggle to swallow other solid oral dosage forms such as tablets; for example, paediatric or elderly patients.

In order to demonstrate the safety of OTFs in newborn infants, a phosphate supplement was formulated as an OTF for the prevention of bone abnormalities in preterm neonates. Characterisation, stability and release studies were carried out and the formulation conformed to ICH standards. In parallel, OTFs containing morphine sulphate were developed for the treatment of neonatal abstinence syndrome.

Ion exchange resins may be ‘loaded’ with drug molecules to form drug 'resinates', which have proved useful in the production of sustained release pharmaceutical formulations. By combining these drug-resin complexes with

OTF technology, several sustained release oral thin films were successfully formulated. An *in vivo* pharmacokinetic study in rats was carried out on one of these formulations - a sustained release morphine sulphate OTF.

The development of a dissolution test method which was more relevant than the Pharmacopoeial designs for oral drug release in neonates was necessary for the characterisation of OTF formulations. Novel apparatus was developed which better mimicked the conditions of saliva production and likely *in vivo* performance.

Oral thin films are proposed as a safe and age-appropriate alternative solid dosage platform for the oral delivery of medicines to both paediatric and elderly patient populations.

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Acronyms and Abbreviations

AAG	α_1-acid glycoprotein
ADI	acceptable daily intake
ALP	alkaline phosphatase
API	active pharmaceutical ingredient
BNF	British National Formulary
BP	British Pharmacopoeia
CHF	chronic heart failure
DL	limit of detection
EMA	European Medicines Agency
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology, and Nutrition
FDA	U.S. Food and Drug Administration
GFR	glomerular filtration rate
GRAS	generally regarded as safe
GTT	gastrointestinal transit time
HPLC	high performance liquid chromatography
HPMC	hydroxypropyl methylcellulose

ICH	International Conference on Harmonisation
IER	ion exchange resin
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KAP	potassium acid phosphate
MHRA	Medicines and Healthcare products Regulatory Agency
MTBE	methyl *tert*-butyl ether
MTDI	maximum tolerable daily intake
NAS	neonatal abstinence syndrome
NICU	neonatal intensive care units
NOEL	no observable effects limit
OTF	oral thin film
$paCO_2$	carbon dioxide partial pressure
Ph. Eur.	European Pharmacopoeia
PIP	Paediatric Investigation Plan
pO_2	oxygen partial pressure
PRM	Princess Royal Maternity
PVP	polyvinyl pyrrolidone
PVPP	polyvinyl polypyrrolidone

QL	limit of quantification
SIDS	sudden infant death syndrome
TGA	thermogravimetric analysis
TPN	total parenteral nutrition
UGT	UDP-glucuronosyltransferases
WHO	World Health Organisation

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Chapter 1 - Introduction

1.1 Paediatric medicine

Currently, there are a limited number of ‘age-appropriate’ formulations available for the safe and effective delivery of medicines to children, both in the UK and internationally. This lack of medicines available in a dosage form suitable for administration to children, and able to deliver the correct dose on a mg/kg basis, has led to the widespread prescribing of medicines to children in an unlicensed or off-label manner.

The International Conference on Harmonisation (ICH) was set up in 1990 with the aim of standardising the format in which pharmaceutical companies were required to submit documentation for registering their products through their respective regulatory bodies, creating international uniformity in product registration practices and allowing easier exchange of information between regulatory authorisations e.g. between the U.S. Food and Drug Administration (FDA) and the European Medicines agency (EMA). The ICH has since developed templates and guidelines to support this harmonised approach (Molzon *et al.*, 2011). Regarding paediatric medicine, the ICH produced a classification system which is now widely used to subgroup children according to developmental stages: preterm newborn infants, term newborn infants (0-27 days), infants and toddlers (28 days to 23 months), children (2-11 years), and adolescents (between 12 and 16 to 18 years; Balakrishnan et al., 2007).

1.1.1 Unlicensed and off-label drug use

Where a medicine is to obtain a marketing authorisation for use in patients, typically only the adult population, rigorous clinical testing must be performed to establish the safety and efficacy of that medicine. However, in children this is not always the case. There is a deficit in the number of licensed medicines available for the paediatric population owing to the limited number of clinical trials and safety data available in these age groups. Performing clinical trials in children can be problematic, with issues over consent, ethics, and recruitment adding additional complexities to trial design. This population also presents limited financial incentive to pharmaceutical companies and performing trials in children can be particularly time consuming (van den Anker, 2005). This has led to prescribers routinely using unlicensed or off-label medicines in children and relying on trial data published in adult populations to extrapolate doses or dosage regimes suitable for use in children.

Unlicensed uses of medicines include extemporaneous compounding of medicines, importing medicines which are licensed for use in children in other countries, and use of chemicals as medicines. Alteration of a formulation to try and make it suitable for use in an infant e.g. crushing a tablet to formulate a liquid suspension or emptying the contents of a capsule into food can drastically alter the rate of absorption, bioavailability, and ultimately the pharmacokinetic profile of the medicine (Conroy and McIntyre, 2005). This can have a profound influence on clinical risk, with the potential for under/overdosing and increased risk of adverse effects. Tablets contain

functional excipients which are not intended for liquid formulations and which may affect the stability of the active compound once in solution/suspension (Glass and Haywood, 2006). Crushing tablets for suspension therefore results in the inclusion of unnecessary ingredients which may have an unwanted effect on the stability of the product or the efficacy of the drug. Interactions with or between excipients are largely unstudied (Woods, 2014). Splitting or crushing tablets can produce inaccuracy in delivered dose and is not recommended in medicines with a narrow therapeutic range. Certain medicines such as those with functional coatings e.g. enteric coating, cannot be further manipulated without the clinical advantage of these preparations being destroyed (Costello, 2007). Off-label use refers to the use of a licensed medicine in a manner which is not covered by the license. This could be for an alternative indication; at a dose outside the marketing authorisation (in children this may be necessary due to the weight of the child); in an age not covered by the license; or by administering the medicine via an alternative route. These uses will not have been studied in clinical trials and so no data relating to their safe application in children will be available (Conroy and McIntyre, 2005).

The use of extemporaneous formulations (i.e. simple pharmaceutical preparations compounded in a ‘custom-made’ fashion by a pharmacist where no commercially available preparation exists) in children is questionable as such products are of unknown safety, quality, stability or efficacy (Conroy and McIntyre, 2005). Few European countries have standards for extemporaneous product preparation, and there is inconsistency even

amongst existing published standards (Ernest *et al.*, 2012). They also lack compatibility study data which may indicate the appropriateness of the compounding of medicines for oral use when consumed with food, drink or other medicines (Standing and Tuleu, 2005). Bioavailability is also rarely investigated for 'specials' products (Ernest *et al.*, 2012).

A review published in the United States reported that up to 80% of prescriptions issued for children are either unlicensed or off-label (Boots *et al.*, 2007). Several studies have been performed which look at unlicensed or off-label use of medicines within neonatal intensive care units and report that as much as 80-93% of neonatal patients in these units receive at least one unlicensed or off-label medicine (Conroy and McIntyre, 2005). The proportion of medicines in the UK which are licensed for children in 2002 was quoted as 58%, which compared to 38% in Australia and 35% in New Zealand (Balakrishnan *et al.*, 2007a). In primary care, this figure is lower but given that the number of children in this sector is greater than in secondary care, and that children in primary care are also under less intensive supervision than in a hospital setting, the use of unlicensed and off-label medicines is still significant. A study of 161 primary care practices in Scotland revealed that 26.1% of children received at least one off-label medicine (Ekins-Daukes *et al.*, 2004).

1.1.2 "Age-appropriate" medicines

There is a global lack of "age-appropriate" medicines for children i.e. medicines available in an appropriate dosage form which allow the correct dose to be delivered and are free from potentially harmful excipients. Some

excipients that are considered inert in adult formulations may cause adverse effects in the paediatric population. Developing physiological differences amongst children can reveal an inability to metabolise or excrete some excipients as effectively as older patients, leading to accumulation of these ingredients to toxic levels (Fabiano *et al.*, 2011). Orally available liquids and suspensions can be considered the "gold standard" formulations suitable for children. However, these formulations present their own problems. For example, pharmacists may be required to further manipulate concentrated liquid formulations, often having to measure very small volumes to dilute in order to obtain the required paediatric dose. This presents a potential source of error as more calculation is required to work out these doses (Uppal *et al.*, 2011). Liquid formulations also present problems in terms of stability, "in use" shelf-life and their ability to support micro-organisms. They may also contain excipients with the potential to cause harm in the paediatric population e.g. benzyl alcohol, ethanol, propylene glycol (van Riet-Nales *et al.*, 2011). Crushing tablets presents problems in terms of compatibility e.g. with foods or drinks, and can also lead to inaccurate dosing. Reformulating a solid powder e.g. a crushed tablet, into suspension to be administered as a liquid is not ideal since poorly soluble drugs may not suspend uniformly without the addition of a suspending agent, and this will result both in residue remaining in the container used for administration and in inaccurate dosing of a non-uniform suspension (Standing and Tuleu, 2005). Inaccuracy or inconsistency in administered dosages can lead to subtherapeutic doses being delivered, or overdosing with the potential for adverse effects or toxicity. As well as

being able to accurately deliver the required dose for the child, an orally administered medicine must also be of an acceptable taste and appearance, ensuring the product is “child friendly” and encouraging compliance. Particular attention should be made, when designing medicines for children, to the rapid developmental changes in gastrointestinal physiology which occur at different ages (see section 1.1.5). Differences in salivary, gastric and intestinal pH, and intestinal transit time may affect the bioavailability of the active pharmaceutical ingredient (Costello, 2007).

1.1.3 Compliance

Compliance within the paediatric population ranges from as low as 5% up to 95% but with a mean of around 50% (Winnick *et al.*, 2005). There are a plethora of factors which can influence compliance in children. The acceptability or palatability of the medicine and any adverse side effects experienced certainly play a significant role. The lack in availability of products in a formulation which will be acceptable to children contributes to this issue. The child’s social or economic circumstances can be another factor, as well as education. A further complication when we consider issues regarding compliance in children is the involvement of third-party members including parents, nurses and other care-givers. Health literacy (i.e. the ability to read information about health and healthcare, and make decisions about treatments and/or follow instructions regarding treatment regimens based on that information) is another influential factor contributing to patient compliance. In paediatrics, health literacy is important to both the patient and the care-giver and information provided must be tailored to suit the level of

understanding and requirements of the particular member of the partnership (Blair, 2010). In terms of the dosage regimen, research looking at compliance within paediatric population has indicated that the simplest effective dosage regimen is best: ideally limiting doses to once daily administration or if this is not possible, then avoiding the hours in the middle of the day to reduce the need for dosing during school hours. Simplifying dosing schedules and improving communication between the physician(s) and the parent(s) or other care giver(s) will improve compliance and continuity of care (Winnick *et al.*, 2005).

1.1.4 Excipient toxicity

Whilst considerable investigation has been directed towards the clinical pharmacology of drugs in paediatric populations, a dearth of information on the extent of exposure and possible toxicological burden of excipients within these medicines exists (Lass *et al.*, 2012). This is despite recorded examples of unintended harm as a result of excipients (Fabiano *et al.*, 2011). This topic is covered in depth in Chapter 3, where the results of an observational study of excipient exposure within a local neonatal intensive care unit are also presented.

1.1.5 Physiological development

Growth and maturation during the course of paediatric life consist of a series of simultaneous physiological processes that result in rapid growth and development. Rapid and continuous changes in body weight, composition, as well as organ maturation all impact on population specific pharmacokinetics

and pharmacodynamics. There exists, within neonatal intensive care, a ten-fold difference in body weight ranging from 0.5 kg to 5 kg. A newborn baby increases in body weight by about 50% across the first six weeks of life and can be expected to triple in weight by its first birthday (Allegaert *et al.*, 2014).

1.1.5.1 Renal development

Renal elimination is affected by diuresis and glomerular filtration rate (GFR) as well as tubular activity (reabsorption and secretion) (Allegaert *et al.*, 2014). Serum creatinine at birth is reflective of the mothers' plasma concentrations. Gallini *et al.* (2000) showed that there is an initial increase in plasma levels within the first few days of life in newborn neonates which then decreases over the next five weeks. In neonates with a lower gestational age, the initial increase is larger and the following decrease is slower. Creatinine clearance is directly correlated to gestational age and increases with postnatal age. There was an element of bias in the study by Gallini *et al.* in that they only considered the renal development of clinically stable neonates, excluding 61% of eligible subjects due to factors which could have affected renal function. However, the study did provide information on the renal development of preterm neonates below 32 weeks gestational age, an ICH subgroup previously not investigated in as much detail. In preterm neonates, the GFR can be as low as 0.6-0.8 mL/min/1.73m^2, reaching around 2-4 mL/min/1.73m^2 by term, and only reaches full maturity (above 90 mL/min/1.73m^2 indicates a normal GFR in adults) by 8-12 months of age (Costello, 2007). Therefore, dosage regimens of drugs cleared renally must be carefully tailored to the rapidly developing and wide-ranging renal

functions of neonates in order to prevent toxic effects. For example, it has been suggested that for the aminoglycoside antibiotic gentamicin, a dosage interval of between 24 and 48 hours is appropriate for neonates to avoid accumulative toxicity (Lanao *et al.*, 2004).

1.1.5.2 Body constitution

Differences in body constitution in terms of percentage body water which is higher in the first few weeks of life, and percentage body fat which is lower, can also influence distribution of drugs in neonates (Strickley *et al.*, 2008). By 26 weeks gestational age, extracellular compartments make up approximately 65% of total body weight. This gradually decreases throughout life to nearer 20% by 10 years of age. In preterm infants, up to 85% of total body weight is water compared to only 55-60% in adults (Modi, 2003; Alcorn and McNamara, 2003). This difference in body composition means that for neonatal infants, doses for highly water-soluble drugs may need to be higher relative to body mass than they would be for adults since a greater proportion of their body is water (Suggs, 2000).

The concentration of total free plasma protein is lower in younger children and significantly lower in preterm neonates and infants. Mostly, protein concentrations increase to mature adult levels over the first six months of life. Whilst the majority of proteins are found at levels much lower than measured in adults, some concentrations are higher amongst neonates than in adults. For example, the protease inhibitors α_1-antitrypsin and α_2-macroglobulin are found at much higher levels in neonatal children reflecting a response to raised protease uptake in this population due to immature intestinal barrier

development (Kanakoudi *et al.*, 1995). In newborn infants, human serum albumin (the most abundant human plasma protein) was found in levels approximately 75-80% of that in adults. In comparison, α_1-acid glycoprotein (AAG), an acute phase protein important for carrying basic drugs, was only found at around 50% of adult levels (McNamara and Alcorn, 2002). Drugs such as those used in post-operative anaesthesia are reliant on free plasma levels of AAG and therefore it is important to take this into account when deciding upon safe doses of basic drugs for use in infants (Stumpe *et al.*, 2006).

1.1.5.3 Gastrointestinal pathophysiology

There are significant differences between the gastrointestinal systems of adults and children which can affect drug absorption and stability within the gastrointestinal tract. Data on gastrointestinal transit can be difficult to obtain since it often involves invasive procedures and carrying out non-essential procedures on children is ethically questionable. However, differences in terms of development of swallowing mechanisms, gastric emptying, gastrointestinal transit times, gastric pH, and gut surface areas have been studied (Costello, 2007). In children below the ages of 4-5 weeks there exists an extrusion reflex which prevents them from swallowing solids. The development of the swallowing mechanism is complex and relies on interactions between the developing central nervous system and the physiological systems (Bowles *et al.*, 2010). During the first 2-3 days after birth, an infant’s stomach does not offer any contractions to empty solids and in fact, gastric emptying in general is slower amongst infants up to about 6

months of age; and is particularly slow in preterm neonates. Gastric emptying in an acutely ill neonate may be virtually non-existent. Therefore, drugs administered to neonates and infants are often preferred in an intravenous or rectal dosage form. The pH of the stomach content is higher at birth (~6-8) due to the alkaline nature of amniotic fluid (pH 6.9-7.9) but this quickly drops to 1.5-3 within the first 6 hours of life. Neonatal stomach content returns to a more neutral pH (6-7) within 1-10 days and then gradually settles to the acid nature seen in adults over the next 2 years. Only small volumes of saliva are produced by neonatal children with minimal enzymatic activity. Approximately 0.03-0.04 ml/min are produced by the neonate compared to 0.3-1.2 ml/min observed in adults (Kaye, 2011). McDonnell and Hector (2001) also noted that of 30 children aged between 6 and 8, 75% showed a preference over which side of their mouths they chewed on. It was previously documented that amongst adults, one sided chewing resulted in a tendency to secrete saliva unilaterally to the same side. The authors concluded that the same effect was observed amongst children, with a greater excretion of saliva seen on the most stimulated side of the mouth. They also found that the distribution of saliva around the whole mouth was not even, with saliva tending to remain on the same side as it is secreted. Not surprisingly, the overall capacity of the stomach changes rapidly in the developing child. In a neonatal baby, the average volume of the stomach can range from 10ml to 100ml and in infancy the capacity can vary from 90ml to 500ml. By adolescence the stomach volume is nearer 1.5 litres compared to the 2-3 litre average adult stomach capacity (Kaye, 2011). Very few studies exploring the

transit time of drugs through the gastrointestinal tract in children exist and even where published studies do exist, differences in terms of age groups, co-morbidities, types of food and drink used, and research methodology make it difficult to compare data. However, it has been observed that preterm infants generally have longer gastrointestinal transit times than babies born at term (Bowles *et al.*, 2010). One cross-over study which looked at the effects of erythromycin on gastrointestinal transit time (GTT) reported an average GTT amongst 21 preterm neonates of 15.9 hours during the placebo period (Costalos *et al.*, 2001). Another reported a median GTT of 19 hours amongst 15 preterm infants fed a standard preterm formula (Mihatsch *et al.*, 2012). This compares to averages of 23.7 and 25.4 hours amongst children aged 2 months to 3 years and 3 to 12 years respectively (Bowles *et al.*, 2010). Transit time through the gut is of clinical relevance since it will affect the absorption of orally available medicines and therefore the bioavailability. A review paper by Kaye (2011) summarises gastrointestinal transit time within different age groups. Generally, neonates and infants up to 6 months old have a slower overall gastric transit time partially owing to a slower gastric emptying time (54-82 minutes in neonates *vs*. 12-70 minutes in infancy). Little difference in transit time through the small intestine is reported across the age range (around 4 hours), however overall transit time through both the small and large intestine is widely variable, ranging from 8-96 hours in infants and 2-48 hours in adults. Although, it is also noted that the surface area of the small intestine within infants is proportionally larger than in adults, which may result in greater opportunity for drug absorption (Kaye, 2011).

1.1.5.4 Ontogeny of hepatic enzymes

A detailed look at the developmental changes in liver enzymes by Hines (2007) followed the ontogeny of the cytochrome P450 enzymes throughout childhood. 240 samples from liver tissue ranging from 8 weeks gestational age to 18 years old were assessed for microsomal cytochrome P450 content. Several significant changes were reported between foetal, neonatal and infant enzyme activity, and described how maturity compared with adult activity. For example, CYP3A7 was by far the most dominant isoenzyme observed in foetal liver samples, observed at levels double that of adult CYP3A4 levels. However, levels dropped by half in the neonatal liver and a further 5-fold reduction was seen by infancy. In contrast, CYP3A4 and CYP2C9 within foetal livers were measured at only 3% and 1% of the levels seen in the mature adult samples respectively. An increase in CYP2C9 was measured in neonatal samples to approximately 25% of the activity in adults, but little further development was seen in either enzyme until much later in life (Hines, 2007). As well as developmental changes in hepatic enzyme activity, genetic factors can also influence interindividual variation in drug response. Visscher *et al.* (2011) reported that 6-10% of individual response to warfarin was attributable to genetic differences in CYP2C9 expression. Genetic testing can allow the prediction of how different patients will respond to certain drugs, adding to the move towards 'individualised' medicine (Evans and Relling, 2004). However, much of the current evidence within the field of pharmacogenomics has been established from studies in adult populations. It is unknown exactly what influence genetic differences will have in paediatric

populations since differences in body weight and organ development may play a greater role, masking the effects of interindividual genetic variations. Genetic influences can account for differences in metabolism resulting in poor metabolisers who are more vulnerable to adverse effects from certain drugs. An example could be genetic differences in CYP3A4 activity between adults being accountable for QT-interval prolongation amongst poor metabolisers of cisapride (a prokinetic drug withdrawn in 2000). However, as activity of this enzyme is low throughout childhood due to developmental differences, the genetic influence is not relevant (Hines, 2013). In contrast, one study which looked at genetic differences amongst children in the Western Cape Province of South Africa (a region with a high prevalence of tuberculosis) found that of 64 children included in the study, 39% were homozygous slow acetylators of isoniazid (an antituberculosis drug), 38% were heterozygous fast acetylators, and 23% were homozygous fast acetylators. Children have a significantly faster rate of elimination of isoniazid than adults and many of the fast acetylators fail to achieve the required target plasma concentrations. It is therefore justified that children should receive greater doses per kilogram body weight than recommended for adults (Schaaf *et al.*, 2005).

Parabens e.g. butyl paraben, are included in many pharmaceutical products and foodstuffs for their antimicrobial actions as preservatives. They are metabolised by hepatic enzymes: primarily undergoing hydrolysis to form 4-hydroxybenzoic acid before undergoing glucuronidation catalysed by UDP-glucuronosyltransferases (UGT) prior to excretion via the kidneys. The main

UGT isoenzymes responsible for the glucuronidation step are UGT1A1, 1A8, 1A9, 2B7, 2B15 and 2B17 (Abbas *et al.*, 2010). Strassburg *et al.* (2002) reported that UGT1A9 and 2B4 showed age dependent increases in activity from 6 months of age up to 24 months of age whereas other isoenzymes did not reveal a statistically significant difference from adult samples. In foetal liver samples, UGT2B7 was measured at 10-20% of adult levels whilst UGT2B17 showed less than 10% of adult activity (Hines, 2008). Toxicity studies have shown an oestrogenic effect of parabens in rats with a similar potential risk in children (Boberg *et al.*, 2010).

Methods for the prediction of drug dosages in children often employ allometric scaling using drug clearance data obtaining from adult populations. However, no scaling technique is effective for estimating clearance for all drugs across all age groups (Mahmood, 2006). Pharmacologically based pharmacokinetic (PBPK) modelling incorporates information about the developing renal and hepatic functions in paediatric populations to provide a better estimation of patient response (Johnson, 2005). Simcyp® is an example of a systematic model which has been able to predict almost 90% of paediatric drug clearances to within 2-fold of the true *in vivo* values by integrating data on developing physiology and hepatic enzyme ontogeny (Johnson *et al.*, 2006). Simulations developed using published *in vitro* data on hepatic enzyme expression and changes in liver weight have been able to calculate the levels of CYP2D6 and CYP3A4 expressed over the first year of life (Johnson *et al.*, 2008). These predictions have correlated well with *in vivo* figures published by Blake *et al*. (2007) and corrected for renal development.

Expression of CYP3A4 at aged 1 year is approximately 72% of that of adults whilst CYP2D6 levels are at 92% of those in adults (Johnson *et al.*, 2008).

1.1.6 Formulations currently available

An appropriate medicinal product for use in children must not only be capable of delivering an accurate dose to the patient, but also must be available in a range of dosage forms suitable for the different requirements of the various age ranges and developmental stages within paediatric populations (Nunn and Williams, 2005). The World Health Organisation (WHO) recommends that dosing in children is done on a mg/kg basis. This allows simple dose calculation and minimises error when compared to more complicated calculations such as body surface area which requires both height and weight. However, occasionally it may be preferable to use body surface area when calculating doses for children as this may better relate to the physiological changes which occur throughout childhood. Within reference sources such as the British National Formulary (BNF), doses for children are also found as age and weight ranges (Lack and Stuart-Taylor, 1997).

As mentioned, liquid formulations are considered the “gold standard” for administration of medicines to children. Other formulations which have been considered suitable for use in patients such as children, the elderly and patients for whom swallowing solid dosage forms is more difficult have included orodispersible or orally disintegrating tablets. These are tablets formulated such that they dissolve rapidly in the mouth within a matter of seconds without requiring water or chewing. Many different techniques for manufacturing rapidly disintegrating tablets have been developed including

the incorporation of effervescent material into tablets by direct compression; use of swelling agents such as microcrystalline cellulose in combination with superdisintegrants such as crospovidone; freeze-drying materials to form porous, water soluble matrices; and using nanoparticles to greatly increase the surface area and thereby the dissolution rate of drug molecules (Badgujar and Mundada, 2011). For example, Orasolv® technology incorporates the active ingredient into a polymer such as methyl cellulose by direct compression. Mannitol and magnesium oxide are included as release agents (magnesium oxide acts as an alkaline diluent), which together with an effervescent base and an effervescent acid, allows rapid disintegration of the tablet when in contact with saliva. Flashtab® is another example of a rapidly disintegrating tablet and uses a swelling agent to promote the release of the active ingredient without the need for chewing and with minimal water requirement (Badgujar and Mundada, 2011).

As mentioned, the simplest dosage regime is the best to encourage compliance in a paediatric population. Therefore, the formulation of an age-appropriate modified-release preparation would be extremely useful. Strategies could include the use of very small solid dosage forms (e.g. minitablets, minicapsules) which children would be able to swallow, or formulation of a modified-release liquid using nano- or micro-particles and polymer coatings (Standing and Tuleu, 2005). Transdermal drug delivery also presents a promising method for modified drug delivery. In the preterm neonate, the structure of the skin is not fully developed with the epidermis and stratum corneum significantly thinner than that of a child born at term.

Although this is detrimental to the neonate in terms of its ability to retain water, heat, and defend against invading pathogens, the immature dermis does present a promising route for drug delivery since transdermal absorption of drugs is increased. However, the risk of toxicity from transdermal drug delivery is also increased and the immature skin is more susceptible to damage (Visscher, 2009).

Presently there is no specific age at which children can be given solid oral dosage forms although the quoted cut-off point is often 6 years of age. However, children younger than this age can swallow solid dosage forms even from as young as two years old with sufficient training. A study carried out in a hospital in London considered the ability of children aged 2 to 6 years old to swallow 'minitablets' – miniature compacts with diameters of between 2 and 5 mm. The results showed that 76% of 4 year old and 87% of 5 year olds could swallow the minitablet. Younger children had a tendency to chew the minitablets before swallowing which could complicate certain formulations e.g. modified release or enteric coated formulations (Thomson *et al.*, 2009). Continuing on from this research, Stoltenberg and Breitkreutz (2011) developed an orally disintegrating mini-tablet formulation using co-processed mannitol variants to provide the rapid disintegration. Focusing on a commonly used diuretic medicine, hydrochlorothiazide, the authors achieved a simulated wetting time of less than 5 seconds and concluded that a rapidly disintegrating mini-tablet represents a solid dosage platform with the potential for use in children even younger than two years of age.

1.1.7 Delivery devices

Another important aspect of drug delivery is the use of delivery devices for accurate administration of medicines to children. For oral liquid formulations, measuring spoons/cups, droppers and oral syringes are the most commonly used delivery devices. Accuracy of dosage varies between devices and relies on correct use of the device for consistent dosing. For example, oral syringes are regarded as the most accurate method of delivering small volumes (< 5mL) of oral liquids. However, some training on correct use of oral syringes is required in order to correctly measure the dosage volume and avoid air bubbles within the syringe. Droppers are used where very small volumes of liquids (drops) are required however aspects such as the angle at which the dropper is held, the viscosity of the liquid, and inclusion of air bubbles can affect the accuracy of the administered dose volume. Measuring spoons are the easiest to use but variability between measured spoonfuls of liquid is a problem (Walsh *et al.*, 2011). Solid dosage forms such as tablets or capsules avoid these difficulties as accurate dosage delivery is achieved by uniformity of content between dosage units. This presents another reason why more age-appropriate solid dosage forms for the paediatric population are required (Walsh *et al.*, 2011). Additionally, the development of an age-appropriate solid dosage formulation for children would allow the omission of potentially toxic preservatives since the water content of solid dosage forms is too low to support micro-organism growth.

1.1.8 Legislation

In 1997 the U.S. Food and Drug Administration (FDA) created the Modernisation Act (1997) to tighten the regulations concerning paediatric medicines. This led to the inclusion of the Paediatric Rule (1997) and the Paediatric Studies Incentive (1998) which offered a six month extension on any new medicine application when studies of that medicine in a paediatric population presented data deemed to be of a benefit to the health of children. This incentive offering extended market exclusivity was expected to increase the numbers of trials performed in children and help fill the gap in safety and efficacy data available for paediatric populations. To evaluate the effect of this, Boots *et al.* (2007) reviewed the drugs granted exclusivity between July 1998 and August 2006. 135 drugs were granted exclusivity of which the authors obtained study information for 118. The results revealed an inconsistency between the drugs granted paediatric exclusivity and the prescribing patterns of physicians in children populations. It was identified that although studies carried out in children did increase during the study period as expected, the drugs for which exclusivity was granted were more commonly prescribed to adults. Therefore the benefits of the legislation in terms of accountability for the healthcare needs of children could be questioned (Boots *et al.*, 2007).

With the positive approaches observed in the U.S., the EMA adopted its own paediatric legislation. This came into effect in 2007 and requires companies applying for a marketing authorisation for any new medicine to submit a Paediatric Investigation Plan (PIP) detailing the steps they will take to study

their product in children. Again, this legislation comes with a 6 month exclusivity incentive which may be granted on submission of a satisfactory PIP. For off-patent medicines without current market exclusivity, there was created a Paediatric Use Marketing Authorisation (PUMA). This incentive offers companies 10 years of exclusivity within the paediatric market in exchange for developing e.g. a new strength, form or route of administration suitable for paediatric use (Stoyanova-Beninska *et al.*, 2011). Overall there has been a positive trend in approval of medicines with study data supporting their safe and efficacious use in children across Europe. There has also been an increase in the availability of evidence regarding pharmacology within children which physicians can refer to when prescribing off-label or unlicensed medicines. An international review of the volume and quality of paediatric research which has been conducted found that studies published in the last decade have shown a significant improvement in quality, indicated by an improvement in the Jadad score (van Riet-Nales *et al.*, 2010). The Jadad scoring system is a widely used method for rating the quality of study methodology. Points are awarded or deducted based on the description and appropriateness of randomisation and blinding, and where the authors account for all patients, including any withdrawals. The final score ranges from 0 to 5 and the higher score indicates a more robust research methodology (Jadad *et al.*, 1996).

Van Riet-Nales and colleagues (2010) also confirmed concerns that there are a lack of trials conducted in pre- and full-term neonates and in infants and toddlers under the age of 2 years. Other trends reported were that patient

preference is growing in importance and that there is still insufficient information included in published studies regarding adverse drug events, palatability and reporting of administration errors.

1.1.9 Palatability/acceptability

There are a limited number of studies published which consider palatability when looking at the suitability of medicines in paediatric populations. However, it has been shown that palatability has an important role to play in compliance within these populations. The numerous ion and G-protein coupled receptors responsible for taste begin functioning well before birth and can be considered structurally mature from 13-15 weeks gestational age. Likewise, the olfactory system is structurally akin to that of an adult by only 11 weeks gestational age. Thus a child only a few hours old can show preference for sweet tastes over more bitter flavours and will reject the latter (Mennella and Beauchamp, 2008). There is currently no universally agreed standard as to how to measure palatability or taste perception in young children. The most frequently used methods for measuring preference and acceptability look at reflex responses such as changes in heart rate, facial expressions, tongue reflex, and sucking inhibition. Facial rating scales have been used to help parents assess medicine acceptability in children. However, there is some debate as to the validity of such a scale in children younger than six. Also, parents may be influenced by their own perceptions of tastes and smells and introduce bias into the process (Cohen *et al.*, 2009).

1.1.10 Adverse drug events and errors

The risk of adverse drug events in children younger than 5 years old is four times greater than in older children. Most adverse reactions occur as skin reactions e.g. rashes, urticaria, and as gastrointestinal upset e.g. diarrhoea, nausea, vomiting. It is difficult to establish exactly what the risk of adverse drug effects will be in a paediatric population since studies often involve relatively small sample sizes and it is impossible to investigate the rarer but still potentially significant side effects in such a small population sample. Also, there is very little data available on the long-term effects of drugs in children calling for more post-marketing research in order to provide a complete safety profile for a drug. This will require a coordinated effort between drug development and post-market research teams. Pharmacovigilance i.e. the collection and evaluation of information regarding potential adverse drug effects with a view to reducing the risk to patients, is an essential but often overlooked process. An Italian study reported that the incidence of 'spontaneous' reporting of information on adverse effects in children is low (1.6-1.8%). There is a lack of understanding regarding the benefits of spontaneous reporting of adverse drug reactions by the general public (Napoleone, 2010). In the UK, the Medicines and Healthcare products Regulatory Agency (MHRA) are responsible for ensuring that medicines and medical devices are 'acceptably safe' and efficacious. In order to promote pharmacovigilance and spontaneous reporting of suspected adverse effects, the MHRA in partnership with the Commission on Human Medicines (CHM) created a nationwide reporting system known as the Yellow Card Scheme.

The scheme allows patients and healthcare professionals alike to quickly and easily report adverse effects and encourages the reporting of all serious adverse events, any event in a closely monitored medicine (known as black triangle), and also *any* adverse effect in a child even if it is a well-established medicine. More than 20,000 Yellow Card reports are submitted every year but its continued success is dependent on voluntary submission of reports and the willingness of people to submit such reports (Medicine and Healthcare products Regulatory Agency, 2011).

1.1.11 Ethical considerations

Conducting clinical trials in children raises a number of legal and ethical issues. Placebo controlled trials have their advantages in that they allow statistically significant results to be drawn from a relatively small sample size. This might seem practical in paediatric trials where the numbers of participants are smaller and also since involving more children than necessary in a trial would be deemed unethical. Use of placebos also eliminates any unnecessary risks from adverse drug reactions. However, placebo controlled trials are not suited to paediatric trials since there could be a therapeutic disadvantage to the children assigned to the placebo which would be equally unethical. The principal of equipoise applies in paediatric research, requiring that both or all arms of any given study should be comparable in terms of risks and benefits (Pinxten *et al.*, 2010). Where it becomes apparent that a particular treatment is obviously superior, equipoise no longer exists and it would be unethical to knowingly continue a less clinically effective treatment in other groups. An alternative model which may

be of interest is that of N-of-1, or single-subject, trials (Adatia *et al.*, 2013). These are multi-cycle, randomised, double blind, crossover studies in single individuals, which can provide information on treatment efficacy in an individual. Multiple measurements in a single individual have less variance and therefore a greater power to detect whether a treatment is beneficial or not (Lillie *et al.*, 2011). N-of-1 trials have already been successfully carried out in children with arthritis, cystic fibrosis and attention deficit hyperactive disorder (ADHD) (Adatia *et al.*, 2013). They are specifically suitable for studying drugs with short half-lives and rapid onset/offset of action, which have a measureable effect on a chronic, stable condition, but do not alter the underlying condition itself (Scuffham *et al.*, 2010). These types of trials may be of value in paediatrics since they offer a personalised approach to a population with small numbers and a limited evidence base, and may help guide clinical practice. Whilst results of a single n-of-1 trial cannot be generalised, summation of results from a series of single subject studies could help complement predictions for the wider population (Duan *et al.*, 2013). The studies are quick and inexpensive, and some studies have suggested that costs may be offset by post-trial savings to the health service (Scuffham *et al.*, 2010).

Prescribing of off-label or unlicensed medicines often represents a reasonable use of medicines in children given that few age-appropriate licensed medicines exist for young children, and that it would be unethical to deny a child the best potential treatment on the grounds that it is unlicensed (Conroy and McIntyre, 2005). However, without an evidence base to support

unlicensed or off-label uses of medicines, there is the potential both for subtherapeutic doses of medicines to be prescribed or increased toxicity in the developing child. This again presents a difficulty in establishing what constitutes an ‘allowable’ risk level within a paediatric clinical trial (Kern, 2009).

In the UK, the law regarding age of consent states that a child (<16 years of age) can give consent to healthcare treatment which cannot be overruled by a parent or guardian where that child is considered competent to do so (known as ‘*Gillick*’ or ‘*Fraser*’ competence). Where a child has not developed the maturity or lacks the understanding to make informed decisions, then parental consent is required to proceed with treatment (Perera, 2008; Payne, 2008).

1.2 Oral thin films

Within the last few years, rapidly dissolving oral thin films have been developed as a novel solid dosage platform for drug delivery. They were first established as breath fresheners and have since progressed towards delivery of active pharmaceutical ingredients (APIs) (Bala *et al.*, 2013; Dixit and Puthli, 2009).

Approximately the size of a postage stamp (see Figure 1), oral thin films dissolve quickly in the saliva, releasing the active ingredient(s) without the need for chewing or water. They are ideally suited to patients who find it difficult to swallow other oral dosage forms such as tablets or capsules. Paediatrics and geriatrics are particularly suited to this dosage design.

Flavourings and sweeteners can be included in the films to mask a bitter tasting active ingredient. The films are discreet, easy to use and convenient.

Figure 1. Oral thin film size. Oral thin films are approximately the size of a postage stamp. Formulations were modelled around a maximum target size of 15 x 15 mm (as shown) for neonates.

Oral thin films (OTFs) have a history of uses in oral hygiene products and medical devices (Arya *et al.*, 2010). In the USA, Novartis marketed several thin film products under the brand Triaminic® which were aimed at children from as young as four for the treatment of coughs, colds, and allergies (in-PharmaTechnologist.com, 2004). In 2010, Zuplenz® became the first FDA approved prescription only medicine in an oral thin film formulation for the delivery of ondansetron in the treatment of postoperative, chemotherapy- and radiotherapy-induced nausea and vomiting (U.S. Food and Drug Administration, 2011). Applied Pharma Research and Labtec later announced the marketing of their own ondansetron based oral thin film product across Europe, under the brand name Setofilm® (Norgine, 2012). The film is licensed in children from 6 months for the management of chemotherapy-induced nausea and vomiting. In February 2012, Applied Pharma Research were successful in marketing a prescription only zolmitriptan based OTF product for the treatment of migraine. Most recently in 2014, BioDelivery Sciences successfully marketed Bunavail™, a sodium carboxymethylcellulose based mucoadhesive film for buccal delivery of

buprenorphine in combination with naloxone (BioDelivery Sciences International, 2015).

A basic oral thin film formulation can contain very few excipients (Arya *et al.*, 2010). Most importantly, a water-soluble polymer is used as the film-forming agent and makes up at least 45% w/w of the dry strip weight. Examples include cellulose derivatives such as hydroxypropyl methylcellulose (HPMC); natural gums, pectin, collagen, gelatin, and polyvinyl alcohol. Pullulan is a natural polysaccharide made up of maltotriose units and derived from the fungus *Aureobasidium pullulans* by simple fermentation processes. Different film formers will produce films with different release profiles and formulators can adjust composition depending on the desired film mechanical strength and dissolution rate required. Secondly, a plasticizer such as glycerol, polyethylene glycol, or propylene glycol is included to give strength and flexibility to the film (normally 0-20% w/w of the films dry weight). Other excipients which can be incorporated into a thin film formulation include sweeteners (3-6% w/w) and flavours (up to 10% w/w) to improve the acceptability and palatability of the product and to mask bitter tasting active ingredients, and colourings (≤1% w/w), which give the end product a uniform appearance adding to the aesthetic acceptability. Emulsifiers (up to 5% w/w), which may be required to allow the dispersion of an oily phase such as a flavouring homogenously throughout an otherwise water soluble mixture, can also be added if necessary (Arya *et al.*, 2010; Dixit and Puthli, 2009). Since oral thin films are formulated as a solid platform for drug delivery, they do not possess a high enough water content to support the growth of

microorganisms and therefore do not require the inclusion of preservatives. The amount of active pharmaceutical ingredient within each dosage unit is mostly limited by the size of the strip, and it can be difficult to incorporate large doses for this reason. Generally, around 5-30% (w/w) drug can be incorporated into the dry weight of each film (Dixit and Puthli, 2009).

A sparse pharmacopoeial monograph now exists with reference to orodispersible thin films under oromucosal preparations (British Pharmacopoeia Commission, 2015b). It defines oral thin films as “single- or multilayer sheets” which “disperse rapidly” in the mouth. No detailed standards are presented; stating only that the films should “possess suitable mechanical strength to resist handling without being damaged” and undergo suitable testing to “demonstrate appropriate release of the active substance(s).” Many studies have used modifications of compendial characterisation tests in order to make them more applicable or relevant to novel formulations such as those for dissolution of orodispersible tablets (Kraemer *et al.*, 2012). Various novel methodologies have been developed to characterise oral thin films including modified disintegration and dissolution apparatuses (Garsuch and Breitkreutz, 2009).

1.3 Summary and thesis overview

A review of the literature revealed that there was a need to increase the number of age-appropriate formulations for providing medicines to the paediatric population. Liquid formulations, although more easily administered to children, were less stable in terms of their ability to prevent microbiological

contamination, and often required the inclusion of potentially toxic excipients such as preservatives and solvents. There was also the potential for dosage errors with the use of liquid medicines since they required accurate measurement of very small volumes of concentrated liquids or manipulation of existing solid dosage forms. Therefore, there was a need for the development of more solid dosage platforms suitable for children.

Orally dispersing thin films are rapidly dissolving polymer films capable of delivering drugs orally without the need to chew or drink water. They have had some success in delivering medicines to older children, but have so far been untried in babies or infants. In order to demonstrate that oral thin films are an age appropriate alternative solid dosage platform for delivering medicines to babies, it was hoped to trial these in a proof-of-concept study. Since placebo studies are unethical in paediatric populations, it was thought that a phosphate salt (potassium acid phosphate) which is routinely given as an oral supplement to neonates to prevent bone abnormalities at birth, would be a suitable active ingredient for demonstrating the safety of the dosage form, whilst also being of therapeutic value and comparatively safe in under/overdose. A clinical audit of phosphate intake in preterm neonates (below 32 weeks' gestational age) was conducted which would inform trial design and also confirmed the safety of an oral potassium acid phosphate supplement in this population. The results of the audit and development of these phosphate films, along with characterisation and release studies are provided in Chapter 2.

It is recognised that 'children are not small adults' and that changes in gastrointestinal physiology and development at various stages of childhood reflect differences in the absorption, distribution, metabolism and excretion of both active pharmaceutical ingredients and excipients within different age groups. Excipient safety in paediatric medicine is one of the key strands of European Paediatric Formulation Initiative (EuPFI) and so in order to establish current excipient exposure in neonates, we conducted an audit of excipient burden within a local neonatal intensive care unit (Chapter 3). The results, which were later presented at the EuPFI 2013 annual conference, revealed that babies were exposed to a high number of excipients during their stay, many of which featured pre-existing toxicity concerns in neonatal populations. The study also provided insight into commonly used excipients within paediatric medicine, which assisted in the design of our oral thin film formulations. Following a discussion with speech and language experts at the Princess Royal Maternity (PRM) in Glasgow, an initial thin film surface area of 15 mm^2 was selected as appropriate for administration to neonates and formulations have been modelled around this as a maximum target size.

Following formulation successes with potassium phosphate, we developed a number of oral thin film formulations containing basic drugs, metoclopramide hydrochloride and morphine sulphate. Both had applications in paediatric health as well as palliative care populations. In neonatology, morphine sulphate is used in the pharmacological treatment of neonatal abstinence syndrome. Currently available marketed liquid formulations of morphine sulphate contain both ethanol and benzoic acid derived preservatives as

functional excipients, but these feature known toxicity concerns in neonates. Therefore, we identified a continued need for the development of solid oral dosage forms of morphine sulphate for this population. Formulations were first designed using metoclopramide as the model drug, since it was similarly basic but featured fewer restrictions on purchase, storage, disposal etc. However, metoclopramide oral thin films too have clinical value in palliative care as well as paediatric populations, and formulations are discussed in Chapter 4.

In parallel to immediate release formulations, it was also observed that there are currently no examples of modified release oral thin films on the market, and that a modified release morphine sulphate film would have value in both paediatric and palliative populations. Therefore, morphine sulphate oral thin films would be formulated both as an immediate release product for the treatment of neonatal abstinence syndrome, and as a modified release formulation for sustained drug delivery (see Chapter 5). Following the initial proof-of-concept study using the phosphate films, the aim would be to demonstrate the value of morphine sulphate oral thin films over existing morphine formulation in paediatrics through a similar small scale clinical trial. By incorporating an established modified release technology, ion exchange resins, within the polymer matrix of the oral thin film, we were able to produce a modified release oral thin film dosage form. Again, we utilised metoclopramide hydrochloride as a model drug in the formulation design (Chapter 4) before replicating the formulation using morphine sulphate (Chapter 5).

Owing to financial barriers, it was not possible to complete the clinical trial of potassium acid phosphate oral thin films within the timeframe of the PhD. However, two small scale *in vivo* studies were performed using immediate release metoclopramide hydrochloride oral thin films and modified release morphine sulphate oral thin films in rats, providing pharmacokinetic data from these two formulations (presented within Chapters 4 and 5).

Alongside formulation design and characterisation, it was necessary to develop a method of dissolution testing was which was more biorelevant, and had greater applicability to oral thin films and low dose drug products designed to dissolve entirely in the oral cavity than traditional compendial methodology. The design and validation of this novel methodology is described in Chapter 6. Finally, in consideration of industrial scalability, and to further demonstrate the versatility of oral thin films and ion exchange resins as a means to produce a modified release OTF, a sustained release diclofenac sodium (an acidic model drug) oral thin film and a combination immediate/modified release film formulation were also designed. These are discussed in Chapter 7.

Chapter 2 - Potassium acid phosphate oral thin films for hypophosphataemia in neonates

2.1 Introduction

2.1.1 Phosphorus

The majority of phosphorus in humans is found in bone (~85%) with the remainder existing as intracellular organic phosphate e.g. nucleic acids, phospholipids, nucleotides. Approximately 15% of serum phosphate is protein bound. As well as its role in skeletal composition, phosphorus has many other important functions. For example, it is a central component of phospholipids, the building blocks of cell membranes; it is contained in the nucleic acids which make up DNA; it is involved in enzymatic activation by phosphorylation, and it is required for production of ATP, an important cellular energy source (Beckett, 2010). Phosphate uptake in humans occurs principally in the proximal region of the small intestine in the duodenum and, to a lesser extent, the jejunum (Marks *et al.*, 2006). Minimal absorption also occurs across the ileum (Walton and Gray, 1979). Absorption occurs through both passive diffusion and via a sodium-dependent active transport mechanism (Borowitz and Ghishan, 1988). Vitamin D has also been shown to stimulate intestinal phosphate absorption; however, the mechanism is unclear and while 1,25-dihydroxyvitamin D_3 has been shown to upregulate the expression of the transporter protein (NaPi-IIb), the effect is both species

and intestinal segment specific (Brown *et al.*, 2012; Marks *et al.*, 2006). Dietary phosphorus exists both in inorganic and organic forms. However, intestinal phosphatases hydrolyse organic phosphates and so it is principally inorganic phosphates which are absorbed (Uribarri, 2007).

2.1.2 Hypophosphataemia

The majority of foetal uptake of calcium and phosphorus occurs during the third trimester of pregnancy. Infants born very prematurely lose this period when these minerals are acquired. Hypophosphataemia increases levels of 1,25-dihydroxycholecalciferol (calcitriol), the active form of vitamin D, which causes demineralisation of bone and hypercalcaemia. A low phosphate level also reduces the tubular reabsorption of calcium producing hypercalciuria. An association between low birth weight and hypercalcaemia and hypophosphataemia was first recognised in the early 1980s. Lyon and colleagues (1984a) were the first to propose that active demineralisation of bone was taking place to maintain necessary plasma mineral levels for cellular function and tissue growth. Reduced bone mineral density can precipitate metabolic bone disease which in very low birth weight premature infants can lead to complications such as fractures, reduced growth and in severe cases, rickets (Mitchell *et al.*, 2009).

There is also an association with hypophosphataemia and nephrocalcinosis where high urinary excretion of calcium coupled with the immature kidney function of neonates leads to deposition of calcium in the kidneys (Hein *et al.*, 2004). One UK study reported the incidence of renal calcification at 26.6% in infants below 32 weeks gestational age whilst another found a lower

incidence in the same age range with only 16% showing nephrocalcinosis (Short and Cooke, 1991; Narendra *et al.*, 2001). There were significant associations with nephrocalcinosis and postnatal treatment with corticosteroids (owing to their mineralocorticoid effect on calcium), furosemide (which causes hypocalcaemia), nephrotoxic antibiotics, and pulmonary surfactant replacement therapies. Nephrocalcinosis can increase the risk of hypertension and impair both glomerular and distal tubular function in children (Schell-Feith *et al.*, 2003).

2.1.3 Phosphorus content of breast milk

A study which looked at the nutritional content of breast milk found that by the end of the first week after giving birth, the phosphorus content of expressed milk was approximately 150 mg/L (Bates and Prentice, 1994). Other authors have quoted similar concentrations. For example, Greer (1989) gave a comparable figure of 140 mg/L, whilst Horsman *et al*. (1989) provided an average phosphorus content (n = 8) of breast milk of 170 mg/L. However, a review of studies looking at the calcium and phosphorus content of human milk revealed a wide range of phosphorus concentrations cited across the literature. The mean phosphorus content of human milk ranged from 17 to 278 mg/L with a median of 143 mg/L (Dorea, 1999).

2.1.4 Calcium and phosphorus solubility in TPN

The two most common routes for providing nutritional requirements to babies born prematurely are enterally by oral administration of supplements or fortification of milk, and parenterally within total parenteral nutrition (TPN).

The main difficulty with providing key minerals parenterally is the limited solubility of calcium and phosphorus in the TPN mixture. Calcium and phosphate form an insoluble salt which precipitates out of the mixture (Dunham *et al.*, 1991). There are many factors that can affect the solubility of these two minerals. It would be normal for a TPN therapy to take up to 24 hours to be administered. During this time, the solution is exposed to both raised temperatures (since a neonatal intensive care unit is kept warm) and light. Where the tubing passes into an incubator, temperatures can reach up to 37°C and solution can spend a prolonged time (up to 2 hours) exposed to this temperature before it reaches the neonate (Wong *et al.*, 2006). Increased temperature results in a greater dissociation of calcium salts resulting in a larger number of calcium ions available to precipitate with phosphate. Solubility also varies between calcium salts e.g. calcium chloride dissociates to a greater extent than calcium gluconate. Phosphate exists in both monobasic and dibasic forms. The monobasic anion is significantly more soluble than the dibasic form. However, at a pH of 7.4 (the physiological pH of blood), phosphate is predominantly in its dibasic form and the resulting dibasic calcium phosphate salt readily precipitates. Formulating the TPN solution at a lower pH improves the solubility of calcium phosphate as more phosphate is available in its monobasic form (Dunham *et al.*, 1991). On the other hand, formulating TPN at an excessively low pH can affect the stability of the lipid emulsion resulting in flocculation (Washington, 1990).

2.1.5 Recommendations and guidelines

Hypercalcaemia responds well to early supplementation with phosphate (Lyon and McIntosh, 1984b) and achieving adequate mineral intake during early developmental stages is shown to reduce the risk of metabolic bone disease (Catache and Leone, 2003). Requirements for neonatal mineral intake are established from *in utero* accretion rates, however figures for both vary between studies. Ryan (1996) reports an *in utero* phosphorus accretion rate of 2.1-2.6 mM/kg/day. Wong (2006) reports a similar *in utero* phosphorus requirement within the third trimester of pregnancy of 2.4 mM/kg/day. With an oral bioavailability of approximately 80%, in order to achieve the required phosphorus retention preterm infants would require a daily phosphorus intake of 3.9-4.5 mM/kg (Trotter and Pohlandt, 2002). In order to match the *in utero* accretion rate, Rigo (2006) recommended that for premature infants the target intake of elemental phosphorus should be 60-75 mg/kg/day. The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee reviewed evidence on nutritional requirements of premature infants up to 1.8 kg body weight and produced guidelines for enteral supplementation (Agostoni *et al.*, 2010). Recommended mineral intakes were 120-140 mg/kg/day of calcium salt (60-90 mg/kg/day calcium) and 60-90 mg/kg/day of phosphorus. The molar ratio of calcium to phosphorus should be between 1.5 and 2. The World Health Organisation published a review in 2006, summarising the evidence and highlighting recommendations for feeding of low-birth-weight infants. Recommended total daily phosphorus intakes for pre-term infants weighing more than 1 kg were

1-1.5 mM/kg/day for the first 7 days, 2.5-3.8 mM/kg/day until term, and 3.4 mM/day (breastfed) or 8.8 mM/day (formula fed) from term until 1 year (Edmond and Bahl, 2006).

