DEVELOPMENT OF A MULTIPARTICULATE-BASED PLATFORM FOR DELIVERING FUNCTIONALISED CAPABILITY AS AN ORAL LIQUID DOSAGE FORM

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PLAGARISM STATEMENT

This thesis describes research conducted in the School of Pharmacy, University College London (formerly University of London School of Pharmacy) between October 2008 and December 2012 under the supervision of Dr Catherine Tuleu. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.
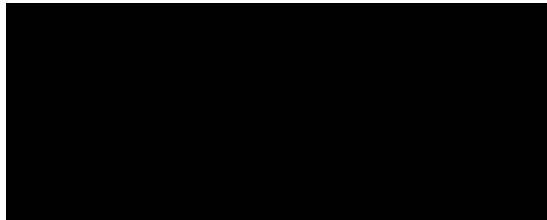
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ABSTRACT

That '*Children are not small adults*' is a commonly quoted adage: nowhere is this more true than in pharmaceutics. When trying to make an "age-appropriate" oral dosage form, a number of patient needs must be met including swallowability, dose-adaptability and acceptability. Acceptability may be enhanced by better tasting, non-gritty medicines: with this in mind this research sought to develop a suspension platform for functionalised multiparticulates, namely for taste-masking.

The rheology of the suspending media and its effect on the suspendability of large (>100 µm) placebo particles was investigated before the influence of particle concentration, size and media viscosity of these suspensions on grittiness and acceptability was assessed in two sensory trials containing young adults. It was found that higher concentrations of hydoxypropylmethycellulose were not well tolerated due to their inherent taste and that their acceptability was improved through the addition of flavouring/sweetening agents. Statistical analysis of the results on the refined media and sensory trial showed that particle size and media viscosity had an effect on grittiness, unlike particle concentration.

Microparticles of Eudragit® E (a reverse-enteric polymer marketed for taste-masking) containing quinine hydrochloride as a bitter drug were prepared by spray-drying without using organic solvents. Initial experiments resulted in many blockages of the spray dryer which were eventually rectified by increased homogenization and a fractional factorial experimental design employed to screen the influence of different levels of excipients. However, even the optimised process suffered from problems with a low feed solids concentration, low spray rate and low yield. Most particles had an aggregated morphology and the formulations which showed the lowest release in salivary pH were the most aggregated with particle sizes >1 mm. These large particles were not easy to uniformly suspend and would have required a large mass to be administered due to low drug loading which made them unsuitable for use as a uniform platform.

ACKNOWLEDGEMENTS

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LIST OF ACRONYMS

Acronym	Description
BNF	British National Formulary
CAB	Cellulose acetate butyrate
CMC	Carboxymethylcellulose
CPL	Cellulose propionate
DL	Drug Loading
EC	Ethylcellulose
EE	Encapsulation Efficiency
EMA	European Medicines Agency
GIT	Gastro Intestinal Tract
HEC	Hydroxyethylcellulose
HPC	Hydroxypropylcellulose
HPLC	High Performance Liquid Chromatography
HPMC	Hypromellose/Hydroxypropyl methylcellulose
HPMCP	Hypomellose phthalate
ICH	International Conference on Harmonisation
IER	Ion Exchange Resin
MC	Methylcellulose
MCC	Microcrystalline cellulose
MeCN	Acetonitrile

MMC	Migrating Motor Complex
NaCMC	Sodium carboxymethylcellulose
O/O	Oil-in-oil
O/W	Oil-in-water
ODT	Orally Disintegrating Tablets
PEG	Polyethylene glycol
SA	Stearic acid
SDS	Sodium dodecyl sulphate
SiO_2	Colloidal Silica
W/O/W	Water-in-oil-in-water

1. INTRODUCTION

1.1. Overview of Multiparticulate Dosage Forms

Multiparticulates or multiparticulate dosage forms can be defined as when "*the dosage of the drug is divided among several discrete delivery entities, in contrast to a classical single-unit dosage form*" (Colorcon, n.d.). The typical size range of multiparticulates can be seen in Figure 1-1.
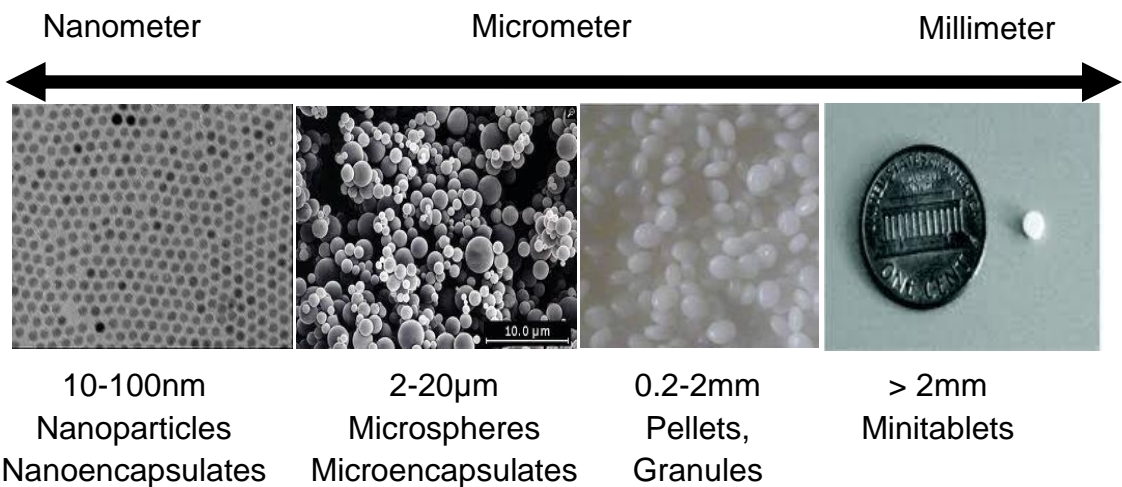
Figure 1-1: Size Range of Multiparticulates (Images from left to right from Thomasson, 2006, Chem List, PharmaTrans Sanaq AG Pharmaceuticals, Thomson et al., 2009b)

Multi-particulates cover a range of different forms and are produced by a number of different methods as discussed in Section 1.4. Different multiparticulates are used to provide a number of different functionalities including:

- Modified Release (Roberts et al., 2012, Shavi et al., 2011)
- pH dependent Release (Alhnan et al., 2010, Nilkumhang et al., 2009, Raffin et al., 2006)
- Bioavailability Enhancement   (Jha et al., 2011, Li et al., 2010)
- Taste Masking (Hu et al., 2009, Shah and Mashru, 2008a, Vaassen et al., 2012)

Multiparticulates are often used in adults due to the ability to blend different multiparticulates of different release rates (e.g. immediate release and modified release) to provide tailored release profiles with multiparticulates transiting more reproducibly through the GI tract than tablet formulations and often associated with less local gastrointestinal irritation (Davis et al., 1986, Newton, 2010, Varum et al., 2010, Zeeshan and Bukhari, 2010).