At the Princess Royal Maternity in Glasgow, it is routine clinical practice to provide all preterm infants born before 32 weeks gestational age with a phosphate supplementation to reduce the risk of osteopenia associated with their prematurity. Supplementation can be provided either by parenteral or enteral nutrition, or both. The normal serum phosphate level should be between 1.8 and 2.6 mM/L and the dose of supplementation will be tailored according to response and blood test results. Most infants will also receive milk (either formula or expressed human milk), which may be fortified with additional vitamins and minerals including phosphate. Oral phosphate supplementation is prescribed in the form of an oral solution containing potassium acid phosphate (1 mM/mL). It is prescribed as per NHS Greater Glasgow and Clyde guidelines at a dosage of 0.5 mM per kg bodyweight twice daily for all babies born before 32 weeks gestational age from the time they are established on enteral feeds until discharge.

2.1.6 Aims, hypothesis and plan

The overall aim of this research was to demonstrate that oral thin films could be used as a safe and effective solid platform for administering active pharmaceutical ingredients to the paediatric population, particularly in preterm and term neonates. It was hoped that investigation through clinical trial would be possible to demonstrate age-appropriateness. Since it is unethical and not permissible to undertake a trial in a paediatric population

without therapeutic benefit to the child (e.g. through use of a placebo), it was decided that formulation of a routinely prescribed mineral supplement (potassium acid phosphate) as an oral thin film would constitute an appropriate intervention for an initial proof of concept study. Therefore the aim was to formulate potassium acid phosphate (KAP) into an oral thin film for use as a source of phosphorus in the treatment and prevention of hypophosphataemia, a condition common to low birth weight infants and associated with osteopenia of prematurity. The objective was to produce a stable, rapidly dissolving oral thin film of appropriate size that adhered to British Pharmacopoeia (BP) specifications for an immediate release solid dosage form. KAP is a food additive as listed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) with a maximum tolerable daily intake (MTDI) of 70 mg/kg body weight (as phosphorus). It has good water solubility (approx. 22 g/100ml) and as a routinely prescribed, essential bone mineral which is already provided to both term and preterm neonates from birth in a variety of forms (e.g. parenteral nutrition, milk fortifiers, oral supplements), it was considered a safe choice for taking forward to clinical trials.

2.2 Materials and methods

2.2.1 Potassium acid phosphate oral thin films

2.2.1.1 Formulation and excipient monographs

Three strengths of potassium phosphate monobasic oral thin films were formulated. Films were cast from stock solutions, the compositions of which

are provided in Table 1. The composition of 0.3 mM and 0.4 mM films was the same and dose was varied by alteration of the individual dosage unit mass only.

Table 1. Formulation details of batch stock solutions.

Ingredient	Manufacturer	Batch No.	0.2 mM	0.3 mM	0.4 mM
			Percentage weight (% w/w)		
Potassium acid phosphate	Sigma-Aldrich, Dorset, UK	031MOO33V	22.5	36.7	
Pullulan	Hayashibara Co. Ltd., Okayama, Japan	9K1612	16.9	13.8	
Polyvinyl pyrrolidone K30	Sigma-Aldrich, Dorset, UK	MKBC3440V	2.2	1.8	
Sisterna SP70	Sisterna, Roosendaal, Netherlands	548Z22	1.6	1.3	
Sucralose	Nantong ChangHai Food Additive Co. Ltd., China	20120715	0.3	0.3	
Lemon flavour 507940 T	Firmenich, Meyrin, Switzerland	1000710486	0.2	0.2	
Glycerol	Melford Labs Ltd., Ipswich, UK	19256	0.1	0.1	
Water, distilled	In house	N/A	56.2	45.9	
Target drug content (mg)			27.22	40.83	54.44
Target mass (mg)			50-60	60-70	85-95

Excipients were weighed on a calibrated analytical balance (A&D, Tokyo, Japan; serial No. 14214367) and homogenised using an Ultra-Turrax (Janke & Kunkel, Staufen, Germany; serial No. 751808). Pullulan and polyvinyl pyrrolidone were weighed separately and combined gradually with other ingredients by hand until homogenously mixed. The stock mixture was then spread onto polymer coated paper at 1.3 mm film thickness using a Micrometer Adjustable Film Applicator (Sheen, Surrey, England; ref. No. 1117; serial No. 100982/7) on an automatic film applicator with built in

vacuum bed (Sheen, Surrey, England; ref No. 1133N) at 50 mm/s, and dried in a cabinet dryer (Mitchell Dryers Ltd., Carlisle, UK; model No. GO3536-010) at 40 °C. Oral thin films were cut by hand using a rotary blade and a 15 mm template on a cutting mat. Strips were then weighed to ensure they were within the target range and finally were individually heat-sealed in multilayer aluminium laminates (Amcor Rayopeel®). A brief monograph for each excipient is provided below.

Pullulan [E1204]

Pullulan is a natural polysaccharide made up of maltotriose units and derived from the fungus *Aureobasidium pullulans* by simple fermentation processes. Although no acceptable daily intake (ADI) has been specified for pullulan by the JOINT FAO/WHO Expert Committee on Food Additives (JECFA), one study in which 8 male adult volunteers consumed 10 g pullulan each day for 14 days reported no adverse effects (Yoneyama *et al.*, 1990). Another cross-over designed study in which 34 adult volunteers consumed a single 50 g dose of pullulan for the purposes of a meal tolerance test revealed a low intensity and frequency of gastrointestinal disturbances (Spears *et al.*, 2005). One study has offered a provisional acceptable daily intake of 45 mg/kg (Kimoto *et al.*, 1997).

Polyvinyl pyrrolidone (PVP) [E1201]

Polyvinyl pyrrolidone (PVP) is a water-soluble polymer of *N*-vinyl-2-pyrrolidone. WHO have provided an ADI of 50 mg/kg. Absorption of povidone from the GI tract was <0.5% in rats. Exact gastrointestinal absorption depends on the molecular weight of the PVP but at low MW (~11,500) there

is no absorption in humans. The only adverse effects reported from oral administration are stool softening and diarrhoea (Joint FAO/WHO Expert Committee on Food Additives (JECFA), 1980).

Sisterna SP70 [E473]

Sisterna SP70 is a water soluble sucrose ester (70% mono-ester) of fatty acid (stearate/palmitate) used as a non-ionic, oil-in-water emulsifying agent. In 2010, an ADI of 30 mg/kg was offered by JECFA for sucrose monoesters of lauric, palmitic or stearic acid. Sisterna SP70 was selected as it is based on natural and renewable raw materials and was included in the formulation to allow the homogenous incorporation of the water insoluble flavour.

Sucralose [E955]

Sucralose is a freely water soluble, artificial sweetener approximately 300-1000 times sweeter than sucrose by weight. WHO have provided an ADI of 15 mg/kg. Sucralose is mainly excreted unchanged in faeces unchanged (~85%) and does not bioaccumulate. It is considered non-toxic and non-irritant and no gastrointestinal side effects have been observed in male adults given 1 g/day. Sucralose does not affect glycaemic control and is not cariogenic (Bowen *et al.*, 1990; Grotz *et al.*, 2003).

Flavouring

Initial discussions were made with Azelis, UK regarding the composition of their flavourings and suitability for use in paediatric formulations. It was desirable that any flavours included in the formulation would be free from propylene glycol, isopropyl alcohol, ethanol, benzyl alcohol and/or benzoic

acid since these are well documented as being harmful in neonates. Several options were confirmed as being suitable, and samples were arranged for testing. Of these options, a lemon flavour (507940 T) was chosen for the formulation since the citrus nature of this flavour could potentially provide some salivary stimulation in the neonate to aid the dissolve of the strip in the mouth. The flavour consists of a mixture of aromatic substances including limonene (80-90%) and citral (7.5-10%). Based on data obtained from mice, rats and rabbits a provisional ADI of 1.5 mg/kg has been suggested for d-limonene (Joint FAO/WHO Expert Committee on Food Additives (JECFA), 1993).

Glycerol [E422]

Glycerol is a clear, colourless and odourless, water soluble excipient which was used as a plasticizer to improve the flexibility of the oral thin films. It is considered in general to be non-toxic and non-irritant although large doses may produce laxative effects and headaches. It is incorporated in metabolic pathways to form glucose and glycogen so may also produce a glycaemic response in large doses. An ADI limit has not been specified by JECFA although a no observable effects limit (NOEL) has been reported at 10 g/kg/day (Hine *et al.*, 1953). Also, the FDA considers glycerol to be ‘generally regarded as safe (GRAS)’ when used as a food additive (Select Committee on GRAS Substances (SCOGS), 2011).

2.2.2 Quantification of potassium phosphate

Several methods were investigated for the assay of potassium acid phosphate: flame photometry, two different UV spectrophotometric methods based on reaction with phosphomolybdate, and two titration methods using sodium hydroxide (NaOH). Flame photometry proved both the simplest and most reliable method and after thorough validation, was employed as the method of assay for the majority of the work presented herein. It was hoped that we would be able to test the formulation through clinical trial in a sample of neonates and for this, the investigational medicinal product would be manufactured at GMP licensed premises. These premises did not have access to a flame photometer and as such, alternate methods of quantification were investigated for batch conformity testing. All methods and subsequent validation, are described below.

2.2.2.1 Flame photometry

For analysis by flame photometry, five standards were prepared in distilled water with KAP concentrations ranging from 10 to 70 mg/L (representing between 16.5-30.1% and 115.7-231.5% of anticipated sample concentrations for dissolution studies). Flame photometry uses filters to select which colour to detect depending on the metal ion being measured (in this case potassium) and quantifies the intensity of the flame colour produced when an atomised sample is introduced. This produces a numeric output without units. The phosphate content of unknown samples can then be determined from

the reading of known calibration standards by rearranging the best fit line equation.

2.2.2.2 Malachite green method

A procedure for phosphate determination based on a coloured complex of phosphomolybdate and malachite green is described by Baykov *et al*. (1988). Briefly, a colour reagent is prepared by mixing an acidified solution of malachite green with an ammonium molybdate solution, along with a surfactant. One part of this colour reagent is mixed with four parts of the analyte solution, and the UV absorbance is measured at 630 nm.

Several attempts were made at replicating this method, however absorbance readings with repeated measurements were inconsistent and linearity was generally poor and not reproducible. In addition, at KAP concentrations of approximately 30 mg/L and above, an insoluble precipitate was formed upon mixing with the colour reagent. Therefore the method was not pursued.

2.2.2.3 Stannous-chloride reduction method

Guidelines on the standard operating procedure for quantifying phosphate in serum have been published by the World Health Organisation (2006). Phosphorus reacts with ammonium molybdate to form phosphomolybdate, which is then reduced by stannous chloride and hydrazine sulphate to molybdenum blue. The intensity of the colour is measured by UV spectrophotometry at 640 nm.

Although an attempt was made to reproduce this methodology, no linear relationship was achieved in the laboratory. Additionally, through COSHH

(Care of Substances Hazardous to Health) assessment, it was decided that alternative methods such as flame photometry would eliminate the necessity for exposure to several harmful and potentially carcinogenic (hydrazine sulfate salt – Cat 1B) substances. Therefore this method was not pursued further.

2.2.2.4 Titration to pH endpoint

A method for assay of potassium acid phosphate is described in the British Pharmacopoeia (British Pharmacopoeia Commission, 2012). 1 g KAP is dissolved in 50 mL carbonate-free water and titrated to a potentiometric endpoint using carbonate-free 1M NaOH. 1 mL of titrant is equivalent to 0.1361 g KAP.

Based on this method, an automated titration using 0.1M NaOH was developed and validated. Standards were prepared by dissolving known amounts of potassium acid phosphate in 50 mL of pure grade (≥ 18.2 MΩ) water. Using a Mettler-Toledo autotitrator, these solutions were titrated to a pH endpoint using 0.1 M NaOH standard solution (Fluka analytical; Sigma-Aldrich 319481; Lot SZBB1870). Linearity was assessed across the concentration range 7-82 mg in 50 mL (representing between 12.86-25.72% and 150.62-301.25% of the anticipated test sample concentration for uniformity tests). Specificity was determined by adulterating one of the standards with excipients at levels exceeding those of the oral thin films. Accuracy was assessed by preparing three KAP concentrations in triplicate and titrating each sample to a pH endpoint. An assay test was then performed by dissolving 5 randomly selected potassium acid phosphate 0.3

mM oral thin films into 50 mL of pure grade water and titrating each sample to a pH endpoint using 0.1M NaOH. The results were compared with previous batch uniformity results as determined by flame photometry. The results were compared statistically by way of a 2 sample t-test using Minitab® statistical software.

2.2.3 Characterisation

2.2.3.1 Dissolution

Dissolution testing was carried out as per the paddle method described in the British Pharmacopoeia for conventional-release solid dosage forms. For each pH, 900 mL of the dissolution medium (prepared as detailed in Table 2) was transferred to each of the 6 dissolution vessels and warmed to 37 ± 0.5 °C. The paddles were set to rotate at a speed of 50 rpm. An oral thin film was placed into each container (n = 6) at time t = 0 minutes and 10 mL samples were taken using a 10 mL glass pipette at t = 5, 10, 15, 20, 30 and 45 minutes. These samples were then assayed by flame photometry to quantify the potassium content at each time point. Solution was not replaced and final results were adjusted for volume.

To comply with the British Pharmacopoeia, all six dosage units must release at least 70% of the stated content of active pharmaceutical ingredient into solution within 45 minutes (British Pharmacopoeia Commission, 2015a).

Table 2. pH buffers prepared for dissolution.

pH	Buffer	Method of preparation
1.2	Hydrochloride	2 L of deionised water was adjusted to pH 1.2 using concentrated (12 M) hydrochloric acid (approx. 10-12 mL).
4.6	Acetate	5.98 g of anhydrous sodium acetate and 3.32 mL glacial acetic acid were mixed with water to obtain 2 L and adjusted to pH 4.6. Formula obtained from USP 29-NF 24 (United States Pharmacopeia Convention, 2006b).
6.8	Tris-hydrochloride	12.114 g TRIS was dissolved in distilled water. Approximately 7.89 mL concentrated HCl was added to this and the solution made up to 2 L with distilled water. The pH was then adjusted to 6.8 using concentrated HCl. Formula calculated using Henderson-Hasselbach equation: pH = pKa + log[A-/HA]

2.2.3.2 Tensile strength testing

A tensiometer (Instron®) was used to study the elongation and load required to break the films. Each film was secured between a set of two 'jaws' and a measurement of the breaking force (N) and the increase in length at breaking point (mm) were recorded. Tests were carried out in triplicate.

2.2.3.3 Weight and content uniformity

The European Pharmacopoeia (Ph. Eur.) requires that for immediate release oral solid dosage forms such as tablets and capsules, the individual drug content of 10 dosage units selected at random is between 85% and 115% of the average content and no more than one unit may fall outside these values. Additionally, no unit may contain <75% or >125% of the average content. Should the content of one unit fall outside the 85-115% margin but still lies within the 75-125% margin, a further 20 units may be selected at random and assayed. No more than 1 unit in 30 may fall outside the 85-115% margin and no unit may fall outside the 75-125% margin. Typically, specific tablet monographs within the British Pharmacopoeia require that tablet content is

limited to 90-110% of the stated content (British Pharmacopoeia Commission, 2015d).

For uniformity of weight, the European Pharmacopoeia requires that no more than 2 out of 20 dosage units selected at random may deviate from the mean weight by more than 10% where the individual units weigh ≤ 80 mg, or more than 7.5% where they weigh between 80 and 250 mg. Also, no unit may exceed twice these values.

In order to evaluate the weight and content uniformity of potassium acid phosphate oral thin films, twenty individually sealed strips were selected at random from a batch of each strength of film. Each strip was removed from its packaging and individually weighed to 1 d.p. on a calibrated analytical balance. Each strip was then dissolved in 1000 mL of distilled water and the solutions were assayed using flame photometry.

2.2.3.4 Thermal stability

In order to assess long-term thermal stability of the potassium phosphate oral thin film formulation, 25 individually sealed oral thin films were selected at random from each batch: 0.2 mM, 0.3 mM and 0.4 mM. These samples were placed in a constant temperature room at 25°C ± 2°C and stored for 12 months as per the ICH Guidelines (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2003) for long-term stability studies. Five strips were removed at time intervals t = 0, 1, 3, 6 and 12 months, weighed and assayed by flame photometry for potassium content. A change

in drug content of more than 5% is considered significant according to the guidelines.

2.2.3.5 Bioburden

Three sample strips were selected from batches of each strength of potassium acid phosphate oral thin film for bioburden analysis. These samples were sent to Andersen Caledonia (Caledonian House, Phoenix Crescent, Strathclyde Business Park, Bellshill, North Lanarkshire, Scotland, UK, ML4 3NJ) for microbial analysis. Analyses were performed according to ISO 11737-1. Tryptone soya agar (TSA 1203444) was used for bacterial colony growth and was incubated for 3 days at 35 ± 1 °C. Sabouraud dextrose agar (SAB TU06/981548) was used for yeast and fungal counts which were cultured over 5 days at 22 ± 1 °C.

2.2.3.6 Thermogravimetric analysis

Thermogravimetric analysis (TGA) is an analytical technique in which the weight of a sample substance is monitored over time in response to heating/cooling. The technique can provide quantitative information on loss of e.g. water, solvent, plasticiser. TGA was carried out on an oral thin film of each strength of potassium acid phosphate (0.2 mM, 0.3 mM and 0.4 mM). Samples were heated from 25-300 °C at 10 °C per minute. Nitrogen was used as the purge gas to control the environment.

2.2.3.7 Raman spectroscopy

Raman spectroscopy is a spectroscopic technique which produces spectral data, complementary to infrared (IR) spectroscopy, about inelastic laser light

scattering. In order to obtain some secondary data on uniformity of API content, a Thermo Scientific DXR Raman Microscope (Serial No. AIY1000276) was used for spectroscopy analysis. A 532 nm laser set at 10 mW power (Serial No. AJC1000278) with a 532 nm filter (Serial No. AJM1000273) and a 400 lines/mm grating (Serial No. ARW1100013) were chosen. Using a 25 µm pinhole, spectra were obtained with a 5 second exposure and 5 exposures per spectrum.

Spectra for each excipient were obtained separately before a 50 x 50 point Raman contour map of the entire surface of a 0.2 mM potassium acid phosphate oral thin film was constructed with a total of 2500 spectra. A peak unique to potassium acid phosphate was identified and using OMNIC Series software, an intensity map was produced using the height of this unique peak which showed the distribution of potassium phosphate across the 15 x 15 mm film (Figure 13).

2.2.4 Clinical audit at Princess Royal Maternity

In order to evaluate daily phosphorus intake in preterm neonates, an audit of phosphorus was conducted in a sample of preterm neonates at the Princess Royal Maternity in Glasgow. During 2011, 106 preterm neonates less than 32 weeks' gestation were admitted to the PRM, approximately one quarter of whom were extremely low birth weight (<1000 g) (n = 27/106). By convenience sampling, we collected data retrospectively from the case notes of a selection of babies of less than 32 weeks' gestation admitted to the neonatal unit between January 1, 2011 and March 21, 2012.

Details of age (in weeks and days), corrected gestational age/post-menstrual age (in weeks and days), weight (kg), serum phosphate (mM/L), serum alkaline phosphatase (U/L), serum potassium (mM/L), serum calcium (mM/L), total parenteral nutrition regimes and any phosphate additives, Intralipid® and Vitlipid® administration, milk types and volumes received, fortification of breast milk, and prescribed oral potassium acid phosphate supplements were recorded daily or as they became available.

Where a duration of breast feeding was recorded in the patients records, it was assumed that they received ~0.6 mL/min (Furman and Minich, 2004) of expressed breast milk allowing an approximate intake to be estimated. From available weights, an average weight per week (168 hours) was calculated, and all sources of phosphorus were converted into mg P/kg/day from these average weights.

A week was defined as a period of 168 hours and was taken to be 7 periods of 24 hours where each day started at 08:00 hours. Day 1 of life was taken to be the period from the time of birth on the date of birth until 08:00.

Table 3. Various sources of phosphorus intake and their content expressed in mM/L. *expressed as median value (Dorea, 1999)

Source	**Manufacturer (proprietary products)**	**Phosphate content (mM/L)**
Expressed breast milk	N/A	4.61*
TPN regimen 0	N/A	10
TPN regimens 2.5, 4, 6 and 8	N/A	12.5
Intralipid®/Vitlipid®	Fresenius Kabi, Cheshire, UK	15
Breast milk fortifier (1 sachet/50 mL)	Cow & Gate, Wiltshire, UK	12.3
C&G Nutriprem 1®	Cow & Gate, Wiltshire, UK	20
SMA Gold Prem 1®	Pfizer Ltd., Berkshire, UK	19.7
SMA First Instant Milk®	Pfizer Ltd., Berkshire, UK	7.7
Pregastimil Lipil®	Mead Johnson, IL, USA	16.5
Neocate LCP®	Nutricia Ltd., Schiphol, Netherlands	16.1
C&G Pepti Junior®	Cow & Gate, Wiltshire, UK	9.03

To compensate for this initial period where day 1 does not equate to 24 hours, the mean values of data collected for week 1 were adjusted to 168 hours. Data was compiled into a Microsoft® Access 2010 database along with patient demographics. Statistical analyses were performed using Minitab® 16.2.1 statistical software.

2.2.5 Clinical trial: oral potassium acid phosphate supplementation for preterm neonates - a comparison of oral thin films and standard oral therapy

Following from formulation successes, a phase II/III clinical trial (EudraCT Number: 2012-003625-19, Protocol ID: UEC1112/65, ClinicalTrials.gov ID: NCT01676844) was designed which would aim to demonstrate therapeutic equivalence of potassium acid phosphate oral thin films compared to standard supplementation with an oral solution. The trial would be conducted in accordance with the recognised best ethical and regulatory practices

governed within the UK and favourable ethical opinion would be sought from NHS West of Scotland Research Ethics Committee (REC1) before patients were enrolled. Additionally, patients would only be allowed to enter the study once their parents or other legal caregivers had provided written informed consent.

Manufacture, packaging and labelling of study treatments was to be carried out within a MHRA licenced facility operating to Good Manufacturing Practices and approved to manufacture clinical trial investigational medicinal products (Pharmacy Production Unit, Western Infirmary, Glasgow; Site ID: 7424; License: MA (IMP) 24712). A comprehensive Statistical Analysis Plan, which would operate to standards of Good Clinical Practice, governing all statistical aspects of the study, would be authored by the Trial Statistician and agreed by the Trial Steering Committee before any unblinded data would be seen. Data management including statistical analyses was to be carried out by an Independent Data Management Centre (The Robertson Centre for Biostatistics, part of the Glasgow Clinical Trials Unit, a fully registered UK CRN Clinical Trials Unit).

Neonates would be randomised at the point of recruitment to receive either OTF or standard therapy for 2 weeks of oral thin films followed by 2 weeks of oral solution or *vice versa*. Randomisation would be carried out by an independent person and at a distal location.

From the results of a pre-clinical audit of phosphate prescribing conducted at the Princess Royal Maternity, it was observed that the mean (SD) plasma

phosphate level for babies prescribed the standard oral solution was 2.10 (0.38) mM/L (Watts *et al.*, 2013). For the purposes of showing equivalence, following a discussion with clinicians at the PRM, a clinically acceptable difference of 20% from the mean plasma phosphate level was decided i.e. 0.42 mM/L. Assuming therapeutic equivalence between the oral solution and OTFs, power calculations as published by Julius (2004) could be applied. If there was truly no difference between control and experimental groups, then 22 patients were required for 90% power and type I error of 2.5%. We therefore aimed to recruit 30 participants to allow for dropouts and/or withdrawals. The primary outcome measure was assessment of plasma phosphate. Secondary outcomes were in terms of age-appropriateness and acceptability of the formulation.

Babies were to be regularly assessed by weight and clinical examination as deemed appropriate by the attending medical team and would have routine (normally weekly) assessment of plasma calcium, phosphate and alkaline phosphatase. A consultant neonatologist was to oversee medical care. No additional blood samples were to be taken for the purposes of the trial; only results obtained from routine clinical sampling would have been used for analysis of treatment affect.

The study received preliminary favourable opinion from various associates with input from the NHS Greater Glasgow & Clyde Research and Development team (Research Coordinators, Joanne McGarry and Mike Barber, and Clinical Trials Pharmacist, Elizabeth Douglas), the West of Scotland Research Ethics Service (Scientific Research Officer, Dr. Judith

Godden), the MHRA (Pharmaceutical Assessor, Elaine Godfrey), and the Western Infirmary Pharmacy Production Unit (Production Unit Manager, Graham Conkie, and Production Pharmacist, Kay Pollock), as well as authorisation from the Princess Royal Maternity Site Manager (Dr. Alan Mathers) and support from consultant neonatologist, Dr. Helen Mactier (Princess Royal Maternity). However, owing to financial barriers, the study could not occur within the timeframe of the PhD. A synopsis of the study taken from the draft protocol is included as Appendix I.

2.3 Results

2.3.1 Potassium acid phosphate oral thin films

2.3.1.1 Formulation development

HPMC E3 (nominal viscosity 3 mPa s) was initially chosen as the film-forming polymer since it is known to exhibit fast dissolving properties. However, when potassium acid phosphate was included in the formulation, an adequate viscosity required for casting the film was not obtainable until HPMC content was increased to ~40% w/v. At this level, the resulting film was too brittle to hold. A higher viscosity HPMC was also tried. HPMC E50 (nominal viscosity 50 mPa s) at a 1:10 ratio to water content produced a film with more favourable mechanical properties, incorporating 10% KAP, but at higher API loading, would not obtain the required viscosity at this ratio. It was suggested that there may have been a saturation issue with the highly water soluble, ionic phosphate salt which displaced HPMC at higher loading. At 20% w/v,

potassium acid phosphate is nearly at its maximum solubility in water of ~22 g/100ml. Additionally, when the phosphate salt was added to the stock mixture containing HPMC, a stable foam was formed which was not removed with vacuum de-gassing or overnight settling. Little improvement was obtained by the addition of simethicone as a defoaming agent.

Pullulan, used as the film-forming polymer, proved more accommodating of the high potassium acid phosphate loading required for this formulation. Approximately 25% (w/w) food-grade pullulan was able to incorporate ~68% (w/w) potassium phosphate into an oral thin film (see Table 1).

PVP K30 (approximate molecular weight 50,000) was selected as a disintegrant to improve the rate at which an oral thin film containing pullulan as the film-forming polymer dissolved. PVP was included in 0.2 mM oral thin films at 5.13% (w/w), and in 0.3 mM and 0.4 mM films at 3.39% (w/w).

2.3.1.2 Particle size reduction

Potassium acid phosphate was purchased from Sigma Aldrich (batch number 031MOO33V) in a crystalline solid form. This product proved problematic since the large particle size distribution resulted in significant sedimentation of KAP when the batch mixture was left overnight to de-gas. Additionally, large crystals were observed in the final film after drying. Crystals were measured under polarising filter using a Reichert-Jung Polyvar microscope. The image was captured and crystal measurements were calculated using Infinity Analyse software version 5.0.3 (see Figure 2).

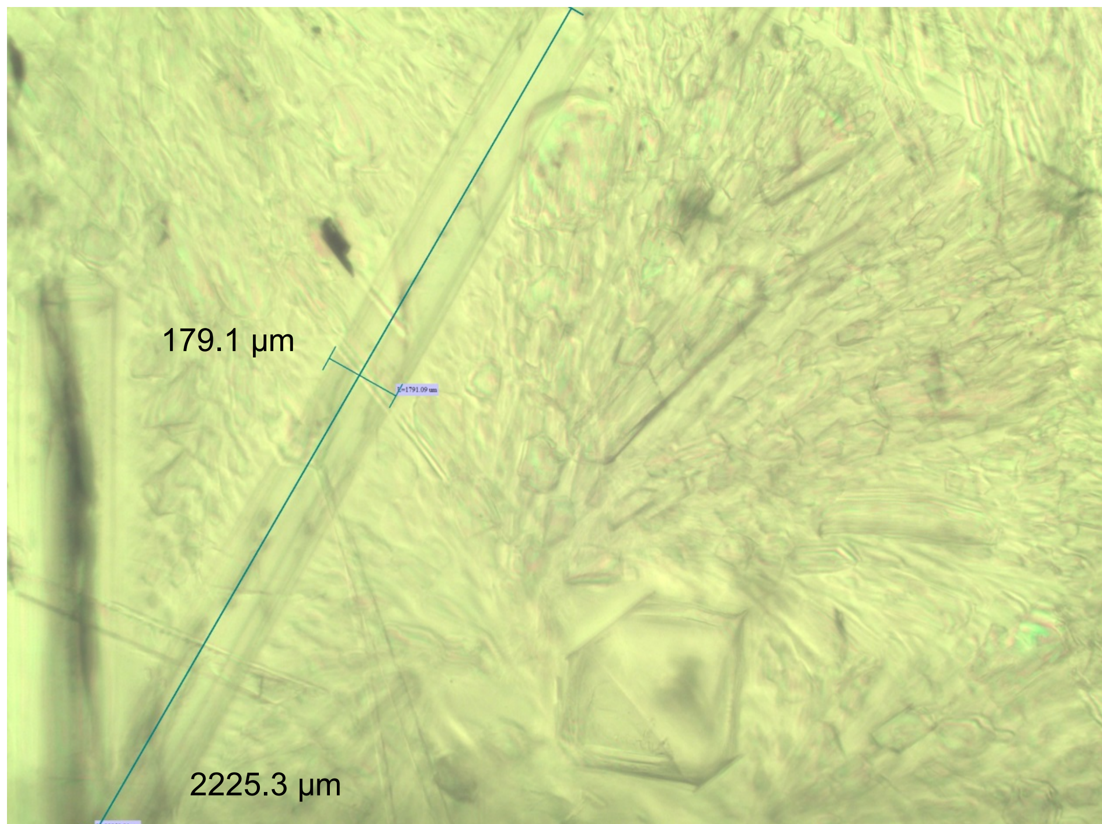


Figure 2. Potassium acid phosphate crystals. Large crystals were observed within the oral thin films upon drying. Image captured using Reichert-Jung Polyvar microscope with a polarising filter and Infinity Analyse software version 5.0.3.

To assess the particle size distribution of the potassium acid phosphate, 50 grams was passed through a series of mesh sieves with pore sizes of 500, 250, 180, 150, 125 and 90 µm. Approximately 50% of the crystalline form was of a particle size greater than 500 µm. The remainder was greater than 250 µm in diameter with only a trace amount (0.86%) found below this size. No material was found to have a particle size less than 180 µm in diameter. In order to overcome this problem, attempts were made with several chemical suppliers to source a powder form of the phosphate salt, or a crystalline form with a reduced particle size. However, no product was readily available so in-house milling of the available crystalline form was required to reduce the particle size. A stainless steel ball mill was used to reduce the particle size of the crystalline form. The product was passed through a series

of mesh sieves and only material found to have a particle size of less than 75 µm was deemed acceptable for formulation.

2.3.2 Quantification of potassium phosphate and method validation

2.3.2.1 Flame photometry

The method showed excellent linearity (R^2 = 0.999) with reproducible results on consecutive days (Table 4 and Table 5 give results on day 1 and day 2 respectively). Calibration curves are shown in Figure 3.

Table 4. Day 1 calibration curve. The method of potassium acid phosphate quantification using flame photometry showed good precision (repeatability and reproducibility) across the concentration range 15-75 mg/L with relative standard deviations ≤1% at all concentrations on consecutive days (n = 6).

Standard (mg/L)	1	2	3	4	5	6	Mean	SD	RSD (%)	Residuals
15	22.0	22.0	22.0	22.0	22.0	22.0	22.00	0.00	0.00	-1.134
30	44.0	43.5	43.0	43.0	43.0	43.0	43.25	0.42	0.97	0.450
45	64.0	64.0	63.5	63.0	64.0	64.0	63.75	0.42	0.66	1.284
60	83.0	83.0	83.0	82.0	82.5	83.0	82.75	0.42	0.51	0.618
75	100.0	101.0	101.0	100.0	100.5	101.0	100.58	0.49	0.49	-1.218

Residual sum of squares = 5.00256

Table 5. Day 2 calibration curve. The method of potassium acid phosphate quantification using flame photometry showed good precision (repeatability and reproducibility) across the concentration range 15-75 mg/L with relative standard deviations ≤1% at all concentrations on consecutive days (n = 6).

Standard (mg/L)	1	2	3	4	5	6	Mean	SD	RSD (%)	Residuals
15	22.0	22.0	22.0	22.0	22.0	22.0	22.00	0.00	0.00	-1.232
30	43.0	44.0	44.0	44.0	44.0	44.0	43.83	0.41	0.93	0.623
45	64.0	64.0	64.0	64.0	65.0	65.0	64.33	0.52	0.80	1.148
60	83.0	84.0	84.0	84.0	84.5	84.0	83.92	0.49	0.59	0.763
75	100.0	101.5	102.0	103.0	102.5	102.0	101.83	1.03	1.01	-1.302

Residual sum of squares = 5.50123

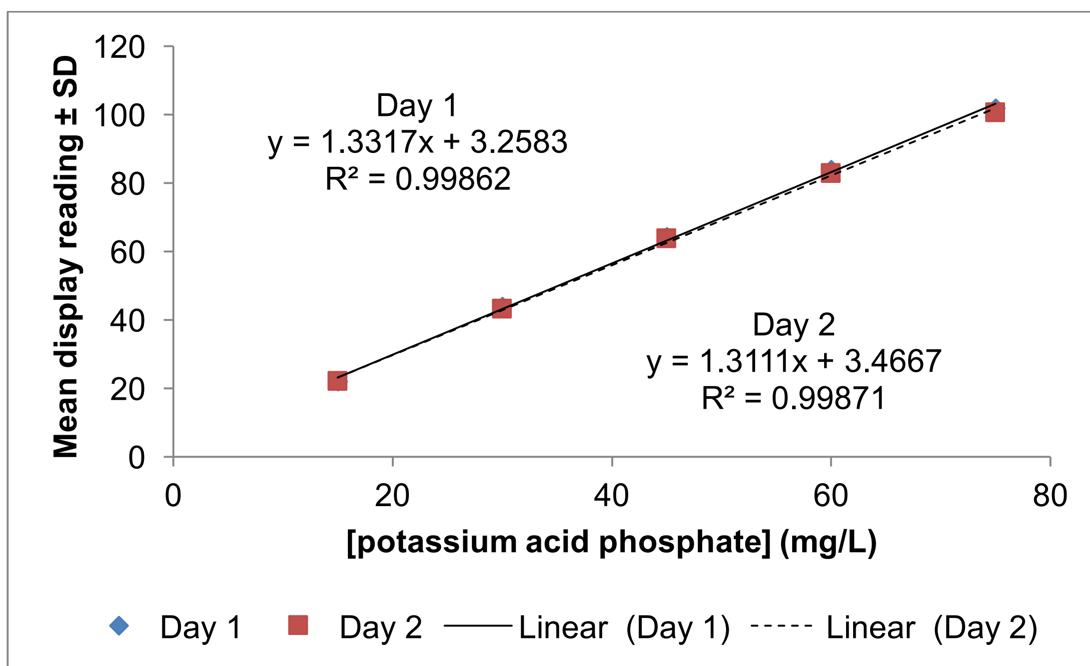


Figure 3. Linearity and intermediate precision. Quantification of potassium acid phosphate using flame photometry showed good linearity (R^2 values > 0.99) and was reproducible on consecutive days.

2.3.2.2 Potentiometric and pH titration

The method showed good linearity (R^2 = 0.9999) across the concentration range 7-82 mg in 50mL. The calibration curve across this range is shown in Figure 4.

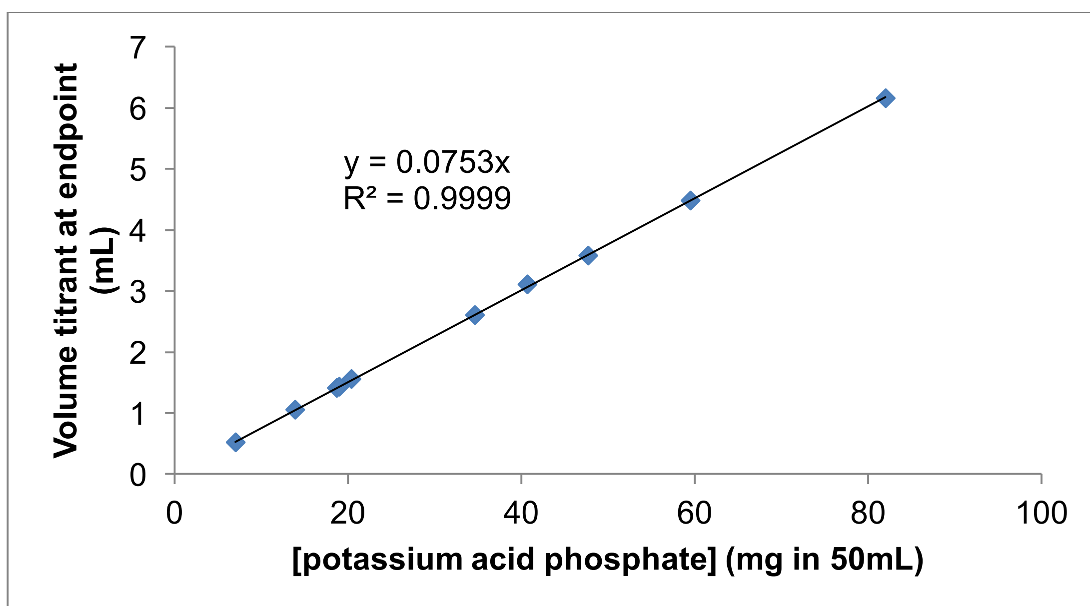


Figure 4. Titration method linearity. Quantification of potassium acid phosphate using an automated titration method with sodium hydroxide showed good linearity across the measured concentration range.

Replicates performed for accuracy test are presented in Table 6. The method was found to be accurate to within 2% of the true values. The addition of excipients did not affect results.

Table 6. Titration method accuracy. The method of quantification of potassium acid phosphate using an automated titration with sodium hydroxide demonstrated a high degree of accuracy with average recovery within 2% of the true value.

KAP concentration (mg in 50 mL)	Titrant volume at endpoint (mL)						Average recovery (%)
	1	2	3	Mean	SD	95% CI	
7.00	0.5219	0.5102	0.5249	0.5190	0.0078	0.4997-0.5383	98.46
13.87	1.0543	1.0679	1.0332	1.0518	0.0175	1.0084-1.0952	100.73
20.37	1.6200	1.5479	1.5098	1.5592	0.0560	1.4202-1.6983	101.67

The average (SD) potassium acid phosphate content of the 5 assayed oral thin films was 44.38 (1.22) mg, range 43.0-45.7 mg. Average (SD) mass was 65.94 (2.10) mg, range 63.9-68.1 mg. All strips were within 10% of the stated target 0.3 mM content (i.e. 40.83 mg) and had good linearity when plotted against film mass (R^2 = 0.9973). Neither weight nor potassium acid phosphate content differed significantly from previous uniformity data collected from this batch, assayed by flame photometry (see section 2.3.3.3, Figure 9). P-values of 0.72024 and 0.75949 were calculated for weight and content respectively (n = 30 *vs*. n = 5).

2.3.3 Characterisation

2.3.3.1 Dissolution

Figures 5-7 show the release profiles of potassium acid phosphate from 0.2 mM, 0.3 mM and 0.4 mM oral thin films respectively. Dissolution was slower than would normally be expected for orodispersible films, which are known to

dissolve in a matter of seconds *in vivo*. However, it was observed that dosage units had a tendency to sink to the bottom of the dissolution vessels and formed a gel mass which then dissolved slowly. This may explain the slower than expected release and further demonstrates that although the pharmacopoeial methodology for dissolution profiling may well be appropriate for other solid dosage forms such as tablets, it is not well suited for characterising oral thin films. Therefore, there is a need to develop more appropriate dissolution methods for these dosage forms.

Despite this, generally, more than 80% of the active ingredient (KAP) was released within the first 10 minutes, and over 90% of the stated content was released within the first 15 minutes from all strips under all pH conditions. The British Pharmacopoeia requires that for immediate release tablets or capsules, no less than 70% of the stated content of active ingredient should be released at 45 minutes (British Pharmacopoeia Commission, 2012). Therefore, all strengths of oral thin films conformed to pharmacopoeial specifications for dissolution testing.

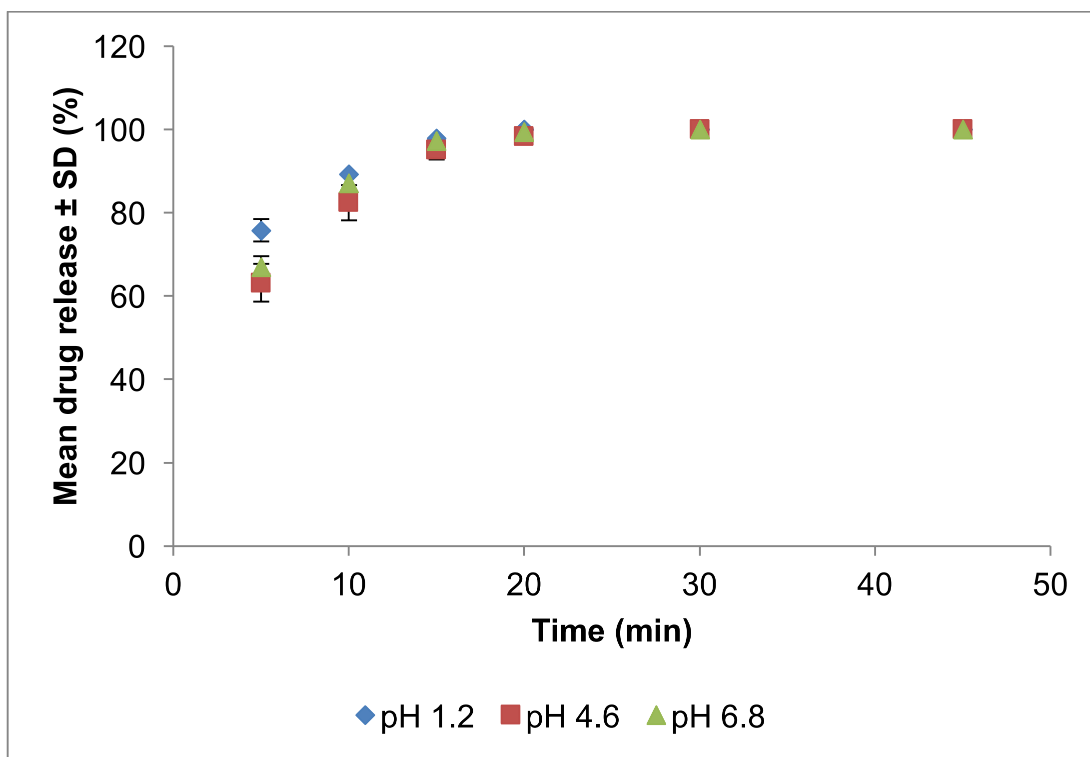


Figure 5. Dissolution profiles for potassium phosphate 0.2mM oral thin films. Drug release was measured using the Type II paddle method in three pH buffers representative of physiological pH range (hydrochloride (pH 1.2), acetate (pH 4.6), and tris-hydrochloride (pH 6.8)) (n = 6 at each).

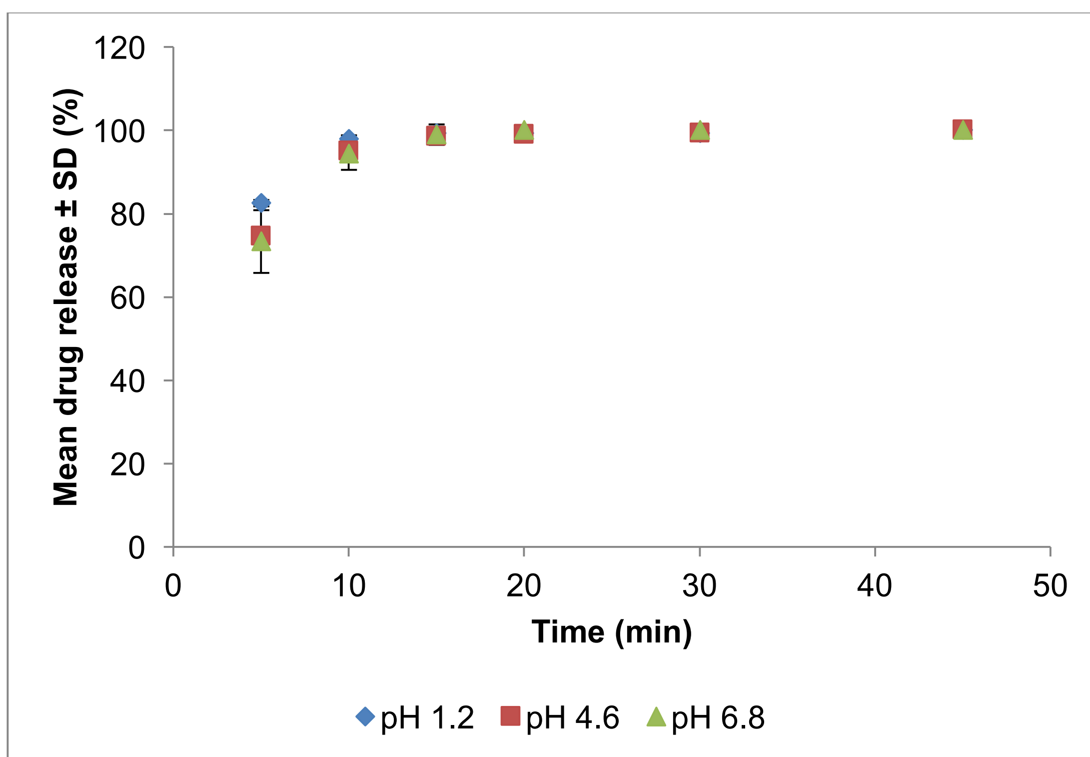


Figure 6. Dissolution profiles for potassium phosphate 0.3mM oral thin films. Drug release was measured using the Type II paddle method in three pH buffers representative of physiological pH range (hydrochloride (pH 1.2), acetate (pH 4.6), and tris-hydrochloride (pH 6.8)) (n = 6 at each).

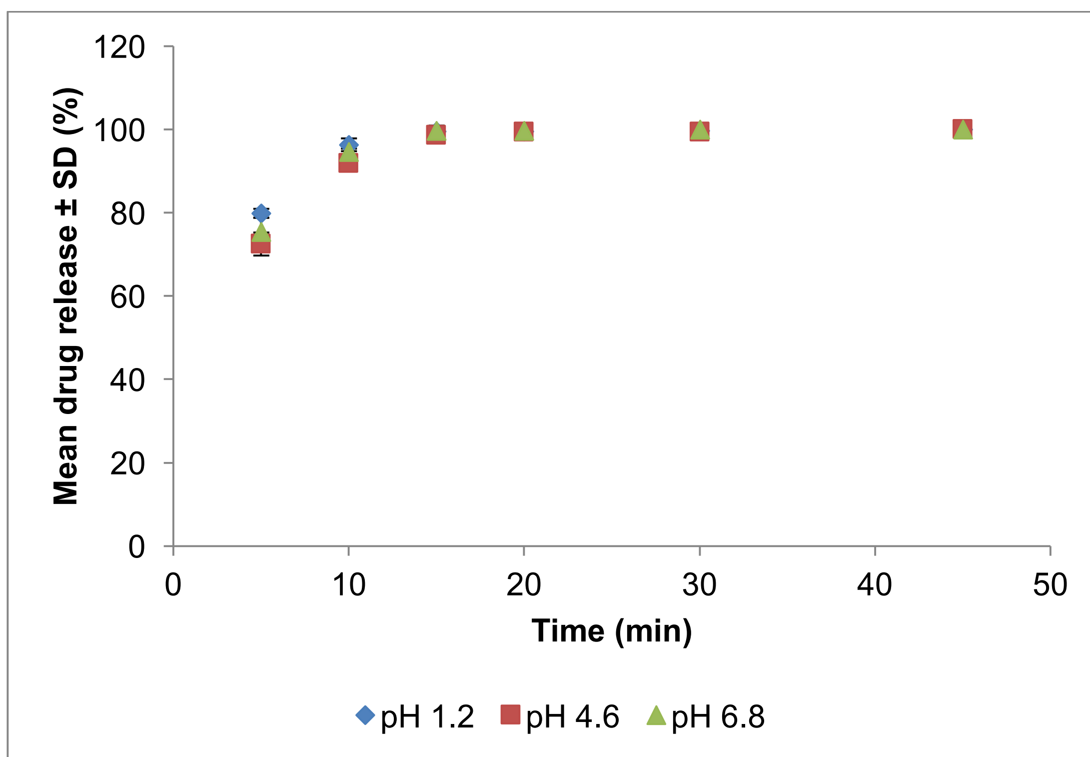


Figure 7. Dissolution profiles for potassium phosphate 0.4mM oral thin films. Drug release was measured using the Type II paddle method in three pH buffers representative of physiological pH range (hydrochloride (pH 1.2), acetate (pH 4.6), and tris-hydrochloride (pH 6.8)) (n = 6 at each).

2.3.3.2 Tensile strength testing

The results of the Tensiometer tensile strength test are presented in Table 7.

Table 7. Tensiometer results showing the mean ± SD elongation (mm) and ultimate tensile strength (N/mm²) required to break the films (n = 3).

Batch	Breaking force (N/mm²)	Increase in length at breaking point (mm)
0.2 mM oral thin films	10.32 ± 1.29	6.12 ± 0.64
0.3 mM oral thin films	7.53 ± 0.57	3.99 ± 0.35
0.4 mM oral thin films	6.56 ± 1.62	4.41 ± 0.43

2.3.3.3 Weight and content uniformity

The average (SD) strip weight for 20 strips selected at random from a batch of 0.2 mM potassium acid phosphate oral thin films (Figure 8) was 58.40 (1.4) mg. The average potassium phosphate content was 28.20 (0.78) mg. All strips were within 90-110% of the target content and therefore conformed to BP and Ph. Eur. specifications.

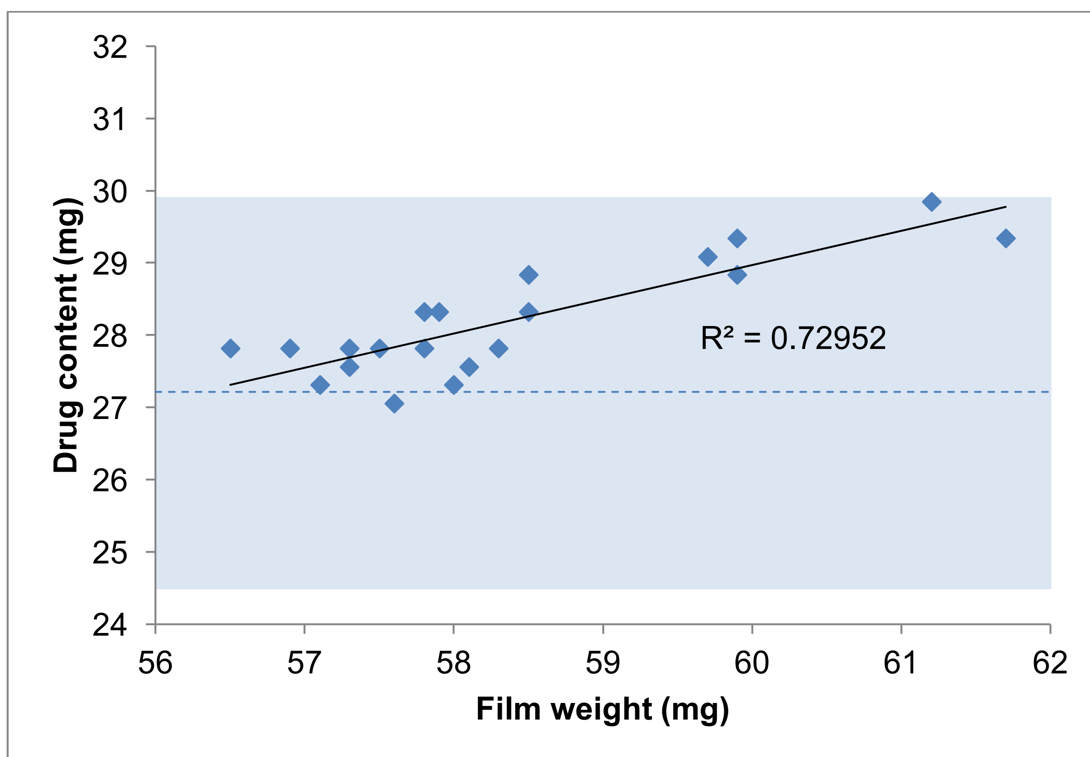


Figure 8. Potassium acid phosphate content uniformity of 0.2 mM batch. Dotted line indicates 0.2 mM target content with shaded area highlighting ±10% acceptance margin.

The average (SD) strip weight for 20 strips selected at random from a batch of 0.3 mM potassium acid phosphate oral thin films (Figure 9) was 65.7(3.7) mg. The average potassium phosphate content was 43.71(2.56) mg. As some strips lay out with 90-110% of the target content, a further 10 strips were assayed. The mean strip weight of all 30 strips was 65.39(3.29) mg whilst the average potassium phosphate content was 44.05(2.27) mg. Only one strip deviated by more than 15% from the target potassium phosphate content. However, since only one strip deviated by more than 15% from the target and no strip deviated by more than 25%, the batch still conforms to Ph. Eur. specifications. The single outlying strip was not of concern since the target strip weight range for this strength was 60-70 mg. Strips were weighed individually during manufacture to ensure they fell within this range, and it is likely that this single strip was passed in error.

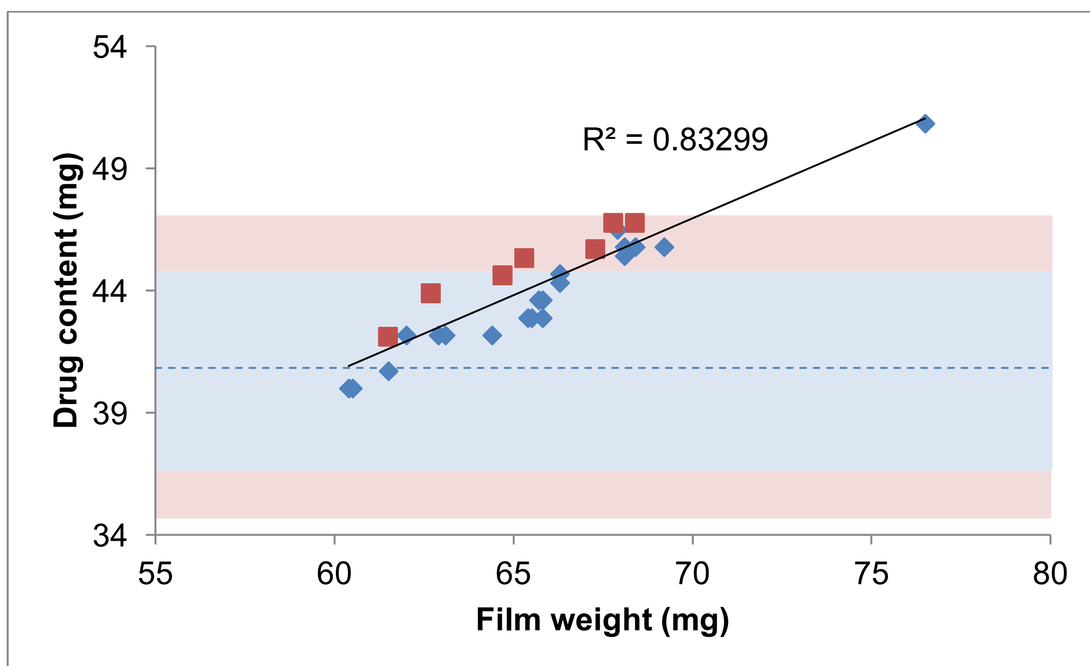


Figure 9. Potassium acid phosphate content uniformity of 0.3 mM batch. Dotted line indicates 0.3 mM target content with blue and pink shaded areas highlighting ±10% and ±15% acceptance margins respectively.

The average strip (SD) weight for 20 strips selected at random from a batch of 0.4 mM potassium acid phosphate oral thin films (Figure 10) was 91.4(2.7) mg. The average potassium phosphate content was 57.2(1.6) mg. All strips were within 92.5-107.5% of the mean weight and within 90-110% of the target drug content therefore conformed to BP and Ph. Eur. specifications.

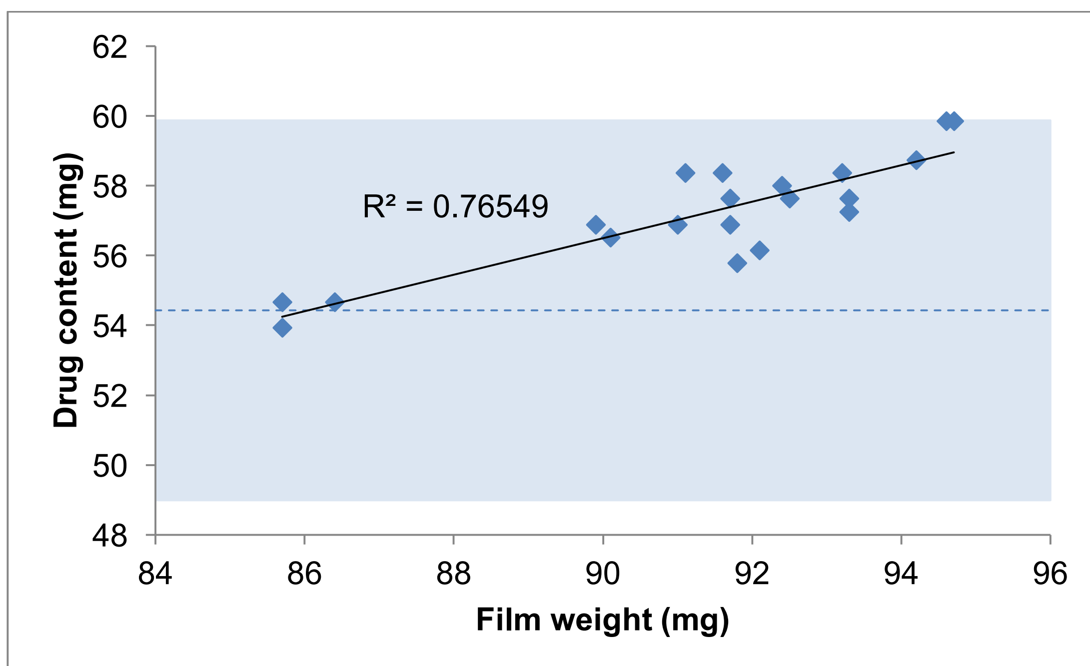


Figure 10. Potassium acid phosphate content uniformity of 0.4 mM batch. Dotted line indicates 0.4 mM target content with shaded area highlighting ±10% acceptance margin.

2.3.3.4 Thermal stability

Figure 11 shows the mean content of potassium acid phosphate obtained by flame photometry for 5 strips removed at time points t = 0, 1, 3, 6 and 12 months for each of 3 strengths of oral thin films (0.2 mM, 0.3 mM and 0.4 mM respectively).

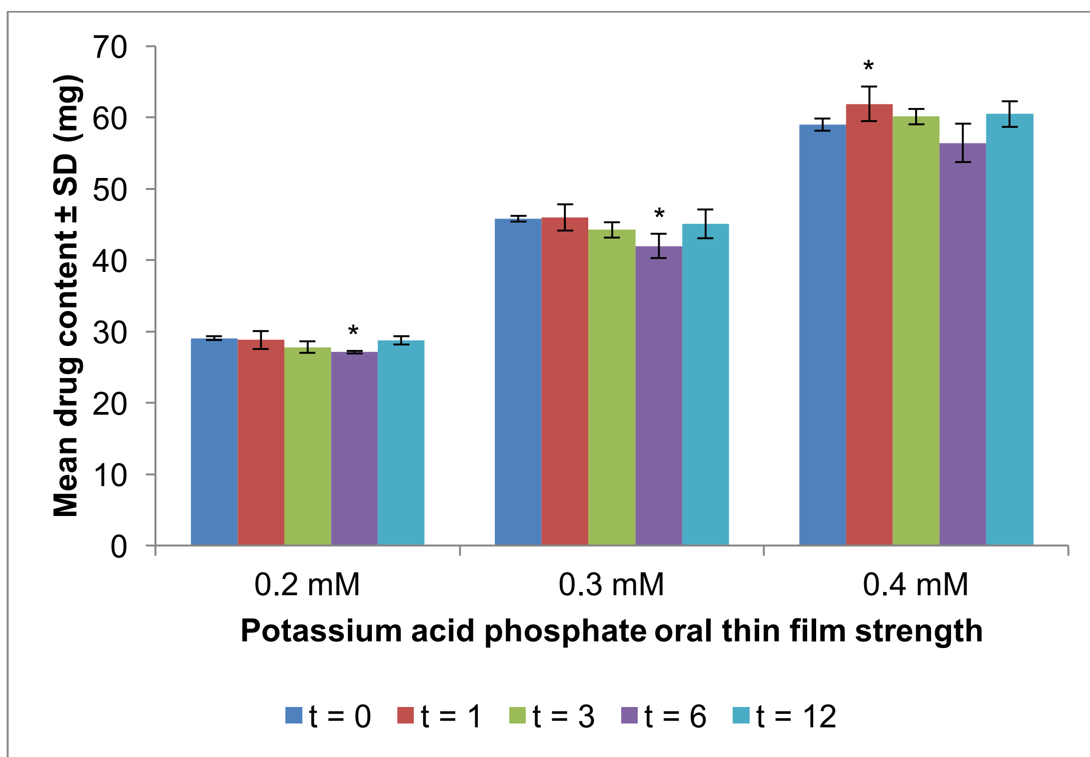


Figure 11. Long-term thermal stability study at 25°C. The * indicates a difference of more than 5% from time zero was observed.

No significant differences in mean drug content from t = 0 were evident for any strength of film at 12 months of storage. A difference of more than 5% from the time zero value was observed at 6 months for the 0.2 mM and 0.3 mM batches and at 1 month for the 0.4 mM batch. However, all films remained within 90-110% of the stated contents as per pharmacopoeial uniformity requirements. The small sample size at each time point could account for the apparent difference, which may have resulted in a Type 1 error.

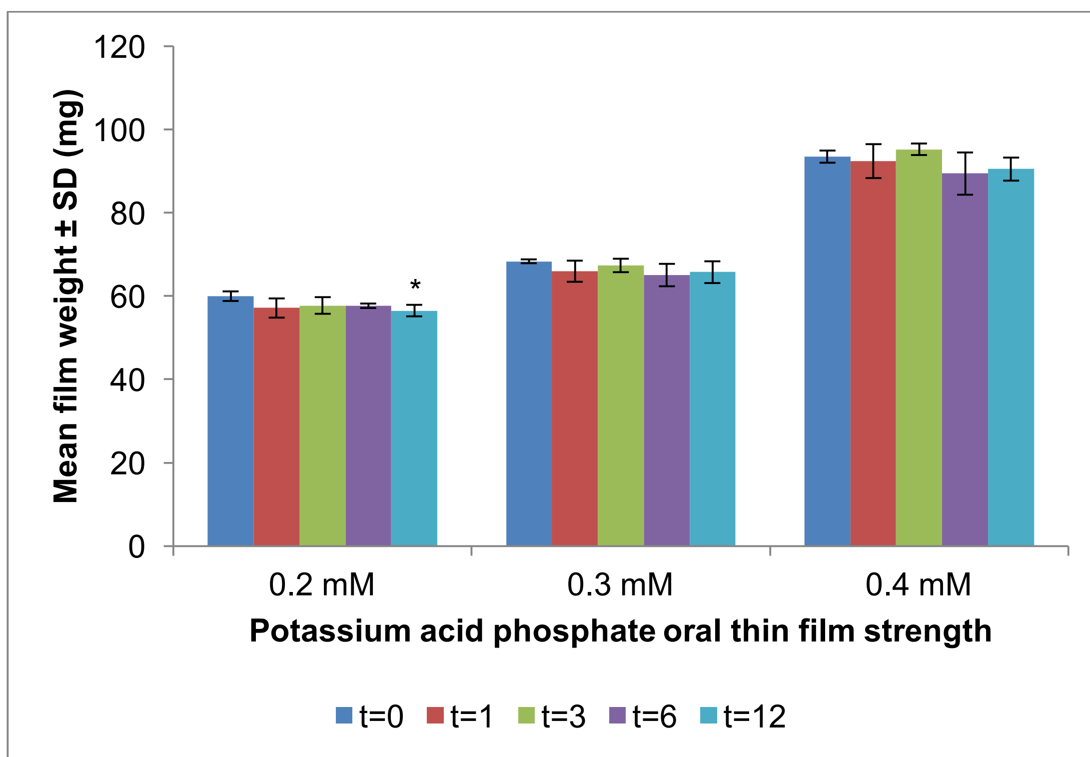


Figure 12. Long-term thermal stability study at 25°C. The * indicates a difference of more than 5% from time zero was observed.

Figure 12 shows the mean weight for 5 strips removed at time intervals throughout the 12 months for each of 3 strengths of oral thin films (0.2 mM, 0.3 mM and 0.4 mM respectively). No significant differences in weight from t = 0 were evident for any strength of film over the first 6 months. A significant decrease in mean film weight was observed for the 0.2 mM batch only after 12 months. However, all strips were still within 10% of the average mass at time zero and therefore continued to conform to pharmacopoeial standards of uniformity.

2.3.3.5 Bioburden

Table 8. Results from microbial analysis carried out by Andersen Caledonia as per ISO 11737-1.

Media type:	Tryptone soya agar			Sabouraud dextrose agar					
Media batch/ lot no:	TSA 1203444			SAB TU06 / 981548					
Culture conditions:	3 days @ 35°C ± 1°C			5 days @ 22°C ± 1°C					
Sample description	Colony type & limits								
	Bacterial	W	A	Yeast	W	A	Fungal	W	A
0.2 mM batch	2	N/A	N/A	0	N/A	N/A	0	N/A	N/A
0.3 mM batch	1	N/A	N/A	0	N/A	N/A	0	N/A	N/A
0.4 mM batch	1	N/A	N/A	0	N/A	N/A	0	N/A	N/A
Abbreviations:	W = warning, A = action								

Assessment of the microbiological quality of oral thin films containing 0.2 mM, 0.3 mM, and 0.4 mM of potassium acid phosphate revealed no fungal or yeast growth, and minimal bacterial growth.

Acceptance criteria according to the British Pharmacopoeia requires that total aerobic microbial (TAMC) and total combined yeasts and moulds (TYMC) counts are less than 2000 and 200 colony forming units per gram respectively.

2.3.3.6 Thermogravimetric analysis

A 14.783 mg sample taken from a 0.2 mM oral thin film and heated from 25°C to 300°C at 10°C per minute incurred a total weight loss of 29.6% across the temperature range, with the greatest loss (23.8%) occurring after approximately 230°C. From the start of the experiment until 230°C, only 5.8% weight loss occurred.

A 17.859 mg sample taken from a 0.3 mM oral thin film incurred a total weight loss of 22.98%. Again, the greatest loss (19.8%) occurred after 230°C

From the start of the experiment until this point, only 3.2% weight loss occurred.

A 21.751 mg sample taken from a 0.4 mM incurred a total weight loss of 22.4% with the greatest loss (19.3%) occurring, again, after 230°C. From the start of the experiment until this point, only 3.1% weight loss occurred.

Differential thermal analysis revealed a significant change in each sample, beginning at approximately 230°C, with the 1st derivative peak around 250°C. This could be explained by the melting point of potassium acid phosphate (252.6°C). Pullulan also degrades at around 250°C so the change could also include the loss of bound water as the polymer matrix degrades. Loss in mass prior to this point could be attributed to loss of unbound water and volatiles.

The results complement the bioburden report (see section 2.3.3.5, Table 8) and show a low free water content (approx. 3-6% w/w) available to support microbial growth. This adheres to the concept of orodispersible thin films as a solid dosage platform, which does not require the inclusion of preservatives to prevent decomposition by microbial growth.

2.3.3.7 Raman spectroscopy

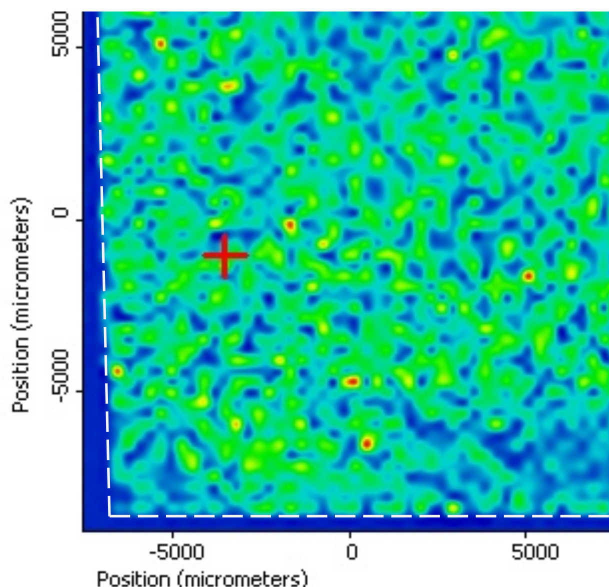


Figure 13. Intensity map by Raman spectroscopy. The map confirms uniform distribution of KAP across the surface of a 0.2 mM oral thin film. Blue indicates absence of drug whilst green through yellow to red show increasing concentrations. White dashed line indicates dosage form edges.

The intensity map produced by Raman spectroscopy (see Figure 13) confirmed that the method used to combine the excipients in the oral thin film batch was effective, with the active ingredient homogeneously distributed throughout the film. Blue indicates the absence of drug, whilst green through to red show varying levels of intensity; the white dashed line has been added to show the edges of the OTF. The method provided a simple, non-destructive way of checking homogeneity of drug across the surface of a single film. However, this was limited to the upper surface of the dosage unit and did not allow a 3-dimensional visualisation.

2.3.4 Clinical audit at Princess Royal Maternity

Data were collected from 31 babies (16 female, 15 male). Birth weight ranged from 0.51-1.86 kg with an average of 1.27 kg (n = 31). Eight infants

(26%) were of extremely low birth weight (< 1000 g). Median gestational age was 29^{+4} weeks, ranging from 24^{+6} to 31^{+6} weeks.

All data were pooled and then separated into weeks of life. ‘Week 1’ refers to data collected from all babies during the first week of life and so on. Across the first eight weeks, of the total number of observations pooled 2, 21, 47, 60, 65, 74, 73 and 74% were prescribed an oral KAP supplement respectively. The average total daily phosphate intakes across the first 8 weeks of life are presented in Table 9.

Table 9. Total daily phosphate intake week-by-week.

	Adjusted mean total daily intake of phosphate							
Week	1	2	3	4	5	6	7	8
Mean PO4 (mg/kg/day)	182	238	280	286	322	314	310	314
mM/kg/day	1.9	2.5	2.9	3.0	3.4	3.3	3.3	3.3
Standard deviation (mg/kg/day)	55	73	71	72	72	76	83	81
n =	30	30	31	30	24	22	18	15

The overall mean (SD) plasma phosphate concentration across all weeks where an oral phosphate supplement was given was 2.10 (0.38) mM/L compared to 1.92 (0.50) mM/L when no supplement was prescribed ($P < 0.001$). Plasma phosphate concentrations plotted according to whether the infant was or was not receiving an oral supplement are presented in Figure 14, with upper and lower target limits for plasma phosphate concentration of 1.8 and 2.6 mM/L. A 2-way ANOVA test was performed for each week with oral KAP supplement and baby as the two factors of interest. Statistically significant differences between the two data sets are highlighted. The P-values for comparisons across the first 8 weeks of life were 0.797, 0.199, 0.257, 0.865, 0.876, 0.002, 0.002 and 0.01 respectively.

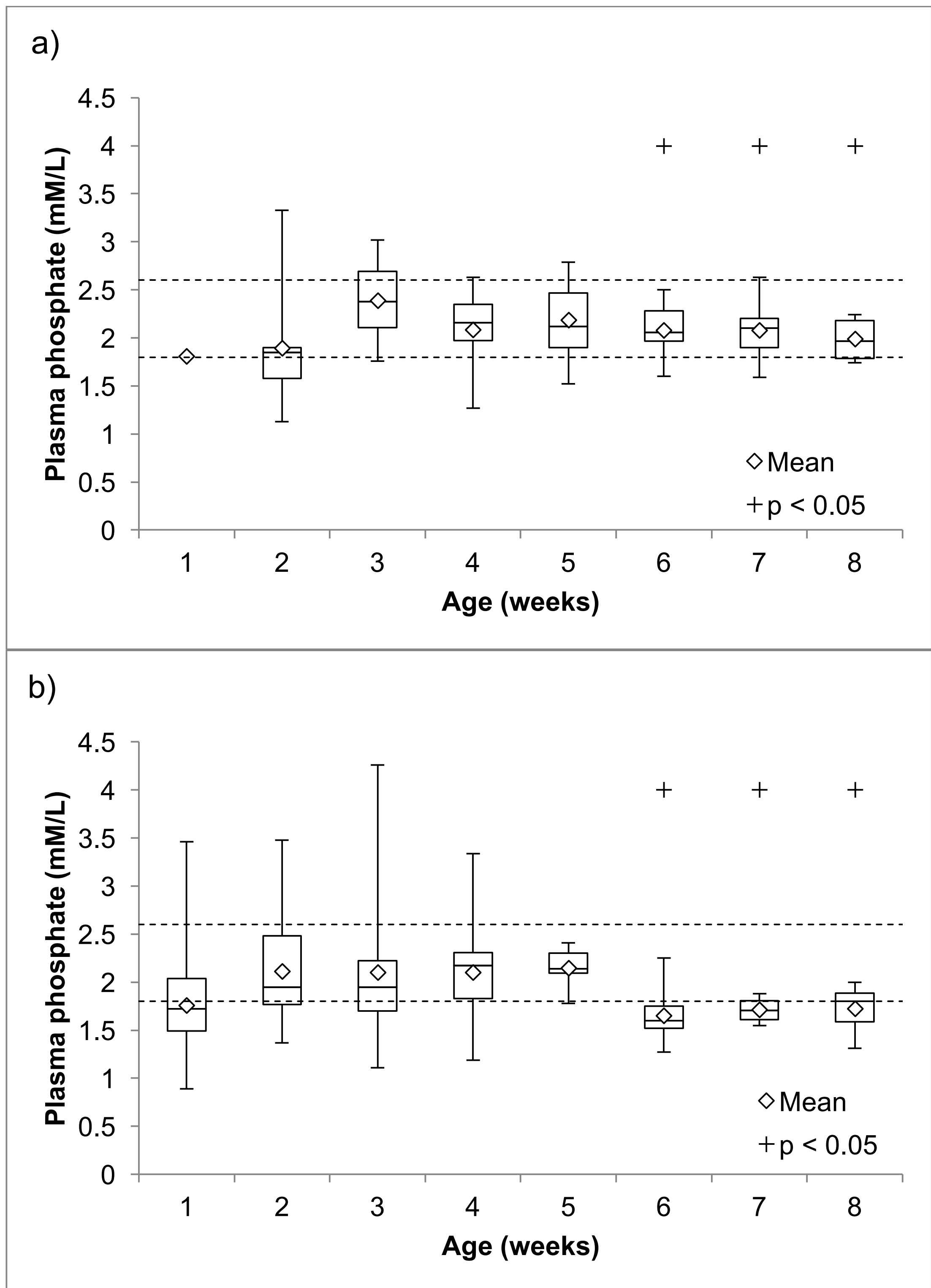


Figure 14. Association between oral KAP supplement and plasma phosphate. Measurements taken on days where an oral KAP supplement was prescribed (a) and where no supplement was given (b). Dashed lines indicate target range (i.e. 1.8-2.6 mM/L).

The overall mean (SD) plasma calcium and alkaline phosphatase (ALP) concentrations were 2.25 (0.35) mM/L and 398.9 (186.7) iU/L, range 1.31-6.60 mM/L and 90-1233 iU/L respectively. Unsurprisingly, regression analysis revealed poor correlation between plasma phosphate and plasma ALP (R^2 = 0.048).

Part of the power of ANOVA is the ability to estimate and test interaction effects. When an interaction effect exists, the impact of one factor depends on the level of the other factor. The interaction test from our analysis gave a non-significant P-value of 0.082. Therefore, there was no evidence to suggest that mean plasma phosphate was not the same for each distinct oral phosphate supplementation-baby combination after the main effects of oral supplement and baby had been accounted for, and so the model was refitted without the interaction factor. Significant inter-individual variations in plasma phosphate concentration existed ($P < 0.001$) and the latter was significantly increased by oral supplementation ($P < 0.001$).

Figure 15 shows the average dosage of KAP oral solution prescribed week-by-week. The overall average (SD) dosage across all babies at all ages was 0.85 (0.31) mM/kg/day. The results show that the prescribed dose of oral KAP varied extensively (range 0.29-2.20 mM/kg/day) but was generally below the recommended dosage according to local guidelines (i.e. 1.0 mM/kg/day). It seemed that while oral KAP was initially prescribed at the recommended dose, this was not adjusted as the weight of the child changed over time.

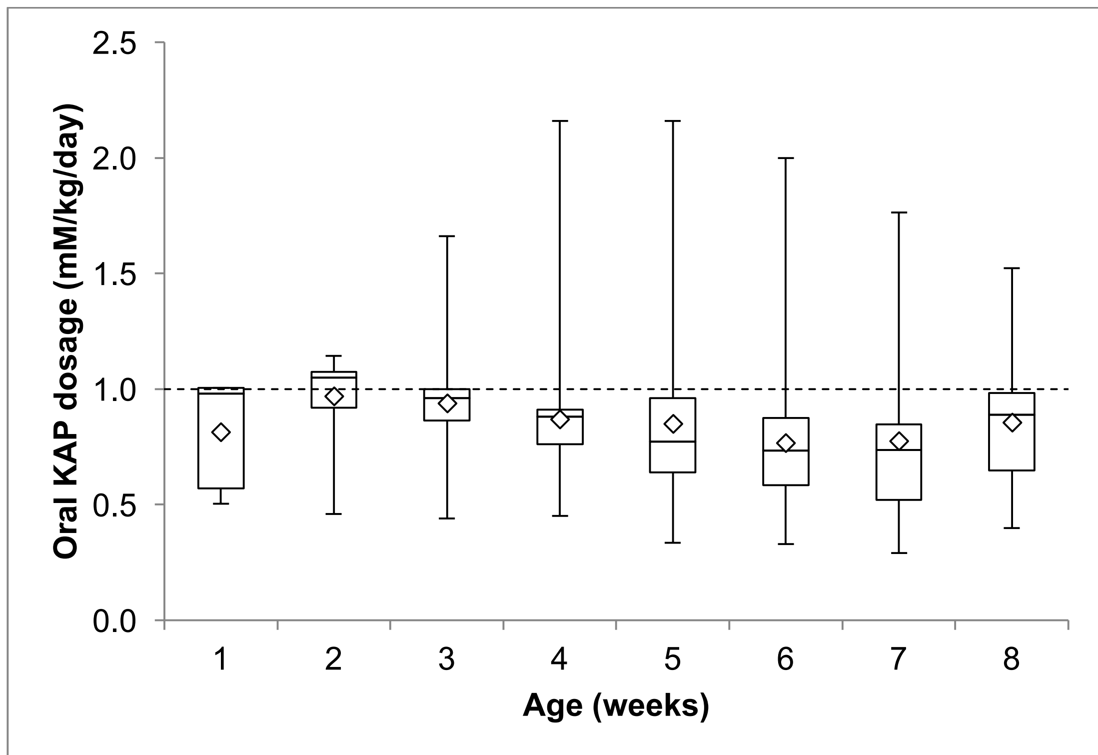


Figure 15. Boxplots of potassium acid phosphate oral solution dosages (mM/kg/day) week-by-week. Dashed line indicates 1 mM/kg/day dosage as per local guidelines. Symbols indicate mean values.

The overall percentage of plasma phosphate readings pooled from all weeks which were below the therapeutic minimum target (Figure 14) was greater amongst those babies who did not receive an oral supplement compared to supplemented babies (45%, n = 106/236 *versus* 18%, n = 21/116). Only 10% of all measurements from supplemented babies were above 2.6 mM/L (n = 12/116). The majority of plasma phosphate concentrations for supplemented babies were within the physiological target range (72%, n = 83/116) whereas less than half of all phosphate levels from non-supplemented babies were within this range (46%, n = 109/236).

The minimum daily phosphate intake as recommended by ESPGHAN was achieved by the second week of life in 24/31 infants overall (Figure 16). However, the maximum recommended daily phosphate intake was exceeded

by week 3 (17/31 infants in week 3 exceeded recommendations). When oral KAP supplement was subtracted from the total daily intake of phosphate, ESPGHAN recommendations were still achieved in 20/31 babies by week 2, and the average daily phosphate intake remained within the target range beyond this period. Table 10 gives the incidence of hyperphosphatemia (plasma phosphate > 2.6 mM/L) and hypophosphatemia (plasma phosphate < 1.8 mM/L) in cases of excessive (> 276 mg/kg/day) and insufficient (< 184 mg/kg/day) total daily phosphate intake respectively. Data is split according to whether or not an oral supplement was provided.

Table 10. High or low plasma phosphate associated with high or low total daily phosphate intake.

	Supplemented	Unsupplemented	Odds Ratio
Total daily PO4 intake > 276 mg/kg/day	77% (89/116)	40% (94/236)	4.98
Plasma phosphate > 2.6 mM/L	9% (8/89)	6% (6/94)	1.45
Total daily PO4 intake < 184 mg/kg/day	15% (17/116)	43% (102/236)	4.43
Plasma phosphate < 1.8 mM/L	29% (5/17)	48% (49/102)	2.22

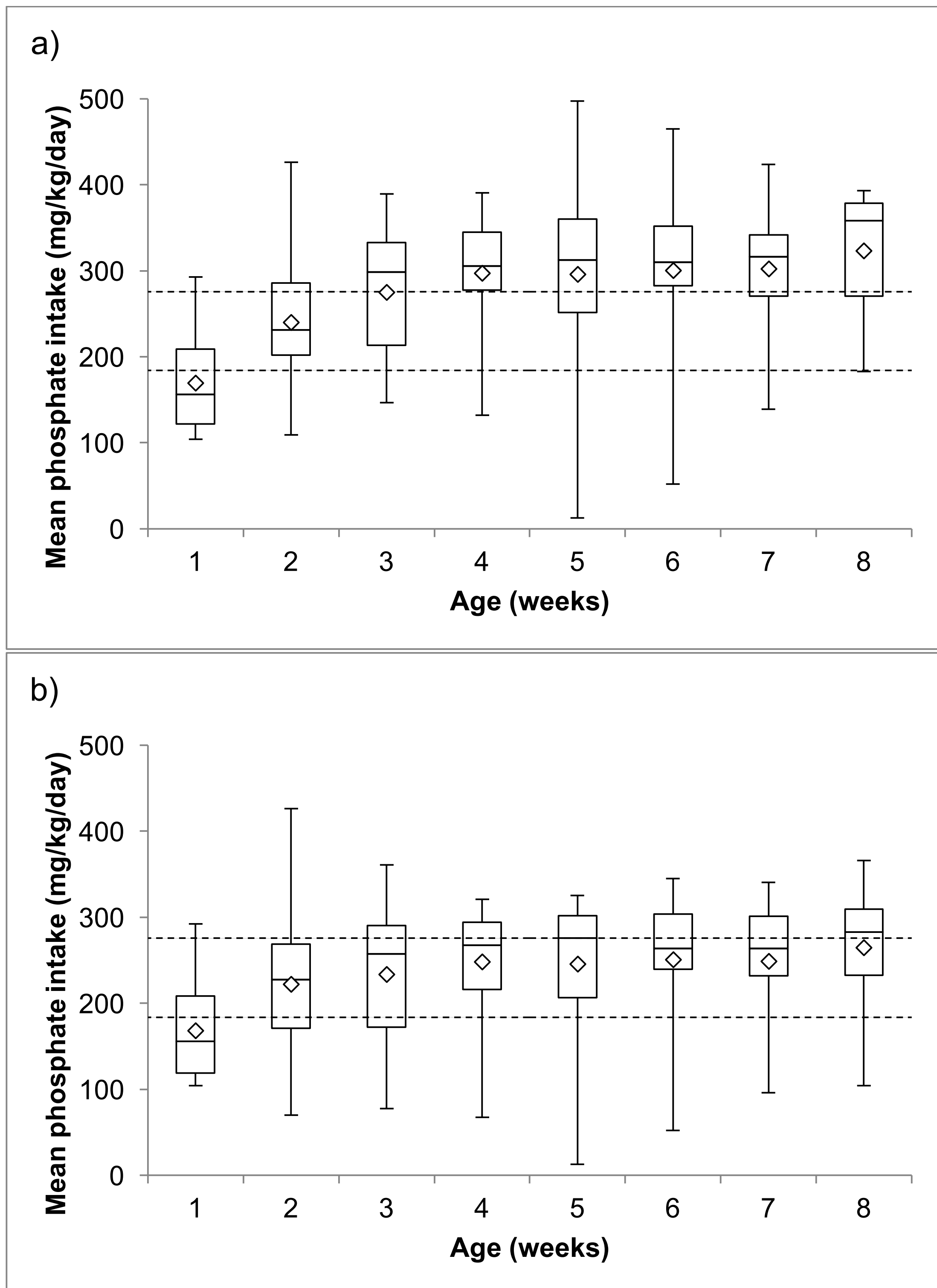


Figure 16. Boxplots of average total daily phosphate intake with (a) and without (b) oral KAP supplement (average weekly weights used and data normalised to 168 hour weeks). Dashed lines indicate ESPGHAN recommended daily phosphate intake (i.e. 184-276 mg/kg/day). Symbols indicate means for each week.

As an example, using the mean birth weight observed from this study, daily excipient exposure for a 1.27 kg neonate given potassium acid phosphate

using oral thin films at the recommended dosage of 1.0 mM/kg/day (i.e. 1.27 mM/day) can be estimated. The total daily dose would be delivered in two instalments, therefore the dose would be rounded to 0.6 mM twice daily. This could be administered as two 0.3 mM films twice a day. Details of subsequent excipient exposure is provided in Table 11, and is based on an average film mass of 65.7 mg.

Table 11. Excipient exposure details for a hypothetical 1.27 kg neonate prescribed potassium acid phosphate at a dose of 0.6 mM twice daily. WHO ADIs provided where available. NS – not specified, QS – quantum satis. *value for d-limonene provided.

Ingredient	Percentage dry weight per film (%)	Content per 0.3 mM film (mg)	Total daily exposure (mg)	Acceptable daily intake
KAP	67.80	44.54	178.17	QS
Pullulan	25.42	16.70	66.81	NS
PVP K30	3.39	2.23	8.91	50 mg/kg
Glycerol	0.17	0.11	0.45	NS
Sucralose	0.51	0.33	1.34	15 mg/kg
Lemon flavour	0.34	0.22	0.89	1.5 mg/kg*
Sisterna SP70	2.37	1.56	6.24	30 mg/kg

2.4 Discussion

2.4.1 Oral thin films in paediatric applications - phosphate

The European Paediatric Formulation Initiative (EuPFI), founded in 2007, is composed of five subgroups which focus on the safety of excipients in paediatric medicines, taste masking technologies, delivery devices for administration of medicines to children, extemporaneous preparations, and the age-appropriateness of formulations (Salunke *et al.*, 2011). Earlier this year, the European Medicines Agency published a draft concept paper

addressing concerns over excipient safety and labelling of medicinal products (Committee for Medicinal Products for Human Use (CHMP), 2012).

It is with these five key areas of interest in mind that current research is focused. Oral thin films could offer a safe alternative for delivering active pharmaceutical ingredients to the paediatric population. As has been demonstrated, OTFs can be formulated using food-grade excipients in safe quantities and free from ingredients with known toxicities in this population group e.g. benzyl alcohol, propylene glycol, ethanol, isopropyl alcohol, or benzoic acid. Using the recommended dosage regime specified in the Children's British National Formulary of 0.5 mM/kg twice daily, a range of oral thin films containing potassium acid phosphate were formulated in increments of 0.1 mM to allow for approximate mg/kg dosing across a 0.4-1.6 kg body weight range if no more than two OTFs are administered as a single dose (Paediatric Formulary Committee, 2011).

The excipients contained within the film formulation are GRAS and of food or pharmaceutical grade. Furthermore, as shown in Table 11, all excipients included in the formulation are well within the acceptable daily intake limits as set by WHO. As the active pharmaceutical ingredient, potassium phosphate monobasic is *quantum satis* i.e. permitted in whatever amount is required therapeutically.

Bioequivalence guidelines published by the EMA state that BCS-based biowaiver (restricted to highly soluble drug substances with known human absorption and considered not to have a narrow therapeutic index) are

applicable for immediate release drug products if more than 85% drug release within 15 minutes is demonstrated and would be considered exempt from *in vivo* pharmacokinetic bioequivalence study requirements by the World Health Organisation (World Health Organization, 2006). More than 95% drug release occurred within 15 minutes in all potassium acid phosphate oral thin film batch strengths across physiological pH range, and the formulation is therefore considered to be ‘very rapidly dissolving’ and an assumption of equivalent *in vivo* performance can be justified. Disintegration/dispersion and drug release was also assessed under conditions reflecting oral drug delivery in neonates, and a comparable release was achieved (see Chapter 6).

Oral thin films batches conformed to pharmacopoeial standards of uniformity in terms of mass and drug content. The results of Raman mapping complimented batch content uniformity results, indicating that the method of manufacture produced a homogenous dispersion of potassium acid phosphate throughout the surface of the oral thin films. This application of Raman spectroscopy proved a simple and effective method for indicating uniform dispersion of the active pharmaceutical ingredient throughout the polymer matrix, and has the potential for use in characterisation of thin films generally.

The oral thin films were flexible and non-brittle. The British Pharmacopoeia states that for oromucosal dosage forms which include sublingual and buccal tablets and orodispersible films “measures are taken to ensure that they possess suitable mechanical strength to resist handling without being

damaged" (British Pharmacopoeia Commission, 2015b). The European Pharmacopoeia also specifies that oromucosal preparations must possess suitable mechanical characteristics to allow them to be handled, although no numerical values are provided in either text specifying the minimum force which the dosage forms must be capable of withstanding (European Directorate for the Quality of Medicines and Healthcare (EDQM), 2002). However, the results of the tensile strength test (see Table 7) are comparable to other oral thin films of similar composition (Garsuch and Breitkreutz, 2009).

The formulation was shown to be thermally stable after 12 months in storage at 25°C. A 'significant' difference (greater than 5%) in potassium phosphate content was observed in the 0.2 mM and 0.3 mM batches after 6 months and in the 0.4 mM batch after 1 month but this could be explained by a random error, which may have occurred when weighing the strips, or during the dilution or analysis by flame photometry. All strips were within 15% of the mean and so continued to comply with content uniformity requirements, and no differences were observed at the final values taken after 12 months. It was not possible to conduct the study under controlled hydrodynamic conditions, however it would be useful to perform humidity testing to allow a full picture of stability testing to be completed.

Results from TGA revealed little significant weight loss (3.1-5.8%) up until the melting point of potassium acid phosphate. The initial loss in weight could be attributable to evaporation of water, or to loss of volatile oils contained within the lemon flavouring. This complimented bioburden test results and was in keeping with general understanding of oral thin films as a solid dosage

platform, featuring minimal available water to support microbial growth. The formulation does not require the addition of preservatives, many of which have known toxicity concerns in paediatric populations e.g. parabens.

A stable OTF of acceptable size, taste and which dissolves rapidly under conditions reflecting those of the oral cavity, was successfully formulated. In collaboration with the Princess Royal Maternity in Glasgow, it is hoped to trial the dosage form in a sample of preterm neonates, demonstrating its effectiveness in clinical practice to treat hypophosphataemia.

2.4.2 Clinical audit

Within this typical population of infants born before 32 weeks' gestation and admitted to a tertiary neonatal unit, 57% did not achieve the recommended intake of phosphate during the first week of life and intake remained low in 23% during week two. Sufficient phosphate to meet internationally recognised guidelines for feeding preterm infants is contained within TPN (including any lipid solution additives), milks and breast milk fortifiers, but calculations assume an intake of at least 150 ml/kg/day. Lower volumes given in the early days of life thus mean that the recommended daily intake of phosphate cannot be met without additional supplementation. Phosphate intake subsequently improved but it is possible that a low intake in the critical first week of extra-uterine life may have implications for the longer term. We noted that 59% of plasma phosphate measurements in those 27 infants who were not receiving additional oral phosphate during the first week of life were below the minimum therapeutic level of 1.8 mM/L. Furthermore, 45% of all plasma phosphate measurements taken from babies on days where no

additional oral KAP supplement had been given over the first 8 weeks of life, were below 1.8 mM/L.

For all babies, additional supplementation with oral KAP significantly increased plasma phosphate levels, and only 18% of measurements taken from infants receiving an oral supplement were below 1.8 mM/L. Therefore, at least for infants of less than 32 weeks gestation, additional phosphate supplementation appears to be necessary to achieve target plasma phosphate concentrations through their stay in the neonatal unit.

Hyperphosphatemia is not often the result of excessive phosphorus intake alone but more frequently occurs in combination with an impaired renal function. Despite higher intake of phosphate than currently recommended, only 9% of all plasma phosphate levels recorded were above the upper therapeutic target of 2.6 mM/L with only 2 out of a total of 351 measurements above 4 mM/L, confirming the safety of a supplemental dose of 1 mM/kg/day.

Both of the infants who showed clinical signs of osteopenia were of extremely low birth weight (birth weights 690 and 510 g respectively). Both infants had low plasma phosphate levels (<1.8mM/L) during at least the first week of life despite receiving an average daily phosphate intake of at least 184 mg/kg/day from birth; and each had received oral KAP supplementation after being stabilised on enteral feeds as per local guidelines.

In this study, we monitored several biochemical markers of bone health including alkaline phosphatase. This hydrolytic enzyme cleaves phosphate groups from a wide variety of molecules and is concentrated at the growing

front, bringing in phosphate required for bone mineralisation. Extended use of TPN is linked with high ALP levels (Tinnion and Embleton, 2012). The results of the present study are in agreement with published data indicating a poor correlation between ALP and hypophosphatemia (Tinnion and Embleton, 2012; Lucas *et al.*, 1989). ALP is therefore not a useful prognostic tool for establishing the need for mineral supplementation. Monitoring blood phosphate levels is however useful since maintaining optimum therapeutic levels of phosphate will enhance bone mineralisation and linear growth, irrespective of serum ALP (Tinnion and Embleton, 2012).

Compliance with local guidelines for enteral supplementation of preterm babies with KAP was variable and the reason for this was not always apparent. Oral KAP was generally initially prescribed according to local guidelines (i.e. 1 mM/kg/day in divided doses) but dosages were frequently not adjusted as the infant's weight increased, resulting in a downward trend in dose per kg over time. Across the first eight weeks of life, 80% of the prescribed doses of KAP oral solution were below 1 mM/kg/day with almost half less than 0.85 mM/kg/day (85% of the guidelines dosage). This is concerning since the correlation between low phosphate intake and osteopenia is so well documented. Few babies were stabilised on enteral feeds before the second week of life, which largely explains why the proportion of additionally supplemented infants was low during the first 2 weeks, however greater attention needs to be paid to local supplementation guidelines to ensure the prevention of metabolic bone disease in developing preterm infants.

Haemolysed blood samples occasionally prevented the ascertainment of plasma phosphate concentrations. The times at which enteral feeds and oral supplements were provided varied, as did the times at which blood samples were taken, and this could have affected the results. Co-morbidities, prescribed medications, and general overall health may also have affected plasma phosphate concentrations. Plasma pH is also known to have an effect on plasma levels of calcium and phosphate. As arterial pH increases, calcium becomes more protein bound and plasma calcium is reduced as a result, causing hypocalcaemia, which subsequently affects phosphate balance. In acidosis, a greater proportion of calcium exists in its ionized form and plasma levels increase (Metheny, 2012). Arterial or capillary pH measurements were only made in ventilated babies, and arterial pH was rarely measured after the first week of life. Finally, the internal validity of our data can be weakened by the fact that plasma phosphate measurements were not independent. Rather, repeated measurements were obtained from several babies. However, our application of General Linear Model ANOVA was able to account for inter-individual effects.

Despite the relatively high phosphate content of commercial breast milk fortifier and preterm formulae, additional oral phosphate supplementation is required to achieve a plasma phosphate level within the therapeutic target range for the majority of infants born before 32 weeks' gestation. A majority of infants born before 32 weeks' gestation do not achieve the recommended phosphate intake in the first week of life. This observational study provided preliminary information on phosphate prescribing and blood phosphate levels

within the neonatal intensive care units which influenced the design of a proposed clinical trial comparing potassium acid phosphate oral thin films to standard supplementation by oral solution. The results demonstrated the wide variance in blood phosphate levels as well as the broad variation in total phosphate intake. This further endorses potassium acid phosphate as a safe initial target compound for assessing the age-appropriateness of oral thin films as a platform for oral drug delivery in this population group.

Chapter 3 - Excipient exposure in the neonatal unit: a prospective case series on an intensive care ward

3.1 Introduction

3.1.1 Excipients in paediatric medicine

Excipients feature in virtually all medicines and serve a variety of essential functions in the manufacturing process (e.g. binders, lubricants, glidants), physical and chemical stability (e.g. preservatives), content uniformity, palatability (e.g. flavourings, sweeteners), colour, and control delivery of the active ingredient (coatings, disintegrating agents). In the past excipients have been thought of as “inert” ingredients i.e. inactive substances with no pharmacological function. However this is not the case. Many excipients have been revealed to have toxic effects and some have even produced allergic reactions (Pifferi and Restani, 2003). For example, solvents such as propylene glycol or ethanol are included in many medicines designed for children to improve the solubility of drugs. However, these excipients have been associated with neurotoxic side effects. Authors of one study concluded that exposure of preterm infants to toxic excipients such as ethanol or propylene glycol was “common” within the neonatal unit (Whittaker *et al.*, 2009). A larger Estonian study also reported exposure of most infants (88%) to at least one excipient known to be toxic in neonates (Lass *et al.*, 2012).

Although information on the extent of excipient exposure and toxicological data from studies of particular excipients in paediatric populations are limited, the following examples indicate some of the hazards of using 'inert' ingredients in medicines for use in children.

3.1.2 Common excipients with known concerns

3.1.2.1 Polysorbate 80 (Tween® 80)

Polysorbate 80 was believed to have contributed to the pulmonary and renal symptoms of E-Ferol syndrome: a condition named after the neonatal vitamin E supplement it was first associated with, and characterised by thrombocytopenia, renal dysfunction, hepatomegaly, cholestasis, ascites, hypotension, metabolic acidosis and pulmonary deterioration (Brown *et al.*, 1986). Polysorbate 80 has also been shown to produce a short-term decrease in blood pressure and tachycardia when administered intravenously in adults, and is known to stimulate histamine release (Munoz *et al.*, 1988).

3.1.2.2 Benzyl alcohol

Benzyl alcohol, commonly used as an antimicrobial preservative, has been associated with respiratory distress and even fatalities within paediatric patients (Fabiano *et al.*, 2011). Benzyl alcohol is oxidised to benzoic acid which then undergoes conjugation with glycine in the liver before excretion via the kidneys as hippuric acid. In neonates, this metabolic pathway is not fully functional and accumulation of benzyl alcohol can result in the aptly named "gasping syndrome" and other neurological toxicities (Hall *et al.*, 2004; Graham and Turner, 2012).

3.1.2.3 Ethanol

Ethanol is included in many medicines designed for children to improve drug solubility. However, alcohols such as ethanol have been associated with neurotoxic side effects. In 1984, the American Academy of Pediatrics recommended that alcohol be removed from liquid formulations intended for use in children (Fiocchi *et al.*, 1999). Yet one recent study at Leicester Royal Infirmary which monitored the routine exposure of preterm infants to excipients during in-patient stay, found that the exposure of 38 low birth weight newborn infants to preparations containing ethanol resulted in them receiving between 1 and 7 units of alcohol per week (Whittaker *et al.*, 2009).

3.1.2.4 Sweeteners

Sweetening agents are widely used in both solid and liquid oral pharmaceutical formulations to enhance flavours and mask the bitter taste associated with many drugs. However, like other excipients, many natural and artificial sweeteners come with inherent risks. For example, aspartame has been associated with granulomatous panniculitis, and renal tubular acidosis (Kumar *et al.*, 1996). Xylitol, sorbitol and its isomer mannitol act as osmotic laxatives (Oku and Nakamura, 2007; Kumar, 2003). One study in adults which looked at the NOEL of xylitol concluded that 0.38 and 0.42 g/kg body weight are the maximum doses for males and females respectively, above which osmotic diarrhoea occurs (Oku and Nakamura, 2007). Rare cases of anaphylactic reactions to mannitol, particularly in young people with asthma, have also been reported (Weiner and Bernstein, 1989). Sorbitol’s osmotic effects can produce abdominal pain and diarrhoea and may reduce

intestinal drug absorption in young children (Payne *et al.*, 1997). Other sweeteners such as sucrose and fructose can affect glycaemic control in diabetic patients. Sucrose is widely known to be cariogenic and therefore its use in paediatric medicines is problematic. Lactose is commonly used in tablet manufacture as a filler to improve compressibility but can cause significant gastrointestinal upset in patients intolerant to this sugar. Glycerin in large amounts can produce electrolyte disturbances, gastrointestinal irritation and even produce adverse CNS effects (Kumar *et al.*, 1996; Weiner and Bernstein, 1989). Although little data is available about specific quantities safe for use in neonates, one study established a NOEL for glycerol of 10 g/kg/day (Organisation for Economic Cooperation and Development, 2002).

3.1.2.5 Parahydroxybenzoates (parabens)

Parabens are included in many pharmaceutical products and foodstuffs as preservatives. As well as exhibiting allergenicity concerns, toxicity studies have revealed an oestrogenic effect of parabens in rats with a similar potential risk in children warranting further research (Boberg *et al.*, 2010). Sodium benzoate is the sodium salt of benzoic acid and undergoes the same glycine conjugation to form hippuric acid (Nair, 2001). It is commonly used as a preservative however its consumption has been associated with hyperactivity amongst children as well as some contact allergic reactions (Bateman *et al.*, 2004; Ernest *et al.*, 2007).

The lesson to be learned is that excipients are not simple “inert” ingredients and care must be taken in considering safe levels and effects in children when formulating medicines targeted at this population.