In the context of this project, multiparticulates were thought to be of interest for use in children to overcome some of the issues discussed throughout this chapter.

1.2.   Medicines for children: what are the requirements and hurdles?

The term "children" covers a large and heterogeneous population who can be loosely grouped based on their biological development stages as shown in Table 1-1. This demonstrates that the term "children" can include the very preterm infant weighing less than a kilogram, all the way through to morbidly obese teenagers. As well as differences in body weight, volume and surface area, this disparate group also covers a wide range of physiological development and in particular enzymatic and liver functions

Table 1-1: International Conference on Harmonisation (ICH) Definitions of Different Periods of Childhood (European Medicines Agency, 2001)

Definitions	Age Range	Biological Stage
Preterm newborn infants	< 37 weeks' gestation	Normal gestation
Term newborn infants	0 – 27 days	Postnatal Changes
Infants and toddlers	28 days to 23 months	Rapid Development Spurt
Children	2 – 11 years	Slower Growth period
Adolescents	12 – 16 or 18 years	Hormonal Changes

Historically, children have been given medicines many of which have not been tested on them due to the ethical considerations of testing medicines in this age group along with the cost and practicalities associated with additional research in paediatrics. This meant that medicines legally used in paediatrics were often either completely unlicensed for this age group or not licensed for the particular condition being treated (e.g. used "off-label") (Conroy, 2011). The proportions of children receiving unlicensed or off-label medication differ depending on where the child is being treated and age of the child, with the youngest and sickest patients, such as those on a neonatal intensive care department, often receiving the most (Whittaker et al., 2009b). As a result of receiving medicines not tested on them, the consequences shown in Figure 1-2 may occur. Children may suffer from adverse events and medication errors may be increased due to the lack of age appropriate dosage forms requiring manipulations of adult dosage forms. Manipulations such as crushing tablets and opening capsules before dispersing the contents and administering a proportion have been shown to be less than reproducible at giving the required dose (Best et al., 2011, Nissen et al., 2009, Richey et al., 2012). Manipulated dosage forms are often poorly accepted resulting in a reduction in concordance (Milani et al., 2010).
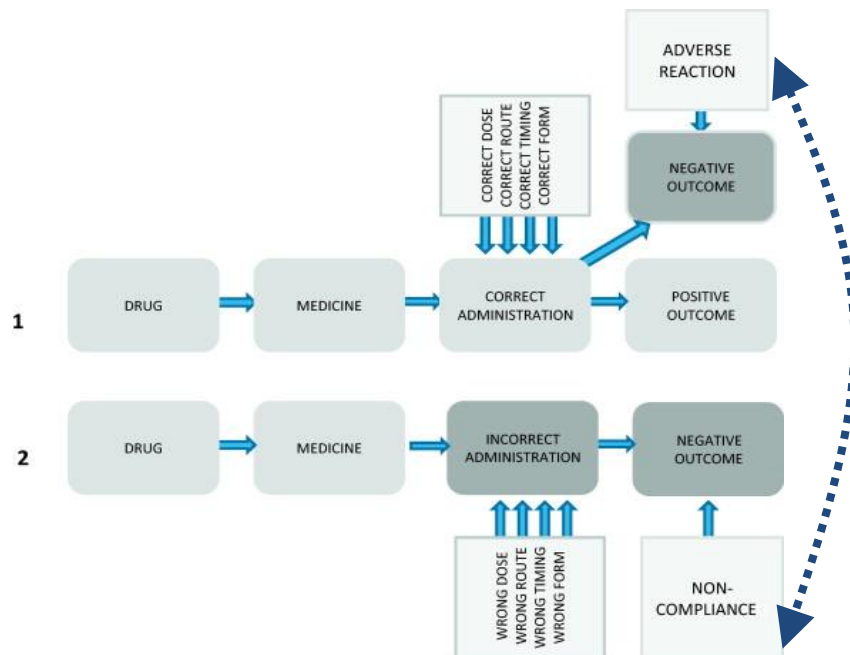
Figure 1-2: Graphical representation of potential negative outcomes caused by 1. Side effects despite correct dosing and 2. Medication error/non compliance with the arrow highlighting that these outcomes can be interlinked (Florence and Lee, 2011)

Since 2007, the advent of the Paediatric Regulation (Regulation (EC) No 1901/ 2006) has meant that companies must consider the need for data from paediatric studies to support a marketing authorization for a new chemical entity. If required, these paediatric studies must be undertaken in accordance with a paediatric investigation plan (PIP) which must be approved by the European Medicines Agency (EMA) Paediatric Committee. The PIP shall specify the timing and the measures proposed to access the quality, safety and efficacy of the medicinal product in all subsets of the paediatric population concerned. As part of the PIP, an age appropriate formulation must be developed meaning that companies now have a legal requirement to develop these which has further increased discussion and research into this area. If there is no therapeutic need for the drug in children, this requirement for data will be waived: The requirement can be deferred if, for example, research is required in adults first to ensure the trials done on children are both safe and ethical. Companies can also benefit from use of the Paediatric Use Marketing Authorisation (PUMA) to undertake research on off-patent medicines with the reward of a ten year period of market exclusivity.

Around 26% of the world's population are under 15 years old, with higher proportions of young people found in developing countries (Population Reference Bureau, 2012). Despite this large patient group their particular formulation requirements are often not recognized nor catered for, many children still lack age-appropriate formulations to meet their specific needs which are discussed throughout Section 1.3.