3.2 Methods

The first twenty consecutive live births that were admitted to the neonatal intensive care unit (NICU) at the Princess Royal Maternity in Glasgow, commencing on the 31st October 2011 were included in the study. In 2010, 266 neonates were admitted to the PRM neonatal unit, ranging from 14-34 admissions per calendar month. Therefore, twenty neonates were not atypical of a four week period and were considered sufficient to capture medicines data representative of the total admissions. Details of all prescribed medicines and parenteral nutrition fluids were documented from birth until discharge. Significant changes in weight and any abnormal biochemical test results were also recorded. Manufacturers were contacted directly to obtain qualitative and quantitative details of the ingredients contained within the prescribed formulations. Daily and cumulative excipient exposures were then calculated for each infant based on their body weight. The project was referred to the West of Scotland Research Ethics Service (Tennent Institute, Western Infirmary, Glasgow, UK). The study was subsequently defined as audit/service evaluation and did not require formal ethics review. In addition, the Caldicott Guardian for NHS Greater Glasgow and Clyde health board was consulted in relation to information governance of data collected during the study, but no concerns were identified. The results of this study were presented as a poster presentation at the 5th European Paediatric Formulation Initiative (EuPFI) annual conference in Barcelona, the abstract for which is provided in Appendix II.

3.3 Results

3.3.1 Demographics

12 males and 8 females were recruited to the study. The gestational age at birth ranged from 26^{+0} to 40^{+0} weeks. The mean birth weight (SD) was 1746 (746) grams and ranged from 740-3510 grams. The average length of stay in hospital (SD) was 26.3 (27.1) days; range 2-94.

3.3.2 Excipient exposure

Neonates were exposed to a combined total of 85 excipients contained within 54 different formulations (see Table 12). A full list of excipients together with acceptable daily intake information, where available, is provided in Table 13. 12% (10/85) of these are known to be potentially hazardous to neonates i.e. benzyl alcohol, ethanol (absolute, 95% and anhydrous), methyl- and propyl-parahydroxybenzoate, polysorbate 80, propylene glycol, saccharin sodium, and sodium benzoate. A further 13% (11/85) have known toxicity concerns in older patients and so have the potential to be unsafe for use in neonates i.e. boric acid, borax, disodium edetate, gelatin, glycine, colloidal silica, sodium acetate trihydrate, sodium bicarbonate, sodium dihydrogen phosphate, sodium metabisulfite, and trometamol (Lass *et al.*, 2012). Table 14 gives details of exposure for a selection of these excipients of concern. Babies were prescribed 12 different drug formulations and were exposed to 23 different excipients on average (range 2-31 and 8-52 respectively).

Table 12. List of therapeutic agents prescribed to neonates. * indicates that the manufacturer failed to disclose quantitative information on composition. ETT – endotracheal tube; ID – intradermal; IV – intravenous, IM – intramuscular, PR – rectal.

Drug formulation
Aciclovir (IV)
Adrenaline 1:10,000 solution for injection (IV)*
Atropine sulphate 0.1 mg/mL solution (IV)*
BCG vaccine (ID)*
Benzylpenicillin sodium (IV)
Caffeine citrate 10 mg/mL solution (IV)
Caffeine citrate 10 mg/mL solution (Oral)
Compound alginate preparation (Oral)
Dexamethasone 0.5 mg/5mL (Oral)
Diphtheria-containing vaccine (IM)
Dobutamine 250 mg/20mL (IV infusion)
Dopamine 40 mg/mL (IV infusion)
Fentanyl 100 mcg/2mL (IV)
Flucloxacillin 250 mg/5mL (Oral)
Fluconazole (IV/Oral)
Furosemide 20 mg/5mL (Oral)*
Furosemide 50 mg/5mL (IV)
Gentamicin 20 mg/2mL (IV)
Glucagon 1 mg/mL (IV)
Glucose 10% (IV infusion)
Glycerin Suppository (PR)
Heparin sodium 10 units/mL solution (IV infusion)
Hepatitis B vaccine (IM)
I2S2 Clinical Trial Study Drug (iodine) (IV/Oral)
Ibuprofen 5 mg/mL solution for injection (IV)
Intravenous TPN emulsion containing 20% purified soybean oil stabilised with purified egg phospholipids
Intravenous TPN emulsion containing 20% Soya Oil, Medium-chain Triglycerides, Olive Oil and Fish Oil
Metronidazole 500 mg/100mL (IV)
Morphine 1 mg/mL (IV)
Morphine sulphate 0.2 mg/mL (Oral)
Multivitamin preparation (vitamins A, B group, C, and D) (Oral)
Palivizumab (IM)*
Phytomenadione 10 mg/mL solution (IV)
Piperacillin and tazobactam (IV)*
Pneumococcal polysaccharide conjugate vaccine (IM)*
Poractant alfa suspension (ETT)
Potassium acid phosphate 1 mmol/mL (Oral)
Ranitidine 25 mg/mL (IV)
Rifampicin solution (IV)*
Sodium bicarbonate 4.2% (IV infusion)
Sodium chloride 0.9% (IV)
Sodium chloride 30% (Oral)
Sodium feredetate elixir (Oral)
Soluble insulin (human) 100 units/mL (IV)
Supplementary emulsion for addition to intravenous infusions
Supplementary solution for addition to intravenous infusions

Suxamethonium chloride 50 mg/mL injection (IV)
Vancomycin (IV)
Varicella-zoster immunoglobulin (IM)*
Zidovudine solution (Oral)*

Some of the neonates in the study were prescribed topically applied creams/ointments such as Sudocrem®. Although it is recognised that these products may contribute to excipient exposure by absorption through the skin, for the purposes of this study such preparations were excluded from the results. Ophthalmic preparations such as chloramphenicol eye drops/ointment were also excluded.

Table 13. Full list of excipients contained within 54 drug formulations. Results from JECFA report provided where available. Where other information on acceptable daily intake was found, or information specific to neonates was available, this has also been provided and cited. * indicates where excipient description was not specific enough for literature search.

Excipient	JECFA evaluation	Other info.	Reference
2-phenoxyethanol		ADI 3 mg/kg/day; CDC 7 ppb; CEDI 0.35 mcg/kg/day	(Hahn *et al.*, 2010; Office of Food Additive Safety (OFAS), 2012)
Aluminium oxide	7 mg/kg/week (aluminium)	FDA restricted aluminium administration in neonates to 5 mcg/kg/day	(Wier and Kuhn, 2012; Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Aluminium phosphate			
Benzoic acid (E210)	ADI 5 mg/kg/day		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Benzyl alcohol	ADI 5 mg/kg/day		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Black cherry flavour*			
Borax	None allocated		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Boric Acid	None allocated		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Cherry flavour containing propylene glycol*	see Propylene glycol (E1520)		
Citric acid	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Citric acid monohydrate (E330)	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Dilute hydrochloric acid	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Disodium edetate	ADI 2.5 mg/kg/day		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Di-sodium hydrogen phosphate anhydrous (E339)	MTDI 70mg/kg (as P)		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Disodium phosphate dihydrate			

Disodium phosphate dodecahydrate			
Egg phospholipids		GRAS at 0.4 g/kg/day for both preterm and term neonates	(FDA (Food and Drug Administration), 2012)
Ethanol (absolute alcohol)		Limited by GMP (use as solvent)	(U.S. Food and Drug Administration (FDA), 2012)
Ethanol 95%		Limited by GMP (use as solvent)	(U.S. Food and Drug Administration (FDA), 2012)
Ethanol anhydrous		Limited by GMP (use as solvent)	(U.S. Food and Drug Administration (FDA), 2012)
Flavour strawberry*			
Flavour white sugar*			
Gelatin	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Gelatin 70% w/v	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Glycerol	Not specified		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Glycine	Acceptable		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Glycocholic acid			
Histidine			
Hydrochloric acid	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Hydrochloric acid 25%	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Lactose monohydrate			
Lecithin	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Liquid maltitol (Hydrogenated glucose syrup E965)	Not specified		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)

Liquid paraffin			
Maltitol solution	Not specified		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Mannitol	Not specified		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Metacresol (Max 3.3mg/ml)	ADI 0.17 mg/kg/day		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Methyl hydroxybenzoate	ADI 10 mg/kg/day		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Methyl parahydroxybenzoate			
Nitrogen	No ADI necessary		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Pectin	Not specified		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Polysorbate 80	ADI 25 mg/kg/day		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Polythene (mw 32000)			
Ponceau 4R (E124)	ADI 4 mg/kg		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Potassium phosphate monobasic	ADI 70 mg/kg (as P)		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Propyl hydroxybenzoate	ADI 10 mg/kg	ADI 5 mg/kg established by European Medicines Agency	(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013; Committee for Medicinal Products for Human Use (CHMP), 2013)
Propyl parahydroxybenzoate			
Propylene glycol (E1520)	ADI 25 mg/kg		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Purified water			
Quinoline yellow E104	ADI 10 mg/kg		(Joint WHO/FAO Expert Committee

			on Food Additives (JECFA), 2013)
Raspberry flavour 545724E*			
Saccharin	ADI 5 mg/kg		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Saccharin sodium	ADI 5 mg/kg		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Silica colloidal			
Sodium acetate	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium acetate trihydrate	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium benzoate (E211)	ADI 5 mg/kg		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium bicarbonate	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium bisulphite			
Sodium carboxymethylcellulose	Not specified		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium chloride			
Sodium chloride 0.9%			
Sodium citrate	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium citrate dihydrate	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium dihydrogen phosphate	MTDI 70 mg/kg (as P)		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium hydroxide	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium hydroxide 1M (E524)	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)

Sodium hydroxide 2M	Not limited		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium metabisulfite	ADI 0.7 mg/kg		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Sodium methyl hydroxybenzoate			
Sodium oleate			
Sodium phosphate dibasic			
Sodium sulfoxylate formaldehyde			
Sorbitol 70%	Not specified		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Soybean oil	Allergenicity not determined		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Succinic acid			
Sucrose			
Sulphuric acid 1M	None allocated		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)
Tocopherol (Max 225mg/1L)			
Trometamol			
Water			
WFI			
Wool fat			
Yellow soft paraffin			
Zinc chloride	ADI 1 mg/kg (as zinc)		(Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013)

All babies received a single dose of phytomenadione at birth to prevent deficiency and haemorrhagic disease of the newborn. The intramuscular vitamin K preparation prescribed contained glycocholic acid, hydrochloric acid, lecithin and sodium hydroxide; therefore it was not surprising that all babies received at least one exposure to these particular excipients.

Table 14. A selection of excipients of concern and details of daily exposure (mcg/kg/day).

Functional Category	Excipient	Exposure incidence (%)	Min	Median	Max	Mean	SD
Solvents	Ethanol (absolute alcohol)	5	6,038	30,189	90,566	40,774	26,574
	Ethanol 95%	25	4.6E-4	1.3E-3	2.4E-3	1.3E-3	4.3E-4
	Ethanol anhydrous	15	9,412	26,471	43,902	28,125	10,291
	Propylene glycol	5	14	68	204	92	60
Antimicrobial preservatives	Benzyl alcohol	25	40	189	316	181	81
	Metacresol	15	5	85	218	96	73
	Methyl parahydroxybenzoate	80	2	58	1,440	449	460
	Propyl parahydroxybenzoate	70	0.80	100	160	73	47
	Sodium metabisulfite	15	30	444	1900	707	743
Sweetener	Saccharin	25	32	86	164	92	30
Emulsifying agent	Polysorbate 80	45	6,098	11,012	24,960	12,035	4,264
Buffering agent	Glycine	80	586	15,588	27,480	14,189	6,582
	Trometamol	10	3,688	3,695	7,560	5,201	2,070
Colouring	Ponceau 4R	25	13	36	69	39	13
Chelating agent	Disodium edetate	80	1	39	112	47	27
Vaccine adjuvant	Aluminium phosphate	20	63	67	73	68	4

Of the excipients with known toxicity concerns in neonates as identified by Lass *et al*. (2012), 80% of infants were exposed to methyl parahydroxybenzoate, 70% to propyl parahydroxybenzoate and 45% to polysorbate 80. Ethanol (absolute, 95% and/or anhydrous) was received by 45% of neonates, whilst propylene glycol, saccharin sodium and sodium benzoate exposures were prevalent in at least 5%. With regards to ethanol,

daily exposure resulted in infants receiving up to 0.01 units of alcohol per day [ethanol 95% (w/v) density = 0.8 kg/L; 1 unit = 8 grams].

Precise excipient quantities contained within drug preparations were not disclosed by manufacturers in a number of cases due to concerns over commercial sensitivity. These were: Adrenaline 1:10,000 solution for injection, Atropine sulphate 0.1mg/mL solution for injection, BCG vaccine, Chloramphenicol 0.5% eye drops, Chloramphenicol 1% eye ointment, Hepatitis B vaccine, Palivizumab 50 mg injection, Pneumococcal polysaccharide conjugate vaccine, Rifampicin 600 mg injection, Piperacillin and tazobactam 2.25 g injection, and Varicella-zoster immunoglobulin. In other cases, manufacturers provided some information regarding specific excipient composition, but declined to provide full formulation details. Excipient quantities were provided in part only for: Furosemide 20 mg/5mL oral solution and Zidovudine 100 mg/10mL oral solution. In cases where manufacturers failed to provide specific information on some or all excipients, the Summary of Product Characteristic (SPC) was used to obtain qualitative information on formulation contents. As a result, 47% of the excipients identified could not be quantified either because they were undisclosed by the manufacturer or because they were used *quantum satis* within the formulation e.g. for pH adjustment. Excipients which could not be quantified were aluminium oxide, borax, boric acid, cherry flavour containing propylene glycol, dilute hydrochloric acid, disodium hydrogen phosphate anhydrous (E339), disodium phosphate dihydrate, flavour strawberry, flavour white

sugar, gelatin, gelatin 70% (w/v), histidine, hydrochloric acid, hydrochloric acid 25%, liquid paraffin, nitrogen, pectin, polythene (mw 32000), purified water, quinoline yellow (E104), raspberry flavour 545724E, saccharin sodium, silica colloidal, sodium acetate, sodium acetate trihydrate, sodium benzoate (E211), sodium bicarbonate, sodium carboxymethylcellulose, sodium chloride 0.9%, sodium citrate, sodium dihydrogen phosphate, sodium hydroxide (E524), sodium sulfoxylate formaldehyde, succinic acid, sulphuric acid, water, WFI, wool fat and yellow soft paraffin.

Of the quantifiable excipients for which acceptable daily intake or accumulative exposure recommendations were available, only three were found to be received in excess of present recommended acceptable daily intakes: sodium metabisulfite, aluminium, and metacresol.

Dopamine (40 mg/mL) solution containing sodium metabisulfite and WFI was received by three infants for the treatment of hypotension. Two infants received doses of sodium metabisulfite (an antioxidant with antimicrobial preservative properties) in excess of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended ADI of 0.7 mg/kg, ranging from 0.735 to 2.032 mg/kg/day.

Accumulative parenteral doses of aluminium greater than 5 mcg/kg/day have been associated with central nervous system as well as bone toxicity in neonates (Wier and Kuhn, 2012). The U.S. Food and Drug Administration (FDA) have placed a restriction on the amount of aluminium which can be contained within large volume parenterals used in neonates, limiting the

aluminium content to 25 mcg/L (Poole *et al.*, 2012). The first dose of a combined diphtheria, tetanus, pertussis, poliomyelitis and Haemophilus Type b conjugate vaccine received as part of a three dose immunisation schedule was administered at 2 months of age. The vaccine was administered to four infants in our sample, resulting in a single exposure of these babies to 126-140 mcg/kg/day of aluminium (mean 136 mcg/kg/day).

The soluble human insulin (100 units/mL) preparation prescribed to neonates contained metacresol as an antimicrobial preservative. Insulin was received by three infants, resulting in one infant's repeated exposure to metacresol doses in excess of the JECFA recommended ADI of 0.17 mg/kg/day, ranging from 0.1716 to 0.2178 mg/kg/day.

3.4 Discussion

Babies admitted to a neonatal intensive care unit were exposed to a high number of excipient during their stay. A minority of the excipients contained within drug formulations used on this neonatal intensive care unit have been assigned acceptable daily intake limits. Much of the available safety data available on excipients was derived from older populations and/or animal studies, and very few have been studied in the neonatal population. There is therefore an urgent need for more pharmacokinetic and toxicological studies in this age group with respect to excipients. It is concerning that some excipients continue to feature in neonatal medicines, despite known toxicity issues in this population. For example, benzyl alcohol has been recognised

as unsafe in newborn infants since the early 1980s, yet a quarter of the babies in the present study were exposed to benzyl alcohol via a heparin preparation (Liston, 1983). In contrast to previous studies, exposure of infants to ethanol was minimal (Whittaker *et al.*, 2009).

Although no data in neonates was available with respect to sodium metabisulfite safety, hypersensitivity reactions to sulfites, which include bronchospasm and anaphylaxis, are relatively common, particularly within asthmatic patients, and there have even been reported fatalities attributed to sulfites in adult populations (Yang and Purchase, 1985). Dopamine (200 mg/5mL) and dobutamine (250 mg/20mL) solutions were both used within NICU to treat hypotension. Both solutions require dilution to 50 mL prior to administration as a continuous IV infusion. However, the dobutamine product contains less than 5% of the sodium metabisulfite content of the dopamine product and therefore a simple substitution to the 2nd line therapy could lower excipient burden.

Concerns regarding aluminium exposure have been expressed for the paediatric population. Of the cases of excessive aluminium intake within this study, babies were at least 2 months of age at the time of exposure. Also, the cases were of single exposure as part of a vaccination schedule whilst accumulative exposure over time is of more concern. The aluminium content was comparable to other diphtheria-containing vaccines. Aluminium salts are important functional ingredients within vaccines, acting as adjuvants upon which the antigen is absorbed (Hunter, 2002). Aluminium salts are highly

water insoluble: only half of the aluminium phosphate received through vaccination is absorbed into the blood over the first four weeks after exposure. Additionally, following public concern, the total aluminium burden in infancy through diet and vaccination was re-evaluated and found to be significantly less than the minimal risk level as set by the Agency for Toxic Substances and Disease Registry (Mitkus *et al.*, 2011). Jefferson *et al*. (Jefferson *et al.*, 2004) reported no severe or long-term adverse effects with the use of aluminium containing diphtheria vaccinations. Given the lifelong health benefits associated with childhood vaccination, the present levels of aluminium received by this sample of infants in NICU through vaccination did not constitute a significant risk and required no further action.

Although no safety concerns were expressed in the JECFA 55th report (2001) with regards to metacresol use as a food additive in humans, one study in rats has revealed greater susceptibility of newborn rats to adverse effects from metacresol compared to older pups (Koizumi *et al.*, 2003; Joint WHO/FAO Expert Committee on Food Additives (JECFA), 2013). However, since insulin administration can be critical for correction of hyperglycaemia associated with TPN use in neonates, and no suitable preservative-free product is currently available, the continued use of the soluble insulin preparation in this age group is warranted.

Almost half of the excipients which neonates in this study were exposed to could not be quantified due to non-disclosure by the manufacturers. This was similar to the study by Lass *et al*. (2012) who also found both qualitative and

quantitative formulation information difficult to retrieve. Although the majority of unquantifiable excipients were used in a buffering or acidity regulation capacity, a number of other excipients had known toxicity concerns e.g. sodium benzoate, borax. Up to 90% of medicines prescribed within neonatal intensive care are unlicensed or off-label and used in a way not covered by the product licence (Conroy and McIntyre, 2005). Due to lack in clarity with regards to pharmaceutical composition of medicines produced by different manufacturers, it is difficult for healthcare professionals to adopt a rational approach to product selection and procurement. In the interim, compulsory disclosure of medicine composition by manufacturers should be enforced by regulators so that cumulative excipient burden can be accurately recorded on an individual patient basis and used to provide an evidence base to inform best practice. Transparency by manufacturers will also allow a better assessment of cumulative excipient burden to be made.

The results of our study provide some information on local prescribing practices and excipient burdens to neonates. Further research is required into the toxicological risk of excipients in paediatric populations and therefore the specific excipients identified in this study may be offered as targets for early toxicological investigation. Qualitative and quantitative data should be made available to pharmacists and neonatologists to aid selection of medicines for neonates and to help in the assessment of risks and monitoring requirements. This information would also be of use to industry and in particular to formulators of paediatric medicines. Topical creams, ointments

and eye drops were excluded from our results as it is difficult to calculate exact excipient burden from these. However, information on such products is sorely lacking in the literature; and since the skin of neonates is fragile, transdermal permeation may be greater than in adults, with a greater risk of adverse or toxic effects from these products. Therefore, age appropriate formulations also need to be designed with respect to these routes of administration.

Chapter 4 – Formulation and characterisation of metoclopramide hydrochloride thin films for immediate and sustained oral delivery

4.1 Introduction

4.1.1 Nausea and vomiting

Nausea and vomiting are thought to be controlled by two discrete regions in the brain. The chemoreceptor trigger zone is situated within the area postrema on the floor of the fourth ventricle, outside the blood brain barrier, making it susceptible to hormonal and chemical stimulation. It contains dopamine, serotonin, acetylcholine, and opioid receptors. The vomiting centre is found in the medulla oblongata, inside the blood brain barrier, and receives input from the cerebral cortex, vagal nerves, peripheral pathways e.g. from the gastrointestinal tract, the chemoreceptor trigger zone, and vestibular signals (Harris, 2010; Gordon *et al.*, 2014).

4.1.2 Aetiology

The sources of nausea and vomiting are extremely variable. For example, drugs such as opioids, cytotoxics, tricyclic antidepressants, digoxin, and some antibiotics feature nausea and vomiting in their side effect profiles. Conditions such as anxiety, raised intracranial pressure (from a tumour or

bleed), gastric stasis, radiation colitis, hypercalcaemia, and infection; as well as vestibular problems such as labyrinthitis, can also result in nausea and vomiting (Harris, 2010). Other sources of nausea and vomiting include bowel obstruction, abdominal or pelvic tumours, and motion.

4.1.3 Nausea and vomiting in advanced cancer

Chronic nausea and vomiting are common in advanced cancer, affecting 30-98% of cancer sufferers (Bruera *et al.*, 1996; Bruera *et al.*, 2000; Davis and Walsh, 2000). Approximately 35-44% of cases in advanced cancer patients are the result of gastroparesis (Harris, 2010). This can be drug induced, or may be caused by conditions such as hepatomegaly (enlargement of the liver), ascites (excess peritoneal fluid), or autonomic dysfunction (Harris, 2010). The afferent vagus nerve and other vagal neurocircuits play a significant role in conveying sensory information from the thorax and abdominal viscera to the CNS (Babic and Browning, 2014). Gastroparesis can also occur when the vagus nerve is damaged by illness or injury. Diabetes is the most common cause of reduced vagus nerve function, as high blood glucose levels causes damage to the nerve over time (The National Digestive Diseases Information Clearinghouse (NDDIC), 2012). Other common causes of nausea in advanced cancer include metabolic anomalies, constipation and cachexia (Bruera *et al.*, 1996).

In cases where the cause of nausea and vomiting is reversible, more than half are the result of a drug effect, with opioids accountable in 83% (Gordon *et al.*, 2014). Pain is experienced by up to 3 out of 4 advanced cancer

patients and the World Health Organisation recommend opioid analgesics; these are prescribed to more than 80% of terminal cancer patients, resulting in reduced gastric motility, nausea and vomiting (Harris, 2010). Although uncontrolled pain can also cause symptoms of nausea and vomiting, opioids are known to be highly emetogenic and antiemetics for opioid induced emesis are frequently required (Hardy *et al.*, 2002).

4.1.4 Metoclopramide

Metoclopramide is a prokinetic antiemetic which is considered the first line drug of choice in advanced cancer patients who present with symptoms of nausea and vomiting caused by gastric stasis (Fass *et al.*, 2009). It acts as a 5HT4 receptor agonist, a D2 receptor antagonist and a weak 5HT3 receptor antagonist to exert its effects. Since its action on 5HT4 receptors is facilitated by acetylcholine at the myenteric plexus, its effects on gastrointestinal motility may be antagonised by antimuscarinic drugs such as cyclizine (Harris, 2010). Metoclopramide readily crosses blood capillary endothelial cells, since the blood brain barrier is less developed at the site of metoclopramide's action, producing extrapyramidal side effects (Jolliet *et al.*, 2007). Acute dystonic reactions are common, especially in children and young adults; and particularly in females (British Medical Association Joint Formulary Committee, 2012; Wynne *et al.*, 1993). Parkinsonian adverse effects are more common in older populations (Wynne *et al.*, 1993). As a treatment for nausea and vomiting resulting from chemotherapy, metoclopramide has been effective as both monotherapy and when used in

combination with dexamethasone, a corticosteroid found to enhance the antiemetic effects of metoclopramide (Bruera *et al.*, 1996).

Metoclopramide has an oral bioavailability of 30-100% (Rao and Camilleri, 2010) and a nasal bioavailability of ~70% compared to intravenous administration (Tomirotti *et al.*, 1994). It is approximately 30% protein bound; undergoes hepatic metabolism primarily via the CYP2D6 isoenzyme to form glucuronide and sulphate conjugates; and in patients with normal renal function, it has a half-life ($t_{½}$) of ~5-6 hours (Bernardo-Escudero *et al.*, 2011; Argikar *et al.*, 2010; Fass *et al.*, 2009). Approximately 20-30% is excreted unchanged in the urine (Rao and Camilleri, 2010). Metoclopramide hydrochloride is a basic molecule with a pKa of 9.27 and a water solubility at room temperature of approximately 17.4 mg/mL (Sweetman, 2011; Shakeel *et al.*, 2014).

In a study by Tam *et al*. (1981), the authors reported a bioavailability for metoclopramide of 0.91 in rats following a 15 mg/kg oral dose, indicating no significant first-pass metabolism. They described dose dependent pharmacokinetics with a prolonged elimination rate, and suggestive enterohepatic recycling. Intravenous doses of the drug up to 15 mg/kg appeared to follow first order kinetics, whereas higher doses of over 25 mg/kg were non-linear.

4.1.5 Routes of administration and associated formulations

Data validating the absorption of metoclopramide across the buccal mucosa is lacking. However, a study which compared an orodispersible tablet preparation of metoclopramide with a conventional tablet found the two formulations to be equivalent in terms of their absorption (Fass *et al.*, 2009). Polyacrylic acids (aka carbomers) are synthetic high molecular weight polymers of acrylic acid which may be cross-linked with either allyl sucrose or allyl ethers of pentaerythritol. They possess strong mucoadhesive properties and have been used in the formulation of metoclopramide 'hydrogels' for buccal administration (García-González *et al.*, 1994; 1993). Cross-linked povidone (polyvinyl polypyrrolidone (PVPP)) is a water-insoluble synthetic cross-linked homopolymer of N-vinyl-2-pyrrolidinone, used commonly as a tablet disintegrating agent. A sublingual tablet formulation of metoclopramide containing 10% PVPP was found to release the drug by zero-order kinetics without affecting the half-life of metoclopramide (Latif, 2012). Nasal delivery of metoclopramide can be as effective as parenteral delivery at controlling nausea and vomiting associated with moderate doses of platinum-based chemotherapy regimens, whilst also showing greater ease of administration and tolerability amongst patients. However, nasal administration of metoclopramide is associated with a delay of up to 3 hours before the maximum plasma concentration is reached (Tomirotti *et al.*, 1994). It has been noted that some adverse effects associated with metoclopramide are related to elevated plasma concentrations (greater than 120 ng/mL). A modified release tablet preparation achieved lower peak concentrations,

which were delayed compared to an immediate release tablet, whilst average plasma concentrations were comparable between the two formulations (Bernardo-Escudero *et al.*, 2011).

4.1.6 Taste masking metoclopramide

A review of the literature uncovered several different approaches for effective taste masking of metoclopramide. For example, formation of a complex between metoclopramide and Eudragit® EPO was achieved by extrusion-precipitation; use of ion-exchange resins such as Indion® 204; or preparation of a drug-glycerol monostearate complex by melt granulation (Ahire *et al.*, 2012; Randale *et al.*, 2010; Rashmi and Rajesh, 2010). Additionally, simple combinations of sweeteners and flavourings have been successful. For example, Rosemont Pharmaceuticals Ltd. have marketed an oral metoclopramide hydrochloride solution containing sorbitol solution in combination with lemon and lime flavours (Rosemont Pharmaceuticals Limited, 2012).

4.1.7 Aims and objectives

A number of target therapeutic agents were identified which would be appropriate for formulation within a thin film for oral drug delivery in end of life care. These included opioid analgesics such as morphine or fentanyl, sedative and antiepileptic drugs such as midazolam or lorazepam, amitriptyline, hyoscine, and metoclopramide. Following discussion with palliative medicine clinicians (Prof. Marie Fallon and Dr. Barry Laird) within the Edinburgh Palliative and Supportive (EPaS) Care Group, regarding the

clinical value of such formulation, metoclopramide hydrochloride was selected as the initial preferred drug. Metoclopramide was selected as the primary active pharmaceutical ingredient for formulation within an oral thin film since it possessed good water solubility and could be easily quantified by simple chromatographic techniques (Harris, 2010). The aim was to produce an immediate release 10 mg film for oral delivery which would be of acceptable palatability and with good mechanical properties. Metoclopramide would also be used as the drug target in the development of a modified release orodispersible thin film formulation.

4.2 Methods

4.2.1 High performance liquid chromatography (HPLC)

4.2.1.1 Method

Based on work by Khan *et al*. (2012), a reverse phase HPLC-UV method of quantification was developed. 20 µL were injected onto an ACE C18 column (4.6 mm diameter x 150 mm length packed with a 5 µm diameter stationary phase) fitted with a guard column of the same material with UV detection at 275 nm. The mobile phase was 0.02 M potassium dihydrogen phosphate buffer solution (pH 3 adjusted with orthophosphoric acid): acetonitrile (60:40), at a flow rate of 1.0 mL/min.

4.2.1.2 Linearity, range and intermediate precision

A 25.8 µg/mL stock solution of metoclopramide hydrochloride dissolved in HPLC grade water was used to prepare standards in the concentration range 0.8-20.0 µg/mL, which represented approximately 7-180% of the anticipated sample concentrations for content assays and dissolution. Three replicate standards were prepared at each of five concentrations and each sample was measured in triplicate. To assess intermediate precision, linearity was repeated using a further five concentration standards in the 0.9-20.0 µg/mL range on a different day using the same equipment.

4.2.1.3 Specificity

A placebo oral thin film was manufactured which contained hydroxypropyl methylcellulose E5 (Dow, Michigan, USA; Batch No. LA03012N21), sucralose (Tate & Lyle, London, UK; Lot XM1D009501), spearmint flavour (Firmenich, Meyrin, Switzerland; Batch No. D0000118864), polyvinyl polypyrrolidone (Sigma-Aldrich, Dorset, UK; Lot KI19107BI), poly(ethylene glycol) average molecular weight 1500 (BDH, Poole, UK; Lot. ZA9535128), glycerol (Melford Labs Ltd., Ipswich, UK; Batch No. 19256), and menthol (Fluka, Buchs, Switzerland; Batch No. 1126763). A 51.1 mg sample of the placebo film was dissolved in HPLC grade water and diluted to 500 mL. A 645 µL filtered sample of this solution (filtered through a Millex®-HP filter unit with 0.45 µm Millipore Express® PES membrane) was vortexed with 355 µL of a 28.1 µg/mL metoclopramide hydrochloride stock solution, and assayed by HPLC (9.98 µg/mL metoclopramide hydrochloride concentration). The

final excipient concentrations (calculated from percentage dry mass) in the placebo film sample were approximately 134-199% of those expected from a typical immediate release oral thin film weighing 40 mg obtained during dissolution analyses (calculated excipient contents are shown in Table 15). Standards were prepared from the same stock solution and all UV measurements were performed in triplicate.

Table 15. Specificity. Excipient content of placebo film. A placebo film was formulated which contained the same excipients as the immediate release metoclopramide hydrochloride oral thin film formulation. The film was dissolved in water and spiked with a known concentration of metoclopramide. The resulting excipient concentrations compared to an example metoclopramide hydrochloride oral thin film are shown.

	Percent dry weight (%)		**Concentration (µg/mL)**		
Excipient	**Placebo**	**OTF**	**Spiked placebo sample**	**Oral thin film assay**	**Percent anticipated (%)**
HPMC E5	75.50	56.27	49.77	25.01	198.99
Sucralose	8.67	6.47	5.71	2.88	198.53
Spearmint	6.97	5.60	4.59	2.49	184.62
PVPP	3.15	2.48	2.08	1.10	188.63
PEG 1500	2.63	2.00	1.73	0.89	194.92
Glycerol	2.03	2.24	1.34	0.99	134.36
Menthol	1.06	0.88	0.70	0.39	178.97

4.2.1.4 Limits of detection (DL) and quantification (QL) using HPLC method

Diluted samples were prepared from a 7.9 µg/mL stock solution of metoclopramide hydrochloride dissolved in HPLC grade water. Limits of detection and quantification were determined using the signal:noise ratio method as described in the ICH guidelines (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 1994).

4.2.2 Immediate release oral thin film formulation

A viscous solution was prepared which was composed of 8.6% (w/w) metoclopramide hydrochloride (BÜFA, Hude, Germany; Batch No. 122906), 20.2% (w/w) hydroxypropyl methylcellulose E5 (Dow, Michigan, USA; Batch No. LA03012N21) as the water-soluble film-forming polymer, 2.3% (w/w) sucralose (Tate & Lyle, London, UK; Lot XM1D009501) as a sweetener, 2.0% (w/w) spearmint flavour (Firmenich, Meyrin, Switzerland; Batch No. D0000118864) to give the films a mild mint flavour and aroma, 0.9% (w/w) polyvinyl polypyrrolidone (Sigma-Aldrich, Dorset, UK; Lot KI19107BI) to improve the disintegration properties of the film, 0.8% (w/w) glycerol (Melford Labs Ltd., Ipswich, UK; Batch No. 19256) and 0.7% (w/w) poly(ethylene glycol) average molecular weight 1500 (BDH, Poole, UK; Lot. ZA9535128) as plasticizers, 0.3% (w/w) menthol (Fluka, Buchs, Switzerland; Batch No. 1126763) as a flavouring agent; and distilled water. The ingredients were weighed on an analytical balance (A&D Instruments Ltd., Abingdon, Oxford, UK; Serial No. 14214367) and combined using an Ultra-Turrax homogeniser (Janke & Kunkel, Staufen, Germany; Serial No. 751808) at 8000 rpm. A film was cast onto polymer coated paper using a Micrometer Adjustable Film Applicator (Sheen; 1117/250mm) at 1.6 mm and dried in a cabinet drier at 40°C for 25 minutes. Oral thin films were cut by hand using a rotary blade and 15 mm cutting template.

4.2.2.1 Content and mass uniformity of the OTF

10 random dosage units were taken from a batch of metoclopramide hydrochloride oral thin films and individually weighed on an analytical balance. Each film was then dissolved in distilled water and the resulting solutions were filtered through Millex®-HP filter units with 0.45 μm Millipore Express® PES membranes (Merck Millipore Ltd., Cork, Ireland) and analysed by HPLC for drug content. The British Pharmacopoeia monograph for metoclopramide hydrochloride tablets stipulates that the drug content of each tablet should not deviate by more than 10% of the stated content.

4.2.2.2 Dissolution of the OTF

Dissolution was assessed using the paddle method as described in the British Pharmacopoeia (British Pharmacopoeia Commission, 2012). Dissolution was assessed at 37°C ± 2°C using three media: hydrochloride buffer (pH 1.2), acetate buffer (pH 4.6) and mixed phosphate buffer (pH 6.8). UV measurements were taken at each time point using a fixed wavelength of 275 nm. Paddles were set at 50 rpm and 900 mL of media were used in each jar. Six repeats were performed for each pH buffer.

4.2.2.3 Oral dissolution

In order to better mimic typical salivary flow rates in a newborn infant, a simple system was designed which introduced dissolution media to the dosage form at a constant fixed rate of 1.8 mL/hour (0.03 mL/min) via a syringe driver. Drug release from immediate release metoclopramide oral thin

films was assessed using this system. Method development and results are covered in depth in Chapter 6.

4.2.2.4 Disintegration of OTF

Five metoclopramide hydrochloride oral thin films were analysed using an Erweka ZT31 tablet disintegrator (Heusenstamm, Germany; Serial No. 100991). The compendial apparatus was modified based on work by Preis *et al*. (2014). A weight was attached to the base of an oral thin film using a small clip, so that the combined weight of the clip was 3 grams. As the authors describe, the weight of 3 grams was selected since 0.03 N (Newton) was identified as the mechanical force applied by the human tongue through licking. The oral thin film was suspended from the arm of the equipment using a second clip. The media (distilled water at 37°C) was added to a level such that when the film reached the lowest point, it was halfway submerged in the media. The endpoint was determined visually as the point at which the film disintegrated, dropping the weight, and was recorded using a stopwatch.

4.2.2.5 Thermogravimetric analysis

Three samples of metoclopramide hydrochloride oral thin film weighing 13.71 mg, 9.37 mg and 12.32 mg respectively, were analysed using a TGA/SDTA851^{e} Mettler Toledo (Switzerland; Serial No. 5125178760) thermogravimetric analyser. Samples were heated from 25-300°C at 10°C min^{-1}. Nitrogen was used as the purge gas at a flow rate of 100 $cm^3\ min^{-1}$ (ATP).

4.2.2.6 Raman intensity map of OTF

Using a Thermo Scientific™ DXR™ Raman Microscope (Serial No. AIY1000276) fitted with a green 532 nm laser (Serial No. AJC1000278), filter (Serial No. AJM1000273), and 400 lines/mm grating (Serial No. ARW1100013), a Raman spectra was obtained for each of the excipients contained within the oral thin film formulation. A peak was identified which was unique to metoclopramide hydrochloride. This peak was then used to produce an intensity map across the surface of an oral thin film, providing an indication of the homogeneity of the drug throughout the film matrix. Spectra were mapped and analysed using OMNIC Software for Dispersive Raman.

4.2.2.7 Accelerated stability study of OTF

For assessment of thermal stability, a batch of oral thin films was produced and stored in sealed polythene pouches at 37°C. Ten random samples were removed at 3 and 6 month time points, and assessed for changes in mass and metoclopramide content. According to the ICH Harmonized Tripartite Guideline for stability testing of new drug substances and products, a change in assay of 5% or more from the initial value is considered “significant” (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2003). 2-sample t-tests were also performed using Minitab® (Version 16.2.4) statistical software to compare groups.

4.2.2.8 *In vivo* pharmacokinetic study of immediate release metoclopramide hydrochloride oral thin film

4.2.2.8.1 Extraction of metoclopramide from rat serum

The method for extraction of morphine sulphate from rat serum (see Chapter 5) was further developed for the extraction of metoclopramide hydrochloride. Diazepam (Sigma, USA; Lot No. 105F0451V) was used as an internal standard and 20 µL of a 93.75 µg/mL diazepam solution (in methanol) was added to 170 µL rat serum containing metoclopramide. The extraction method continued as described in Chapter 5 (5.2.2.4.1) using 0.2 M borate buffer and methyl *tert*-butyl ether (MTBE). The residue was reconstituted in 150 µL mobile phase (0.02 M potassium dihydrogen phosphate buffer solution (pH 3 adjusted with orthophosphoric acid): acetonitrile (60:40)) and 20 µL were injected onto the HPLC column in triplicate.

4.2.2.8.2 HPLC-UV method

20 µL were injected onto an ACE C18 column (4.6 mm diameter x 150 mm length packed with a 5 µm diameter stationary phase) fitted with a guard column of the same material with UV detection at 275 nm. The mobile phase (isocratic) was 0.02 M potassium dihydrogen phosphate buffer solution (pH 3 adjusted with orthophosphoric acid): acetonitrile (60:40), at a flow rate of 1.0 mL/min.

4.2.2.8.3 Single dose pharmacokinetic study design

A single dose pharmacokinetic study in twelve healthy male Sprague Dawley® rats weighing 336-425 g; average (SD) weight 359.8(25.6) g. Each

rat was administered an orodispersible thin film which contained 5 mg metoclopramide hydrochloride (11.8-14.9 mg/kg bw) under isoflurane (IsoFlo®, Abbott Laboratories Ltd., Berkshire, UK) anaesthesia. The oral thin film was placed inside the cheek using forceps and wetted by the administration of approximately 100 µL of water. For each animal, 0.4 mL blood samples were then taken from the tail vein (under anaesthesia) at a maximum of four time points within an 8 hour period such that across the 12 animals, three samples were obtained at 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 minutes. Blood samples were collected in clean 1.5 mL PPE Eppendorf® tubes and allowed to coagulate at 3-5°C in an upright position. Samples were then centrifuged at 9503 g for 10 minutes to separate the serum which was transferred to clean 2 mL Eppendorfs® and frozen at −80°C prior to analysis. After the terminal blood samples were taken, the rats were euthanized using carbon dioxide. Analyte extraction and assay by HPLC was carried out as described above.

The study was carried out in the Biological Procedures Unit (Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow). All procedures were carried out under animal license 7008973 and were approved by the local animal welfare committee. The procedures had a severity rating of moderate.

4.2.3 Modified release metoclopramide oral thin films

4.2.3.1 Preparation of drug resinates

10 g Dowex® 50WX8 (50-100 mesh i.e. 149-297 μm), a strong acid cation ion exchange resin with 8% divinylbenzene cross-linking, was dispersed in 100 mL of a 5% (w/v) solution of metoclopramide hydrochloride (BÜFA; Lot No. 13C12-B02) in distilled water, and stirred by magnetic stirrer (250 rpm) for 24 hours. The drug-resin complex was then filtered through a 47 mm 0.2 μm nylon membrane (Phenex™, Phenomenex Inc., Cheshire, UK) and washed with distilled water. The filtrate was diluted and assayed by UV spectrophotometry at 275 nm to indirectly determine the extent of drug loading. The drug-resinate was dried at 60°C in a cabinet drier.

A second drug-resinate was prepared using a higher mesh size for comparison. Dowex® 50WX8 (200-400 mesh i.e. 37-74 μm) was dispersed in a solution of metoclopramide prepared in distilled water such that the ratio of metoclopramide to resin was 1:3 by weight, and stirred by magnetic stirrer (250 rpm) for 24 hours.

4.2.3.2 Drug release from resin complex and dissolution

Assessment of drug release from the drug-resin complexes and from the finished oral thin film dosage form units was carried out using the paddle dissolution method as described in the European Pharmacopoeia (Council of Europe, 2007). Dissolution jars each contained 900 mL of media maintained at 37°C and paddles were set at 50 rpm. Dissolution media used were hydrochloric acid buffer (pH 1.2) and phosphate buffer solution (pH 6.8) as

described in Ph. Eur. (Council of Europe, 2007). Six replicates were performed with each media. Drug concentrations of metoclopramide were determined by UV spectrophotometry at 275 nm. Measurements were taken every 20 minutes until completion (i.e. at least 80% drug release achieved).

4.2.3.3 Preparation of modified release oral thin films

An oral thin film formulation was developed containing 10 mg metoclopramide hydrochloride (BÜFA; 13C12-B02) loaded within an ion-exchange resin complex (Dowex® 50WX8, 50-100 mesh (i.e. 149-297 μm), prepared as above). A viscous stock mixture was prepared which included 14.0% (w/w) drug-resinate; 18.3% (w/w) pullulan (Cornelius; 1E0712) which was included as the film forming polymer; 2.4% (w/w) polyvinyl polypyrrolidone (Sigma; K11107B1) as a disintegrating agent; 0.1% (w/w) glycerol (Melford Labs Ltd.; 19256) as a plasticiser; 1.1% (w/w) sucralose (Tate & Lyle; XM1D009501) as a sweetener; 1.0% (w/w) lemon 507940T (Firmenich; 1000710486) to give the oral thin film a citrus flavour and aroma; 1.7% (w/w) Sisterna SP70 (Sisterna; 548Z22) as an emulsifying agent; and distilled water. All ingredients were weighed on an analytical balance (A&D; 14214367) and combined using an Ultra-Turrax homogeniser (Janke & Kunkel; 751808) at 8000 rpm. The films were cast on polymer coated paper using a Micrometer Adjustable Film Applicator (Sheen; 1117/250mm) to a thickness of 1.4 mm, and dried in a cabinet drier (Mitchell Dryers Ltd.; G03536010) at 40°C for 20 minutes. Oral thin films were cut to a target weight using a rotary blade.

4.2.3.4 Homogeneity of drug-resin distribution within oral thin films

It was observed that the ion-exchange resin beads were sufficiently distinct from the polymer film background in colour that they could be identified and counted when viewed under a microscope or when scanned or photographed at a sufficiently high resolution. To assess the uniformity of the resinate distribution throughout the oral thin film matrix, a sheet of oral thin film material containing the drug-resin complex was scanned using an EPSON Stylus SX515W scanner at 2400 dpi. Using the GNU Image Manipulation Program (GIMP) (version 2.8.2) a 10 mm square grid was superimposed over the image and the cells numbered. Using an online random number generator (http://www.randomizer.org/), a set of twenty unique random cells was identified for analyses. These samples were cropped from the image and saved individually as jpeg files. Manual counts of drug-resin beads were then performed for each square sample using *ImageJ* (Maryland, USA) image processing software. Partial beads which appeared on the edges of the image were included in the count. Additionally, an 'automated count' method was developed using the software's 'Analyze Particles' function. This automated method was verified and validated against the data from the manual count.

To ensure that the manual count was reasonable and accurate, a single sample was independently counted manually by ten individuals and checked for closeness.

4.2.3.5 Content and mass uniformity of modified release OTF

For assessment of content and mass uniformity, a random sample of 10 oral thin films were selected from a batch of modified release metoclopramide oral thin films. Each film was weighed and dispersed in 100 mL 0.1 M HCl. The samples were stirred by magnetic stirrer at room temperature for 24 hours. Solutions were filtered, diluted and assayed by UV spectrophotometry at 275 nm. Standards were prepared in 0.1 M HCl.

4.2.3.5 Thermogravimetric analysis of modified release OTF

Two samples of metoclopramide hydrochloride oral thin film weighing 11.273 mg and 9.977 mg, were analysed using a TGA/SDTA851^e Mettler Toledo (Switzerland; Serial No. 5125178760) thermogravimetric analyser. Samples were heated from 25-300°C at 10°C min^{-1}. Nitrogen was used as the purge gas at a flow rate of 100 cm^3 min^{-1} (ATP).

4.3 Results

4.3.1 HPLC validation

4.3.1.1 Linearity, range and intermediate precision

The method showed good linearity across the concentration range (see Figure 17) with an R^2 > 0.999 on both days. The relative standard deviations were < 2% at all concentrations on both days (see Table 16).

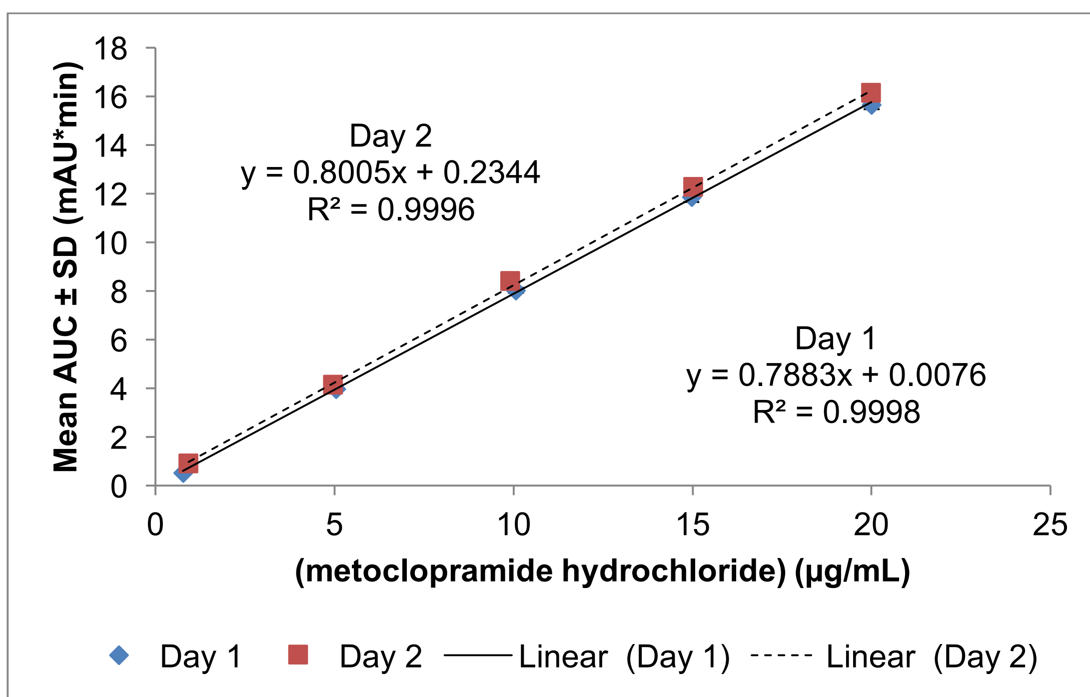


Figure 17. Linearity and intermediate precision. Quantification of metoclopramide hydrochloride using HPLC-UV showed good linearity (R^2 values > 0.999) and was reproducible on consecutive days.

Table 16. Intermediate precision. Relative standard deviations (RSD) were <2% on both days across the concentration range, indicating method precision.

Day 1			Day 2		
Metoclopramide hydrochloride conc. (μg/mL)	**Mean AUC (mAU*min)**	**RSD (%)**	**Metoclopramide hydrochloride conc. (μg/mL)**	**Mean AUC (mAU*min)**	**RSD (%)**
0.774	0.5415	0.97	0.93	0.9114	1.62
5.031	3.9825	1.07	4.96	4.1409	1.88
10.062	8.0378	1.26	9.92	8.3934	1.23
14.964	11.8755	1.72	15.035	12.2776	1.51
19.995	15.6683	1.15	19.995	16.1436	0.80

4.3.1.2 Specificity

An average (SD) metoclopramide recovery of 101.99(0.32)% was obtained from assay of the adulterated solution. The excipients contained within the placebo film did not interfere with the metoclopramide peak and no additional peaks were observed at 275 nm (see Figure 18).

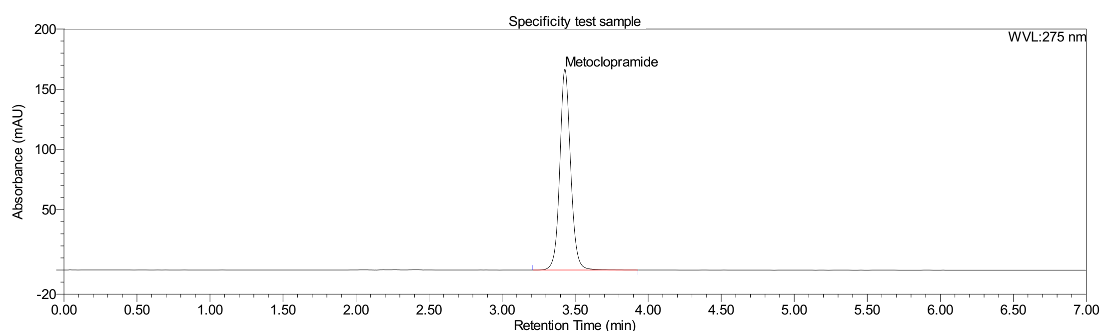


Figure 18. Chromatogram from excipient adulterated metoclopramide sample.

4.3.1.3 Limits of detection (DL) and quantification (QL)

A limit of detection (DL) of approximately 80 ng/mL was estimated based on the signal-to-noise ratios observed at low metoclopramide concentrations (see Table 17). This allowed for a 2:1 ratio of signal to noise. A quantification limit (QL) of approximately 200 ng/mL was also predicted which was estimated to allow for a 10:1 signal strength compared to the baseline noise.

Table 17. Signal-to-noise ratios. The area under the curve of the metoclopramide hydrochloride peak was compared to that of a typical peak from the baseline noise and a ratio was calculated. A 2:1 ratio was used to estimate the limit of detection and a 10:1 ratio was used for the limit of quantification.

(metoclopramide hydrochloride) (ng/mL)	AUC	Noise	Signal:noise ratio
39.5	0.0028	0.0057	0.49
79	0.0146	0.0074	1.97
158	0.0468	0.0064	7.31
316	0.1228	0.0076	16.16
632	0.2727	0.0052	52.44
790	0.3410	0.0080	42.63

The method also showed good linearity across this low concentration range (39.5-790 ng/mL, R^2 = 0.9993).

4.3.2 Immediate release oral thin film characterisation

4.3.2.1 Content and mass uniformity

The batch of oral thin films conformed to British Pharmacopoeia requirements as no single strip deviated from the target metoclopramide

content of 10 mg by more than 10% (see Table 18). Based on the requirement for tablets weighing less than 80 mg, the batch also conformed in terms of mass uniformity as the films were all within 10% of the mean weight (i.e. 33.5-41.0 mg).

Table 18. Mass and content uniformity (n = 10).

	Weight (mg)	Drug content (mg)
Mean	37.23	9.84
SD	1.91	0.51
Median	37.05	9.79
Min	34.8	9.27
Max	40.5	10.77

4.3.2.2 Dissolution

Under acidic pH conditions (pH 1.2 and pH 4.6), more than 80% release was achieved in less than 10 minutes. At pH 6.8, more than 80% release was achieved by 20 minutes. As a basic molecule, metoclopramide (pKa 9.27) is predominantly unionised at high pH, which explains the retarded release profile at pH 6.8. Figure 19 shows the release profiles across the pH range.

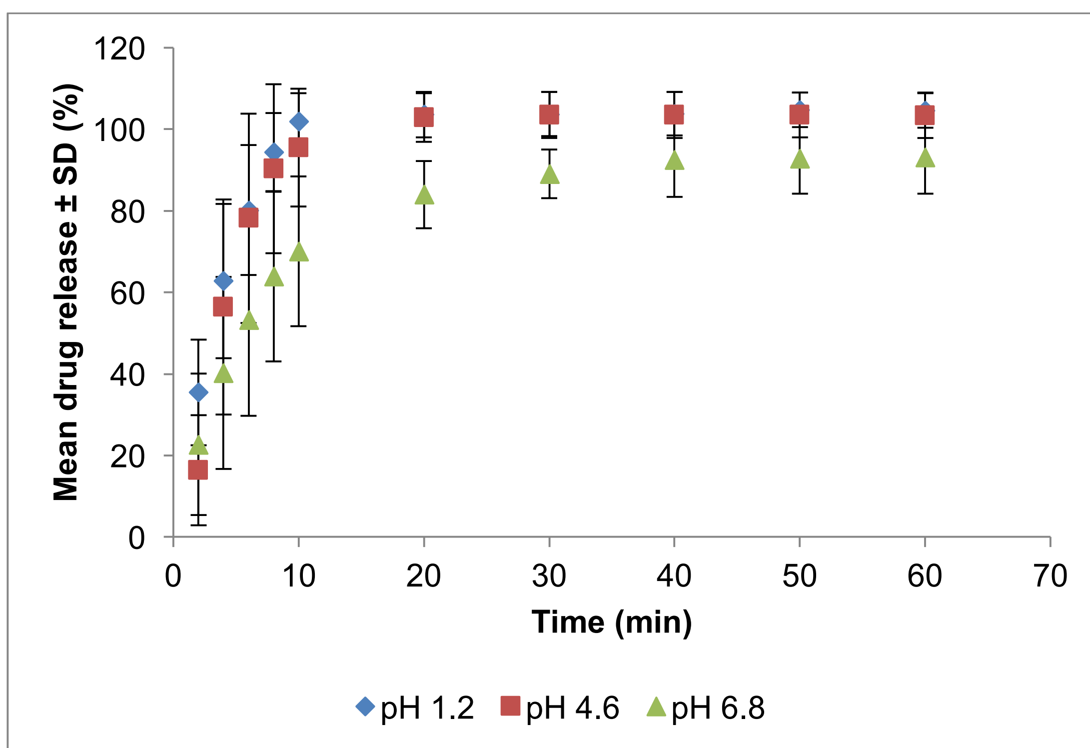


Figure 19. Drug release profiles for immediate release metoclopramide oral thin films. Dissolution was assessed using the Type II paddle method across physiological pH range. As a basic molecule, metoclopramide (pKa 9.27) is predominantly unionised at high pH and this is evidenced by a retarded release profile at pH 6.8.

4.3.2.3 Disintegration

The oral thin films disintegrated at 26, 21, 18, 23 and 20 seconds respectively. The mean (SD) time to disintegration was therefore 21.6 (3.05) seconds.

4.3.2.4 TGA

A gradual mean (SD) loss in mass of 7.61(1.68)% was observed across the first 180°C which may be attributed to evaporation of unbound water and volatiles e.g. flavouring agents. A sharp loss of mass was then observed with a first derivative peak measured at 193°C. This could be the result of thermal degradation of the film forming polymer (HPMC) with subsequent release of

bound water. HPMC features a glass transitional temperature of 170-180°C, browns at 190-200°C and chars at 225-230°C (Rowe *et al.*, 2009).

4.3.2.5 Raman intensity map

A peak was observed at 1594.79 cm^{-1} which was unique to metoclopramide hydrochloride and could be attributed to the amide group (see Figure 20). Using the peak height at this band, an intensity map was produced (see Figure 21).

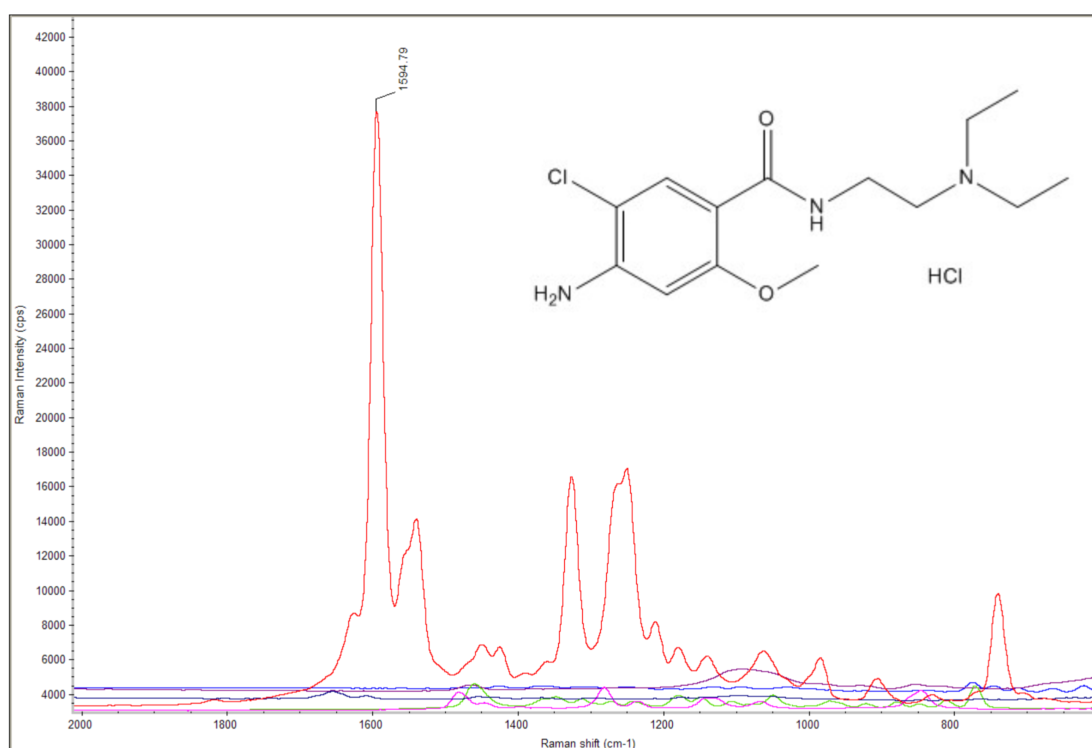


Figure 20. Raman spectra of metoclopramide (red) and other excipients, indicating distinct API peak. The chemical structure of metoclopramide hydrochloride is shown, and the peak of interest (attributable to the amide functional group) is highlighted.

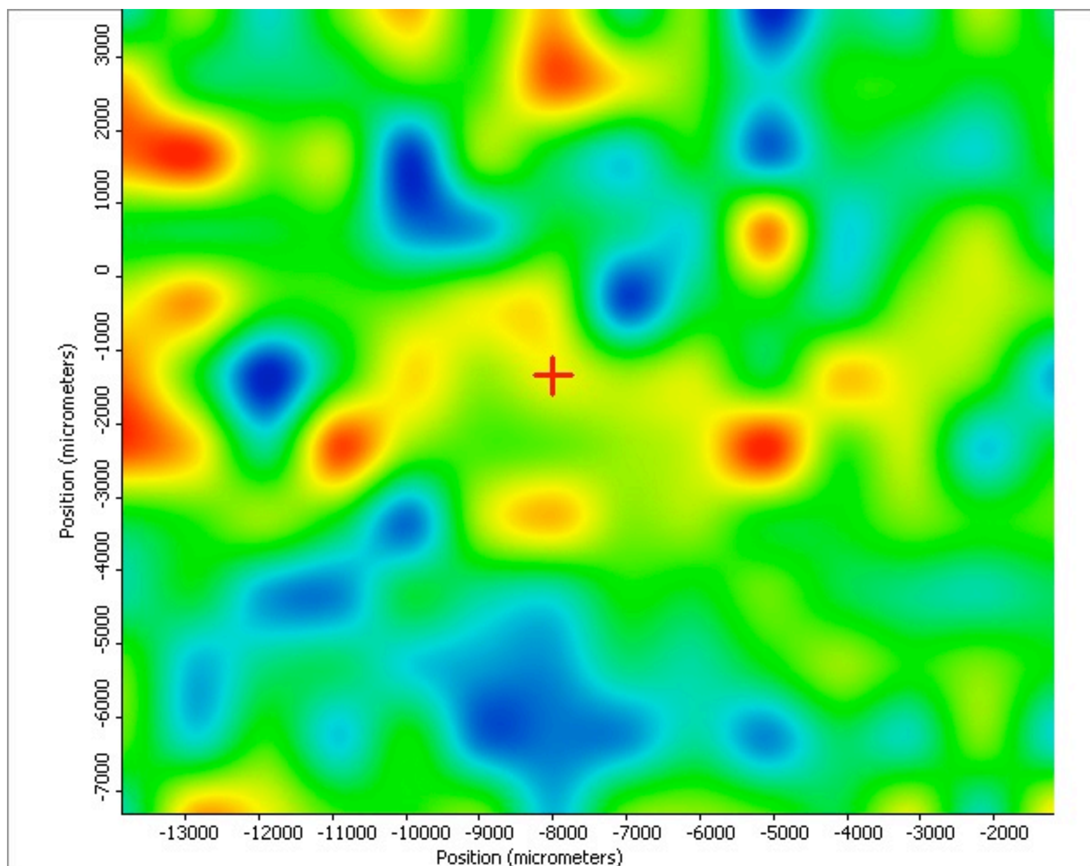


Figure 21. Raman intensity map. The map confirms uniform distribution of metoclopramide hydrochloride across the surface of an immediate release oral thin film. Blue indicates absence of drug whilst green through yellow to red show increasing concentrations.

4.3.2.6 Accelerated stability study

No statistically significant changes in metoclopramide hydrochloride content were observed after 3 months or after 6 months (P-values 0.188 and 0.331 respectively) and differences were less than 5% as accepted by the ICH guidelines. Although no change in mass was observed after 3 months (P-value = 0.935), a modest decrease was observed by 6 months (P-value = 0.03). However, this loss in mass was less than 5% from the original mean at t = 0 so the batch conformed to ICH requirements.

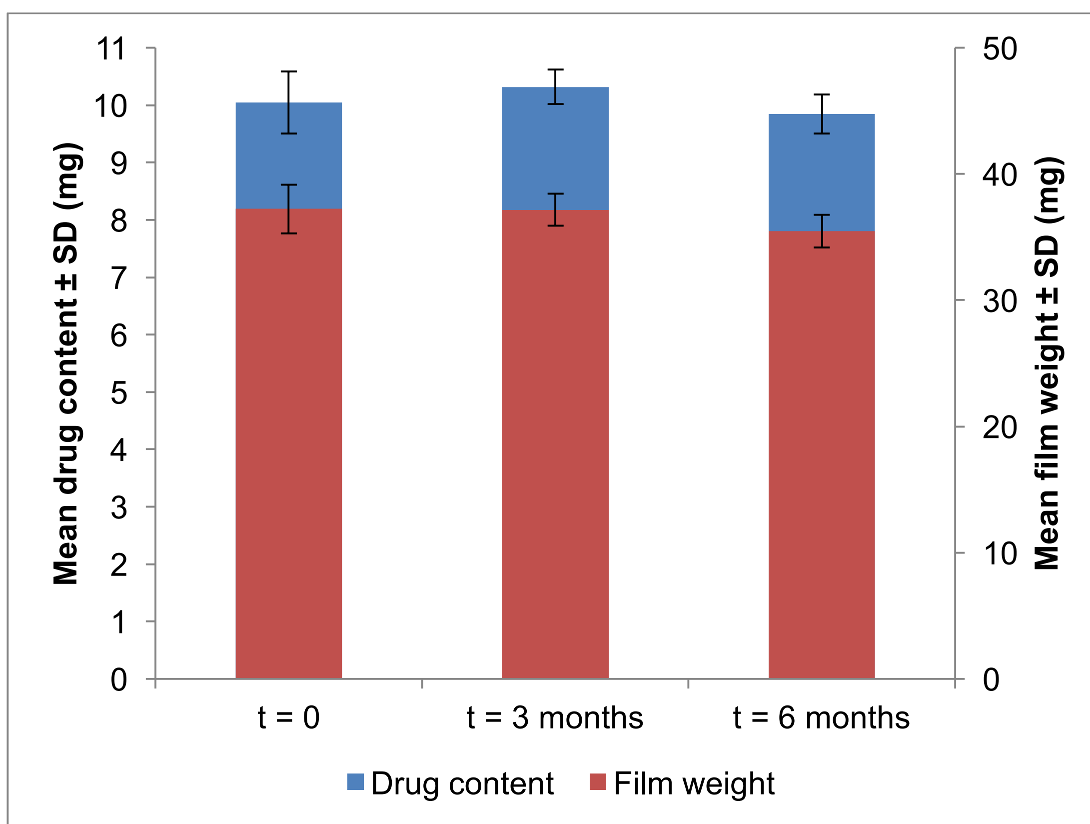


Figure 22. Accelerated thermal stability study. Oral thin films were stored at 37°C for a period of 6 months. Changes in mass and API content were assessed at 3- and 6-month time points (n = 10). No significant changes were found.

4.3.2.7 *In vivo* pharmacokinetic study of immediate release metoclopramide hydrochloride oral thin films

4.3.2.7.1 Extraction and assay method validation

The high performance liquid chromatography method for assay of metoclopramide in rat serum, using diazepam as an internal standard, yielded a linear calibration curve (see Figure 23) across the concentration range 0.116-3.016 µg/mL.

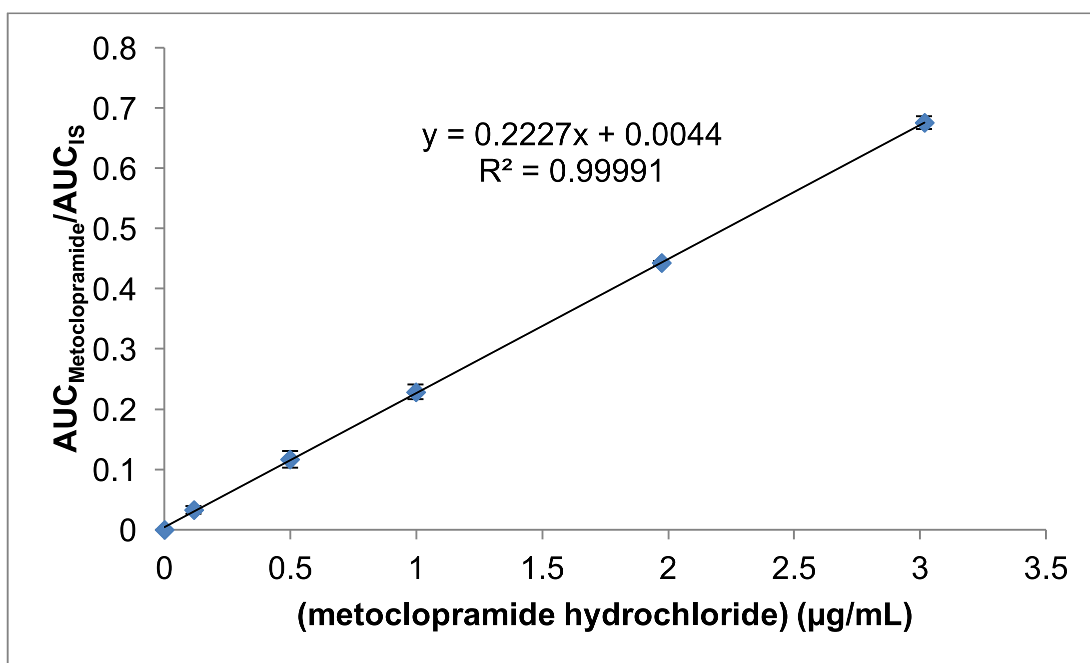


Figure 23. Calibration standard curve. Area of metoclopramide peak to internal standard (n = 3, mean ± SD). The method of extraction of metoclopramide hydrochloride from rat serum showed excellent linearity across the concentration range.

4.3.2.7.2 Pharmacokinetic results

Figure 24 shows the serum concentration-time profile following administration of metoclopramide hydrochloride orodispersible thin films to rats. A typical pattern of immediate drug release can be observed showing rapid absorption of metoclopramide hydrochloride. A maximum serum concentration (C_{max}) of 2.16(0.83) µg/mL was reached by 1 hour (T_{max}) following a 5 mg metoclopramide hydrochloride dose to rats. These results closely compared to results by Tam *et al.* (1981) who reported a T_{max} of 30-60 minutes and a comparable C_{max} of approximately 2 µg/mL following the oral administration of a 15 mg/kg dose of metoclopramide hydrochloride to rats as an oral solution.

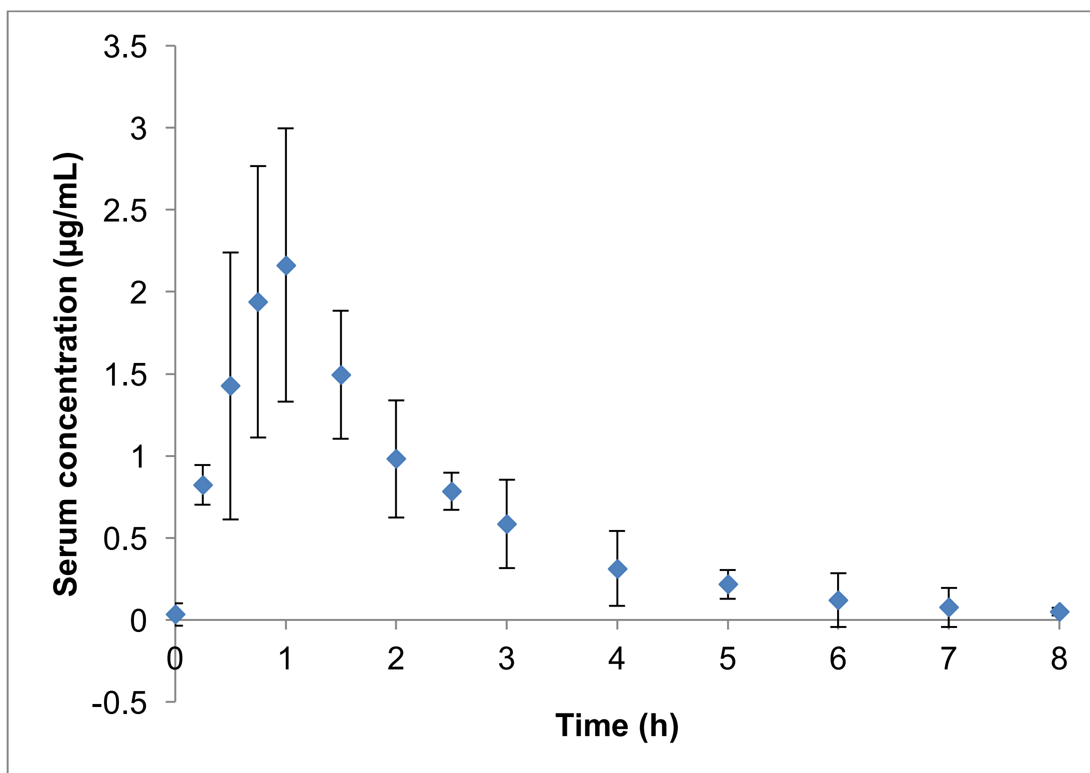


Figure 24. Serum concentrations of metoclopramide hydrochloride after oral administration of metoclopramide hydrochloride oral thin films to rats (n = 3, mean ± SD). The concentration-time profile shows a typical pattern of absorption and elimination as expected from an immediate release oral dosage form.

A linear elimination phase is shown in Figure 25 and calculated pharmacokinetic parameters are summarised in Table 19.

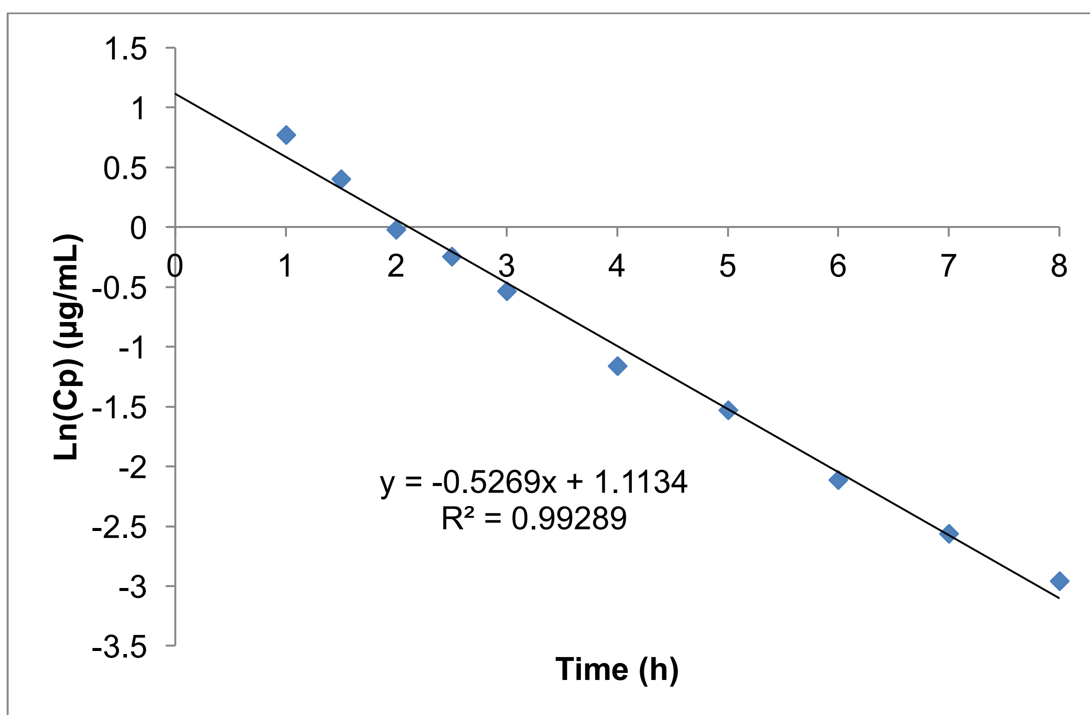


Figure 25. Elimination phase plotted on natural logarithmic scale. A linear relationship is observed ($R^2 > 0.99$). The slope of the line provides the elimination rate constant k_e.

Table 19. Pharmacokinetic parameters.

Parameter		**Unit**	**Equation**
$AUC_{0\text{-last}}$	4.69	mg.h/L	Linear trapezoidal method
k_e	0.53	h^{-1}	k_e = -slope
$AUC_{0\text{-inf}}$	4.79	mg.h/L	$AUC_{t\text{-inf}} = Cp_{last}/k_e$ $AUC_{0\text{-inf}} = AUC_{0\text{-last}} + AUC_{t\text{-inf}}$
CL	1.04	L/h	$CL = Dose/AUC_{0\text{-inf}}$
V_d	1.98	L	$V_d = CL/k_e$
$t_{½}$	1.32	h	$t_{½} = \ln(2)/k_e$
F	1.00		$F = V_d.k_e.AUC_{0\text{-inf}}/dose$

4.3.3 Modified release oral thin films

An oral thin film which dissolved rapidly to release a metoclopramide-loaded resinate, which then released the active pharmaceutical ingredient over a prolonged period, was successfully formulated. The oral thin film was flexible and non-brittle, disintegrated rapidly upon moistening, and had a sweet, citrus aroma.

4.3.3.1 Resinate drug loading and release

The method for assay of metoclopramide hydrochloride by UV spectrophotometry at 275 nm showed good linearity across concentration range 6-29 µg/mL (R^2 = 0.9999) which represented approximately 54-261% of the test concentrations. With a total exchange capacity of 1.7 mEq/mL (2.1 mEq/g) for Dowex® 50WX8, 10 g of resin could be expected to trap approximately 7.1 g of metoclopramide hydrochloride (MW 336.3). Analysis of the filtrate revealed 1.33% recovered metoclopramide, therefore an entrapment efficiency of 70% was achieved with 98.67% drug loading by the ion exchange resin. The resulting dried drug-resin complex had a metoclopramide content of 33.15% (w/w). 50% release from the ion exchange resin was reached at 1.2 and 1.7 hours at pH 1.2 and 6.8 respectively; 80% drug release was achieved at 3.0 and 4.7 hours (see Figure 26).

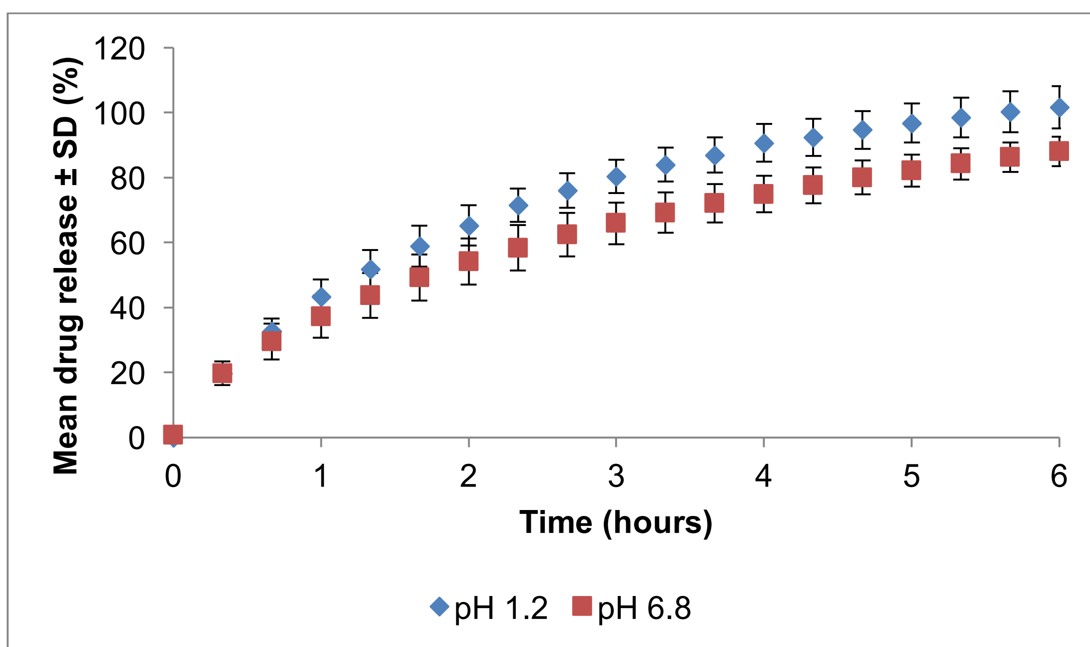


Figure 26. Drug release from metoclopramide resinate. Drug release from the metoclopramide-ion exchange resin complex was assessed at pH 1.2 (hydrochloric acid buffer) and pH 6.8 (phosphate buffer) using the Type II paddle method (n = 6).

Using Dowex® 50WX8, 200-400 mesh (i.e. 37-74 μm), a similar drug loading of 98.72% was achieved. The resulting drug resinate had a metoclopramide content of 24.8% (w/w). Assessment of metoclopramide release was performed at pH 1.2 and results are presented in Figure 27. A sustained but earlier release was observed with the higher mesh size, with 80% release attained after only 1 hour.

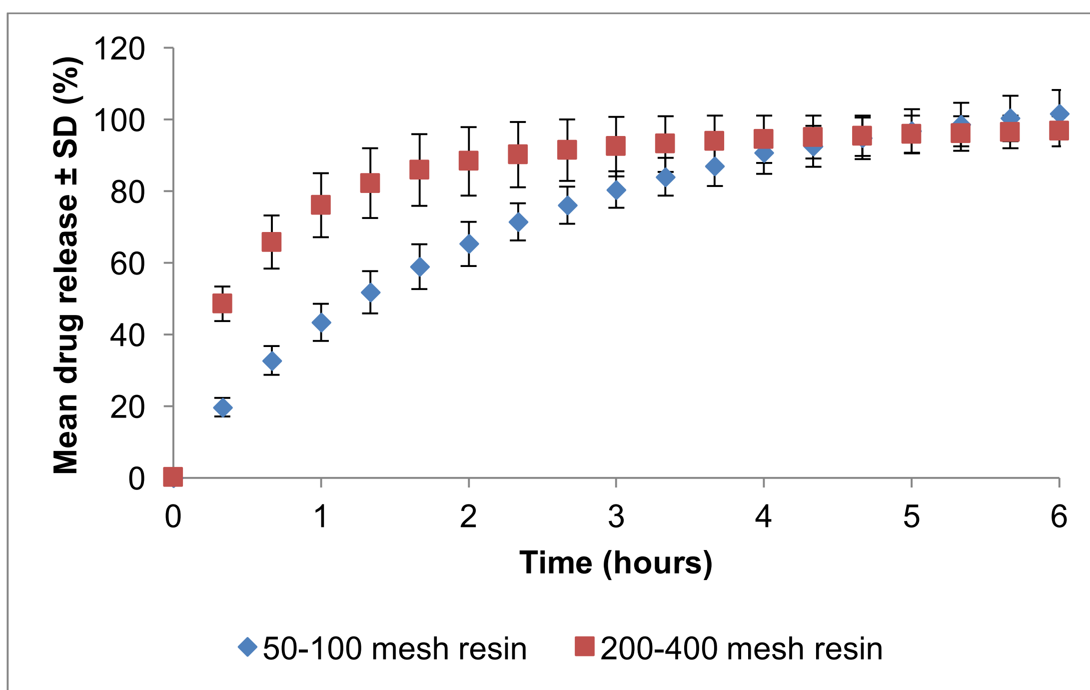


Figure 27. Effect of mesh size on drug release. Release of metoclopramide hydrochloride from an ion exchange resin complex at pH 1.2 (hydrochloric acid buffer) was assessed, comparing two different particle sizes of the same resin (Dowex® 50WX8). A greater ion exchange resin mesh size (smaller particle size) produced a faster rate of drug release.

4.3.3.2 Dissolution from oral thin films

Drug release profiles obtained from dissolution of oral thin films revealed 50% drug release at 1.6 and 2.5 hours at pH 1.2 and 6.8 respectively; 80% release was achieved at 3.9 and 6.4 hours (see Figure 28). An observed f2 value, as determined by the Bootstrap method, of 46.3 implies that the mean dissolution profiles for the two pH values are dissimilar.

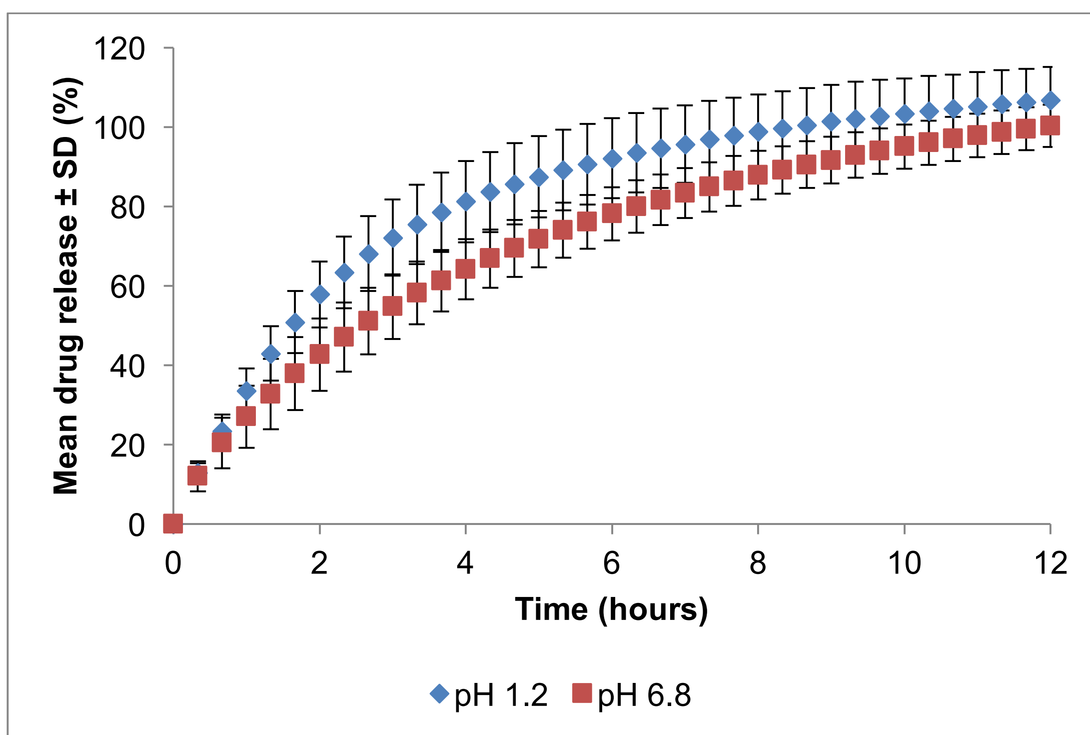


Figure 28. Dissolution results from modified release oral thin films at pH 1.2 and 6.8. Drug release from modified release metoclopramide hydrochloride 10 mg oral thin films was assessed using the Type II paddle method (n = 6) in hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 6.8).

4.3.3.3 Uniformity of resinate dispersion

The mean (SD) number of drug-resin beads contained within the images (as determined by manual counting) was 276(14); range 255-299. The automated method produced a consistently higher result with a mean (SD) of 299(21); range 264-334. However, the majority of automated results were within 10% of their corresponding manual values. In terms of uniformity of distribution, count variability between samples via the manual and automated methods did not exceed 8.5 and 11.6% of the means respectively. Inter-individual variability by independent manual counts did not differ by more than 5% from the mean.

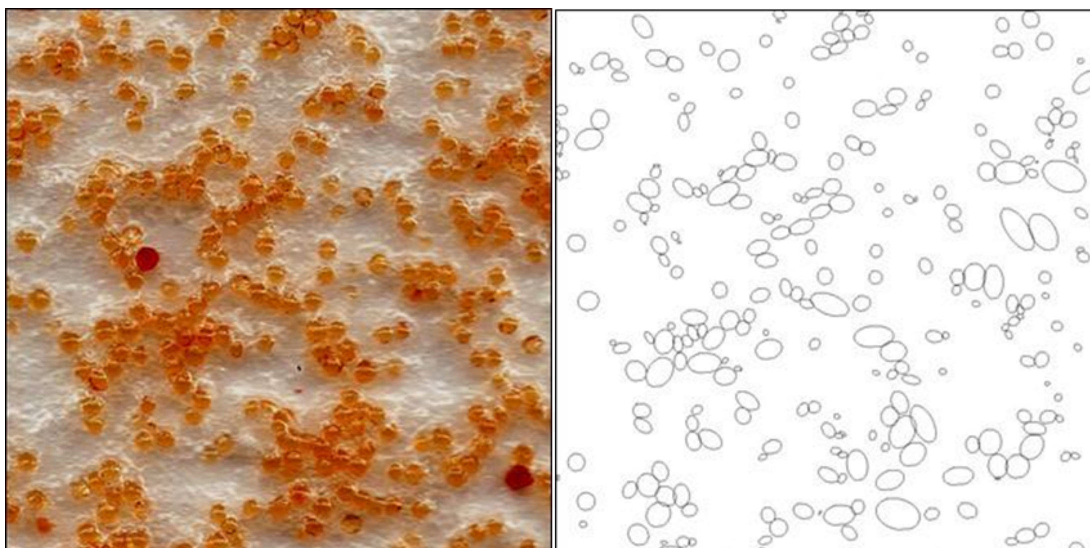


Figure 29. Assessment of resinate dispersion homogeneity. Sample scanned image (left) of drug resinate within polymeric film (10 mm^2 cropped image) *vs*. processed image (right) for automated particle count.

4.3.3.4 Content and mass uniformity

Results of the batch uniformity test are presented in Table 20. The batch conformed to pharmacopoeia requirements as all units were within 90-110% of the stated metoclopramide content (10 mg). The average film weight was more than 80 mg but less than 250 mg, therefore the batch also conforms to the requirement that no individual unit deviates by more than 7.5% of the average mass (i.e. 80.3-93.3 mg).

Table 20. Metoclopramide content and film mass uniformity (n = 10).

	Weight (mg)	**Drug content (mg)**
Mean	86.8	10.4
SD	4.2	0.3
Median	85.2	10.5
Min	81.6	9.9
Max	93.1	10.8

4.3.3.5 Thermogravimetric analysis

A gradual 8.9-10.3% loss in mass was observed across the first 140°C (example curve shown in Figure 30). A significant decrease occurred with the first derivative peak measured at 184°C (onset-endset 173-196°C).

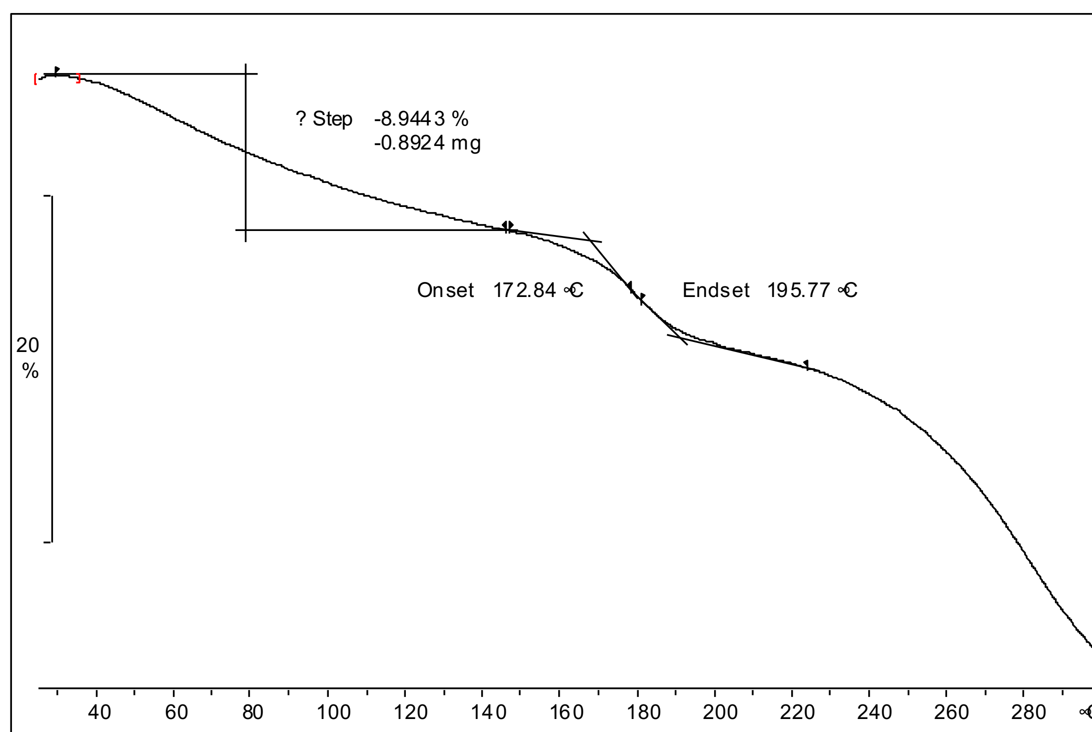


Figure 30. Thermogravimetric analysis of modified release metoclopramide hydrochloride OTF example. Samples were heated at 10°C per minute. Change in mass as a percentage of original is shown.

4.4 Discussion

An orodispersible thin film which contained approximately 10 mg of metoclopramide hydrochloride was successfully formulated. Rapid release of the active ingredient was achieved with more than 80% released from the immediate release oral thin film within 10-20 mins across a pH 1.2-6.8 range. The rapid drug release which can be achieved with immediate release oral thin films (complete drug release within 10 minutes at gastric pH) is

analogous with that of a liquid formulation and is of benefit to patients suffering from nausea and vomiting who require a fast onset of action to manage their symptoms.

Although no standards exist for orodispersible thin films with regards to disintegration, the British Pharmacopoeia states that for orodispersible tablet (ODT) disintegration, units must disintegrate within 3 minutes (British Pharmacopoeia Commission, 2015c). *In vitro* disintegration times for oral thin films using conventional disintegration methodology have been reported at around 39-47 seconds which is comparable to a commercially available ODT preparation (Liew *et al.*, 2012; Yan *et al.*, 2010). Preis *et al*. (2014) reported disintegration times of 17.3-21.1 seconds using their modified method, and our results employing this method were similar (21.6 ± 3.05 seconds).

In 2013, the European Medicines Agency's Committee on Medicinal Products for Human Use (CHMP) made recommendations, changing the use of metoclopramide for licenced indications (European Medicines Agency's Committee on Medicinal Products for Human Use (CHMP), 2013). These recommendations were based on evidence which demonstrated that the risk of well-documented neurological side effects associated with long term use of metoclopramide outweighed the benefits. For adults, the normal dose of 10 mg up to three times a day continues to be safe and effective for short-term use (no longer than 5 days), with caution advised in patients with known neurological problems. Use of metoclopramide in palliative care is not a licensed indication, and therefore its off-label use by specialists in palliative

care (an unapproved patient group) is not covered by the CHMP review. Palliative care often requires the use of medicines out with their standard licensed indications since few medicines are specifically studied and licensed for use in terminally ill patients, in whom the balance of risks and benefits may differ from other patient groups. Therefore the off-label use of metoclopramide in palliative medicine can be justified and continues with due care and careful monitoring.

Oral thin films are easy to swallow as they dissolve rapidly in saliva without a requirement to chew or drink water. This makes them particularly appropriate for patients with swallowing difficulties, such as the elderly or in end of life, or cancer. Simple flavour and sweetener combinations can be included for effective taste masking of bitter active ingredients, and this, along with their rapid rate of dissolve, gives oral thin films good palatability and acceptability as a medicine. They are ideally suited to palliative populations as they require very little available water to disperse, making them useful in xerostomia which can affect as much as 78-82% of patients in advanced cancer (Wilberg *et al.*, 2012; Davies *et al.*, 2010).

Metoclopramide has also been used off-label in neonates in the treatment of gastro-oesophageal reflux since the 1980s (Kearns *et al.*, 1998). Although not licensed for use as a prokinetic in neonates, the BNF for Children provides a dose of 100 μg/kg body weight for metoclopramide every 6-8 hours for neonates, either oral or intravenous (Paediatric Formulary Committee, 2011). However, commercially manufactured formulations of

metoclopramide currently available in the UK are not age-appropriate for neonates. Oral liquids and solutions for injection are mostly produced in a 5 mg/mL concentration. For administration of neonatal doses, this would require accurate measurement of very small volumes of liquid, or further dilution, both of which increase the risk of dosing error. Rosemont Pharmaceuticals Ltd. manufacture a 1 mg/mL oral solution which is licensed as a second line therapy in children (aged 1-18 years) for the prevention of delayed chemotherapy induced nausea and vomiting, however this product is formulated using methyl (5mg/5mL) and propyl (1mg/5ml) hydroxybenzoates, as well as propylene glycol, all of which are known to have toxic effects in paediatric populations (Rosemont Pharmaceuticals Limited, 2012). Since oral thin films are formulated as solid single-dose units, they do not require the addition of excipients such as preservatives or alcohols, unlike many liquid formulations.

An oral thin film capable of sustained release of metoclopramide hydrochloride from within an ion exchange resin complex, was successfully formulated. The film had good mechanical properties and the drug release profiles obtained *in vitro* were reproducible. Ion exchange resin technology was easily incorporated within a polymer film matrix to control drug release. Sustained release of metoclopramide was achieved with more than 80% release reached within 4-6 hours at physiological pH and complete release over 12 hours. Some side effects associated with metoclopramide are known to be related to high plasma concentrations (observed with standard 8 hour

dosing of immediate release metoclopramide) and so a sustained release formulation, capable of delivering the daily dose gradually and avoiding large peaks in blood concentration, could be expected to avoid some of these (Rao and Camilleri, 2010). As shown in Figure 27, a change in the mesh size of the ion exchange resin affected the release rate, with a higher mesh size producing a controlled but earlier release of metoclopramide. A similar effect has been observed in other studies of ion exchange resin drug complexes, where a faster release has been observed with smaller particle size fractions of resin (Sriwongjanya and Bodmeier, 1998). This is most likely due to a surface area effect on exchange kinetics: smaller particles (greater mesh size) have a larger surface area but a reduced internal volume for ions to diffuse resulting in a more rapid diffusion from within the resin (The Dow Chemical Company, 2006). A resin with a smaller particle size reaches equilibrium more rapidly, demonstrating increased drug loading and also faster drug release (Jeong and Park, 2008). There are several other factors that affect the properties of drug loading and release from ion exchange resins. For example, Jeong and Park (2008) showed that multi-batch drug loading produced a greater drug loading compared to single batch, increasing the ratio of drug to resin, whilst the degree of cross-linking within the ion exchange resin had a greater effect than particle size in respect to moisture content. In contrast, the effect of particle size on the release profile was not seen with comparatively small variations in particle size, whilst variations in affinity for the ion exchange resin over different ions had a greater effect (Torrado and Valiente, 2004). This demonstrates how the

release kinetics can be easily manipulated depending on clinical requirements. Our results demonstrate that oral thin films are a suitable platform for the oral administration of sustained release drug delivery technologies. Ion exchange resins proved a simple and effective option for controlling drug release, with predictable and reproducible release profiles, and these can be easily formulated within an oral thin film. They are insoluble in all solvents, regardless of pH, and are not absorbed by the body, making them non-toxic and safe for oral administration (Hughes, 2004). A wide variety of different cation and anion exchange resins are commercially available, including some with pharmacopoeial monographs e.g. Amberlite™ IRP69 (sodium polystyrene sulfonate) (British Pharmacopoeia Commission, 2014b). These provide the formulator with a range of release rate options, which may be of value for different APIs. Another formulation option which can be easily undertaken is to include an amount of free drug in addition to the resinate complex within the oral thin film, allowing for a rapid, immediate release of an active ingredient followed by a continued, sustained release. An example of this type of combination oral thin film was developed, the details of which, along with discussion of many other immediate/sustained release combinations, will be covered in Chapter 7. Following successes in producing a sustained release oral thin film using metoclopramide in combination with ion exchange resin technology, a modified release morphine sulphate oral thin film formulation was developed with both paediatric and palliative applications, and will be detailed in Chapter 5.

Chapter 5 - Formulation and characterisation of morphine sulphate thin films for immediate and sustained oral delivery

5.1 Introduction

Morphine is an opioid analgesic, first discovered in the early 1800s by a German pharmacist's assistant named Friedrich Sertürner, and is still widely recognised as the gold standard treatment for severe pain worldwide (Booth, 1996). Morphine salts such as morphine sulphate are well absorbed from the gut following oral administration although significant first pass metabolism occurs (Hoskin and Hanks, 1990). It is approximately 35% protein bound and has a short half-life of 2 hours. Morphine sulphate has a pKa of 7.9 and a water solubility at 25°C of 64.5 mg/mL (Sweetman, 2011).

5.1.1 Neonatal abstinence syndrome

Babies born to drug-misusing mothers have been associated with a higher risk of prematurity, stunted intrauterine growth, and withdrawal symptoms after birth (Dryden *et al.*, 2009). In 2007, a U.S. study estimated that 5.2% of women used illicit drugs during their pregnancy. Of the babies born to these women, up to 90% experienced withdrawal to the illicit drug. This withdrawal is known as neonatal abstinence syndrome (NAS) and requires pharmacological treatment in approximately 70% of cases (D'Apolito, 2009). As well as irritability, sleep disturbance, sweating and fever, symptoms of

NAS can include problems with weight gain, tremors, seizures, and is also associated with an increased risk of sudden infant death syndrome (SIDS) (Liu *et al.*, 2011). Foetal exposure to opiates during pregnancy is thought to increase the risk of SIDS by as much as 10-fold. However, many other variables including life-style factors, prematurity, low birth weight and maternal abuse of more than one drug during pregnancy, may also contribute to this risk (Kandall and Gaines, 1991).

Pharmacological treatment of withdrawal symptoms depends on the drug of misuse (Hudak *et al.*, 2012). For example, barbiturate withdrawal is treated with phenobarbital, whereas withdrawal resulting from intrauterine benzodiazepine exposure is treated with diazepam. Whilst cocaine and amphetamine misuse can produce sympathomimetic effects in newborns, there is no evidence of a withdrawal syndrome. There is no data to support pharmacological treatment and instead management is based on prolonged nursing, comforting techniques and swaddling (Mactier, 2013). Previous pharmacological treatments of NAS due to opiate misuse have included paregoric (tincture of opium), benzodiazepines and antipsychotics such as chlorpromazine, but growing evidence in this field has revealed opioids such as morphine, buprenorphine and methadone, or barbiturates such as phenobarbital to be the first-line treatments of choice. Morphine is the most commonly used drug for managing NAS and aims at slowly weaning the child off the drug over weeks or months (Liu *et al.*, 2011). However, there are several drawbacks to using commercially available opioids. One significant

problem is that currently available marketed liquid preparations in the UK are formulated in high concentrations of alcohol (e.g. Oramorph® oral solution contains 10% v/v of 96% ethanol) and contain benzoic acid derived preservatives. As described in Chapter 3, ethanol and parabens have been associated with neurological, allergenic and other adverse effects in infants. Additionally, the use of currently available marketed liquid morphine preparations requires accurate measurement of small volumes of liquids (or further manipulation by dilution) for administration of neonatal doses, which presents an opportunity for dosing error e.g. a 60 mcg/kg dose equates to 30 mcL of Oramorph oral solution. As with the majority of neonatal medicines, there are no oral liquid morphine preparations licensed for use in the pharmacological treatment of neonatal abstinence syndrome and so clinicians must rely on the off-label use of available preparations or the unlicensed use of an extemporaneous or 'specials' manufactured product.

Sleep disturbance has been found to correlate with the severity of the withdrawal syndrome. Babies with NAS who require pharmacological intervention show a reduction in 'quiet' sleep and sleep efficiency, with an increase in 'indeterminate' sleep i.e. sleep which can be classed as neither active nor quiet. The degree of sleep disturbance may therefore be a good indicator of the need for pharmacological treatment (O'Brien and Jeffery, 2002). Philip Lipsitz developed a diagnostic tool which has been used in an attempt to standardise treatment of NAS. A score ranging from 1 to 3 is assigned against the presence or nature of tremors, irritability, reflexes,

stools, muscle tone, skin abrasions, respiratory rate, repetitive sneezing/yawning, vomiting or fever (Lipsitz, 1975). The American Academy of Paediatrics recommends the Lipsitz tool as a simple method with high sensitivity for assessing the severity of the withdrawal syndrome (American Academy of Pediatrics, 1998). Treatment is then started if a threshold score is reached. The NAS guidelines for NHS Greater Glasgow and Clyde state that treatment should be commenced where the Lipsitz score is >4 on two occasions, twelve hours apart despite attempts to relieve these symptoms by nursing the child (Mactier, 2009). These guidelines recommend that initial pharmacological treatment should consist of morphine solution at a dosage of 60 μg/kg every four hours titrated upwards gradually according to response. Infants are then weaned by 10 μg/kg morphine per dose each day with an aim to withdraw treatment entirely by the 10th day of life. Data collected in 437 babies born to drug-misusing mothers receiving methadone substitution therapy over a 3 year period at the Princess Royal Maternity in Glasgow, revealed a mean birth weight of 2.71 kg with an average gestational age at birth of 37.8 weeks. Pharmacological treatment was required in 45.5% of babies who developed symptoms of NAS and was started according to the NHS Greater Glasgow and Clyde guideline criteria described above on the third day of life on average, and lasted a median of 11 days. The prescribed maternal dose of methadone was found to significantly correlate with the infant likelihood of requiring pharmacological treatment for NAS (Dryden *et al.*, 2009).