The production of any dosage form is always a balance between what the pharmaceutical industry require and what the patient needs from a medicine, Figure 1-3 summarises the general requirements which need to be met for any medicine to satisfy all parties and that these requirements are interlinked. It is worth remembering that in paediatrics this will generally involve a third party as a caregiver as well. It can be seen from this figure that there is the potential for all of these requirements to be addressed through the use of multiparticulate dosage forms as will be highlighted throughout this thesis.
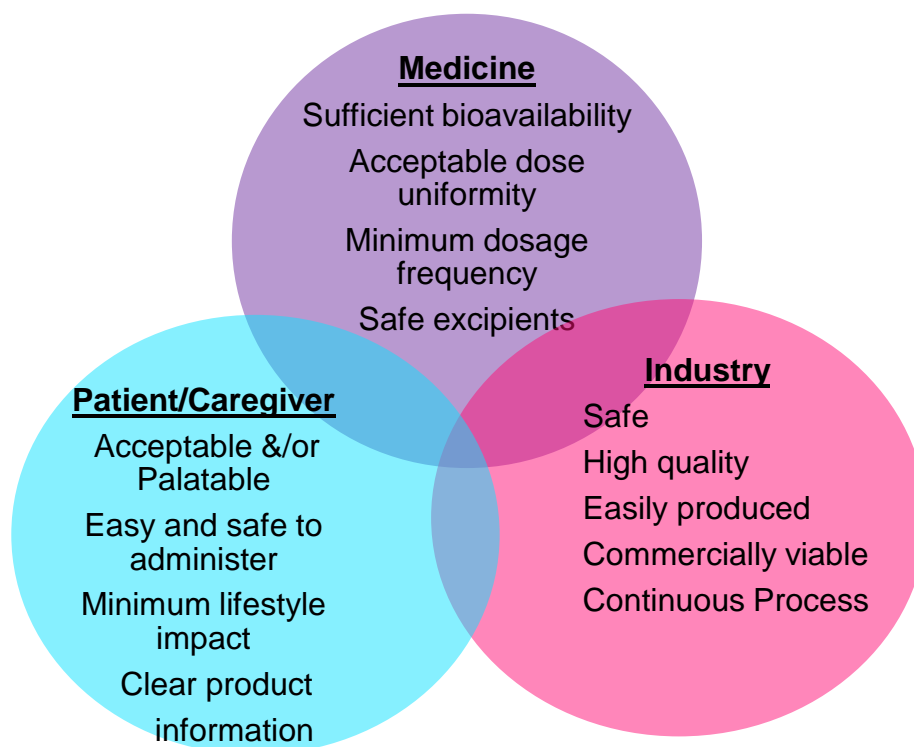
Figure 1-3: General Requirements for Medicines showing the Key Requirements for each Stakeholder with the areas of colour crossover highlighting the importance of considering all three stakeholders adapted from European Medicines Agency, 2005 and Krause & Breitkreutz 2008

1.3.   Challenges associated with Paediatric Formulations

1.3.1. Adherence

Medicine compliance is a problem in paediatric therapy with compliance rates ranging anywhere from 11-93% depending on many factors including frequency of therapy and taste (Matsui, 2007). When 88 US paediatricians were asked about barriers to treatment completion: frequency of dosing 96%/91% and unpleasant taste 91%/84% were reported as the top two barriers in children with acute/chronic illnesses respectively (American Academy of Pediatrics, 2000). These reasons for the lack of compliance are important since they may be overcome through the use of age appropriate taste-masked or modified release formulations such as functionalised multiparticulates.

1.3.1.1   Swallowability and Saliva pH

In general, children can swallow semi-solid foods from around six months so may be able to include multiparticulates in their favourite food, yet they cannot swallow conventional tablets until much older (Delaney and Arvedson, 2008, Rogers and Arvedson, 2005, European Medicines Agency, 2011). There is no clear evidence based answer as to at which age children can swallow monolithic dosage forms but is it is often considered to be around six years (Meltzer et al., 2006, Yeung and Wong, 2005). Paediatric patients with chronic conditions may be trained in order to swallow tablets by using sweets, a flavoured lubricant spray or head positioning techniques (Diamond and Lavallee, 2010).

Children aged between six months and two years of age can swallow minitablets (2 and 3 mm) which are a form of multi-particulate but only single minitablet administration has been tested (Thomson et al., 2009a, Spomer et al., 2012). Dysphagia (the inability to swallow) additionally affects many old people, who report difficulty in swallowing solid dose medication at some time so the geriatric population would benefit from multiparticulates as would many patients who require tube feeding (Stegemann et al., 2012, Stegemann et al., 2010). Predominantly, medicines are designed for oral administration and this will be the only route focused on in this thesis.

Chewable tablets exist which are most commonly available for children in the form of chewable vitamins bought over-the-counter by parents which may be "candy-like" with fears of potential overdoses (Lam et al., 2006). Chewable dosage forms have not been extensively used for prescription medicines and as many of them are both a chewable and dispersible form, the age range of which chewable tablets are accepted from is unclear (Strickley et al., 2008). There have been safety concerns over using chewable tablets in younger patients however, these tablets have not been seen be a major contributor to aspiration injuries (Michele et al., 2002) . Due to the mechanical nature of chewing increasing the surface area of the tablet, it would be difficult to

control drug release from the tablets in terms of modified release or taste masking.

The pH of the saliva is an important part of dosage form design, especially in the development of taste masked formulations. If a drug is coated with a polymer to prevent the bitter taste of the drug being detected by the taste buds, the polymer must not be soluble at the pH values found within the mouth – otherwise the drug will be released and the taste masking capacity of the polymer will be lost. Unstimulated salivary pH values have been seen to range between 7-7.5 in children aged between 3 and 13.years which is similar to that of adults (Sanchez and Fernandez De Preliasco, 2003, Wu et al., 2008). Unstimulated pH values were seen to be slightly lower in infants aged from 3 days to 12 months ranging from 6 – 6.74 (Ben-Aryeh et al., 1984). When the saliva is stimulated, for example by drinking soft drinks, the pH values of saliva from healthy children/adults was not seen to drop below pH which is likely to be due to the buffering capacity of saliva (Sanchez and Fernandez De Preliasco, 2003, O'Sullivan and Curzon, 2000, Meurman et al., 1987).

1.3.1.2.   Tolerance of Poor Taste/Acceptability

Taste can be defined as "*the sensation of flavour perceived in the mouth and throat on contact with a substance*" (Oxford Dictionary, 2012). It can be seen from Figure 1-4 that the tongue contains three types of gustatory papillae (circumvallate, foliate and fungiform) which are largely on the upper surface of the tongue (Jacob, n.d.). On these papillae are taste buds which contain taste receptor cells (shown by sensory cells in Figure 1-4), supporting cells, basal cells which are developing into taste cells and the gustatory afferent axon (nerve). The taste receptors have projections into the lumen of the taste buds which detect dissolved compounds. Taste signals are relayed from the receptor cell synapse at the base of the sensory cell to the brain via the cranial nerves IX and VII (in addition to X with signals from the extraoral receptors) to the nucleus tract solitarius in the medulla oblongata. From here, the signal is further relayed to the somatosensory cortex which is responsible for taste perception and the hypothalamus, amygdale and insula which cause, for example, aversions.
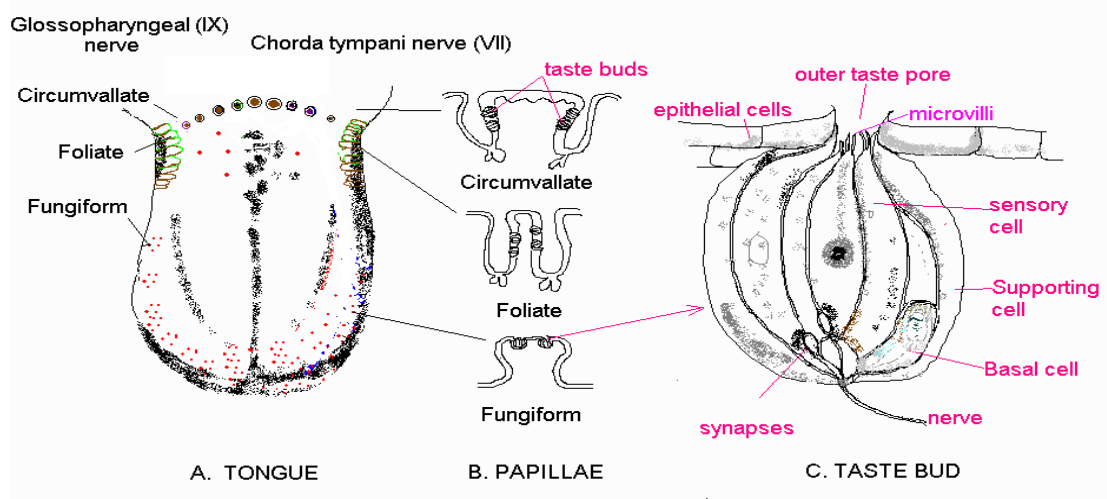
Figure 1-4: Representation of the Types and Location of Papillae on the Tongue with the Location and Structure of Taste Buds (Jacob, n.d.)

Different types of molecule illicit different taste responses and there are five primary taste sensations of sweet, sour, salty, bitter and unami (known as a savoury taste or the taste of certain amino acids) although overall taste is likely to be a blend of these. Signal transduction of taste stimuli is still not

fully understood but sweet, bitter and unami tastes are thought to be mediated largely via g-protein coupled receptors whereas salt is via sodium channel and sour by acid sensing channels, all causing depolarisation. Different types of g-protein coupled receptor exist where sweet substances binding to receptors known as T1R2 and T1R3 receptors, bitter substances bind to T2Rs and unami substances to T1R1 and T1R3 receptors. Bitter receptors known as T2Rs have more subtypes than other taste sensations which may be due to the large variety of different chemical structures that are detected as bitter.

There are around 25 members of the bitter receptor family known as TAS2R with one subtype, TAS2R38, having two different genetic forms where those with an inactive form are insensitive to the bitter taste of propylthiouracil. When children (range 3.1-10.9 years, n=448) were assessed for these forms, it was found that those who were sensitive to the taste were more likely to have tried solid dosage forms (52% vs. 36%) which are less in contact with the taste buds than liquid forms and those who were bitter sensitive preferred higher concentrations of sucrose (Lipchock et al., 2012). Similarly in young patients with HIV (median 2.9 years, Interquartile range: 2.5-3.26), where many of the medicines are known to be bitter, taste issues and vomiting were reported more often with syrup than tablets. By 8 weeks of therapy, tablets were preferred by the majority of patients and caregivers, despite the low age of patients (Nahirya-Ntege et al., 2012).

While often reported taste thresholds are shown in Table 1-2, taste perception differs depending on the individual's genetics and where they live - therefore not everyone is equally sensitive to tastes and these thresholds have not been validated in all the paediatric spectrum (Mennella et al., 2005). When 8-9 year boys and girls were compared to adult male and females, it was seen that the boys had a poorer ability to detect sucrose, sodium chloride, and citric acid than adults (e.g. higher concentrations were required before the chemicals were detected) along with a poorer ability than female adults to detect caffeine. However the responses of 8-9 year old girls to the chemicals were similar to those of adults suggesting that their gustatory

response was fully mature (James et al., 1997). In contrast, when 8-9 year old children were compared with adults looking at the sweetness of sucrose, similar responses were seen between adults and children for simple stimuli such as sucrose alone but not for complex stimuli like orange juice suggesting that taste perception was still developing (James et al., 2003).

Table 1-2: Thresholds for Common Tastes (Cardello, 1998)

	Substance	Threshold for Detection (M)
Salt	Sodium Chloride	0.01
Sweet	Sucrose	0.01
Sour	Hydrochloric acid	0.0009
Bitter	Quinine	0.000008
Unami	Glutamate	0.0007

The taste system of an infant develops throughout pregnancy and postnatally with taste cells first appearing in the foetus at 8-9 weeks of gestation and appearing mature by 13-15 weeks of gestation while the tongue continues to grow until it reaches adult dimensions at 15-16 years. Within a few hours of birth, infants prefer sweet/unami tastes and reject bitter substances – this trend of taste preference continues until adolescence (Mennella and Beauchamp, 2008). Children were seen to have higher optimal preferred sucrose concentrations than both adolescents and adults (De Graaf and Zandstra, 1999).

Many medicines by virtue of being external noxious substances to our body taste bitter in a physiological attempt to prevent administration which may be harmful and evoke a gustatory response e.g. vomiting (Shi et al., 2003). This is obviously problematic for children when they prefer sweet tastes and have a poor tolerance for bitterness.

Most medicines for adults are available as solid oral dosage forms such as capsules and coated tablets. They have fewer issues with any bitter tasting drugs as the dosage form stays intact within the mouth and hence the drug does not become in contact in solution with the taste receptor and therefore

no adverse taste is recognised. Even if taste is recognised as an issue in a dosage form, the medicine can be encapsulated or coated and adults are able to logically determine that they need to take their medicine for the desired therapeutic outcome, even if they do not like the taste of it and anecdotally may even believe that a worse tasting medicine is stronger!

There are two main formulation methods of overcoming the problem of objectionable taste: either to obscure it by the addition of various additives or to prevent/reduce the concentration of the drug coming into contact with the taste buds (Ayenew et al., 2009). Even where approach of preventing/reducing the contact of the drug with the taste buds is used, it formulation will still probably need a degree of flavouring/sweetening to aid acceptability.

Historically, medicines were made to taste better "*with a spoonful of sugar to make the medicine go down*". The problem of administration of unpleasant tasting medicines has been around for centuries with advice from the 1800s recommending the use of sweeteners such as sugar or jam to make medicines palatable (Churchill, 1883). This attempt to obscure the taste of the medicine was used with commonly used suspensions such as Calpol® (containing the bitter drug paracetamol) containing sugar, sweeteners and flavouring agents. In the United Kingdom, this was known to be very much liked in children and anecdotally evokes fond memories amongst adults when remembered. Over the years, this product has been reformulated into a sugar-free and subsequently a sugar-/colour-free suspension as there becomes increased awareness of the lack of inertness of excipients yet there is a lack of evidence showing improved safety from the removal of these excipients (Fabiano et al., 2011).