5.1.2 Palliative care

It is recognised that oral morphine is the 'gold standard' treatment for severe pain. It continues to be recommended by national health organisations and the World Health Organisation as the first line treatment choice for severe pain in advanced cancer in adults (Scottish Intercollegiate Guidelines Network, 2008) and for children with persist moderate to severe pain due to medical illness (World Health Organization, 2012).

5.1.2.1 Low dose morphine sulphate: dyspnoea

Breathlessness (dyspnoea) is a common and often distressing symptom of many respiratory and cardiac diseases, as well as many malignancies and neuromuscular disorders. The underlying mechanism which causes dyspnoea is complex and, as with pain, is likely to consist of both sensory and non-sensory influences (Nishino, 2011). Factors which may contribute to the sensations of breathlessness include a rise in breathing effort, irritation of thoracic receptors, a reduction in oxygen partial pressure (pO_2) or rise in arterial carbon dioxide partial pressure ($paCO_2$), or the manifestation of symptoms of fear or anxiety (Clemens and Klaschik, 2007). Chemoreceptors are especially sensitive to changes in $paCO_2$, and also to pO_2 and pH. These chemoreceptors communicate with the respiration centre in the medulla oblongata to regulate breathing. Opioids are able to increase the tolerance of peripheral chemoreceptors to increases in $paCO_2$, and thereby improve symptoms of dyspnoea (Clemens and Klaschik, 2007).

As well as its application as an analgesic in moderate- to severe pain, morphine is also commonly used in the treatment of breathlessness as part of palliative care with around half of general cancer sufferers experiencing dyspnoea as a symptom (Dudgeon *et al.*, 2001). In terminally ill patients, there are many factors which influence the manifestation of breathlessness. Many existing cardiac and respiratory diseases can result in dyspnoea e.g. chronic obstructive pulmonary disease, congestive heart failure, pulmonary embolism. Other conditions can also exacerbate symptoms of breathlessness such as anaemia, infection and anxiety. The latter of these is particularly problematic since anxiety is both a cause and a symptom of breathlessness. This vicious cycle can be broken through the use of anxiolytics such as low dose benzodiazepines (Davis, 1997). Up to 70% of cancer patients in the final six weeks of life suffer from dyspnoea which accompanied with symptoms of anxiety and fear can be particularly distressing and reduce quality of life significantly (Reuben and Mor, 1986).

There is some ethical controversy over the use of morphine in terminal cancer patients stemming from common misconceptions regarding tolerance with morphine use, and also since there is no significant reduction in mortality with morphine use. Additionally, the use of opioids is considered controversial amongst patients of some ethnic backgrounds e.g. Chinese, which may originate from the Opium Wars of the 19th century (Hu *et al.*, 2004). It is suggested that opioids reduce the central perception of dyspnoea and also reduce anxiety to relieve breathlessness. Mechanisms of action also

include an increase in cardiovascular function and a reduction in oxygen consumption associated with opioid use. There is evidence that oral and parenteral opioids are useful in dyspnoea whereas nebulised opioids do not show a significant effect (Jennings *et al.*, 2001). There is little evidence to support the use of opioids in palliative care to improve exercise tolerance. Non-pharmacological measures can also help relieve symptoms of breathlessness (Jennings *et al.*, 2001).

Opioids can be effective at relieving breathlessness in end of life. Current palliative care guidelines produced by NHS Greater Glasgow and Clyde recommend the use of opioids in the management of dyspnoea. For opioid naïve adult patients, a starting dose of 2.5mg morphine every four to six hours is recommended. This can be titrated upwards slowly if necessary. For frail or elderly patients, or those with renal impairment, a lower initial dose of 1-2mg every six to eight hours is recommended (NHS, 2010).

5.1.2.2 Chronic heart failure

Histories of myocardial infarction, coronary heart disease or long-lasting hypertension are the main causes of chronic heart failure (CHF). Palliative care is becoming more prevalent and important in the management of CHF. Of the symptoms of CHF, fatigue and breathlessness can be considered the most troubling and affect up to 90% of patients already receiving optimal drug treatment for heart failure. Evidence for the use of opioids for breathlessness in chronic heart failure is conflicting. One small scale study (n = 10) concluded that low-dose morphine given four times daily provided significant

relief of dyspnoea, with four patients continuing to take the treatment 1 year after the study due to this benefit (Johnson *et al.*, 2002). However, a second study of the same treatment in 35 patients concluded no benefit over placebo (Oxberry *et al.*, 2011). Despite this, the off-label use of low dose opioids for the treatment of dyspnoea in CHF is recommended within national guidelines as part of palliative treatment (SIGN, 2007). Opioid doses equivalent to 2.5-5mg morphine four times daily (titrated as per response) are suggested as appropriate for breathlessness in CHF (Hochgerner *et al.*, 2009). Alternatively, 10mg morphine administered once daily as a modified release preparation and increased weekly as per response has also been suggested in CHF (Currow *et al.*, 2011).

Chronic heart failure patients are often limited by exertional breathlessness. There is evidence that dihydrocodeine, which is often used in the palliative treatment of breathless in chronic obstructive pulmonary disease patients, is beneficial in improving exercise tolerance for CHF patients. It is suggested that an upregulation of arterial chemoreceptors is responsible for the increased exercise ventilation requirements of these patients and that dihydrocodeine exerts its respiratory depressant effects through a reduction in chemosensitivities. Opioids such as morphine are also known to have a venous and systemic arteriolar dilatory effect which may also contribute to the relief of respiratory symptoms. It is also suggested that a change in central perception of discomfort or distress associated with opioid use may also play a role (Chua *et al.*, 1997).

5.1.3 Buccal morphine

Oral bioavailability of morphine is reduced by first pass metabolism. Also, oral absorption is dependent on a functional gastrointestinal tract. Pain or anxiety, abdominal surgery, peritonitis or pancreatitis may suppress intestinal peristalsis resulting in gastric stasis (Jooste *et al.*, 1999). It would therefore be advantageous to administer morphine via the buccal route. However, low systemic availability of morphine has been reported with use of buccal preparations, with poor analgesic efficacy post-operatively (Simpson *et al.*, 1989; Manara *et al.*, 1990). Acceptability of some buccal preparations was also poor, with patients frequently reporting a bitter or metallic taste (Manara *et al.*, 1989; Manara *et al.*, 1990; Simpson *et al.*, 1989). Because of its low lipophilic properties, absorption through the buccal mucosa is inadequate and it is likely that buccal preparations largely dissolve in saliva and are absorbed via the gastrointestinal route. There have been some successes at permeating the buccal mucosa using ester pro-drugs to improve lipophilicity and bioadhesive polymers to control drug release (Christrup *et al.*, 1997; Anlar *et al.*, 1994). It is also recognised that absorption through the buccal mucosa improves as pH increases, since ionisation of the amino group of morphine occurs to a greater extent at low pH (Christrup *et al.*, 1997). Under normal clinical use the absorption of morphine via the buccal mucosa is negligible and may be excluded. Therefore, this route of delivery was not pursued during formulation development.

5.1.4 Modified release morphine

In order to optimise the blood concentration-time profiles and/or reduce the frequency of dosing of some drugs with short half-lives, formulators have sought to develop novel technologies which delay or prolong drug release from dosage forms within the gastrointestinal tract. Some examples include the capture of drugs within slowly dissolving matrices, addition of coatings to tablets with pH dependent solubility, and osmotically controlled systems which use semipermeable membranes to control drug release (Rathbone *et al.*, 2002). Morphine is an example of a drug which has a short elimination half-life, requiring frequent 'around-the-clock'-dosing to achieve effective pain control (Amabile and Bowman, 2006). The systemic bioavailability of morphine following oral administration of an aqueous morphine sulphate solution is variable but has been reported in healthy subjects to be around 24% on average (Hoskin *et al.*, 1989; Masood and Thomas, 1996). The relative bioavailability of modified release morphine tablets (MST® Continus®) when compared to an oral solution has been reported between 80 and 100% (Hoskin *et al.*, 1989; Poulain *et al.*, 1988; Kealey *et al.*, 1990).

Prolonged release oral morphine preparations have been demonstrated to be of benefit in the discontinuation of high dose morphine since they are able to maintain a steady blood concentration, thus avoiding withdrawal symptoms (Ahmed *et al.*, 2010). The convenience of twice daily dosing with many sustained release oral morphine products encourages patient compliance whilst also sustaining blood levels, enabling better pain management and

control of symptoms (Amabile and Bowman, 2006). There is also some evidence that extended release opioids may achieve a more favourable side effect profile compared to immediate release (Balch and Trescot, 2010).

Available licensed oral dosage forms of morphine have their limitations. Solid forms such tablets (Sevredol®, Morphgesic®, MST® Continus®) or capsules (Zomorph®, MXL®) can be problematic for patients with swallowing difficulties. Dysphagia is a common problem amongst palliative patients, and an important aspect of end of life care. Paediatric populations too have more difficulty swallowing solid oral dosage forms; the European Medicines Agency do not consider tablets/capsules acceptable or applicable to children under the age of 2 (Committee for medicinal products for human use (CHMP), 2006). Children aged 2-6 have variable acceptability which depends on development as well as tablet size (Zajicek *et al.*, 2013). Oral liquids (Oramorph®) can be helpful in some dysphagic patients but require accurate measurement of volumes. In paediatrics, the lack of age appropriate licensed drug formulations has led to the widespread off-label use of adult medicines in children. The need for measurement of very small volumes of concentrated liquid preparations or dilution or reconstitution of available products can result in inaccurate dosing in this population. In addition, many liquid drug formulations e.g. Oramorph® require the inclusion of excipients such as alcohols or preservatives, which can have undesirable or toxic effects in paediatric patients (Fabiano *et al.*, 2011). MST® Continus® (Napp Pharmaceuticals Ltd., Cambridge, UK) is available as granules for

reconstitution as a suspension for modified release administration of morphine. Although an attractive twice daily preparation, this formulation again requires additional manipulation prior to administration. The manufacturer's instructions advise dispersion of the granules in at least 10-30 mL of water (Napp Pharmaceuticals Limited, 2014). For smaller doses e.g. in paediatrics, smaller aliquots may be required, however not only would this would rely on accurate measurement of small volumes, sedimentation or non-uniform suspension of the granules could result in dose inaccuracy. For these reasons, there is a continued need for innovative formulation strategies to deliver morphine in a controlled release manner.

5.1.5 Ion exchange resins

Ion exchange resins (IERs) are water insoluble, polymeric compounds containing either positively or negatively charged sites capable of trapping ions with an opposing charge in an exchange reaction. Through this process, ion exchange resins may be 'loaded' with drug molecules to form drug-resin complexes known as resinates, which have proved useful in the production of sustained release pharmaceutical formulations (Shamma *et al.*, 2011). Ion exchange resins have been extensively used within pharmaceutical formulations for many years: as active pharmaceutical ingredients e.g. cholestyramine functions as a bile acid sequestrant; in the purification of water or active ingredients; and as excipients used for taste-masking, modified release properties, tablet disintegration, or to improve bioavailability or chemical stability of active compounds (Bhise *et al.*, 2008; Guo *et al.*,

2009; Srikanth *et al.*, 2010). Ion exchange resins are available in a range of particle sizes, and exhibit good mechanical strength and physicochemical stability. Drug release profiles obtained under different physiological conditions are reliable and reproducible, avoiding 'dose dumping' associated with some other modified release options.

5.2 Methods

5.2.1 Immediate release oral thin films for NAS

5.2.1.1 Oral thin film formulation development

With awareness of the study data collected by Dryden *et al*. (2009), and following discussions with a consultant neonatologist at the Princess Royal Maternity in Glasgow, it was decided that four strengths of morphine sulphate oral thin films would be formulated which would permit a wide range of dosages to be prescribed. Through the administration of up to 2 strips per dose, 4 oral thin film strengths permitted up to 14 different dose bands. After some deliberation, it was decided that 20, 50, 140 and 200 µg morphine sulphate oral thin films would be suitable as these would permit dosing at approximately 60 µg/kg (as per initial dose recommended by NHS Greater Glasgow and Clyde Neonatal Guidelines) across the weight range 0.3-6.6 kg; which was felt to adequately cover both pre-term and full-term birthweights as well as the weeks after birth required for weaning treatment.

Several polymer blends and flavour combinations were investigated during the formulation development and details of the two lead formulations are described in Table 21. All ingredients with the exception of hydroxypropyl methylcellulose were weighed on an analytical balance (A&D; 14214367) and homogenised using an Ultra-Turrax homogeniser (Janke & Kunkle; 751808) at 8000 rpm. The film forming polymer was weighed separately and then combined gradually with the wet mixture by hand. The final batch mixture was cast onto polymer coated paper using a micrometre adjustable film applicator (Sheen; 1117-250) at thicknesses of 1-1.6 mm, and dried at 40°C in a cabinet drier (Mitchell Driers Ltd.; G03536-010) for 20-30 minutes. The dry films were removed from the paper and cut by hand to different sizes using cutting templates, tablet and craft paper hole punches. Oral thin films were stored in resealable polythene sachets for short term storage.

Table 21. Immediate release morphine sulphate oral thin film batch formula.

Ingredient	**Manufacturer**	**Batch number**	**Percentage dry weight (% w/w)**			
Morphine sulphate	Macfarlan Smith	08-00044	0.6	0.6	0.6	2.3
HPMC E3	Dow	YC1301DLLI	79.5	79.5	79.5	
HPMC K4M	Colorcon	WG0212NO2				69.0
PVP K30	Sigma	MICBC3440V	14.5	14.5	14.5	
Sucralose	Fit Ltd.	XMOF016101	2.9	2.9	2.9	
Peppermint flavour	Symrise	SY/200809	1.8	1.8	1.8	
Spearmint flavour	Givaudan					9.2
Sisterna SP70	Sisterna	548Z22				16.1
Glycerol	Melford Labs Ltd.	19256	0.7	0.7	0.7	2.3
Xanthan gum	Azelis	1D1771K				1.1
Target dose (μg)			20	50	140	200
OTF dimensions (mm)			1.4 x 6π	1.4 x 10π	1.0 x 15^2	1.6 x 15^2

5.2.1.2 HPLC method development

Based on work by Lee and Sabra (2006), a reverse phase HPLC method was developed. A Zorbax Sil C18 250 x 4.6 mm x 5 µm column fitted with a column guard was used, with a column temperature of 25°C. A 1 mL/min flow rate was set and a wavelength of 237 nm for UV absorbance. The mobile phase consisted of ammonium acetate 1% (w/v) and acetonitrile (55:45) pH adjusted to 3.6 using orthophosphoric acid.

Standards were prepared using morphine sulphate (Macfarlan Smith; 08-00044) dissolved in mobile phase and three repeats were performed at each concentration.

5.2.1.3 UV quantification method development

Calibration standards were prepared in Simulated Gastric Fluid USP without enzymes (pH 1.2) and Phosphate Buffer BP (pH 6.8) using morphine sulphate (Macfarlan Smith; 08-00044) in the concentration range 1-20 µg/mL. Each media was also used as blank. The most concentrated sample in each media was first analysed by UV spectrophotometry over the range 200-350 nm. The point of maximum absorbance was then used for obtaining subsequent spectra.

5.2.1.4 Characterisation

5.2.1.4.1 Content and mass uniformity

Ten randomly selected oral thin films from each batch were individually weighed and dissolved in mobile phase. Solutions were filtered through 0.22

µm syringe filters into vials for assay by HPLC. Standards were prepared from a stock solution of morphine sulphate, dissolved in and diluted with mobile phase.

5.2.1.4.2 Oral dispersibility

In order to better mimic typical salivary flow rate in a newborn infant, a simple system was designed which introduced dissolution media to the dosage form at a constant fixed rate of 1.8 mL/hour (0.03 mL/min) via a syringe driver. Method development and results are covered in depth in Chapter 6.

5.2.1.4.3 Thermogravimetric analysis

Thermogravimetric analysis was carried out on samples (n = 3) of the immediate release morphine sulphate oral thin film using a TGA/SDTA851^{e} Mettler Toledo (Switzerland; Serial No. 5125178760) thermogravimetric analyser. Samples were heated in 70µL alumina crucibles from 25-300°C at 10°C per minute. Nitrogen was used as the purge gas to control the environment.

5.2.2 Prolonged release morphine sulphate oral thin films

5.2.2.1 Drug loading of ion exchange resin

A strong acid cation ion exchange resin with 8% divinylbenzene cross-linking (Dowex® 50WX8, 149-297 µm (BDH Laboratory Supplies, Poole, UK; Lot K18954850 429)) was dispersed in a 4.0% (w/v) aqueous solution of morphine sulphate (kindly donated by Macfarlan Smith, Edinburgh, UK; Lot 13-00396) such that the ratio of morphine sulphate to ion exchange resin was

1:1.5 by weight, and stirred by magnetic stirrer (250 rpm) for 24 hours at room temperature, protected from light. The drug-resin complex was then filtered through a 47 mm 0.2 μm nylon membrane (Phenex™, Phenomenex Inc., Cheshire, UK) and rinsed twice with 250 ml distilled water. The filtrate was diluted and assayed by high performance liquid chromatography (method described below) to indirectly determine the extent of drug loading onto the exchange resin. The drug-resinate was dried at 70°C in a cabinet drier.

5.2.2.2 Formulation of oral thin film

Three orodispersible thin films were produced containing morphine sulphate loaded within an ion-exchange resin complex (prepared as above). The relative compositions of the formulations are described in Table 22. The oral thin films were prepared from a solution composed of the drug-resinate; pullulan (Cornelius, Hertfordshire, UK; Lot 1E0712) as the film forming polymer; polyvinyl polypyrrolidone (Sigma-Aldrich, Dorset, UK; Lot KI19107BI) as a disintegrating agent; Sisterna SP70 (Sisterna, Roosendaal, Netherlands; Batch No. 548Z22) as an emulsifying agent; lemon flavour 507940T (Firmenich, Meyrin, Switzerland; Batch No. 1000710486) to give the films a citrus flavour and aroma; sucralose (Tate & Lyle, London, UK; Lot XM1D009501) as a sweetener; glycerol (Melford Labs Ltd., Ipswich, UK; Batch No. 19256) as a plasticiser; and distilled water. All ingredients were weighed on an analytical balance (A&D Instruments Ltd., Abingdon, Oxford, UK; Serial No. 14214367) and combined using an Ultra-Turrax homogeniser

(Janke & Kunkel, Staufen, Germany; Serial No. 751808) at 8000 rpm. Films were cast from the solution on to a polymer coated paper using a Micrometer Adjustable Film Applicator (Sheen; 1117/250mm) at 1.4 mm and dried in a cabinet drier (Mitchell Dryers Ltd., Carlisle, UK) at 40°C for 30 minutes. Oral thin films were cut to a target weight using a rotary blade.

Table 22. Composition of oral thin film stock solutions.

Ingredient	**Manufacturer**	**Batch number**	**Percentage weight (% w/w)**		
			5 mg OTF	**10 mg OTF**	**20 mg OTF**
Drug-resinate	See above	N/A	10.2	10.2	13.8
Pullulan	Cornelius, Hertfordshire, UK	1E0712	19.2	17.3	17.4
Polyvinyl polypyrrolidone	Sigma-Aldrich, Dorset, UK	KI19107BI	2.6	2.4	2.3
Glycerol	Melford Labs Ltd., Ipswich, UK	19256	0.2	0.2	0.2
Sucralose	Tate & Lyle, London, UK	XM1D009501	1.0	1.0	0.9
Lemon flavour	Firmenich, Meyrin, Switzerland	1000710486	1.0	1.1	1.0
Sisterna SP70	Sisterna, Roosendaal, Netherlands	548Z22	1.8	1.7	1.6
Water, distilled	In house	N/A	64.0	66.2	62.8
Target oral thin film mass (mg)			40	90	150

5.2.2.3 Characterisation

5.2.2.3.1 High performance liquid chromatography (HPLC) method

Due to the loss of a previous column, further development of our chromatographic method was required. An ACE C18 column (4.6 mm diameter x 150 mm length packed with a 5 μm diameter stationary phase) fitted with a guard column of the same material was used. A 1.5 mL/min flow rate was set and a wavelength of 237 nm for UV absorbance. An isocratic

mobile phase consisting of an aqueous 1% (w/v) ammonium acetate and acetonitrile (90:10 v/v) was used for elution. 20 µL samples were injected onto the column. Standards were prepared using morphine sulphate (Macfarlan Smith, Edinburgh, UK; Lot 13-00396) dissolved in HPLC grade water and all measurements were taken in triplicate.

Validation of the new method was carried out according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 1994). Linearity was assessed over the concentration range 5-30 µg/mL which represented approximately 22.5-135% of the test concentration for anticipated content assays. Three replicate samples for each of five concentrations were prepared and measured in triplicate using HPLC. The limits of detection and quantification were determined using the signal-to-noise approach. Samples were prepared in the 50-1000 µg/L concentration range. Intermediate precision was determined by assessment of day-to-day variation on consecutive days. For assessment of specificity, a placebo oral thin film was prepared which contained (dry mass) 73.0% (w/w) pullulan, 9.8% (w/w) polyvinyl polypyrrolidone, 7.6% (w/w) Sisterna SP70, 4.6% (w/w) lemon flavour 507940T, 3.9% (w/w) sucralose, and 1.1% (w/w) glycerol. A 58.6 mg placebo OTF sample was dispersed in distilled water and diluted to 500 mL. A 500 µL aliquot was vortexed with 500 µL of a 133 µg/mL morphine sulphate stock

solution and assayed by HPLC. Standards were prepared from the same stock solution in a 33.25-133 μg/mL concentration range.

5.2.2.3.2 Homogeneity of drug-resin dispersion and uniformity of content

A sheet of oral thin film material containing the drug-resin complex was scanned using an EPSON Stylus SX515W scanner at 2400 dpi. Using the GNU Image Manipulation Program (GIMP, version 2.8.2; GIMP Development Team, California, USA) a 5 mm square grid was superimposed over the image and the cells numbered. Using an online random number generator (http://www.randomizer.org/), a set of twenty unique random cells was identified for analyses. Manual counts of distinct drug-resin beads were performed for each square sample using *ImageJ* (Maryland, USA) image processing software. Partial beads which appeared on the edges of the square were included in the count.

For assessment of content uniformity across the batch, we applied the requirements of the pharmacopoeial monograph for prolonged release morphine tablets. That is, the morphine content of 10 randomly selected dosage units from a batch should lie within 5% of the stated content (British Pharmacopoeia Commission, 2014d). Using compendial Type II dissolution equipment, each film was dispersed in 900 mL 0.1N HCl at 37°C and stirred using the paddle method at 50 rpm for 48 hours. An aliquot of each solution was filtered through a 0.22μm syringe filter and analysed by HPLC for morphine sulphate content.

5.2.2.3.3 Thermogravimetric analysis

Thermogravimetric analysis was carried out on samples (n = 3) of the modified release morphine sulphate oral thin film using a TGA/SDTA851^{e} Mettler Toledo (Switzerland; Serial No. 5125178760) thermogravimetric analyser. Samples were heated in 70µL alumina crucibles from 25-300°C at 10°C per minute. Nitrogen was used as the purge gas to control the environment.

5.2.2.3.4 Tensile strength and disintegration

The British Pharmacopoeia features a general monograph for orodispersible films which declares that dosage units should be of “suitable mechanical strength to resist handling without being damaged” (British Pharmacopoeia Commission, 2014c). A tensiometer (Instron, High Wycombe, UK) was used to study the elongation and load required to break the films. Each film was secured between a set of two ‘jaws’ and a measurement of the breaking force (N) and the increase in length at breaking point (mm) were recorded.

For assessment of dosage form disintegration, a novel methodology published by Preis *et al*. (2014) was applied. The method utilised an adaptation of an existing compendial apparatus for dynamic assessment of tablet disintegration. Each oral thin film (n = 6) was suspended from the arm of a disintegration tester (Copley Scientific, Nottingham, UK; Serial No. 21169) using a small clip at the top edge. At the lower edge, a second clip was attached with an additional weight added such that the total weight of the clip came to 3 grams. Distilled water maintained at 37°C was used as the

disintegration media. The water level was set such that when the suspending arm reached its lowest point, the oral thin film was halfway submerged. A timer was started and the endpoint was visually recorded as the point at which the oral thin film disintegrated, dropping the weight.

5.2.2.3.5 Dissolution

Dissolution testing was carried out on oral thin films (n = 6 for each pH) using the paddle method as described in the British Pharmacopoeia. Dissolution media used were hydrochloric acid media (pH 1.2) and phosphate buffer solution (pH 6.8), as described in British Pharmacopoeia (British Pharmacopoeia Commission, 2014a). Each dissolution vessel contained 900mL of media maintained at 37°C, with a paddle speed of 50 rpm. Drug concentrations were determined by HPLC as described above. Samples were extracted through 0.22µm filters at 0 mins, 10 mins, 20 mins, 30 mins, 1 hour, 1.5 hours, 2 hours and then hourly until 6 hours. MST® Continus® 20 mg suspension (Napp Pharmaceuticals Ltd., Cambridge, UK; Batch No. 169935) was used as a comparator product and dissolution was performed by the same method. Release profiles were also obtained from a sample of MST® Continus® 10 mg tablets (Napp Pharmaceuticals Ltd., Cambridge, UK; Batch No. 176133) using the same dissolution method; samples were measured by UV spectrophotometry at 210 nm.

5.2.2.3.6 Statistical analyses

Dissolution profiles were compared for statistical similarity using the f2 statistic. The Microsoft Excel add-in program, DDSolver, was used to

calculate the f2 similarity factor using the bootstrap method (Zhang *et al.*, 2010). An f2 value above 50 indicated similarity between dissolution profiles.

5.2.2.3.7 Thermal stability

In order to assess thermal stability of the modified release morphine sulphate oral thin films, a batch of 5 mg m/r films were heat sealed in aluminium laminated pouches and stored in a MIR-154-PE incubator (Panasonic Healthcare Co. Ltd., Japan; Serial No. 12050038) at 40°C ± 0.5°C for 6 months. The humidity within the chamber was not controllable but varied between 80% R.H. and 50% R.H. At time intervals (0 months, 3 months and 6 months) samples were removed for weight and drug content analyses. At each time point 10 randomly selected films were individually weighed and dispersed in 900 mL of 0.1 M HCl at 37°C (stirred by paddles at 50 rpm) for 24 hours. The solutions were filtered through 0.22 µm syringe filters into vials for assay by HPLC. As per the ICH Guidelines, a change of 5% was considered significant (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2003).

5.2.2.4 *In vivo* pharmacokinetic study of modified release morphine sulphate oral thin films

5.2.2.4.1 Extraction of morphine sulphate from serum

Based on a method published by Mahdy *et al*. (2012) on the detection of heroin, tramadol, and their metabolites in whole blood, a method for the

extraction and subsequent quantification of morphine sulphate from rat serum was developed.

Rat serum for use in the method development and validation was isolated from whole blood provided by the Biological Procedures Unit, Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow. Whole blood was collected (from healthy adult rats) in non-heparinised 15 mL polypropylene centrifuge vials and allowed to coagulate for 1 hour in an upright position at 3-5°C. The vials were then centrifuged using a Mikro 20 centrifuge (Hettich Lab Technology, Tuttlingen, Germany) at 9503 g for 10 minutes. 600 μL aliquots of serum were transferred to 1.5 mL Eppendorf tubes and frozen at -80°C. The samples were thawed immediately prior to use.

A 0.2 M borate buffer (pH 9.0) was prepared by dissolving 6.183 g boric acid in distilled water and diluting to 500 mL (pH adjusted with sodium hydroxide).

Extractions were carried out in clean 2 mL Eppendorf tubes. 20 μL of a 5 μg/mL nalorphine hydrochloride (Sigma, USA; Lot No. 059H0819V) (internal standard) solution (in methanol) was added to 170 μL rat serum containing morphine and vortexed for 30 seconds. 85 μL of 0.2 M borate buffer (pH 9.0) was added and vortexed for 30 seconds. 1 mL of extraction solvent (MTBE) was added and vortexed for 30 seconds. The sample was then shaken via vial rotation (40 rpm) for 10 minutes and centrifuged for 10 minutes at 9503 g. The organic layer was transferred to a clean 2 mL Eppendorf tube and evaporated under nitrogen in a water bath at 40°C. Meanwhile, a further 2 x

1 mL extractions were performed with MTBE. The organic layers were combined in the same Eppendorf tube each time after centrifuging and were evaporated to leave a single residue. The residue was reconstituted in 150 µL mobile phase (ammonium acetate 1% (w/v): acetonitrile (90:10 v/v)), and 100 µL was injected onto the HPLC column.

To assess the recovery achieved through this method of extraction, 10 repeats were carried out on blank serum samples spiked with known concentrations of morphine sulphate and internal standard to provide morphine concentrations within a 500-700 ng/mL range. In addition, further extractions (n = 5) were performed using small volumes of serum (50-150 µL) to determine accuracy in the event of insufficient blood volumes during sampling.

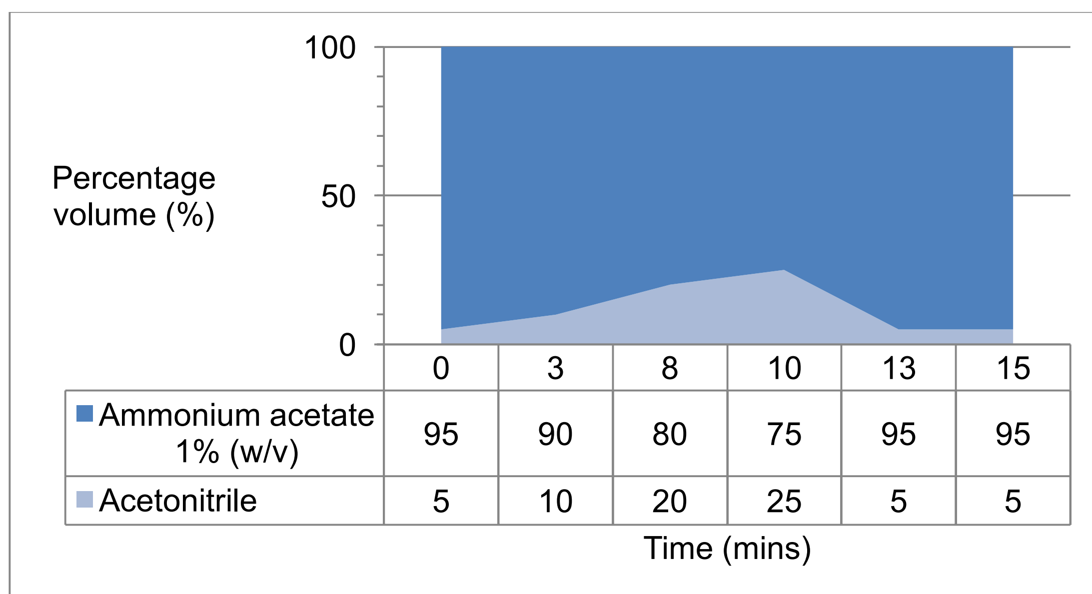
Linearity was assessed for nalorphine through the concentration range 0.3-3.3 µg/mL. Three repeats were performed at each concentration and the standards were prepared in mobile phase (90% aqueous).

Lower limits of detection and quantification were estimated based on the signal-noise ratio method as described in the ICH guidelines on validation (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 1994). Samples were diluted from a 5.3 µg/mL stock solution of morphine sulphate, again using mobile phase (90% aqueous). Repeat extractions (n = 5) were performed in samples at the limit of quantification to determine accuracy.

The ratio of the area under the curve for the morphine sulphate peak to that of the internal standard was used to plot calibration standards across the concentration range 0.1-0.8 µg/mL. Standards were prepared in mobile phase (90% aqueous).

5.2.2.4.2 HPLC-UV method

HPLC-UV detection of morphine sulphate and nalorphine at 237 nm was carried out using an ACE C18 4.6 x 150 mm x 5 µm column fitted with a guard column. The mobile phase consisted of acetonitrile-ammonium acetate 1% (w/v) and analyses were performed in gradient mode at a flow rate of 2 mL/min. Figure 31 describes the composition of the mobile phase throughout the gradient method.



	0	3	8	10	13	15
Ammonium acetate 1% (w/v)	95	90	80	75	95	95
Acetonitrile	5	10	20	25	5	5

Figure 31. Gradient method. A mobile phase consisting of ammonium acetate 1% w/v (aqueous) and acetonitrile (organic) was used. The ratio of aqueous:organic changed stepwise to achieve an optimum separation of components. The relative percentage changes are shown over time.

5.2.2.4.3 Single dose pharmacokinetic study design

A pharmacokinetic study by Nakamura *et al*. (2007) evaluated a once daily, modified release formulation of morphine hydrochloride based on a swelling, layered polymer system. A daily dose of 160 mg/kg morphine was administered as controlled release granules to rats and yielded a plasma concentration greater than 250 ng/mL 24 hours after administration. The authors also noted that during their study design, it was observed that a 40 mg/kg dose of an aqueous morphine solution was required to achieve a 500 ng/mL plasma concentration.

Based on this design, we conducted a single dose pharmacokinetic study in twelve healthy male Sprague Dawley® rats weighing 342-371 g; average (SD) weight 359.3(9.8) g. Each rat was administered a sustained release orodispersible thin film which contained 50 mg morphine sulphate (135-146 mg/kg bw) under isoflurane (IsoFlo®, Abbott Laboratories Ltd., Berkshire, UK) anaesthesia. The oral thin film was placed inside the cheek using forceps and wetted by the administration of approximately 100 µL of water. For each animal, 0.4 mL blood samples were then taken from the tail vein (under anaesthesia) at four time points within a 24 hour period such that across the 12 animals, three samples were obtained at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16 18, 20, 22, and 24 hours. Blood samples were collected in clean 1.5 mL PPE Eppendorf® tubes and allowed to coagulate at 3-5°C in an upright position. Samples were then centrifuged at 9503 g for 10 minutes to separate the serum which was transferred to clean 2 mL Eppendorfs® and frozen at

−80°C prior to analysis. After the terminal blood samples were taken, the rats were euthanized using carbon dioxide. Analyte extraction and assay by HPLC was carried out as described above.

The study was carried out in the Biological Procedures Unit (Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow). All procedures were carried out under animal license 7008973 and were approved by the local animal welfare committee. The procedures had a severity rating of moderate.

5.3 Results

5.3.1 Immediate release morphine sulphate oral thin films for NAS

5.3.1.1 HPLC method validation

The method showed good linearity (R^2 = 0.9997) over the concentration range 1-10 µg/mL.

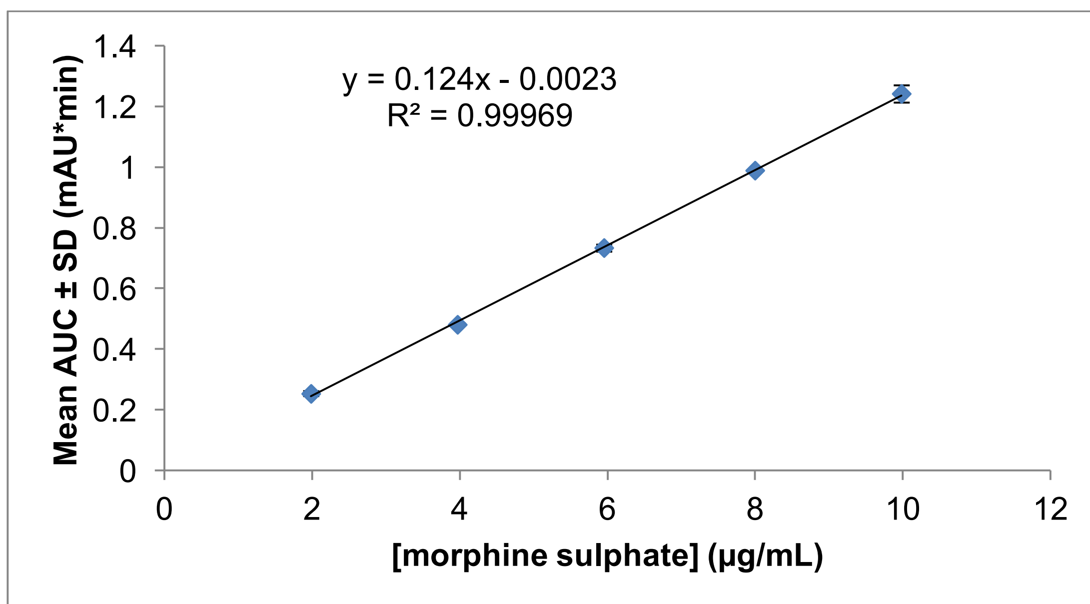


Figure 32. Calibration standard curve using HPLC method. Three repeats were performed at each concentration. The method showed excellent linearity across the concentration range ($R^2 > 0.999$).

5.3.1.2 UV spectrophotometry

Maximum absorbance was observed at 210 nm for both media. pH had no effect on UV absorbance nor linearity, which was excellent within the concentration range ($R^2 = 1$).

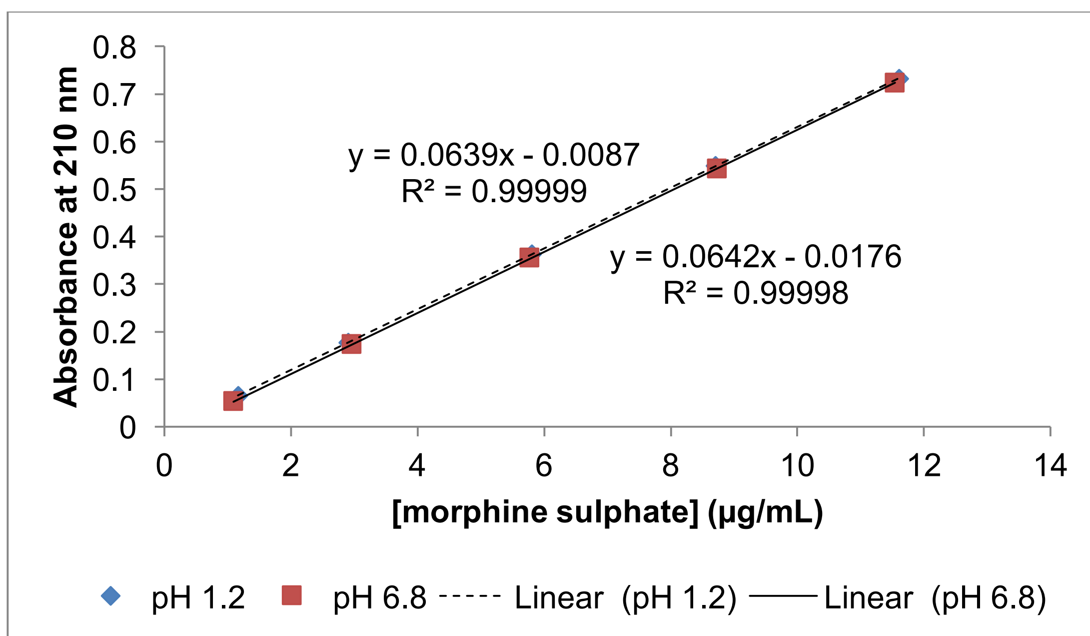


Figure 33. Calibration standard curve using UV method. The method of quantification of morphine sulphate using standard UV showed excellent linearity at a fixed wavelength of 210 nm and this was unaffected by change in pH.

5.3.1.3 Characterisation

5.3.1.3.1 Content and mass uniformity

The results of batch uniformity tests are summarised in

Table 23. Batches conformed to pharmacopoeial standards of uniformity of drug content for immediate release solid dosage forms as individual strips lay within 90-110% of the stated target contents. Strips also conform to mass uniformity as no individual unit deviated from the mean weight by more than 10%.

Table 23. Immediate release morphine sulphate OTF mass and content uniformity (n = 10).

Target dose (µg)	20	50	140	200
Mean film mass (mg)	5.5	13.0	32.8	7.8
SD (mg)	0.2	0.3	0.9	0.4
RSD (%)	3.8	2.6	2.8	5.1
Min (mg)	5.1	12.6	31.8	7.3
Max (mg)	5.8	13.7	34.0	8.5
Mean drug content (µg)	20.7	53.8	141.1	194.2
SD (µg)	0.6	0.8	3.2	8.7
RSD (%)	3.1	1.5	2.3	4.5
Min (µg)	19.0	52.5	138.7	184.8
Max (µg)	21.7	54.7	145.4	209.0

5.3.1.3.2 Oral dispersibility

Results are presented in Chapter 6.

5.3.1.3.3 Thermogravimetric analysis

An average (SD) loss in mass of 10.45(1.88)% (range 8.8-12.5%) was observed across the first 200°C. Extensive loss in mass was then observed above approximately 215°C. A sharp decrease in mass was observed at approximately 215-220°C, with a first derivative peak calculated at

218.05(0.33)°C. This could be attributed to the approximate boiling point of PVPP at atmospheric pressure. An example TG curve is presented in Figure 34.

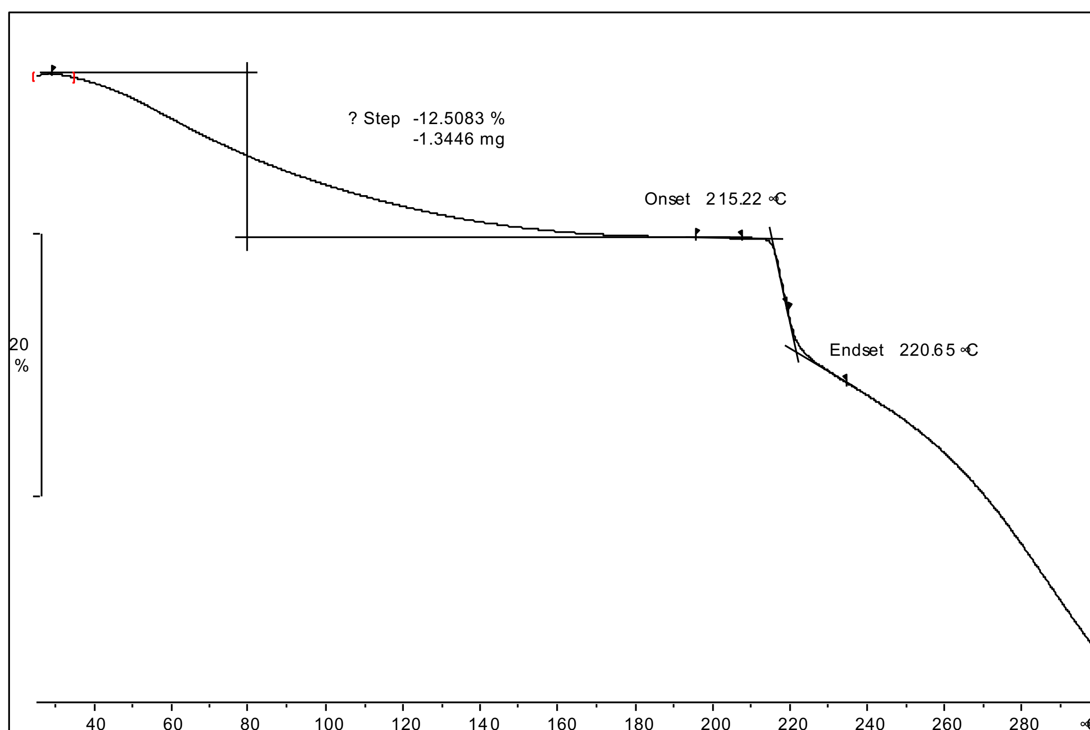


Figure 34. Example Thermogravimetric (TG) curve for immediate release morphine sulphate oral thin films. Samples were heated at 10°C per minute. Change in mass as a percentage of original is shown.

5.3.2 Prolonged release morphine sulphate oral thin films

5.3.2.1 Loading of IERs

The HPLC method for assay of morphine sulphate at 237 nm showed good linearity across concentration range 1-30 µg/mL (n = 5; R^2 = 0.9998) which represented approximately 5-135% of the test concentrations. Additionally, two morphine sulphate samples adulterated with Ponceau 4R (a colorant present in MST® Continus® suspension which interfered with standard UV

spectrophotometric measurements) at concentrations of 2.57 and 12.85 µg/mL revealed no change in linearity.

Analysis of the drug-resinate filtrate revealed that 2.81% (112.55 mg) of the available morphine sulphate did not complex with the ion exchange resin. Therefore the resulting dried drug-resin complex had an API content of 39.519% (w/w).

5.3.2.2 Characterisation

5.3.2.2.1 HPLC method validation

Figure 35 shows the regression analyses for calibration standards. This method gave good linearity (R^2 = 0.9999) across the concentration range. The relative standard deviations (RSDs) were <2% at all concentrations on both days.

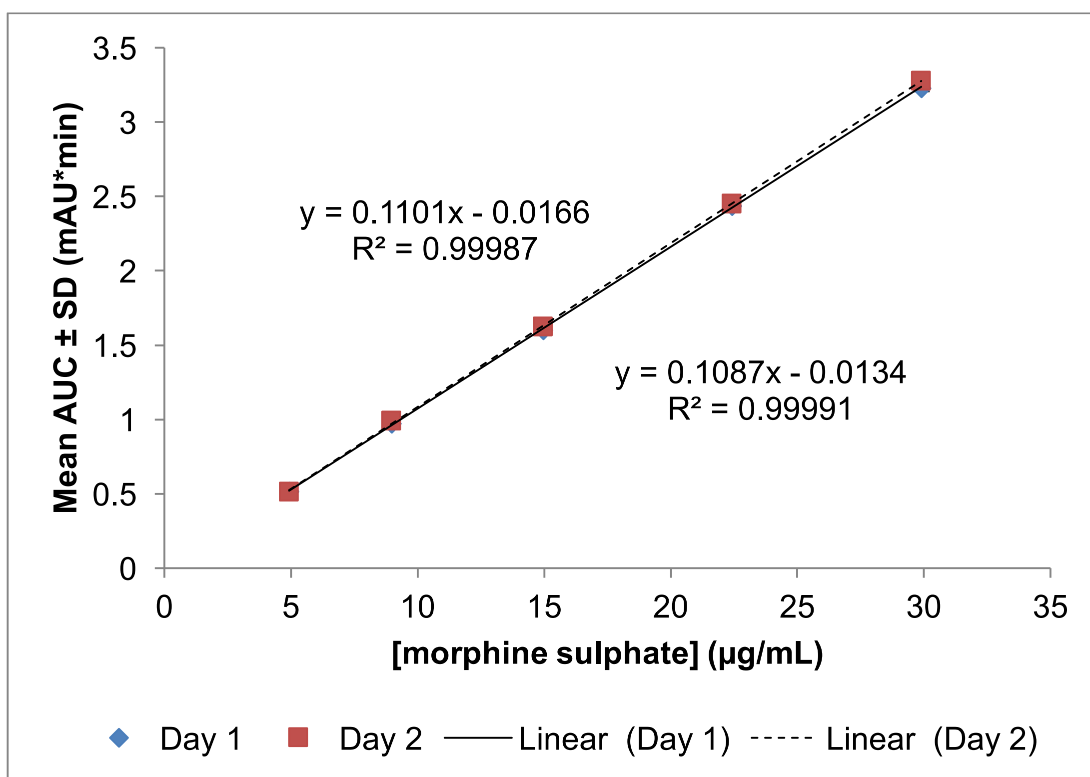


Figure 35. HPLC method validation. Linearity, repeatability and between-day variation. Linearity was assessed on consecutive days using standard solutions and conformed to ICH guidelines.

Table 24 shows the signal-to-noise ratios for samples, calculated using area under the curve. From these results, a limit of detection of 250 μg/L was established for morphine sulphate using our HPLC method. This allowed for a signal:noise ratio of at least 3:1. A limit of quantification was set at 1000 μg/L, allowing for a signal:noise ratio greater than 10:1.

Table 24. Limits of detection (DL) and quantification (QL) using signal:noise ratios. The area under the curve of the morphine sulphate peak was compared to that of a typical peak from the baseline noise and a ratio was calculated. A 3:1 ratio was used to estimate the limit of detection and a 10:1 ratio was used for the limit of quantification.

[morphine sulphate] (μg/L)	AUC (mAU*min)		Signal:noise
	Sample	Baseline noise	
49.3	Below DL		
98.7	0.0081	0.0066	1.23
246.7	0.0184	0.0059	3.12
493.4	0.0492	0.0065	7.57
986.7	0.1028	0.0082	12.54

Table 25 shows the results from the specificity test. From the calibration curve, the calculated percent recovery of morphine sulphate was (67.0/66.5)*100 = 100.8%. Therefore the assay result was not affected by the presence of excipients, and no additional peaks were observed on the chromatogram (see Figure 36).

Table 25. Specificity test of placebo adulterated morphine sulphate sample.

[morphine sulphate] (µg/mL)	Mean AUC (mAU*min)	Relative standard deviation (%)
33.25	4.3521	1.92
46.55	6.0909	1.14
66.5	8.8407	0.68
99.75	12.9980	1.42
133	17.7581	0.36
Sample (66.5 µg/mL)	8.8454	1.40

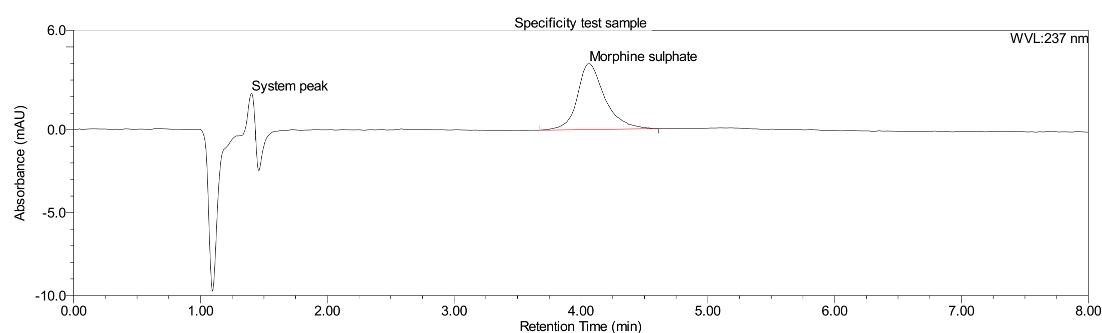


Figure 36. Example chromatogram showing morphine sulphate peak of adulterated sample. No additional peaks were observed with inclusion of formulation excipients.

5.3.2.2.2 Homogeneity of dispersion

The mean (SD) number of IER beads per 0.5 x 0.5 cm oral thin film sample was 60.6 (5.65); range 51-70.

5.3.2.2.3 Content uniformity

Random samples of 10 oral thin films taken from formulation batches were found to comply with pharmacopoeial standards as no individual film deviated from the respective target drug content by more than 10%. Mean (SD)

morphine sulphate contents were 4.81(0.20) mg, range 4.51-5.12 mg; 9.91(0.39) mg, range 9.43-10.18 mg; and 20.17(0.52) mg, range 19.27-20.86 mg. The batches also conformed in terms of mass uniformity as no individual dosage unit deviated from the mean mass by more than 7.5%. Mean (SD) oral thin film masses were 37.02(0.90) mg, range 35.7-38.4 mg; 89.98(3.55) mg, range 85.6-94.3 mg; and 151.19(4.29) mg, range 145.3-157.9 mg.

5.3.2.2.4 Thermogravimetric analysis

Cation exchange resins can be safely dehydrated by heating at temperatures up to 120°C without destroying the resin (Polyanskii and Tulupov, 1971). An average (SD) loss in mass of 11.55(1.13)% range (10.2-12.2%) was observed across the first 145°C. A sharp decrease in mass was observed at approximately 175-200°C.