There are a variety of "additives" to be used in taste masking such as sweetening agents, flavouring agents, salts and viscosity enhancers which have been employed with varying degrees of success

The sweeteners used can either be 'natural' chemicals such sucrose, which has fallen out of favour due to its impact on dental caries, or artificial sweeteners, which despite having enhanced sweetness intensity can have a metallic/bitter aftertaste and increased toxicity concerns (Feigal et al., 1981). High concentrations of sweeteners may be required to try to taste mask which may be associated with adverse effects and many sweetening agents are short acting so do not cover the after taste associated with a medicine. As an example, epinephrine was unable to be taste masked through the addition of aspartame or acesulfame potassium but the acidic nature of added citric acid imparted an acceptable "lemon like" flavor (Rachid et al., 2010).

Flavouring agents such as fruit flavours can suffer from poor stability due to the volatile components of the essential oils used in them and can show limited acceptability dependent upon cultural and individual tastes (e.g. cherry and bubble gum flavour are common in the United States whereas less common in the United Kingdom). A flavouring agent cannot cover the taste of all drugs and must be determined on a case-by-case basis which may require time consuming sensory trials though these may now be supported through the use of an electronic tongue. An undisclosed drug had its taste improved by the addition of a cherry or lemon flavour whereas grape and vanilla flavours were found to make the taste worse (Campbell et al., 2012)! The addition of sodium chloride and glutamate were seen to improve the taste of a range of drugs including pseudoephedrine and quinine but the effect of the salt on the overall dosage form must also be considered (Campbell et al., 2012, Rachid et al., 2010).

The taste of a drug can be masked through the addition of a chemical that interacts with the taste buds so that it is not able to combine and be tasted: an example of this is of enterodiol (25 mg/L) which interacted with the protein model of the bitterness receptor hTas2R10 to reduced the caffeine (500 mg/L) bitterness intensity by 30% in an adult sensory trial (n=22) but so far has been of limited pharmaceutical use (Ley et al., 2012, Ley, 2008).

Not having the drug in an aqueous solution is a way to reduce the bitter taste of a drug, this can be achieved by preparing the drug e.g. in a lipid vehicle such as medium chain triglycerides or by preparing the drug in a suspension (which may be beneficial if the drug already has low solubility) (Bahal et al., 2003). However, even in a non-solution dosage form, the concentration of drug in solution, even if low, might reach the taste threshold (Lorenz et al., 2009). Drug modification to reduce solubility such as adding a prodrug moiety works by a similar effect but would be labour intensive and may modify the release/therapeutic nature of the compound (Hejaz et al., 2012).

An additional way to reduce the bitter taste is by either molecular or inclusion complexation of the drug so that it is unable to interact with the taste buds. This approach is very drug specific since it is dependent upon the interaction between the drug and the complexing agent so would need to be individually assessed. Common agents used in this type of complexation include modified cyclodextrins and a cationic polymer, Eudragit® E as described later in Section 1.4 and Chapter 4 (Orlu-Gul et al., 2012, Szejtli and Szente, 2005, Randale et al., 2010, Khan et al., 2007). Ion Exchange Resins work on this principle and are discussed in Section 1.5.

A final approach to reducing the problem of bitter taste is to apply a barrier between the drug and the taste bud. In adults this would be achieved by coating a tablet, coating the dosage form is still an option but difficult with smaller dosage forms that may be desired for children for ease of swallowability. Encapsulation of the drug within a barrier/polymer is a way to produce particles which are taste masked as has been achieved by extrusion of lipid components or through microencapsulation (Krause et al., 2009).

The most commonly used forms of microencapsulation (particle size <100 µm) used for taste masking are:

- Emulsification/solvent evaporation (Gao et al., 2006, Hashimoto et al., 2002, Chiappetta et al., 2009)

- Spray drying (Xu et al., 2008a, Sollohub et al., 2011, Bora et al., 2008)
- Co-acervation (Shah et al., 2008, Yoshida et al., 2009)

Commonly used polymers include the poly (meth) acrylates and cellulosic derivatives as discussed in Section 1.4. and Chapter 4.

The approach of encapsulating a drug to prevent release is desirable because it can be used as a drug independent platform for taste masking rather than other types which depend on individual research. This encapsulation depends upon the drug not releasing an appreciable drug concentration at the near neutral pH in the mouth or for the time it is being administered as is discussed in Section 1.5. Moreover, the particles produced must not be gritty otherwise this can adversely affect acceptability of the dosage form as discussed in Chapter 3.

1.3.1.3. *Need for Medicines at School*

The vast majority of oral medicines on the market are available as immediate-release capsules and tablets (British Medical Association and the Royal Pharmaceutical Society of Great Britain, 2008a). Although these monolithic, solid dosage forms are successfully used in the pharmacological management of a variety of conditions, drug delivery systems which are able to offer modified drug release may offer a number of benefits over immediate release preparations. One of the most notable is increased patient compliance due to a decreased dosing frequency down to once daily dosing in an ideal situation as illustrated in Figure 1-5. Once daily dosing would be especially desirable for groups such as school children who are often unable to receive medicines whilst at school, or elderly patients who may have difficulty in remembering to take medicines more frequently or at different times of the day (Wong et al., 2004). Modified release preparations can also offer reduced adverse drug effects associated with high plasma concentrations and maintaining stable concentrations is of benefit in many chronic conditions or where constant drug levels are required (National Prescribing Centre, 2000).
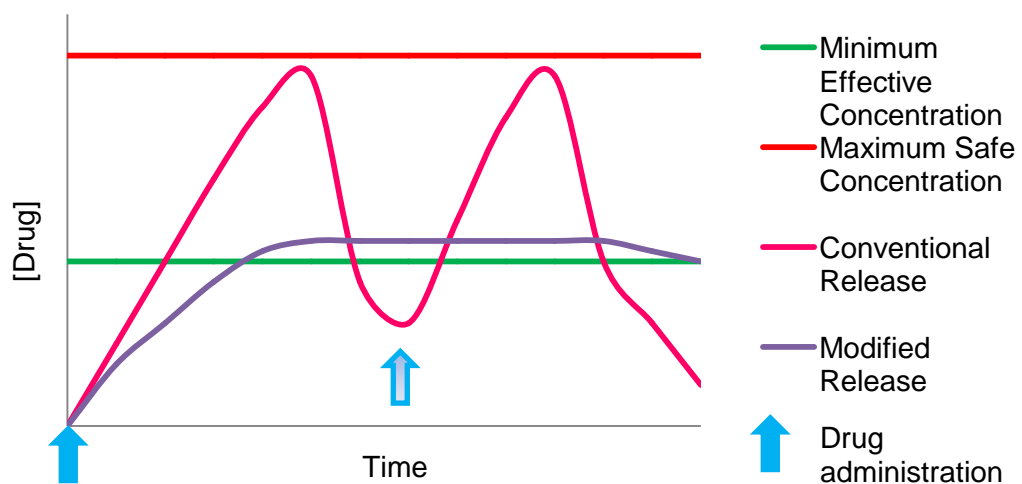
Figure 1-5: Schematic Representation comparing Conventional and Modified Release Drug Profiles

It can therefore be seen that taste masking by preventing the release of the drug in the mouth may be classed as delayed or modified drug release but may also be classed as immediate release if 80% of the drug is released within 45 minutes in the gastric contents. This will depend on the intent of the formulator and the target product profile desired.