5.3.2.2.5 Oral thin film tensile strength and disintegration

The average (SD) load in Newtons required to break the films was 25.6 (6.3); range 15.5-37.2. The average (SD) increase in length at the breaking point in millimetres was 4.8 (1.1); range 3.7-6.6. The average (SD) time to disintegration was 6.15 (0.98) seconds; range 5.0-7.7.

5.3.2.2.6 Dissolution

Figure 37 shows the dissolution profiles of 20 mg modified release oral thin films compared to MST® Continus® suspension and MST® Continus® tablets at pH 1.2 and 6.8. The oral thin film formulation produced a sustained release of morphine sulphate, achieving >80% release within 3-6 hours across the pH range. A more rapid release was observed at pH 1.2 compared to pH 6.8,

for all formulations. As a basic molecule (pKa 7.9), morphine sulphate will exist principally in its unionised form at higher pH values, which may explain the slower rate of dissolution at pH 6.8. The release profiles of the oral thin film formulation were found to lie between the two commercial comparator products at both pH values, with a slower release than MST® Continus® suspension but faster release than the controlled release tablets.

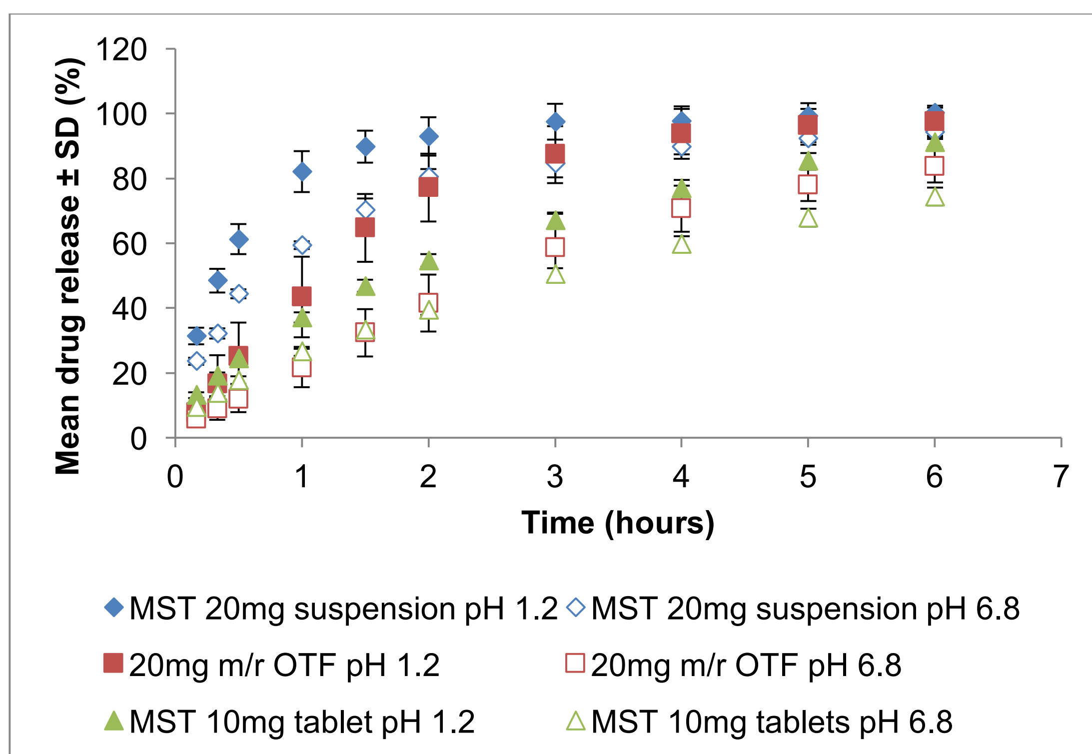


Figure 37. Release of morphine from oral thin films, MST® Continus® suspension and MST® Continus® tablets (n=6). Dissolution was assessed under two pH conditions (pH 1.2, hydrochloric acid buffer and pH 6.8, phosphate buffer) using the Type II paddle method. Samples were assayed by HPLC. The drug release profile of modified release morphine sulphate oral thin films was found to lie between those of the two commercial products: MST® Continus® suspension and MST® Continus® tablets.

5.3.2.2.7 Thermal stability

The batch conformed to ICH requirements for thermal stability as no changes in mass or morphine sulphate content greater than 5% were observed with 6 months of storage at 40°C. In addition, 2-sample t-tests revealed no statistically significant differences in mass (P-values 0.308 and 0.860 after 3

and 6 months respectively) or drug content (P-values 0.168 and 0.681 after 3 and 6 months respectively) compared to time zero. Figure 38 summarises the uniformity analyses through the accelerated thermal stability study.

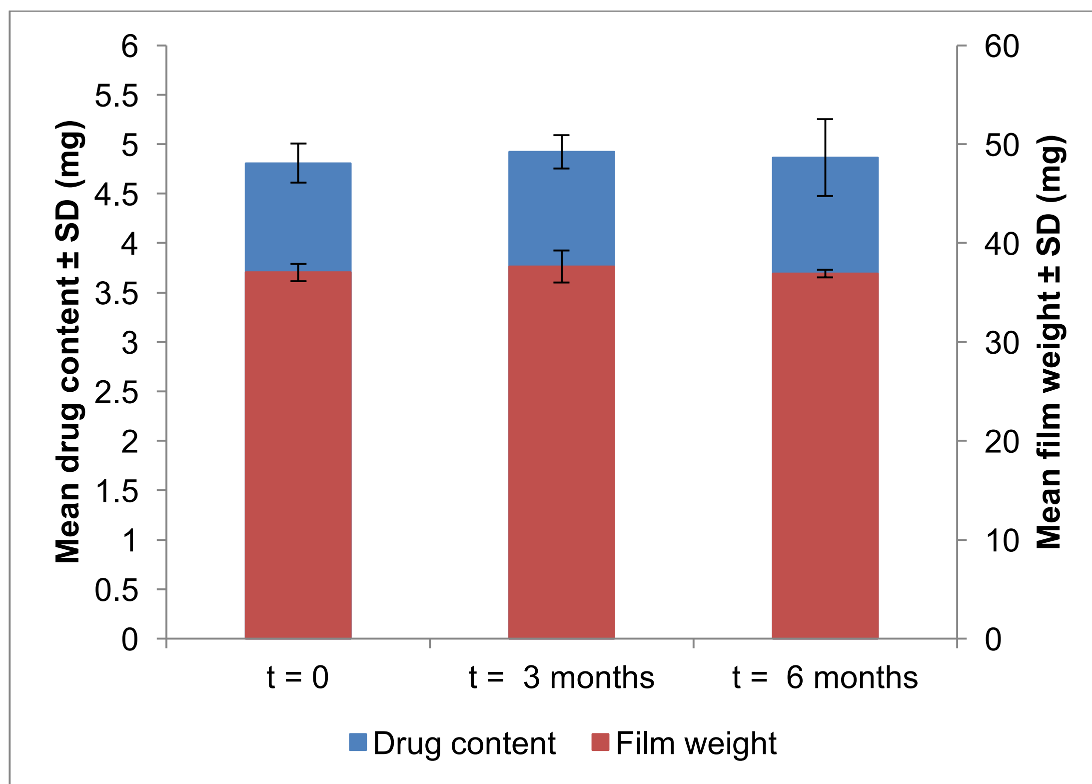


Figure 38. Accelerated stability study at 40°C. Oral thin films were stored in an incubator at 40°C for a period of 6 months. Changes in mass and API content were assessed at 3- and 6-month time points (n = 10). No significant changes were observed.

5.3.2.4 *In vivo* pharmacokinetic study of modified release morphine sulphate oral thin films

5.3.2.4.1 Extraction and assay method validation

The HPLC-UV method showed excellent linearity for the detection of nalorphine hydrochloride across the 0.3-3.3 µg/mL concentration range (R^2 = 0.9999). An example chromatogram of a calibration standard is presented in Figure 39. Linearity using the ratio of peak areas was also good (R^2 = 0.998)

across the morphine sulphate concentration range 0.1-0.8 µg/mL (see Figure 42).

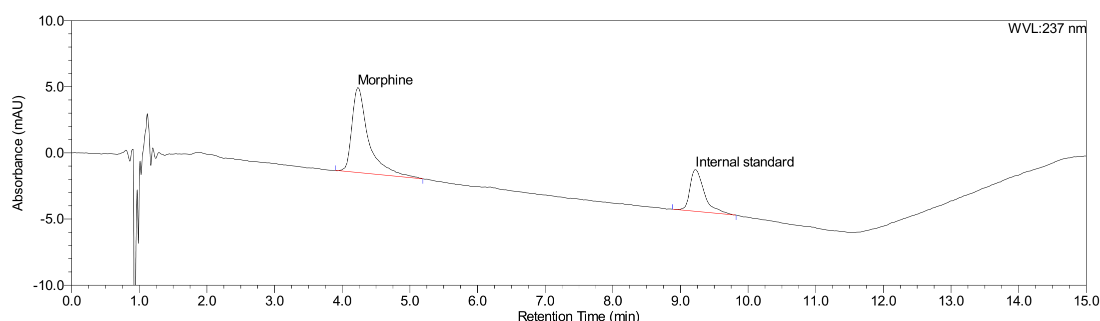


Figure 39. Chromatogram of calibration standard. The HPLC-UV method was able to separate the two analytes as distinct peaks.

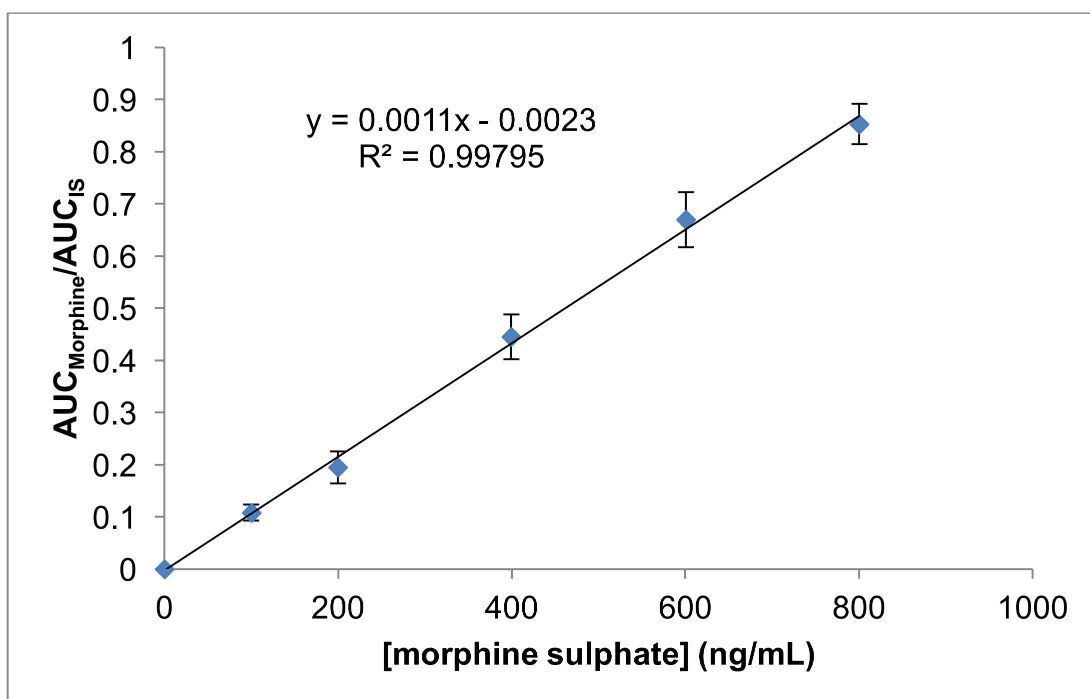


Figure 40. Calibration standard curve. Ratio of area of morphine peak to area of internal standard peak (n = 3, mean ± SD).

Repeat extractions achieved an average (SD) recovery of 97.5(4.6)%. Figure 41 shows an example chromatogram from a spiked serum sample post-extraction. The extraction method was applied to a blank serum sample (without morphine or internal standard) for comparison and the chromatogram is also presented. Peaks for the two analytes, morphine (M)

and nalorphine (IS), were sufficiently separated from impurities which appeared in the blank matrix (B) to avoid interference.

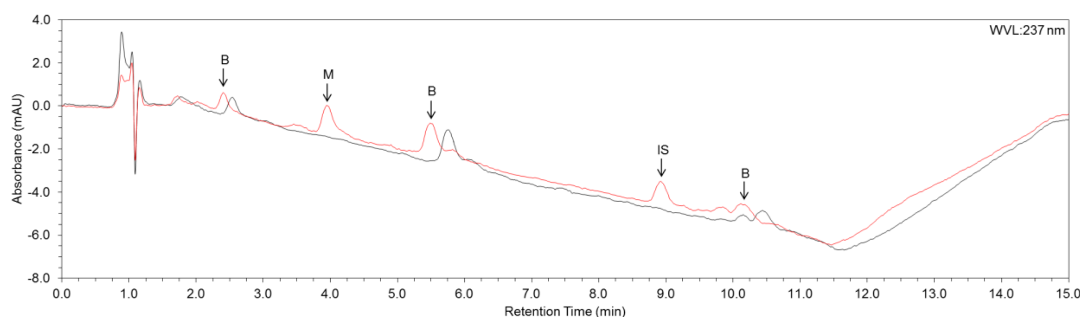


Figure 41. Example chromatogram from serum sample containing morphine with internal standard (red) compared to blank matrix (black) post-extraction. Impurities in the blank (B) were sufficiently separated from the distinct morphine (M) and nalorphine (IS) peaks and so did not interfere.

Table 26 shows the signal-to-noise ratios for samples, calculated using area under the curve. From these results, a limit of detection of approximately 40 ng/mL was estimated for morphine sulphate to achieve detection at three times the baseline noise. A limit of quantification was estimated at 150 ng/mL, which would allow for a signal:noise ratio greater than 10:1. Additionally, it was noted that linearity across this low concentration (53-265 ng/mL) was good (R^2 = 0.9993).

Table 26. Limits of detection (DL) and quantification (QL) estimated based on the signal:noise ratios. The area under the curve of the morphine sulphate peak was compared to that of a typical peak from the baseline noise and a ratio was calculated. A 3:1 ratio was used to estimate the limit of detection and a 10:1 ratio was used for the limit of quantification.

[morphine sulphate] (ng/mL)	Mean AUC (mAU*min)		Signal:noise
	Sample	Baseline noise	
53	0.0173	0.0045	3.85
79.5	0.0247	0.0034	7.27
106	0.0307	0.0038	8.08
159	0.0468	0.0035	13.37
212	0.0621	0.0031	20.02
265	0.0765	0.0034	22.49

Repeat extractions (n = 5) at the limit of quantification (148.4 ng/mL) in spiked rat serum samples achieved an average (SD) morphine sulphate

recovery of 91.2(12.1)%. Extractions (n = 5) performed using small volumes of serum (50-150 µL) resulted in an average (SD) recovery of 94.1(1.4)%.

5.3.2.4.2 Pharmacokinetic results

The serum profile of morphine oral administration of modified release morphine sulphate oral thin films to rats is shown in Figure 42. A maximum (SD) serum concentration (C_{max}) of 337.6(220.7) ng/mL was obtained by 12 hours (T_{max}). The $AUC_{0\text{-}24h}$ calculated using the linear trapezoidal method was 6.18 µg·h/mL.

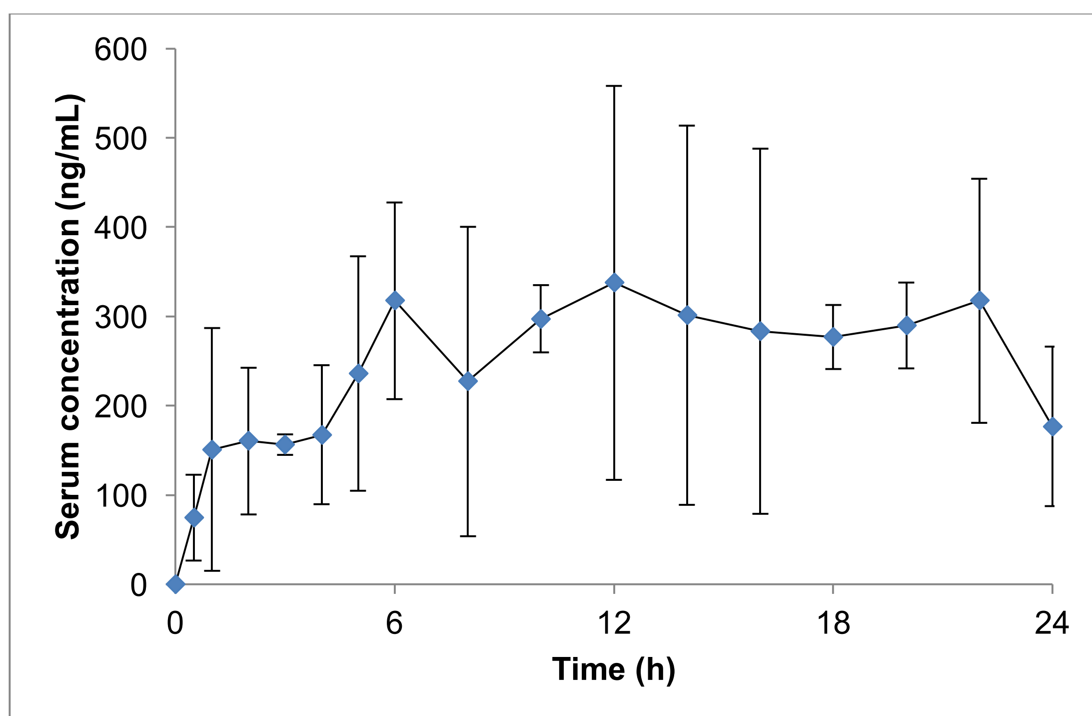


Figure 42. Serum concentrations of morphine sulphate after oral administration of modified release morphine sulphate oral thin films to rats (n = 3, mean ± SD). Serum morphine concentration can be observed to increase steadily over the first 12 hours, reaches a plateau and then remains elevated and begins to decrease after 22 hours.

5.4 Discussion

5.4.1 Immediate release morphine sulphate oral thin films

Oral thin films containing paediatric doses of morphine sulphate for the treatment of neonatal abstinence syndrome were successfully formulated. The films were of an appropriate size for neonatal delivery and manufactured batches passed tests of uniformity in terms of morphine sulphate content and mass variation. The films dissolved under conditions reflective of neonatal salivary flow (see Chapter 6 for detailed results) with more than 90% drug release achieved within 1 hour.

The formulation provides a rapid dissolve with subsequent release of the active ingredient, even under 'stressed' conditions i.e. under very limited water availability and without agitation (mastication was not represented in our model). The excipients contained within the formulation were of food or pharmaceutical grade and the formulation was free from alcohol and preservatives. Several of the ingredients have been granted GRAS status by the FDA as food additives.

The strengths formulated provided reasonable dose banding options across the anticipated weight range for preterm and term newborns for the treatment of neonatal abstinence syndrome. It is hoped that, following a successful initial proof of concept study in neonates using oral thin film technology to deliver an oral phosphate supplement (as described in Chapter 2), and demonstrating the age appropriateness of this technology as a safe delivery

platform for this age group, it will be possible to pursue morphine sulphate oral thin films as a candidate for a controlled trial in neonates in the treatment of neonatal abstinence syndrome. As a solid dosage platform, oral thin films remove the requirement for accurate measurement of small volumes of liquids, improving dose accuracy and reducing the risk of error.

5.4.2 Prolonged release morphine sulphate oral thin films

A sustained release oral thin film capable of delivering 20 mg morphine sulphate over a 6 hour period (above 80% release within 3-6 hours) was successfully formulated using ion exchange resin technology. The film was flexible, non-brittle and had a sweet, citrus taste and aroma. The method of manufacture described herein produced an even distribution of the drug-resin complex throughout the oral thin film (relative standard deviation 9.3%), resulting in a dosage form which complied with pharmacopoeial standards in terms of mass and content uniformity. Oral thin films provide an alternative solid dosage platform for drug delivery, and can be formulated without the need for preservatives due to its low available water content. As well as the greater stability associated with solid dosage forms, this is an attractive option for paediatric drug delivery where many antimicrobial preservatives have known toxicity concerns in children e.g. benzoates (Lass *et al.*, 2012). Our bioburden results reiterate the low capability for oral thin films to support microbial growth. Thin films offer a discreet and convenient alternative to other solid dosage forms such as tablets with improved stability over liquid formulations. Unlike liquid preparations, which often require the accurate

measurement of small volumes or reconstitution before administration, oral thin films offer accurate dosing and require no additional water for administration. They dissolve immediately upon contact with saliva and therefore remove choking risks for patients who may not be able to swallow other solid dosage forms (Bala *et al.*, 2013). Composed of minimal food or pharmaceutical grade excipients, they are cheap to produce and can be easily scaled up. The provision of morphine sulphate as a controlled release oral thin film in this manner may also offer reduced potential for opioid abuse since the product dissolves rapidly but then releases the active ingredient slowly over a prolonged period, maintaining a steady blood concentration. The ion exchange resin complex avoids the possibility of tampering with the formulation via grinding or chewing to achieve rapid release, unlike some other prolonged release alternative e.g. enteric coating.

When compared to MST® Continus® suspension, our results showed comparable, but not statistically identical ($f2 < 50$), dissolution profiles between the two formulations. The oral suspension product utilised the same ion exchange resin technology for its release mechanism, however we found that our thin film preparation achieved a slower drug release profile compared to the commercial suspension. This was likely due to a surface area effect on exchange kinetics since the commercial product contained the same ion exchange resin form, however with a greater mesh size (i.e. smaller particle size), resulting in a more rapid diffusion from within the resin (The Dow Chemical Company, 2006). MST® Continus® tablets incorporate an

ethyl cellulose swelling matrix as opposed to ion-exchange technology for their controlled release. They produced a significantly slower release profile *in vitro*. Although no data exists in the public domain comparing MST® Continus® suspension with other formulations, pharmacokinetic data collected by Napp Pharmaceuticals Ltd. show MST® Continus® suspension to have an equivalent systemic bioavailability to an immediate release morphine sulphate solution and result in an *in vivo* plasma profile comparable to MST® Continus® tablets (Napp Pharmaceuticals Limited, 2014). Our results indicate that the dissolution profile of our oral thin film formulation lies between that of MST® Continus® suspension and MST® Continus® tablets. At pH 6.8, the release profiles of our formulation were found to be statistically similar to the tablet preparation (observed f2 = 57.775). It would therefore be reasonable to assume that the human plasma profile of our formulation *in vivo* would also be comparable.

The results of our *in vivo* study in rats compliment the *in* vitro dissolution profiles, demonstrating the controlled and sustained release of morphine sulphate achievable though ion exchange resin technology, delivered in an oral thin film dosage design. Serum concentrations, after a single oral dose of approximately 140 mg/kg to rats, increased steadily across the first 6 hours and plateaued around 300 ng/mL with the maximum concentration achieved by 12 hours. The $AUC_{0\text{-}24h}$ of 6.18 μg.h/mL was slightly lower than the results reported by Nakamura *et al.* (2007) who achieved an $AUC_{0\text{-}24h}$ of 8.88 μg·h/mL with the same oral dose of 160 mg/kg. However, the authors

reported that a sustained plasma concentration >250 ng/mL was achieved across the 24 hours, and our results are comparable, with plasma morphine levels elevated above 250 ng/mL until after 22 hours. The mechanism of sustained release was different in the study by Nakamura *et al.* (2007), utilising a swelling polymer incorporation layer system rather than ion exchange technology. Their study was also carried out in fasted rats, whereas we did not strictly control animal diet and therefore food effects could also account for the differences observed. The developed protocol of extraction of morphine from rat serum was straightforward and provided a robust, effective (>90% recovery) method for quantification of morphine sulphate using simple HPLC-UV. Although minimal, trace loss of morphine could have occurred at a number of steps in the extraction method e.g. residue left on pipettes, drug retained within the aqueous phase, non-reconstituted material on final Eppendorf tubes, or failure to transfer the organic phase entirely. However, through careful control of pH and repeated extractions, these losses were minimal and a good, reproducible and predictable recovery was obtained.

Chapter 6 - A biorelevant dissolution method for oral thin films

6.1 Introduction

6.1.1 Dissolution

Dissolution testing, measuring the release profile of one or more active pharmaceutical ingredients from a drug formulation, is an *in vitro* technique and a fundamental part of drug development and quality control. It is required for all solid dosage forms in an attempt to predict *in vivo* drug release profiles and show batch compliance with product profile specifications. In certain circumstances, under strictly defined and specific physicochemical and hydrodynamic conditions, it can be used as a substitute for *in vivo* studies as part of bioequivalence testing (Committee for Medicinal Products for Human Use (CHMP), 2010).

A number of standard tests for dissolution exist but the two most commonly used are the 'rotating basket' (Type I) and 'paddle' (Type II) methods (United States Pharmacopeia Convention, 2006a). The FDA dissolution database details recommended dissolution methods for 23 drugs formulated as orodispersible tablets. The rotating basket method is recommended for 3 of these products, with the remainder recommended using the paddle method (Kraemer *et al.*, 2012).

Although a useful indicator of *in vivo* release, compendial methods for *in vitro* dissolution testing do not account for factors such as the presence of physiological levels of enzymes or surfactant (bile) in the gut, or the presence of food, which may interact or otherwise influence drug release. They also do not reflect movements such as intestinal peristalsis, appropriate to the different stages of digestion (Cardot *et al.*, 2007). Although some systems, such as the TNO Simulated Gastro-intestinal Tract Model 1 (TIM-1), have been developed which aim to mimic the human gastrointestinal tract more completely, these are not without their own flaws and may be too complex for consideration as useful quality control tools (Dickinson *et al.*, 2012).

Standard pharmacopoeial dissolution methods employ a large volume (900 mL) of dissolution medium. This is not an appropriate volume for evaluation of solid oral dosage forms designed for paediatric administration. The overall capacity of the stomach changes rapidly in a developing child. In a neonate, the average volume of the stomach can range from 10-100 mL and in infancy the capacity can vary from 90-500 mL. By comparison, the average adult stomach volume can be 1-3 litres (Kaye, 2011). This volume is also irrelevant from a physiological perspective for dosage forms designed to dissolve completely in the oral cavity. Stimulated salivary production has been quoted as ranging from 0.1-6.0 mL/min in children, although considerable variation is found between studies (Leonor *et al.*, 2009). There is currently no published data on stimulated salivary rates in newborns. Non-stimulated salivary production is equally varied, but can range from as little as 0.03-0.04 mL/min

in neonates to 0.3-1.2 ml/min in adults (Kaye, 2011). Additionally, low-dose oral drug products such as those used in paediatric populations can be excessively diluted in large volumes of medium, and therefore analytical methods may not be sensitive enough to detect or quantify drug present in solution at low concentrations (Emmanuel *et al.*, 2010). Oral thin films can present an additional problem as they have the tendency to float in the dissolution medium thereby providing erroneous results (Dixit and Puthli, 2009). Hence, there is a need for the development of a more biorelevant method to measure dispersibility and dissolution.

Many studies have applied modifications to the various pharmacopoeial dissolution apparatuses in order to make the tests more applicable to specific formulations (Kraemer *et al.*, 2012). For example, small volume vessels and flow cells are available for low-dose oral drug products (Zheng, 2009). Klein (2006) described such a method which employs a mini paddle and vessel set. The author also identified that use of large volumes of biorelevant media can be expensive in early stage development. For very rapidly dissolving drug products, conventional dissolution tests can result in an apparent immediate 100% release of the drug, and so differences in release with respect to, for example, pH or temperature can be difficult to determine. Therefore, slower stirring speeds may be more appropriate for very rapidly dissolving oral preparations in order to obtain a release profile (Klancke, 2003). However, adjustments of the hydrodynamic conditions in dissolution apparatus can create concerns around physiological relevance. Specific to

orodispersible thin films, other published methods with compendial modifications have included the use of a stainless steel weight alongside the paddle method to help hold the film at the bottom of the vessel, and adjustment of the basket method to include forceps, which hold the strip in place (Mahajan, 2012; Dinge and Nagarsenker, 2008).

6.1.2 Aims and objectives

The British Pharmacopoeia now possesses a sparse monograph for orodispersible films which states only that “suitable” dissolution tests should be carried out to demonstrate appropriate drug release from the product (British Pharmacopoeia Commission, 2015b). Although various techniques have been developed to evaluate the release profiles of orally disintegrating dosage forms, no standardised methods are available. The aim of this project was to develop a method which better reflected the conditions of the oral cavity, particularly within paediatric populations, and could be used to assess the dissolution profiles and/or dispersibility of oral thin films under these conditions.

6.2 Materials and methods

6.2.1 Oral dispersibility

Since standard tests for dissolution involve the use of a large volume of medium (900 mL), which is not a relevant or suitable representation of the volume of saliva in the oral cavity where oral thin films are designed to dissolve, a number of different experiments were designed to test the rate at

which oral thin films dispersed and released the active pharmaceutical ingredient in a small volume of media.

6.2.1.1 Low volume dispersion test

A single 0.2 mM potassium acid phosphate oral thin film (see Chapter 2 for details of formulation development) was placed in a beaker containing 5 mL of deionised water at room temperature and allowed to dissolve on its own without agitation. Time-lapse photography was used to monitor the strip's progress.

6.2.1.2 Low volume release test

In another method, based on a design by Shukla *et al*. (2009), seven randomly selected oral thin films from a batch of 0.2 mM potassium acid phosphate OTFs were weighed on an analytical balance and then transferred to seven individual sample bottles. 5 mL of tris-HCl buffer pH 6.8 (used to reflect salivary fluid pH) were added to each sample container and a timer was started. At each time point (t = 1, 3, 5, 10, 15, 20 and 30 minutes) one of the seven samples was filtered through type 1 Whatman filter paper. A 50 µL aliquot of the filtered solution was then diluted to 10 mL and assayed by flame photometry for potassium content. Although the authors used phosphate buffer for their experiment, tris-HCl buffer was selected since potassium acid phosphate was already being used as the active ingredient and tris-HCl was considered to adequately reflect the pH of saliva.

6.2.1.3 Orodispersibility and biorelevant dissolution of potassium phosphate oral thin films

A novel apparatus was developed which better mimicked the conditions of saliva production (see Figure 43). Setup initially included a peristaltic pump, which was attached to a plastic sample bottle lined with moistened filter paper. The pump was set to 0.04 mL/minute to represent the average non-stimulated salivary flow rate of neonates (Seidel *et al.*, 2001), and allowed to draw up a solution of distilled water (pH 7.3) heated to 37°C ± 1°C. A 0.3 mM potassium acid phosphate oral thin film was placed onto the filter paper at t = 0 minutes. The solution dripped into the container, flowed across the thin film and the filter paper, and was collected in clean 1.5 mL Eppendorf tubes over each 2 minute period for 30 minutes. 10 µL aliquots of each sample were then diluted to 10 mL with distilled water and assayed by flame photometry for potassium content. An accumulative release of potassium acid phosphate could then be calculated to establish percentage release.

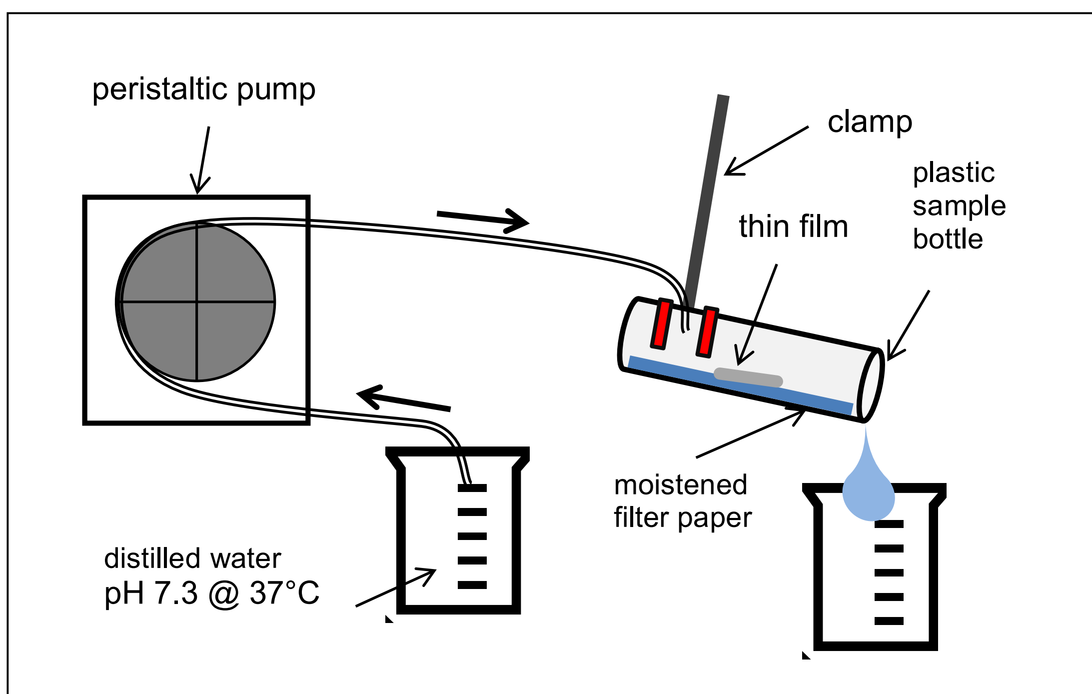


Figure 43. A novel method for evaluation of dissolution/disintegration was constructed using a peristaltic pump to mimic salivary flow rate.

6.2.1.4 Further method development: oral dissolution model

The method was later developed further and used to assess two oral thin film formulations – an immediate release metoclopramide hydrochloride film and a low dose, immediate release morphine sulphate film. In order to mimic typical salivary flow rate in a newborn infant more closely, the design was altered such that the dissolution media was introduced to the dosage form at a constant fixed rate of 1.8 mL/hour (0.03 mL/min) via a syringe driver (see Figure 44 for schematic). This represented the salivary flow rate of a newborn infant. A BD Plastipak syringe (1) loaded with dissolution media was fitted to the syringe driver (2) which introduced the media to the dosage form (3) at a controlled rate. The dosage form (in this case an oral thin film) was placed in a glass container (4) lined with moistened filter paper (included to represent the moist mucosal lining of the oral cavity with residual oral saliva)

at t = 0 minutes, and the syringe driver was started. The media passed through the dosage container, flowed across the oral thin film and was collected in volumetric measures (5) which were replaced at time intervals. The drained solutions were diluted to a known volume with media and assayed by HPLC-UV. In order to maintain a temperature which was representative of body temperature, the apparatus was assembled and run within a constant temperature room at 37°C.

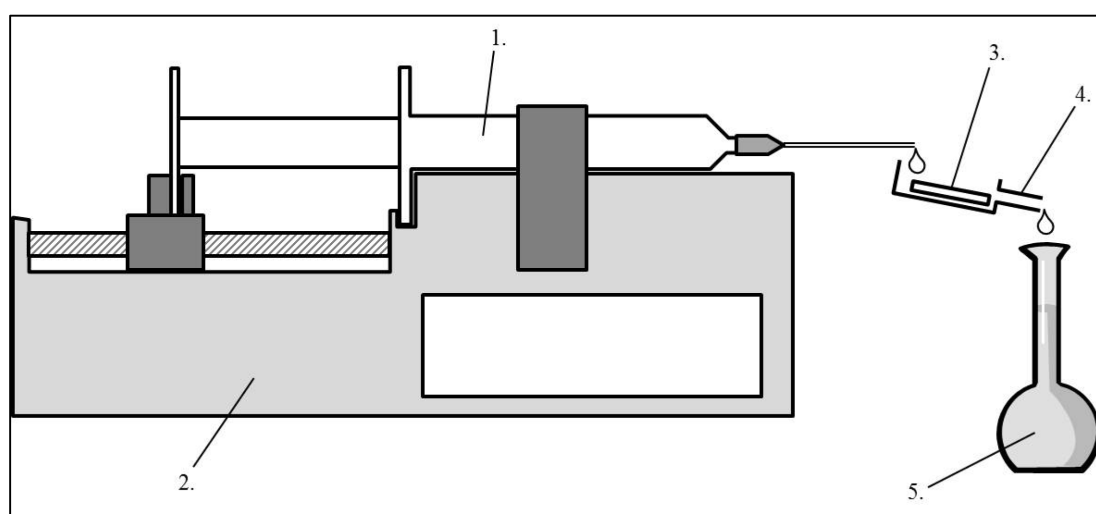


Figure 44. A biorelevant dissolution model for oral thin films. The method utilises a syringe driver to introduce media to the dosage form at a fixed rate, representative of salivary flow rate.

6.2.2 Metoclopramide

6.2.2.1 Oral thin film manufacture

An oral thin film was prepared from a viscous solution consisting of 8.4% (w/w) metoclopramide hydrochloride (BÜFA, Hude, Germany; Batch No. 122906), 19.4% (w/w) hydroxypropyl methylcellulose E5 (Dow, Michigan, USA; Batch No. LA03012N21), 2.2% (w/w) sucralose (Tate & Lyle, London, UK; Lot XM1D009501), 1.8% (w/w) spearmint flavour (Firmenich, Meyrin, Switzerland; Batch No. D0000118864), 0.8% (w/w) polyvinyl polypyrrolidone

(Sigma-Aldrich, Dorset, UK; Lot KI19107BI), 0.7% (w/w) poly(ethylene glycol) average Mw 1500 (BDH, Poole, UK; Lot. ZA9535128), 0.5% (w/w) glycerol (Melford Labs Ltd., Ipswich, UK; Batch No. 19256), 0.3% (w/w) menthol (Fluka, Buchs, Switzerland; Batch No. 1126763) and distilled water, homogenised at 8000 rpm using an Ultra-Turrax (Janke & Kunkle; Serial No. 751808) homogeniser. The mixture was spread onto polymer coated paper using a Micrometer Adjustable Film Applicator (Sheen, Surrey, England; ref. No. 1117; Serial No. 100982/7) at 2 mm thickness and dried at 40°C in a cabinet drier. The resulting film was removed from the paper and oral thin films were cut to 15 mm^2 using a rotary blade and template.

6.2.2.2 Characterisation of oral thin films

A reverse-phase high performance liquid chromatography method was developed based on work by Khan *et al*. (2012). An ACE C18 column (4.6 mm diameter x 150 mm length packed with a 5 µm diameter stationary phase) fitted with a guard column of the same material, was used at a column temperature of 30°C. The mobile phase used was 0.02 M potassium acid phosphate (pH 3.0 adjusted with orthophosphoric acid): acetonitrile in the ratio 60:40 v/v. The flow rate was set at 1.5 mL/min and 20 µL injections were measured by UV at a wavelength of 275 nm. Further details of the method development and validation are covered in Chapter 4.

To assess content uniformity, ten random oral thin films were individually weighed on an analytical balance (A&D; Serial No. 14214367) and each was

dissolved in 200 mL of HPLC grade water. Aliquots were filtered through 0.22 µm syringe filters into vials for analysis by HPLC.

6.2.2.3 Oral dissolution

Two buffers were prepared which were used to represent gastric and intestinal pH conditions. A hydrochloride buffer (pH 1.2) was prepared by mixing 50 mL of 0.2 M potassium chloride with 85 mL of 0.2 M hydrochloric acid and diluting the resulting solution to 200 mL with distilled water. The pH was adjusted using 10 M sodium hydroxide. Phosphate buffer (pH 6.8) was prepared by mixing 100 mL of 0.1 M potassium acid phosphate with 44.8 mL of 0.1 M sodium hydroxide and diluting the resulting solution to 200 mL with distilled water. The pH was adjusted using 10 M sodium hydroxide. At t = 0, an oral thin film was placed onto moistened filter paper as shown in Figure 44. Solutions were collected in 10 mL volumetric measures which were replaced at 15, 30, 60, 90, 120, 180 and 240 minutes. 30 µL aliquots of each sample were diluted to 1 mL and assayed by HPLC. Three repeats were performed for each pH media.

6.2.3 Morphine sulphate

6.2.3.1 Oral thin film manufacture

0.1% (w/w) morphine sulphate (Macfarlan Smith, Edinburgh, UK; Lot 13-00396), 21.7% (w/w) pullulan (Cornelius, Hertfordshire, UK; Batch No. 1E0712) , 2.9% (w/w) polyvinyl polypyrrolidone (Sigma-Aldrich, Dorset, UK; Lot KI19107BI), 2.0% (w/w) Sisterna SP70 (Sisterna, Roosendaal, Netherlands; Batch No. 548Z22), 1.2% (w/w) sucralose (Tate & Lyle, London,

UK; Lot XM1D009501), 1.1% (w/w) lemon flavour (Firmenich, Meyrin, Switzerland; Batch No. 1000710486), and 0.2% (w/w) glycerol (Melford Labs Ltd., Ipswich, UK; Batch No. 19256) were dispersed in distilled water and homogenised. The batch mixture was spread onto polymer coated paper at 1.6 mm film thickness and dried at 40°C. The oral thin films were cut using a 10 mm diameter tablet punch.

6.2.3.2 Characterisation of oral thin films

Based on work by Lee and Sabra (2006), a reverse phase HPLC method was developed. An ACE C18 150 x 4.6 mm x 5 µm column fitted with a column guard was used, with a column temperature of 30°C. A 1.5 mL/min flow rate was set and a wavelength of 237 nm for UV absorbance. The mobile phase employed consisted of ammonium acetate 1% (w/v) and acetonitrile (90:10 v/v). Details of the method development and validation are covered in Chapter 5.

6.2.3.3 Oral dissolution

Two media were prepared which were representative of gastric and intestinal pH. Phosphate buffer, mixed BP was prepared at pH 6.8 by dissolving 2.88 g sodium phosphate dibasic and 1.15 g potassium dihydrogen phosphate in distilled water and diluting to 1 L. Hydrochloride buffer, pH 1.2 was prepared by mixing 25 mL of 0.2 M sodium chloride with 42.5 mL of 0.2 M hydrochloric acid and diluting to 100 mL. The pH was adjusted using concentrated HCl (37% w/v). At t = 0, an oral thin film was placed onto moistened filter paper as shown in Figure 44. Solutions were collected in 5 mL volumetric measures

which were replaced at 15, 30, 60, 90, 120 and 180 minutes. Samples were made up to the 5 mL mark with the corresponding media and then filtered through Millex®-GP filter units with Millipore Express® 0.22 μm PES membranes into vials for assay by HPLC. Three repeats were performed for each pH media. HPLC standards were prepared in the relevant buffer.

6.3 Results

6.3.1 Potassium acid phosphate oral thin films

6.3.1.1 Low volume dispersion test

Figure 45 demonstrates the dispersion of a 0.2 mM potassium acid phosphate oral thin film at room temperature in a small volume of distilled water (5 mL) and without stirring or other mechanical manipulation. A series of photographs are shown over the first 10 minutes demonstrating disintegration of oral thin films under extreme conditions.

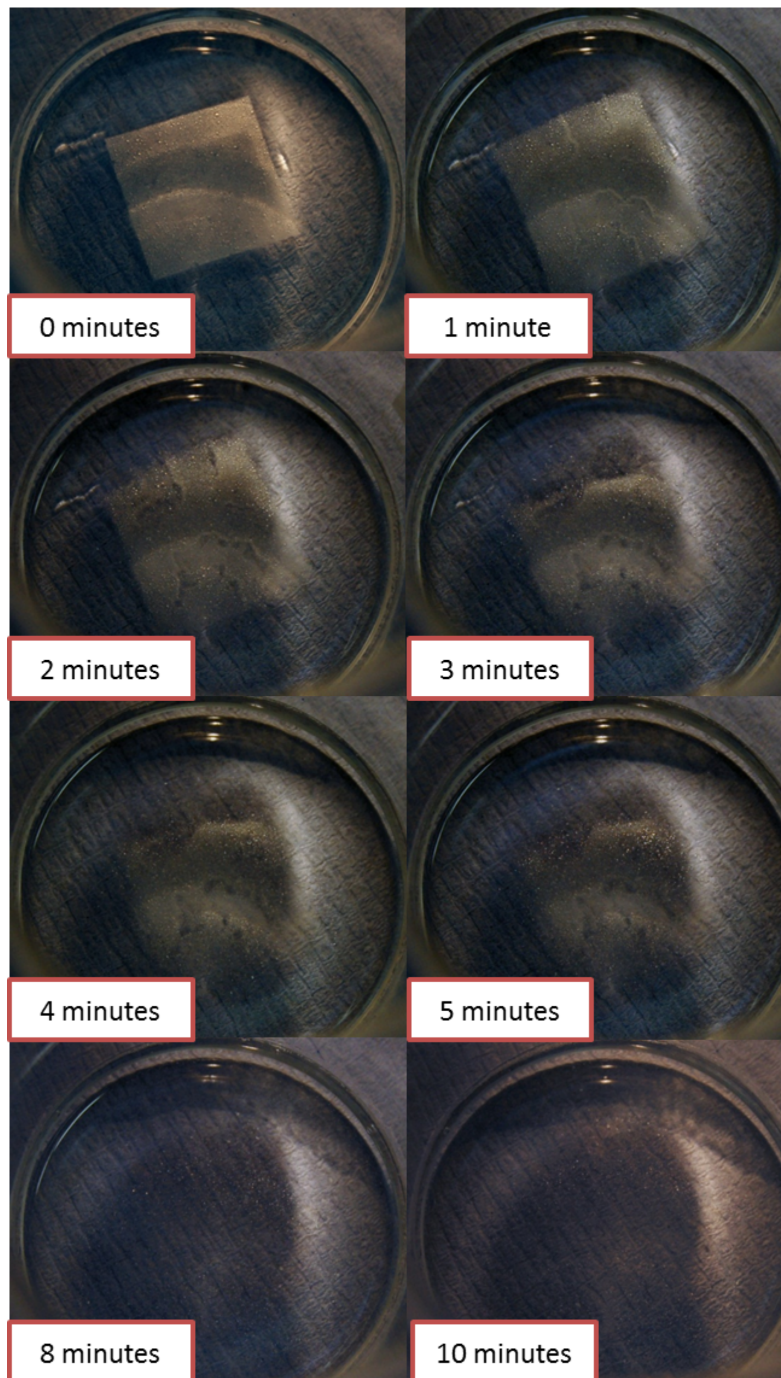


Figure 45. Time lapse photography series showing an oral thin film dissolving without agitation in 5 mL distilled water at room temperature.

6.3.1.2 Low volume release test

Figure 46 shows the release profile of 0.2 mM potassium acid phosphate oral thin films in 5 mL tris-HCl buffer (pH 6.8) without agitation, at room temperature. A logarithmic line of best fit was added as indication of the

predicted dissolution pattern under these extreme conditions (without hydrodynamic influence and at a lower temperature than observed *in vivo*). Approximately 70% release was observed within 10 minutes.

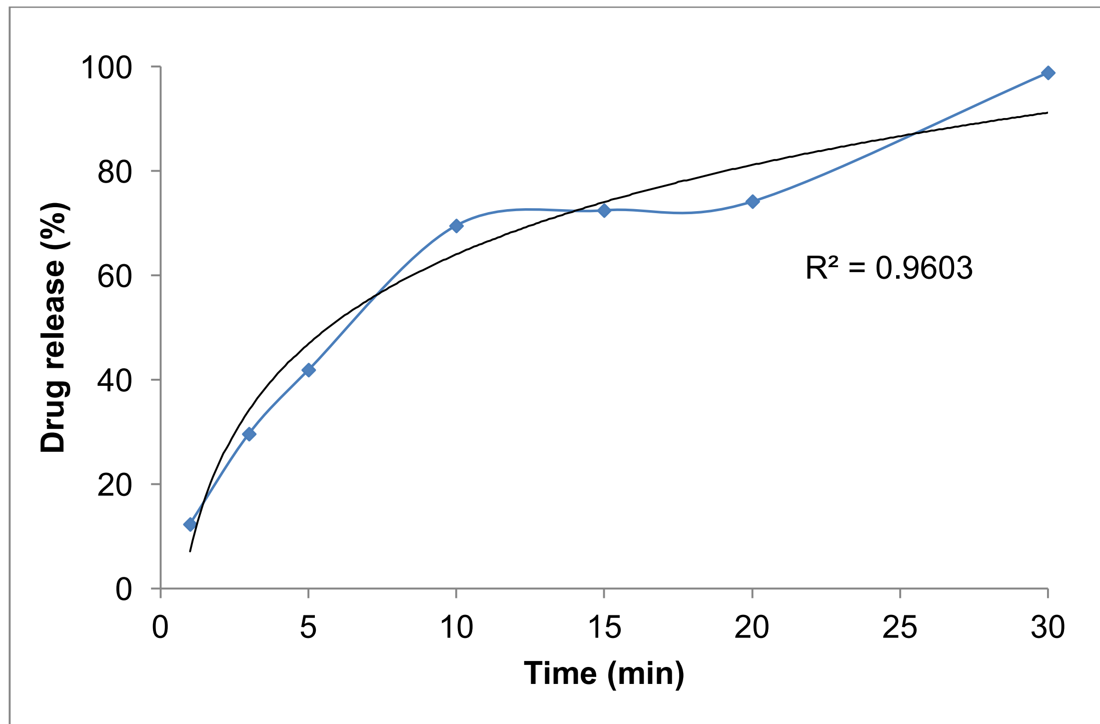


Figure 46. Release profile of a potassium acid phosphate oral thin film using method by Shukla *et al.* (2009); solid black line indicates logarithmic best-fit. Oral thin films were allowed to dissolve at room temperature in 5 mL tris-HCl buffer (pH 6.8) without mechanical disturbance. N = 1 at each time point.

6.3.1.3 Orodispersibility/dissolution

Figure 47 shows the accumulative percentage release of potassium acid phosphate from a 0.3 mM KAP oral thin film over time using the salivary flow apparatus depicted in Figure 43. Results showed that ~80% release of the active ingredient was achieved within 12 minutes. Photographs taken over the time course of the experiment are presented in Figure 48.

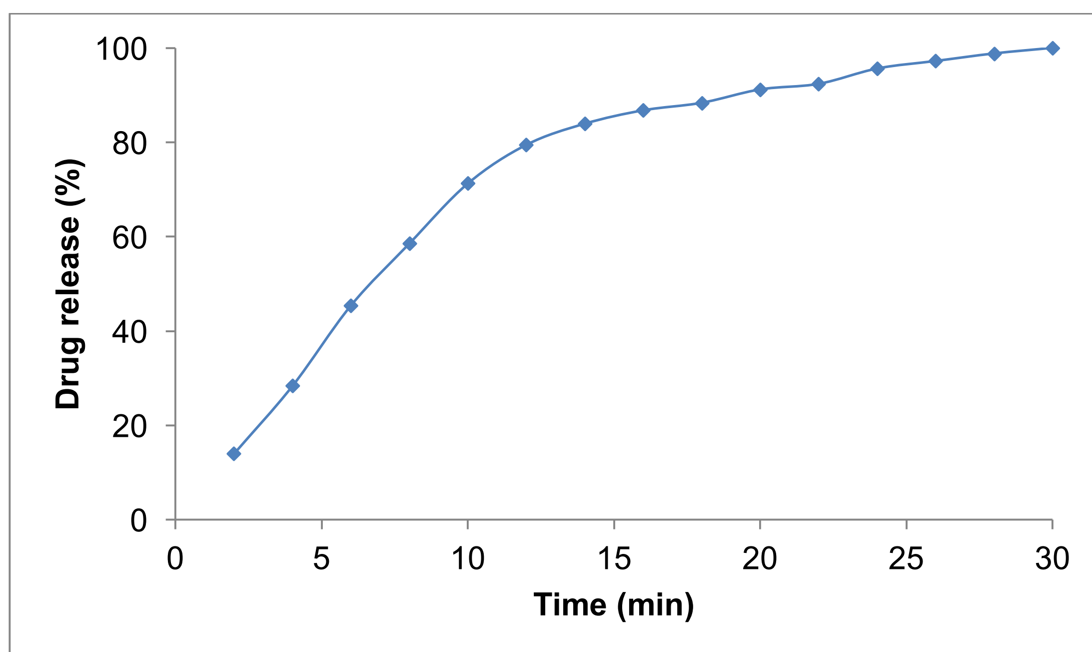


Figure 47. Release profile from novel dissolution/disintegration technique using peristaltic pump.