The terms delayed or modified release includes the pH dependent release of drugs, either to:

- Target the dosage form to a specific area of the gastro-intestinal tract (GIT) for example budesonide to the colon in ulcerative colitis (Varshosaz et al., 2011)
- Prevent release in certain areas e.g. enteric coating of non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac to protect the gastric mucosa (Rattes and Oliveira, 2007)
- Protect the drug from releasing in the stomach if it is acid-labile (e.g. omeprazole) (Ponrouch et al., 2010)

There are a number of solid oral dosage forms that provide controlled drug release on the market (95 individual drugs contained within 218 formulations in the British National Formulary 2008 when the terms sustained, delayed, repeat prolonged, controlled, modified and enteric were searched) (British Medical Association and the Royal Pharmaceutical Society of Great Britain, 2008a). Modified release dosage forms contain a larger dose of drug to allow for less frequent dosing and additional excipients to control release when compared to immediate release tablets. Due to this increased drug and excipient load in modified release tablets, they are often of a larger size than those tablets designed for immediate drug release. These large, modified release formulations may be difficult to swallow, especially for paediatric and geriatric patients but also other patients with dysphagia. Tablets have an additional drawback for these patient groups in that, they are unable to provide the range of doses required in heterogeneous population. Dosing needs based on age, weight, surface area or adapted to hepatic or renal

function cannot be met as it is recommended that controlled release formulations are not split or crushed. Using a small in sized dosage form such as a functionalised multiparticulate would allow the benefits of modified release dosage forms to be experienced by those patients who struggle to or are unable to swallow larger dosage forms such as tablets and capsules.

1.3.1.4.   Industrial Need for a Universal Platform Approach

As the Paediatric Rule discussed in Section 1.2. only came into existence within the past ten years, companies still have limited experience of formulating for children. This combined with the consequence of the increasing cost of drug development makes it of benefit for industry to use formulations known as "platform formulations" which are base formulations without drug into which a variety of drugs can be added without needing to develop a formulation from scratch for each individual drug. There is clinical, scientific and commercial value of a platform formulation including quicker access of the paediatric patients to an effective treatment, better scientific understanding of formulation to tailor release to requirements and reduced formulation cost allowing better patient access with the potential for industry collaboration.

This need for a universal platform includes the requirement that dosage forms researched can potentially be scaled up and made available for clinical practice. In terms of manufacturing multiparticulates, the pharmaceutical industry already use and have products on the market produced by large scale granulation, pelletization and spray drying whereas other laboratory scale techniques such as emulsification/solvent evaporation would be more difficult to try to scale up.

In terms of considering cost and developability from the industrial prospective, organic solvents should be avoided as discussed in Chapter 4 and the minimum required levels/numbers of excipients used which comes in line with excipients not being inert from the patient perspective.

1.3.2. Possible Approaches to Meet Paediatric Medicine Requirements

1.3.2.1 Liquids

Liquid dosage forms are still considered the "gold standard" by many for children largely due to the ease with which they can be swallowed and the ability to give a range of different doses as the child matures and dose weight/volume increases. However despite these key advantages, liquids have a number of disadvantages.

Liquids can be difficult to administer in the range of different implements used shown in Figure 1-6: it can be seen from these that the potential for spilling the medicine if trying to administer to a reluctant child is high and volume errors, which may be as high as a factor of 10 may occur due to high drug concentrations (Yin et al., 2010, McMahon et al., 1997, Madlon-Kay and Mosch, 2000).

Figure 1-6: A Selection of Administration Devices for Liquid Medications

It is difficult to achieve functionalised capacity in a liquid in terms of taste masking or controlled release. Few controlled release liquid forms exist that are based on ion exchange resins or multiparticulates as described in Section 1.6. In situ gelling systems seem an interesting approach to controlled release liquids but due to the intricacies of gelation, must be formulated on a drug-by-drug basis (Itoh et al., 2011, Itoh et al., 2010).

Often the need for excipients in liquid is higher in quantity than those in solid dosage forms (e.g. in mg/ml compared with a few mg in a tablet) and in a larger variety to address the additional taste & stability challenges as discussed in Section 1.3.2.1. and Chapter 2 respectively.

Many of the excipients currently in use in products designed for paediatric use are deemed to be acceptable due to their long standing use in children. However some excipients are known to cause problems in specific subsets of the paediatric population such as preservatives, co-solvents, and sweeteners. Some examples of problematic excipients are shown in Table 1-3 (Breitkreutz and Boos, 2007b, Whittaker et al., 2009a).

Adverse events are even more common in younger children due to the immaturity of their renal and hepatic clearance as well as physiological differences such as altered body composition and a higher likelihood of allergies due to their developing immune system.(Whittaker et al., 2009a, Alcorn and McNamara, 2003)

Table 1-3: Examples of Excipients and their Adverse Effects in Children from Ernest et al., 2007

Excipient	Use	Adverse Effect
Aspartame	Sweetener	Potential issues in phenylketonurics
Polyols	Bulking agent/ vehicle	GI disturbances
Benzyl alcohol	Antimicrobial preservative	Gasping syndrome which can cause fatalities
Carrageenan	Suspending agent	Induces inflammatory responses in animals
Diethylene glycol	Co-solvent/Vehicle	Poisoning
Docusate sodium	Wetting agent	Diarrhoea
Ethanol	Co-solvent	Neurotoxicity
Propylene glycol	Co-Solvent/Anti-microbial Preservative	CNS adverse events

Due to the limited evidence base surrounding the use of many excipients in children, a risk vs. benefit analysis must underlie the decision to give any excipient to a child (Salunke et al., 2012). The excipient used, in order to minimise risk, should be technically necessary, those that we know the most about (which may be in relation to patient metabolic activity) and in the lowest dose/exposure possible as "*the only difference between a cure and a poison is the dose*" Paracelsus (1493-1541). Nevertheless the advantages of a liquid dosage form means that many formulations for paediatrics are liquids containing a myriad of technically necessary excipients.

1.3.2.2.   *Solid Oral Dosage Forms*

It terms of multiparticulates it can be seen from Table 1-4 that powders or multiparticulates are accepted by most age groups except preterm newborn infants for whom the oral route is not usually used. While this table from the EMA is used to define acceptability of various dosage forms in different aged children, it is important to recognize that the table was developed after asking only around 40 patients, health care professionals and parents and these mainly German, which dosage forms they thought each age range would be able to accept. This highlights the importance of the need for more research into the acceptability of dosage forms in children as discussed further in Chapter 3.

Table 1-4: Acceptability of Dosage Forms for Different Ages of Children (European Medicines Agency, 2005)

Oral Dosage Form	Preterm newborn infants	Term newborn infants (0d-28d)	Infants and Toddlers (1m-2y)	Children (pre school) (2-5y)	Children (school age) (6-11y)	Adolescent (12-16/18y)
Solution/ Drops	2	4	5	5	4	4
Emulsion/ Suspension	2	3	4	5	4	4
Effervescent Dosage Forms	2	4	5	5	4	4
Powders/Multi-particulates	1	2	2	4	4	5
Tablets	1	1	1	3	4	5
Capsules	1	1	1	2	4	5
Orodispersable Dosage Forms	1	2	3	4	5	5
Chewable tablets	1	1	1	3	5	5
Key: 1 = not accepted, 2 = accepted under reserve/reluctantly, 3 = acceptable, 4 = preferred acceptability, 5 = dosage form of choice						

In addition, an expert panel found that there was a "*general acceptance of the benefits of flexible solid dosage forms over liquid dosage forms for stability, dosing and administration issues*" with less excipients in quantity (dependent upon dose volume), especially when used in developing countries - these forms include multiparticulates with benefits such as: (World Health Organisation, 2008)

- ✓ Ease of Swallowing
- ✓ Stability
- ✓ Dose Flexibility
- ✓ Dose Uniformity
- ✓ Ease of Administration

- ✓ Range of Types Available
- ✓ Taste masking
- ✓ Modified Release
- ✓ Excipients
- ✓ Price

These flexible oral dosage forms are recommended as most suitable for developing countries due to the difficulty and high cost of transporting liquids, difficulty in obtaining clean water and for those medicines where precise dose measurement or titration are required to allow production of 'tailored' doses and strengths. Flexible oral dosage forms allow for the preparation of a range of dosage form e.g. by using one multiparticulate formulation in a variety of ways such as granules to be compressed into tablets for adults and administered as a sprinkle on food for children. Multiparticulates offer benefits over liquids for substances that are not stable or cannot be taste masked in liquid preparations or to provide controlled release although the high surface area to volume ratio may mean make it difficult to control release using multiparticulates (European Medicines Agency, 2005).

There are some potential disadvantages to using multiparticulates with one being the risk of choking/aspiration of dosage forms however no literature was found on this and multiparticulates are often used in food on children who are already weaned. It is not known what size of multiparticulate would be assessed as gritty and there may also have to be considerations as to what dose can be administered in this way as investigated in Chapter 3. Lastly the method of administration needs to be decided – often this is as a sprinkle on food where issues of uniformity and compatability may arise – different method of administering multiparticulates are examined in Section 1.5.

In summary, multiparticulates provide an excellent formulation for children due to their small size for swallowability, dose adaptability and ability to provide taste masking/controlled release functionalities (they provide many of the advantages of tablets without the disadvantages). However for ease of administration/compatability and to provide a dosage form for pre-weaning children, multiparticulates could be suspended in a liquid.

1.4.   Preparation of Multiparticulate Dosage Forms

The most common ways to prepare commercial multi-particulate dosage forms are to prepare granules or produce pellets by extrusion/spheronisation, or coat drug onto ready prepared spheres which could them be functionalized by coating. Lately minitablets (tablets usually smaller than 5 mm) have been proposed as a multiparticulate dosage form of interest for children since the small mass of drug which can be contained within each minitablet would necessitate the dosing of multiple minitablets for all those but the most potent drugs (Thomson et al., 2009b, Tissen et al., 2011).

As the suspension of multiparticulates in a suspending vehicle would be desirable for administration, it is thought given the size range of granules, pellets and minitablets are often in the hundreds of microns to millimeters in size that all of these approaches will make particles too large to suspend. Hence the production of microparticles by solvent evaporation, coacervation and spray drying which can produce small (<100 μm particles) are considered.

1.4.1. Controlling Drug Release

Multiparticulates are typically used to control drug release to achieve an appropriate pharmacokinetic profile. Controlling drug release depends on the matrix the drug is entrapped in and the physicochemical properties of both the drug and polymer as shown for Eudragit® L or S particles containing dipyridamole, cinnarizine, amprenavir, bendroflumethiazide, budesonide and prednisolone (Alhnan et al., 2010). Polymer molecular weight/blending and crystallinity are important in controlling drug release profiles (Alhnan and Basit, 2011). Drug release can be triggered by a range of variables as well covered in a review by Freiberg and Zhu, 2004 including pH (Rattes and Oliveria, 2007, Xu et al., 2008b), ionic strength (Asare-Addo et al., 2013, Qiu and Park, 2012) and enzymes (Hu et al., 2012, Thornton et al., 2007). Once the polymer/matrix reaches the appropriate conditions for drug release the

drug may diffuse through pores or be released by degradation/erosion of the polymer or by osmosis (Rizi et al., 2011).

There is often an initial burst release of drug from the surface (presumably due to drug that has not been successfully captured by the control mechanism) followed by a more constant release phase as seen for ibuprofen microparticles coated with ethylene vinyl acetate, ethyl cellulose, ethyl cellulose aqueous dispersion, polyethacrylate or Eudragit® NE 30D or carnauba (Sriamornsak et al., 2011). Drug release can be modified by the location of drug in the multiparticulate – if it is uniformly dispersed it may have a larger initial burst than if most of the drug is encompassed in the core of the microsphere; if it is encompassed mainly in the core, release will be retarded. In microspheres where the drug is variably and unpredictably dispersed, the release will be variable and difficult to control – the location of drug can be assessed by confocal laser scanning microscopy as in the case of Eudragit® L containing riboflavin, dipyridamole and acridine orange (Nilkumhang et al., 2009).

In light of this, microspheres can be further modified by the addition of an outer layer or shell to enhance controlled release and reduce the initial burst effect. For example by coating highly porous particles or in pH-dependent delivery where the shell will degrade at a specific pH value and leave the core to provide prolonged release as in the case of erodible microcapsules of diclofenac coated with CAP and EC, or to encompass two different drugs such as codeine and chlorpheniramine (Biju et al., 2004, Zeng et al., 2007). However as coated microspheres will be larger than uncoated microspheres, it may be that the larger size of coated microspheres are more difficult to suspend if they are desired to be administered in a suspension formulation. Washing the microspheres to remove surface drug or curing the microspheres may further modify drug release after manufacture as in the case of ibuprofen loaded poly(D,L-lactic acid) microspheres washed with sodium carbonate solution (Leo et al., 2000).