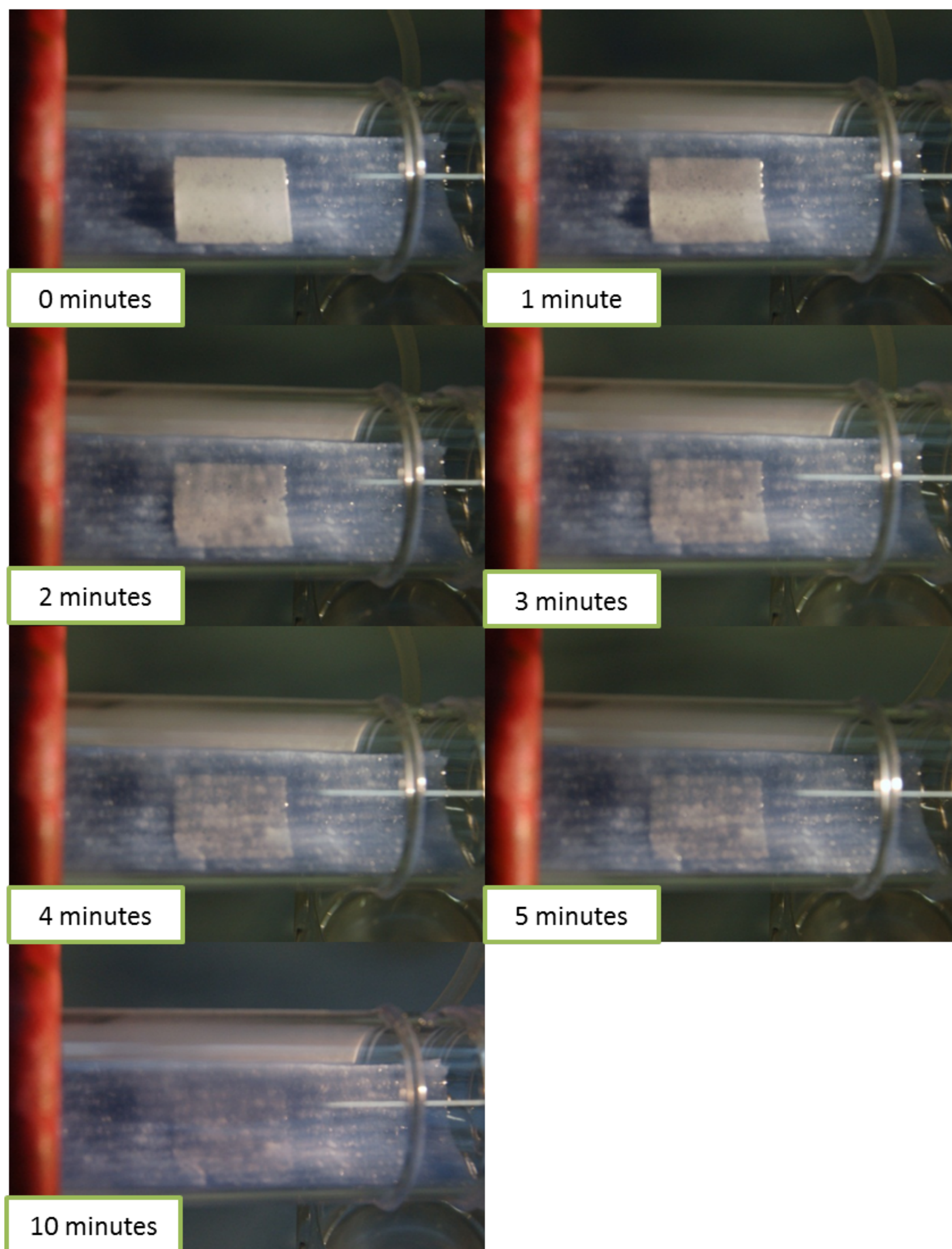


Figure 48. Photos taken from novel dissolution/disintegration technique using a peristaltic pump.

6.3.2 Metoclopramide hydrochloride oral thin films

6.3.2.1 Oral thin film content and mass uniformity

The mean (SD) drug content of the oral thin films was 10.6 (0.76) mg, median 10.5 mg. The mean (SD) mass was 45.78 (3.29) mg, median 45.05 mg. Regression of weight (mass) against metoclopramide content also showed good linearity (R^2 = 0.9599).

6.3.2.2 Oral dissolution

Under conventional dissolution methodology, the films exhibited very rapid drug release *in vitro* with more than 80% release within approx. 10-20 mins. More than 85% drug release occurred within 30 minutes and the formulation is therefore considered to be 'rapidly dissolving' and would be considered exempt from *in vivo* pharmacokinetic bioequivalence study requirements by the World Health Organisation (World Health Organization, 2006). However, using the novel, biorelevant oral model, a much slower rate of dissolution is predictably observed, approaching complete release (generally understood to be more than 80% release) by 90 minutes (see Figure 49).

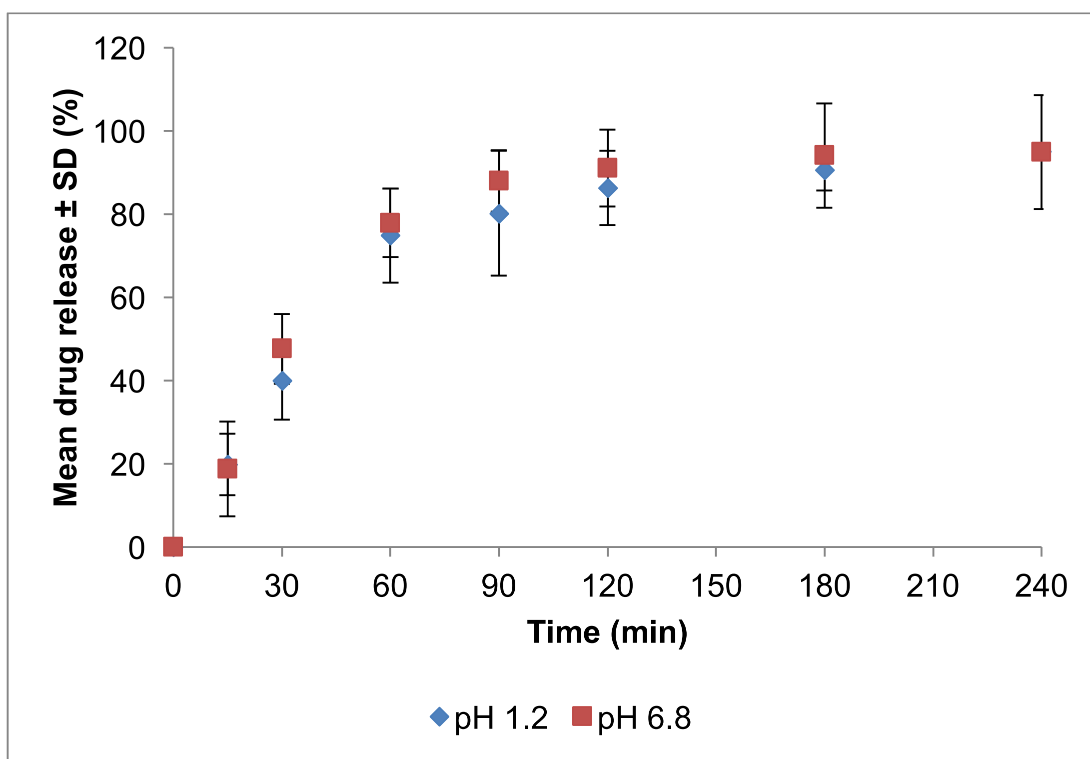


Figure 49. Metoclopramide dissolution results using biorelevant model at pH 1.2 and 6.8 (n = 3). Flow rate was controlled using a syringe driver and represented salivary flow rate in neonates. Profiles were statistically similar at both pH values.

An observed f2 value of 66.791, as determined by the bootstrap method, indicate that the profiles are similar for the two pH values.

6.3.3 Morphine sulphate oral thin films

6.3.3.1 Oral thin film content and mass uniformity

The mean (SD) morphine sulphate content of the oral thin films was 39.6(1.7) µg, median 39.5 µg. The mean (SD) mass of the films was 11.8(0.4) mg, median 11.9 mg.

6.3.3.2 Oral dissolution

Figure 50 shows the release profiles as determined by HPLC analysis, in both HCl buffer BP (pH 1.2) and phosphate buffer BP (pH 6.8).

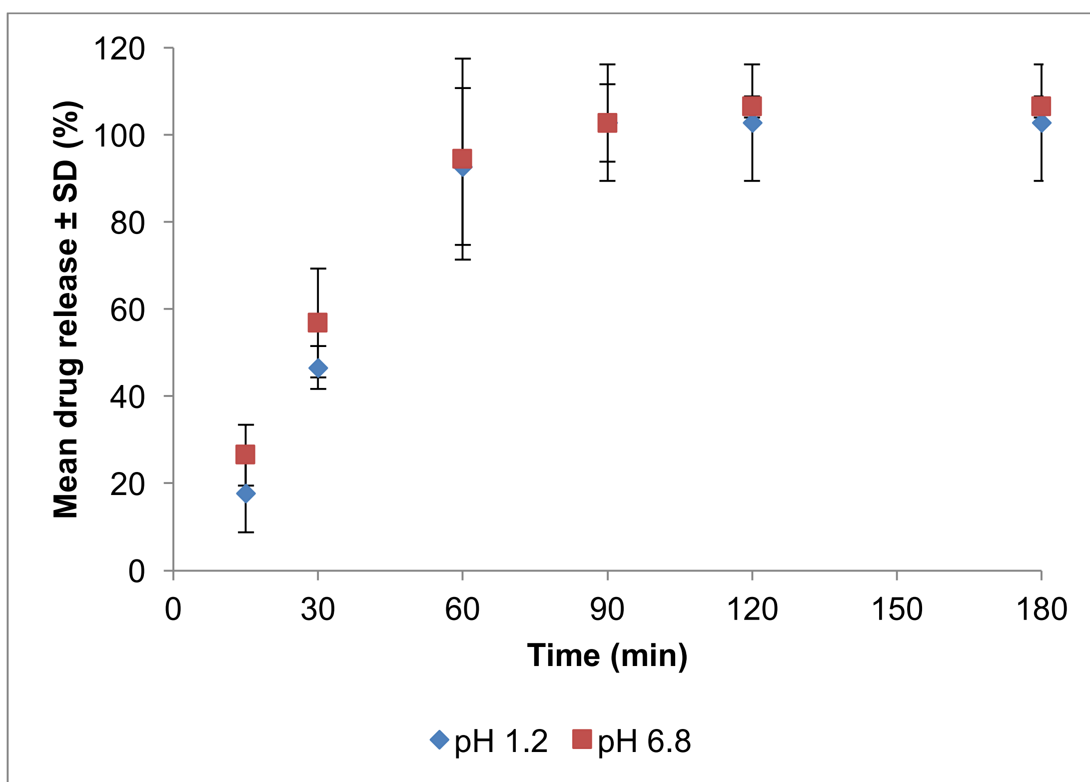


Figure 50. Oral dissolution model. Assessment of morphine sulphate oral thin films at pH 1.2 and 6.8 (n = 3). Flow rate was controlled using a syringe driver and represented salivary flow rate in neonates. Profiles were statistically similar at both pH values.

An observed f2 value of 61.051, as determined by the bootstrap method, indicate that the profiles are similar for the two pH values. An average release of above 90% was achieved within 60 minutes using our novel syringe driver set-up.

6.4 Discussion

Drug release data obtained for potassium acid phosphate oral thin films using the salivary flow method depicted in Figure 43 and described in Figure 47 complemented the results achieved using the conventional dissolution method described in the British Pharmacopoeia (see Chapter 2, section 2.3.3.1). Even under minimal water availability and without agitation, complete release (≥85% drug release) was achieved by 15 minutes and the

formulation would therefore be considered ‘very rapidly dissolving’ (Committee for Medicinal Products for Human Use (CHMP), 2010).

Moistened filter paper was used to represent the lining of the mouth with residual saliva present. There is the potential to improve this aspect of the design, perhaps using some inert hydrogel or similar material to better reflect the oromucosal lining. No method exists to date which reflects the mechanical effects possible within the mouth. A search of the literature reveals a plethora of different formulae for the preparation of simulated salivary fluid and artificial saliva (Preetha, 2005; Preis *et al.*, 2014).

Results obtained using this novel dissolution system, for metoclopramide oral thin films (Figure 49), complemented release results observed using normal compendial equipment. Over 80% drug release was achieved within 90 minutes, and this was independent of pH. This supports the notion of oral thin films as an age-appropriate drug delivery platform for patients with limited salivary flow such as in neonates or in the elderly, since the formulation showed excellent release under limited water availability and without physical agitation. The effect of pH on drug release was not seen ($f^2 > 50$) with the novel dissolution apparatus, unlike conventional dissolution results (see Chapter 4). As the formulation is designed to dissolve entirely in the oral cavity before swallowing as a solution, the effect of pH observed with conventional dissolution methodology may not be relevant.

With conventional dissolution methodology, to comply with the British Pharmacopoeia, immediate release solid oral dosage formulations must

release at least 70% of the stated content of active pharmaceutical ingredient into solution within 45 minutes (British Pharmacopoeia Commission, 2015a). From dissolution results presented in Figure 50, using a novel methodology with limited water availability and no agitation, morphine sulphate oral thin films achieved this target of 70% approximately within the required time. This further demonstrates the ability of the oral thin films to release in a timely manner and their age-appropriateness as an alternative solid dosage form for use in paediatric populations.

The method described herein provided a more biorelevant alternative to the standard dissolution apparatus, and better reflected the conditions of the oral cavity in neonates in terms of available fluid, salivary flow rate and physiological pH. The test confirmed that oral thin films dissolve and release the active ingredient when water availability is extremely limited and without physical agitation. The method could be useful for other dosage forms which are designed to disperse entirely in the oral cavity and describe any initial drug release which occurs there. From organoleptic evaluations of orodispersible thin films in adults, the dosage forms can be expected to dissolve within 25-60 seconds *in situ* (Dinge and Nagarsenker, 2008; Liew et al., 2012). Although the suck reflex is not fully developed in premature infants, there is evidence that stimulation of the lips, gums or tongue can evoke sucking behaviours in preterms and even accelerate maturation of the suck reflex (Fucile *et al.*, 2002; Poore *et al.*, 2008; Bache *et al.*, 2014). Whilst no literature exists on *stimulated* salivary flow rates in neonates, one study

reports a 2-fold increase in salivary flow rate following stimulation in older children (aged 6-12) (Anderson *et al.*, 2001). As discussed in Chapter 1, the unstimulated salivary flow rate in neonates has been reported at around 0.03-0.04 ml/min and it was around this rate that our method was designed (Kaye, 2011). This *in vitro* model for dissolution in the oral cavity is reflective of extreme conditions, without any agitation and with very limited water availability. In reality, it can be expected that there will be some degree of movement in the oral cavity and potentially stimulation of salivary flow to assist in an even faster dissolve.

As discussed in Chapter 5.1.3, there is limited evidence of buccal absorption of morphine sulphate. Metoclopramide too is lacking data to confirm buccal absorption. However, other drugs including some opioids (buprenorphine, fentanyl) have been successfully formulated as oral thin films for buccal delivery (Finn and Vasisht, 2012; BioDelivery Sciences International, 2012). There have also been some successes in using small concentrations of menthol to enhance the mucosal permeation of drugs (Shojaei *et al.*, 1999). As shown, oral thin films have the propensity for rapid dissolution in small volumes of media. They dissolve quickly in saliva, releasing the active ingredient which is swallowed as a solution and can be expected to behave as such. Oral thin films have achieved in vivo pharmacokinetic profiles akin to oral solutions (Choudhary et al., 2012).

Chapter 7 - Modified release oral thin films: industrial scale up and combination drug products

7.1 Introduction

7.1.1 Industrial scale up – delayed casting

Several methods exist for manufacturing orodispersible thin films including solvent casting, semi-solid casting, hot melt extrusion, solid dispersion, rolling and spray drying. Solvent casting is the most commonly used and involves homogenisation of an aqueous stock solution containing the active pharmaceutical ingredient, film-forming polymer(s) as well as other excipients, followed by casting and drying of the film, which is then cut into dosage units (Irfan *et al.*, 2015).

Utilising an established modified release technology, ion exchange resins, within an oral thin film matrix, it has been possible to formulate thin films capable of delivering an active ingredient over a sustained period. Manufacture of oral thin films can be easily scaled up to an industrial level. However, given the combination of technologies included in the novel modified release formulation, it was questioned as to whether release of the active pharmaceutical ingredient could occur if the drug-resinate lay suspended within the viscous stock solution for a sustained period of time, prior to film casting. For example, a large volume of stock mixture may be left

to rest overnight to de-gas. Through delayed casting and subsequent characterisation, using the modified release morphine sulphate oral thin film formulation as an example, we aimed to determine whether drug release could be affected in this way, which could potentially complicate manufacture at an industrial level downstream.

7.1.2 Diclofenac sodium modified release oral thin films

Diclofenac is one of the most commonly prescribed anti-inflammatory agents, used in pain and inflammatory conditions since the 1970s. Like all non-steroidal anti-inflammatory drugs (NSAIDs), diclofenac exerts its effects through the inhibition of prostaglandin synthesis by blocking cyclooxygenases, COX-1 and COX-2. It is available in two salt forms: diclofenac sodium and diclofenac potassium, with the sodium salt being the most commonly prescribed (Moore *et al.*, 2011). Indications for its use include rheumatic disease, gout, migraine, and musculoskeletal and post-operative pain. It is also used topically in actinic keratosis, *ophthalmically* for peri- and post-operative inflammation and seasonal allergic conjunctivitis, and has been associated with an opioid-sparing effect in cancer pain (Dodds *et al.*, 2014; Palmero *et al.*, 1999; Bjorkman *et al.*, 1993). Diclofenac is very well absorbed via the oral route, undergoes extensive first-pass metabolism, binds almost entirely to plasma proteins at therapeutic levels and has a half-life of approximately 1-2 hours (Davies and Anderson, 1997).

The combination of ion exchange resin technology within an orodispersible thin film matrix to control drug release was successful through the formulation

of modified release metoclopramide hydrochloride and morphine sulphate oral thin films. Both of these pharmaceutical compounds are basic in nature and therefore a strong acid cation resin (Dowex® 50WX8) was selected for the exchange reactions. In order to demonstrate that this method of manufacturing a modified release oral thin film can also be applied to acidic drugs, diclofenac sodium was selected as a target compound and an anion exchange resin (Dowex® 1X8) was chosen with the aim of producing a prolonged release diclofenac sodium oral thin film.

7.1.3 Combination oral thin films

Novel formulations that feature both immediate release components as well as extended release have a number of practical applications. Avinza™ (Elan Pharmaceuticals Research Corp., Gainesville, GA) once-a-day capsules utilise ammonio methacrylate copolymer coated beads to provide a pH independent initial immediate release of morphine sulphate followed by an extended release (Portenoy *et al.*, 2002). This allows a rapid achievement of steady state and maintenance of therapeutic concentrations whilst avoiding peak/trough fluctuations. In 2004, Sanofi- Aventis U.S. LLC (Bridgewater, NJ) marketed a combination antihistamine/decongestant product under the brand name Allegra-D for the treatment of allergic rhinitis (Mansfield, 2006). This formulation provides immediate release fexofenadine with sustained release pseudoephedrine in a once-a-day tablet form. Once a day dosing is convenient for patients and encourages compliance.

Ion exchange resins consist of an insoluble polymer matrix (typically cross-linked polystyrene) featuring charged sites, capable of complexing with molecules of an opposing charge in an exchange reaction. As demonstrated in Chapters 4.3.3 and 5.3.2, these drug-resinate complexes can be easily incorporated within a water soluble polymeric film to produce orally dispersing thin films for sustained oral drug delivery. There is also the potential for formulating an orodispersible thin film containing an amount of free drug alongside the resinate complex, for initial immediate release followed by sustained release. The aim was to manufacture an oral thin film that demonstrated proof of this concept. Therefore, a combination oral thin film that delivered an immediate release of free metoclopramide hydrochloride and a sustained release of morphine sulphate within an ion exchange resin complex would be formulated.

7.2 Methods

7.2.1 Batch solution storage

To assess the effect of delayed casting on drug release, a viscous stock solution was homogenised in a 100 mL aluminium beaker which contained 63.7% (w/w) distilled water, 19.1% (w/w) pullulan (Cornelius, Hertfordshire, UK; Lot 1E0712), 10.2% (w/w) drug-resinate containing morphine sulphate (prepared as described in Chapter 5, section 5.2.2.1), 2.5% (w/w) polyvinyl polypyrrolidone (Sigma-Aldrich, Dorset, UK; Lot KI19107BI), 1.8% (w/w) Sisterna SP70 (Sisterna, Roosendaal, Netherlands; Batch No. 548Z22),

1.1% (w/w) sucralose (Tate & Lyle, London, UK; Lot XM1D009501), 1.0% (w/w) lemon 507940T flavour (Firmenich, Meyrin, Switzerland; Batch No. 1000710486), and 0.5% (w/w) glycerol (Melford Labs Ltd., Ipswich, UK; Batch No. 19256). This solution was allowed to stand at room temperature and oral thin films were cast from the mixture after 0, 24 and 48 hours. The mixture was re-homogenised by hand before each casting. Dissolution was assessed at pH 6.8 in phosphate buffer, BP using the paddle method and samples were assayed by HPLC (methods described in Chapter 5.2.2.3.1 and 5.2.2.3.5). Similarity (f2) tests were performed using the Bootstrap method to assess any differences in release.

7.2.2 Diclofenac sodium modified release oral thin films

7.2.2.1 UV method

A UV-spectrophotometric method for quantification of diclofenac sodium was developed based on work by Khaskheli *et al*. (2009). Diclofenac sodium standards were prepared in phosphate buffer BP (pH 6.8) in 5-35 µg/mL concentration range, which represented 36-252% of the anticipated sample concentrations for dissolution studies. UV absorbance was measured at a fixed wavelength of 276 nm. Phosphate buffer was also used as the blank.

7.2.2.2 Preparation of drug resinates

Dowex® 1X8 (149-297 µm), a strong base anion ion exchange resin with 8% divinylbenzene cross-linking, was dispersed in a 25 mM solution of diclofenac sodium in distilled water, such that the ratio of diclofenac to resin was 1:1.5 by weight, and stirred by magnetic stirrer for 24 hours. The drug-resin

complex was then filtered through a 47 mm 0.2 μm nylon membrane (Phenex™, Phenomenex Inc., Cheshire, UK) and washed with distilled water. The filtrate was diluted and assayed by UV spectrophotometry at 276 nm to indirectly determine the extent of drug loading. The drug-resinate was dried at 50°C.

7.2.2.3 Preparation of oral thin films

An oral thin film formulation was produced containing 12.5 mg diclofenac sodium loaded within an ion-exchange resin complex (Dowex® 1X8, 149-297 μm, prepared as above). A viscous stock mixture was prepared containing 15.9% (w/w) drug-resinate; 17.9% (w/w) pullulan (Cornelius, Hertfordshire, UK; Lot 1E0712) which was included as the film forming polymer; 2.4% (w/w) polyvinyl polypyrrolidone (Sigma-Aldrich, Dorset, UK; Lot KI19107BI) as a disintegrating agent; 0.2% (w/w) glycerol (Melford Labs Ltd., Ipswich, UK; Batch No. 19256) as a plasticiser; 1.0% (w/w) sucralose (Tate & Lyle, London, UK; Lot XM1D009501) as a sweetener; 1.0% (w/w) lemon 507940T (Firmenich, Meyrin, Switzerland; Batch No. 1000710486) to give the oral thin film a citrus flavour and aroma; 1.7% (w/w) Sisterna SP70 (Sisterna, Roosendaal, Netherlands; Batch No. 548Z22) as an emulsifying agent; and distilled water. All ingredients were weighed on an analytical balance (A&D; Serial No. 14214367) and combined using an Ultra-Turrax homogeniser (Janke & Kunkel; Serial No. 751808) at 8000 rpm. The films were cast on polymer coated paper using a Micrometer Adjustable Film Applicator (Sheen; 1117/250mm) to a thickness of 1.4 mm, and dried in a cabinet drier (Mitchell

Dryers Ltd.; G03536010) at 40°C for 20 minutes. Oral thin films were cut using a rotary blade and template.

7.2.2.4 Dissolution

Drug release was assessed using the paddle method as described in the British Pharmacopoeia. Dissolution was assessed at 37°C ± 2°C in phosphate buffer BP (pH 6.8). UV measurements were taken at 20-minute intervals using a fixed wavelength of 276 nm. Paddles were set at 50 rpm and 900 mL of media were used in each jar. Six repeats were performed. Diclomax Retard® 100 mg capsules (Galen Ltd., Craigavon, UK; Batch no. E066) were used as a commercially available product for comparison and dissolution was performed by the same method.

7.2.3 Combination prolonged release morphine sulphate with immediate release metoclopramide hydrochloride oral thin films

7.2.3.1 Formulation

Dowex 50WX8 (149-297 μm) was dispersed in a 4% (w/v) morphine sulphate aqueous solution, such that the ratio (by weight) of morphine sulphate to resin was 1:1.5, and stirred by magnetic stirrer for 24 hours, protected from light. The resulting drug-resin complex was filtered, washed twice with deionised water and dried. The filtrate (and washings) was diluted and assayed by UV spectrophotometry at 237 nm to indirectly determine the extent of drug loading.

An orodispersible thin film was produced from a stock mixture that contained 11.1% (w/w) drug-resinate containing morphine sulphate, 5.3% (w/w) metoclopramide hydrochloride, 21.9% (w/w) pullulan, 0.3% (w/w) glycerol, 0.9% (w/w) sucralose, 1.2% (w/w) lemon flavour, and distilled water. The mixture was homogenised by hand and cast onto polymer coated paper using a micrometer adjustable film applicator at a thickness of 1.6 mm. The film was dried at 40°C for 25 minutes and cut to 30 x 15 mm using a rotary blade and cutting template.

7.2.3.2 Dissolution

Drug release was assessed using Type II dissolution apparatus (paddle method) as described in the British Pharmacopoeia. The dissolution media (900 mL) was phosphate buffer BP (pH 6.8) which was stirred by paddles at 50 rpm, at a temperature of 37°C. Filtered 1 mL samples were taken at 10, 20, 30, 45, 60, 120, 180, 240, 300 and 360 minutes and transferred to HPLC vials for assay. The samples were analysed twice according to the HPLC method for morphine sulphate described in 5.2.2.3.1 and for metoclopramide as described in 4.2.1.1. Sample volumes (1 mL) were not replaced and final results were volume adjusted.

7.3 Results

7.3.1 Batch solution storage

Dissolution profiles for OTFs cast after 0, 24 and 48 hours are presented in Figure 51. Similarity tests, comparing the release profiles, gave f2 factors of

59.6 (0 *vs*. 24 hours), 73.2 (0 *vs*. 48 hours) and 63.8 (24 *vs*. 48 hours). An f2 value above 50 indicates statistical similarity between profiles. Therefore, storage of stock solution for up to 48 hours did not have a significant effect on drug release from the ion exchange resin.

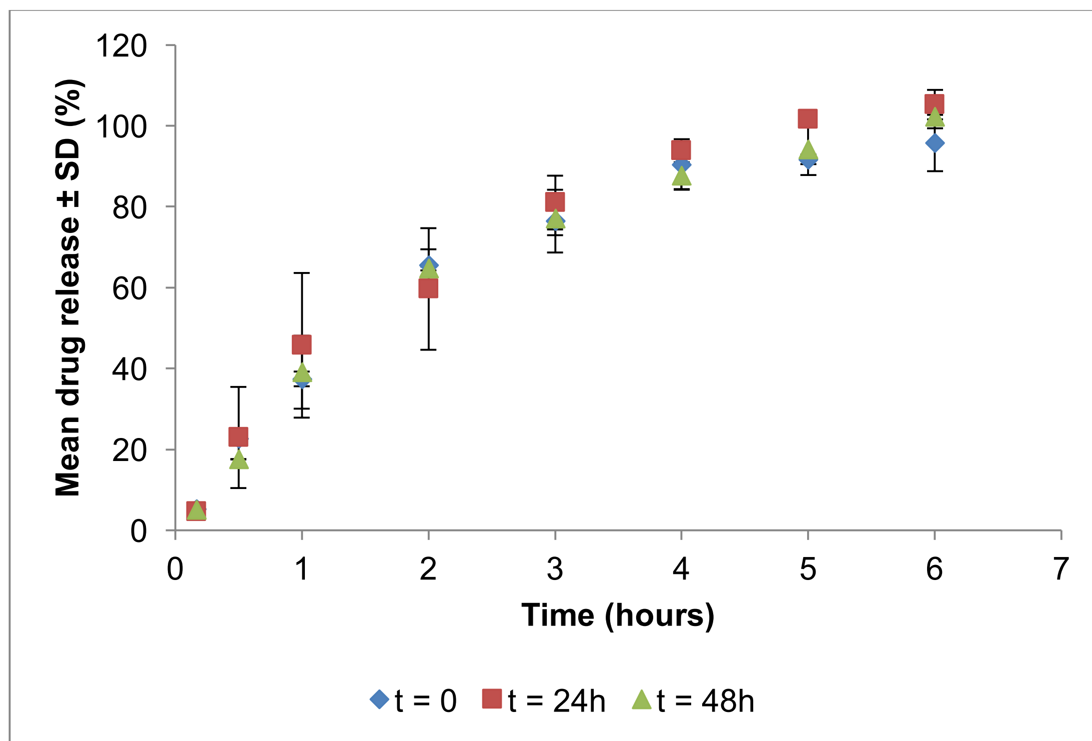


Figure 51. Drug release profiles after delayed casting. Modified release morphine sulphate oral thin films were prepared from a stock suspension containing drug resonate. The films were cast from the stock mixture after 0, 24 and 48 hours. No statistically significant differences in the resulting dissolution profiles were observed at pH 1.2.

7.3.2 Diclofenac sodium modified release oral thin films

The method showed excellent linearity (R^2 = 0.99997) across the concentration range studied. A calibration curve is presented in Figure 52.

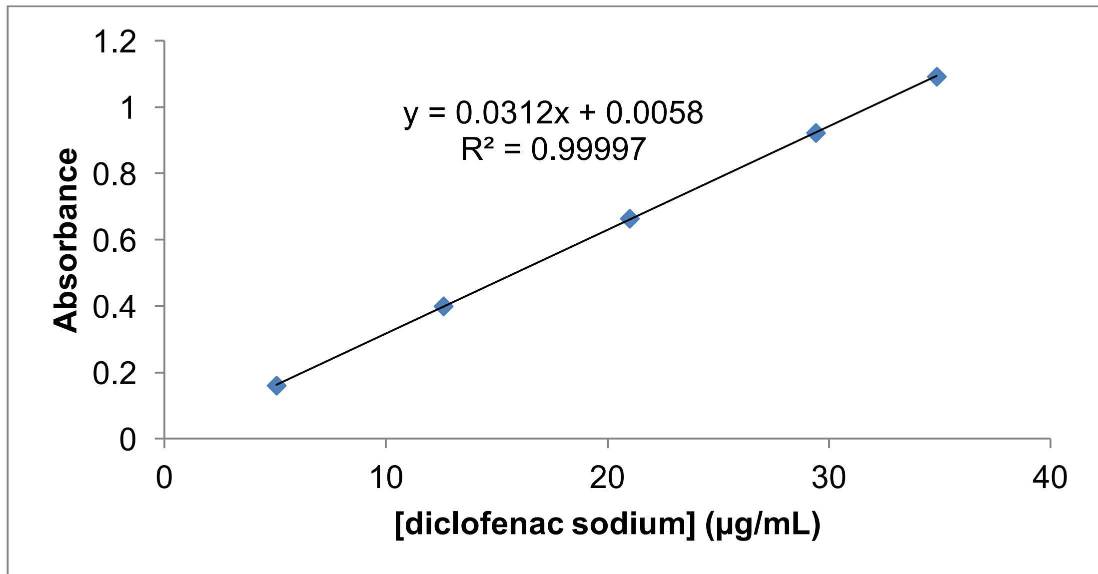


Figure 52. Diclofenac sodium UV absorption. Calibration curve. The method showed good linearity across the concentration range at a fixed UV wavelength of 276 nm (R^2 > 9.999).

A 50% entrapment efficiency was achieved with 41% drug loading resulting in a resinate containing 21% (w/w) diclofenac sodium. Drug release from the modified release diclofenac oral thin films (n = 6) is presented in Figure 53.

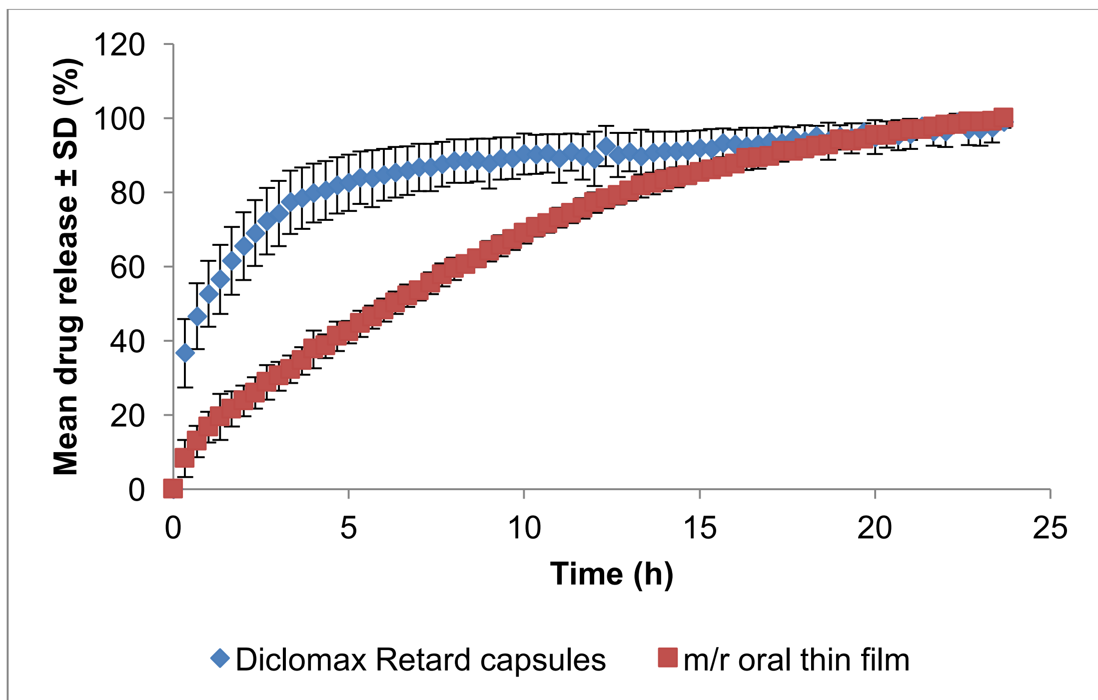


Figure 53. Dissolution results at pH 6.8 (n = 6). Diclofenac oral thin films and a commercial comparator (Diclomax Retard capsules) were compared in phosphate buffer (pH 6.8) using the Type II paddle dissolution method. The films showed a slower sustained release compared to the once-a-day capsule formulation.

7.3.3 Combination prolonged release morphine sulphate with immediate release metoclopramide hydrochloride oral thin films

7.3.3.1 Dissolution

A rapid immediate release of metoclopramide hydrochloride was achieved with more than 80% drug release within 10-20 minutes. A sustained release of morphine sulphate was observed across the 6 hour period, with 80% release observed within the first 3-4 hours. Figure 54 shows the release profiles of the combination oral thin films at pH 6.8 (n = 6). A random sample of oral thin films (n = 10) had an average (SD) mass of 105.4(2.14) mg, range 103.1-108.3 mg, and contained 9.26(0.17) mg, range 9.04-9.49 mg morphine sulphate and 12.78(0.26) mg, range 12.50-13.13 mg metoclopramide hydrochloride respectively.

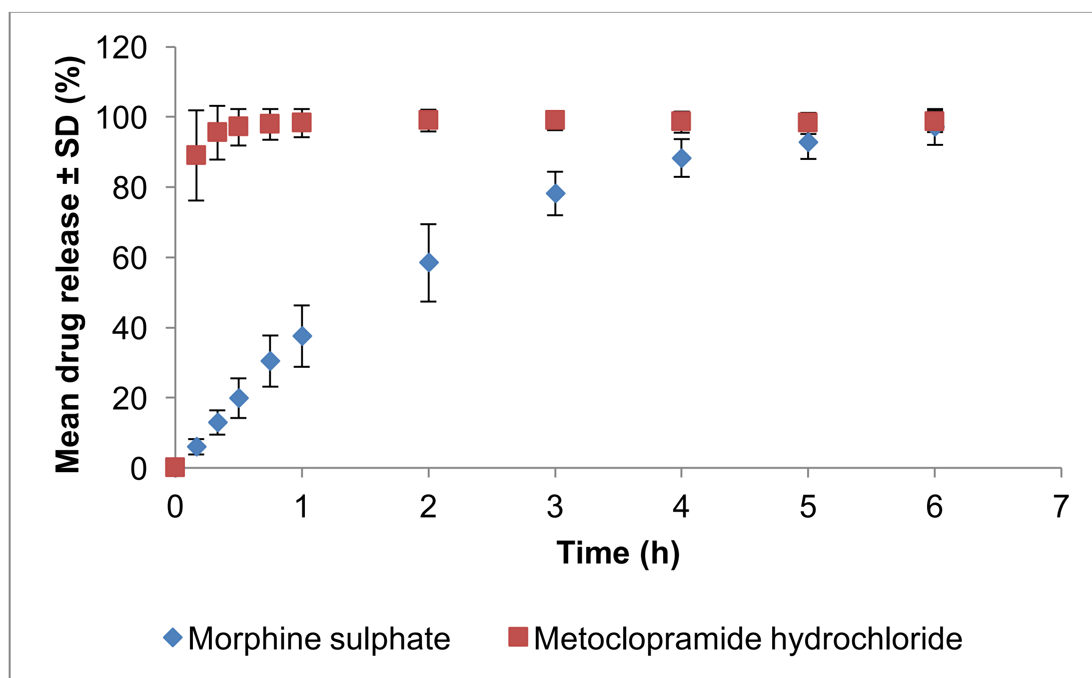


Figure 54. Combined immediate release metoclopramide with controlled release morphine oral thin films. Drug release profiles at pH 6.8. Results showed an immediate release of metoclopramide hydrochloride (red markers) with almost 90% release by the first time point (10 minutes), and simultaneous sustained release of morphine sulphate (blue markers) across a 6 hour period.

It can also be observed that both drugs achieved complete release (approaching 100% at 6 hours) and therefore there was no exchange reaction between morphine sulphate and metoclopramide hydrochloride in solution i.e. morphine was not replaced by metoclopramide to form another drug resinate.

7.4 Discussion

7.4.1 Industrial scale up

Storage of the ion exchange resin complex within a stock solution did not result in drug release prior to production of the oral thin films. It would appear that there is insufficient ionic availability within the highly viscous stock media to support an exchange reaction for drug release. This is reassuring and further demonstrates that ion exchange resin technology is appropriate for providing controlled release from an oral thin film formulation, and lends itself to this element of commercial scale up.

Orodispersible thin films are well suited to commercial manufacture, with much of the equipment required for the solvent casting method of manufacture (mixing vessels, coaters, drying ovens, packaging equipment etc.) already commonplace within the pharmaceutical industry. The rates at which the films can be cast and dried have the greatest impact on overall production speed. However, oral thin film thicknesses can be easily adjusted to help optimise these processes, and use of continuous coating/drying lines can be beneficial (Greb, 2009).

7.4.2 Diclofenac sodium modified release oral thin films

A rapidly dissolving prolonged release diclofenac sodium 12.5 mg oral thin film formulation was successfully produced. Furthermore, a simple and sensitive UV-spectrophotometric method was successfully used for the determination of diclofenac sodium in an oral thin film. Dissolution results revealed a sustained release of diclofenac sodium from the oral thin film over a 24 hour period. The release was more gradual with the oral thin film formulation than the commercial comparator (Diclomax Retard), reaching above 80% release within 13.1 ± 0.9 hours compared to 4.8 ± 2.4 hours with the capsule formulation. Dowex® 1X8 was an effective anion exchange resin for providing controlled release of an acidic drug from an oral thin film. This formulation demonstrates the potential for both acidic and basic drugs to be formulated as controlled release oral thin films using ion exchange resin technology.

7.4.3 Combination immediate and sustained release oral thin films

A combination orodispersible thin film was successfully formulated which provided immediate release of metoclopramide hydrochloride alongside synchronous sustained release of morphine sulphate. Morphine sulphate, like other opioids, has an emetogenic effect, which often requires concurrent treatment with antiemetic therapies (Smith and Laufer, 2014). Some studies also suggest that anti-emetics such as metoclopramide can be effective in controlling nausea and vomiting resulting from opioid use in advanced cancer patients, although more research is needed (Laugsand *et al.*, 2011). Oral thin

films that provide immediate release of an anti-emetic such as metoclopramide alongside controlled release morphine could potentially help suppress this anticipated and limiting side effect. As a single, combined dosage form, the thin films could also help with compliance.

This combination film demonstrates the ability of oral thin films to be produced with both immediate release and controlled release components. A similar effect to Avinza™ may also be achievable with free morphine alongside the ion exchange resin complex to provide a rapid onset of analgesia and achievement of steady state followed by continued sustained release from the drug resinate. Another potential formulation strategy could be to mix two different drug resinates within a single oral thin film to provide simultaneous controlled release of two drugs, which could also aid compliance. For example, Diclopram (licensed in the UK in October 2014 by PharmaSwiss) provides modified release diclofenac and omeprazole in a single unit dose (Medicines and Healthcare Products Regulatory Agency (MHRA), 2014). Figure 55 summarises how various controlled and immediate drug release patterns may be obtained through different combinations of ion exchange resin complexes and free drugs. For example, two drugs with the same basicity/acidity may be complexed on the same resin (a) or different resins if they are of different charges (b) to create a combination sustained release product; or a resinate could be mixed with an uncomplexed drug (c) to create an immediate and sustained release combination. Uncharged drugs can also be easily included in any of these combination oral thin films as an

immediate release element. The formulator has many other variables such as the surface area of the resin, or the degree of cross-linking within the resin, which may be carefully controlled to alter the release profile(s) of the active ingredient(s), as discussed in Chapter 4. Controlled release of uncharged drugs from within an oral thin film may also be possible through polymer coating of drug microparticles prior to oral thin film formulation, or through a suspension of micelles within the polymer matrix, and could be an interesting area for further research (Capece *et al.*, 2015; Paulsson and Edsman, 2001).

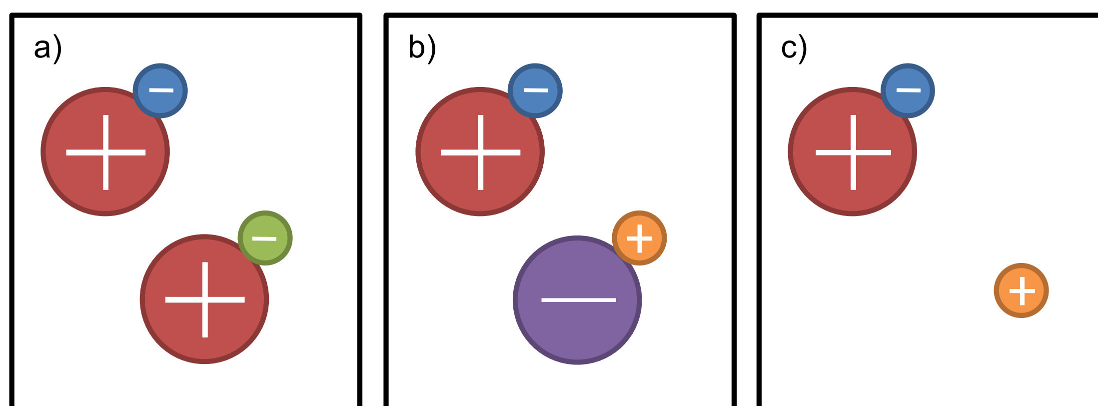


Figure 55. Drug release formulation options. Drug molecules (smaller circles) may be complexed with ion exchange resins (larger circles) according to their charge, and integrated within oral thin films in different combinations to produce various patterns of sustained and/or immediate release. The colours in the diagram represent different drugs and/or resins.

7.5 Final conclusions and future work

There is a continued need to develop more age-appropriate formulations for the oral delivery of active pharmaceutical ingredients to paediatric populations. Oral thin films offer several advantages over other solid dosage forms. As shown in our small volume dispersion tests, and through application of our novel oral dissolution model, OTFs dissolve in very small volumes of fluid, and without mechanical intervention (e.g. chewing).

The results of our excipients exposure study revealed that neonates continue to be exposed to a large number of excipients within intensive care units, despite many of which having known toxicity concerns in this population. OTFs consist of relatively few ingredients, of food or pharmaceutical grade, many of which have been granted GRAS status. As an alternative solid dosage platform, they may be formulated without the inclusion of preservatives and other excipients with known toxicity concerns in neonates. Going forward, there is a need to increase the number of toxicological studies of excipients in paediatric populations. Excipient exposure and accumulative excipient burden data will be useful to clinicians in neonatology to help inform medicine selection, and also to industry and regulators to assist in the formulation and development of safe medicines for children.

The manufacture of modified release oral thin films was achieved through the use of ion exchange resin technology. The inclusion of an ion exchange resin-drug complex within an oral thin film polymer matrix, produced a reproducible and predictable sustained drug release from a versatile dosage form. The method of manufacture was simple, cost effective and could be easily scaled up to an industrial level.

Two successful *in vivo* studies were achieved in rats using our immediate release metoclopramide hydrochloride, and sustained release morphine sulphate oral thin film formulations. These studies provided pharmacokinetic data to supplement our *in vitro* characterisation work, and complemented dissolution results. It would be useful, for comparison, to repeat these studies

using commercially available comparator products as controls e.g. MST® Continus® suspension. Additionally, we would like to perform similar PK studies using the modified release metoclopramide hydrochloride, and the immediate release morphine sulphate OTFs.

There is a deficit in the number of biorelevant test methods for oral thin films and other dosage forms designed to dissolve entirely in the oral cavity. Compendial methodology such as the paddle method are also difficult to apply to low dose drug products such as those used in paediatric medicine, without adaptation. We were able to design a simple method for determining drug release from OTFs under conditions reflective of the oral cavity. This method was more applicable than the compendial dissolution methodology since it employed a more relevant fluid volume and used a flow rate typical of neonatal salivary flow. The method could be further developed to even better reflect these conditions. For example, the dosage container could contain some inert gel to represent the oromucosal lining and/or an artificial saliva could be used in place of the dissolution buffer solution. The method may also prove useful as a biorelevant model for studying food interactions in neonates, using milk or formula as the dissolution media.

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Appendix I

Study Synopsis

Title: Oral potassium acid phosphate supplementation for preterm neonates; a comparison of oral thin films and standard oral therapy

Short title: Oral thin films - novel delivery of medicines to infants

Phase: II/III

Design: Randomised control trial; crossover

Study centre: Princess Royal Maternity, Glasgow

Duration of the study: Approx. 9 months

General objective: Demonstration of non-inferiority between two formulations of a routinely prescribed oral phosphate supplement – an oral thin film and an oral solution, in the treatment and/or prevention of hypophosphataemia. In doing so, establishing the age-appropriateness of oral thin films as a drug delivery option for neonates.

Number of subjects: 20-30

Main inclusion criteria: *Sex*: Male or female. *Age*: Born <32 weeks' gestation. *Consent*: Parent(s) demonstrate understanding of the study and willingness to consent to their child's participation as evidenced by voluntary written informed consent. *Medications and treatments*: Participants must

have been established on oral feeds (as defined by as > 75% of predicted volume enterally for three consecutive days).

Study product, dose, route, and regimen: *Test IMP*: Potassium acid phosphate oral thin films 0.5 mmol/kg body weight twice daily. *Comparator*: Potassium acid phosphate oral solution 0.5 mmol/kg body weight twice daily.

Duration of administration: Infants randomised to either receive 2 weeks of oral thin films followed by 2 weeks of oral solution or *vice versa*.

Statistical methodology: A paired t-test will be used to compare plasma phosphate levels between the two treatments. The two treatments will be considered equivalent if the 95% CI for the difference between the means lies within a predetermined region of clinical indifference (i.e. 1.696-2.544 mM/L). Additionally, individual plasma phosphate levels must lie with an acceptable physiological range i.e. ≥1 mM/L and ≤4 mM/L.

Appendix II

Paper abstract

Is additional oral phosphate supplementation for preterm infants necessary: an assessment of clinical audit

Abstract Adequate phosphate intake is important for the prevention of metabolic bone disease in preterm infants. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition recommends a daily phosphate intake of 184-230 mg/kg/day, which should be met by standard feed volumes of either commercially fortified breast milk or preterm formulae. We sought to investigate whether our local practise of providing supplemental oral phosphate for all infants born before 32 weeks' gestation continues to be necessary. Details of parenteral and milk feeding and both oral and parenteral phosphate supplementation from birth until 8 weeks of age were collected retrospectively from the case notes of 31 preterm infants. Routinely collected biochemical markers of bone mineral status were also recorded. Mean (SD) plasma phosphate concentration was higher when oral phosphate supplementation was given [2.10 (0.38) versus 1.92 (0.50) mM/L without supplement ($P < 0.001$)]. A minimum average phosphate intake of 184 mg/kg/day was achieved by 47 and 77% of babies in weeks 1 and 2, respectively, and by 84-100% of infants from week 3. The percentage of plasma phosphate measurements below the minimum target of 1.8 mM/L was greater amongst unsupplemented babies (45 versus 18%). *Conclusion*:

A majority of infants <32 weeks' gestation did not achieve the recommended phosphate intake during the first week of life. Despite achieving the recommended phosphate intake from week 3, many infants did not have plasma phosphate concentrations within the accepted normal range. Additional oral supplementation may help to achieve blood phosphate concentrations within this target range.

Conference poster abstract

Excipient exposure in the neonatal unit – a prospective case series on an intensive care ward

European Paediatric Formulation Initiative (EuPFI) 5th conference 'Formulating Better Medicines for Children'

18th-19th September 2013, Barcelona, Spain

Introduction: Many of the advances realised in paediatric medicine are reliant on the unlicensed or off-label use of adult medicines (Conroy and McIntyre, 2005). Whilst considerable investigation has been directed towards the clinical pharmacology of drugs in paediatric populations, a dearth of information on the extent of exposure and possible toxicological burden of excipients within these medicines exists (Lass *et al.*, 2012). This is despite recorded examples of unintended harm as a result of excipients (Fabiano *et al.*, 2011). **Purpose:** The aim of this study was to identify and quantify neonatal excipient exposure within an intensive care unit. The results were

compared with current recommendations on excipient intake levels where available. **Materials and Methods:** Twenty consecutive live births admitted to the neonatal intensive care were included in the study. Details of all prescribed medicines and parenteral nutrition fluids were documented from birth until discharge. Manufacturers were contacted to obtain quantitative details of medicine composition. Daily and cumulative excipient exposures were then calculated for each infant based on their bodyweight. **Results:** Neonates were exposed to a total of 85 excipients contained within 54 formulations. Babies were exposed to 23 different excipients on average (range 8-52). In a few cases, excipient exposure exceeded recommended acceptable daily intake limits. Almost half of the identified excipients could not be quantified either due to non-disclosure by the manufacturer or because they were used *quantum satis* within the formulation. **Conclusions:** There is still a high prevalence of unlicensed and off-label medicine use in neonates despite current incentives for industry to develop age-appropriate formulations. It remains concerning that neonates continue to receive excipients with known toxicity concerns. In the interim, compulsory disclosure of medicine composition by manufacturers should be enforced by regulators so that cumulative excipient burden can be accurately recorded on an individual patient basis and used to provide an evidence base to inform best practice.

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