Many of the microparticles prepared by various methods have the disadvantage of a low mass of drug relative to that of polymer with drug to polymer ratios of 1:10 not uncommon (Al-Zoubi et al., 2008b). This high mass of polymer is less acceptable in paediatrics as it may result in a proportionally higher dose-volume which is more difficult to give a child. It also means more polymers which may not necessarily have much relevant toxicity information in the younger age groups e.g. phtalates.

The use of lower ratios of polymer to drug has found incomplete microparticle formation with drug crystals not only on the microparticles but also existing as discrete crystals. This may be due to how the particles are prepared e.g. from the solution or solid drug dispersion: As a result of crystal growth, an uneven, aggregated product can be formed as seen for Eudragit® RS or Kollicoat SR microparticles containing buspirone or paracetamol containing particles produced using various cellulose derivatives (Al-Zoubi et ail., 2008a, Billon et al., 1999). This impacts on drug release rates as well as make the microparticles more difficult to handle due to their impaired flow properties.

1.4.2. Polymers

Polymers can be water soluble or insoluble. They can also exhibit pH dependent or independent solubility. In the course of trying to make a functionalised suspension from multi-particulates, insoluble polymers or pH sensitive polymers are likely to have increased release retardation in liquid compared with hydrophilic polymers. Two of the larger classes of polymers are those of the cellulose derivatives and methacrylates with the former summarised in Table 1-5 .

Table 1-5: Summary of Commonly Used Cellulose Derivatives and their Solubility

Water Soluble	Water Insoluble	pH Sensitive
Hydroxypropylmethylcellulose Hydroxypropylcellulose Hydroxyethylcellulose Sodium Carboxymethylcellulose	Ethylcellulose Cellulose acetate	Cellulose acetate phthalate HPMC acetate succinate Cellulose acetate trimellitate Hydroxypropylcellulose phthalate

Eudragit® polymers are methyacrylic acid copolymers available in a variety of different grades for different purposes. The different grades can be used to provide enteric-coating, immediate release or sustained release. Varying the ratios of the different polymers and the film thickness can customize drug release profiles. The different polymers are described in Table 1-6

Other polymers such as chitosans, alginates and gelatin along with lipid components such as wax have also been used for multiparticulate preparation. There was insufficient time to explore the utility of all of these polymers in the course of this thesis work. There may be value in exploring these polymers in a blend with the Eudragit® E since the polymer used did not achieve the required robust encapsulation of the trial drugs used but this may be aided by polymers of possessing difference characteristics such as different glass transition temperatures (discussed in Chapter 4).

Table 1-6: Description of the Composition and Functionalities of Different Eudragit®
Polymers (Evonik Industries, n.d.)

Polymer	Description	Formulation	Dissolution Properties	Use
L 100-55 L 30 D-55	Methacrylic Acid - Ethyl Acrylate Copolymer (1:1)	Powder Aqueous dispersion 30 %	Dissolves at < pH 5.5	Enteric Coating
L 100 L 12.5	Methacrylic Acid - Methyl Methacrylate Copolymer (1:1)"	Powder Organic solution 12.5 %	Dissolves at < pH 6	
S 100 S 12.5	Methacrylic Acid - Methyl Methacrylate Copolymer (1:2)	Powder Organic solution 12.5 %	Dissolves at < pH 7	
FS 30D	Anionic copolymer based on methyl acrylate, methyl methacrylate and methacrylic acid	Aqueous dispersion 30 %	Dissolves at < pH 7	
RL 30D RL PO RL 100 RL 12.5	Ammonio Methacrylate Copolymer Type A	Aqueous dispersion 30 % Powder, Granules Organic solution 12.5 %	Insoluble High permeability PH independent swelling	Sustained Release
RS 30D RS PO RS 100 RS 12.5	Ammonio Methacrylate Copolymer Type B	Aqueous dispersion 30 % Powder ,Granules Organic solution 12.5 %	Insoluble Low permeability pH independent swelling	
NE 30D NE 40D	Polyacrylate (ethyl acrylate and methyl methacrylate) Dispersion 30 %	Aqueous dispersion 30% 40 %	Insoluble Low permeability pH independent swelling	
E 100 E PO E 12.5	Basic butylated methacrylate copolymer	Granules Powder Dispersion	Soluble in gastric fluid up to pH 5, swellable and permeable above Ph5	Protection/ Taste Masking

1.4.2. Production Methods

The three ways most commonly used to produce multiparticulates of a particle size <100 μm are emulsification/solvent evaporation, co-acervation and spray drying as detailed below.

1.4.3.1.   Emulsification and Solvent Evaporation

The technique of emulsification/solvent evaporation depends upon the aqueous solubility of the drug to be entrapped. If the drug in question is hydrophobic then it can be combined with the polymer in an organic solvent (known as the disperse phase), this is then emulsified by the addition of an aqueous continuous phase. Following the formation of this oil-in-water emulsion (o/w), the solvent in the disperse phase diffuses into the continuous phase so that the disperse phase forms solid particles which can be removed and undergo further drying to remove the residual organic solvent as summarised in Figure 1-7
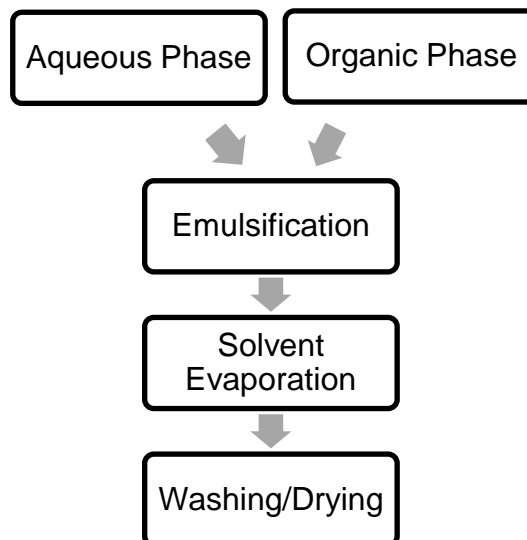
Figure 1-7: Overview of a Simple Emulsification/Solvent Evaporation Process

The situation is not as straight forward for hydrophilic drugs since if the o/w emulsification method is used then the hydrophilic drug would leech out into the aqueous continuous phase and hence the drug would be lost. This

means that there are four different ways in which to entrap hydrophilic drugs: by preparing a water-in-oil-in-water (w/o/w) double emulsion, using a co-solvent to dissolve the drug in an organic disperse phase, dispersing the drug as a powder in a polymer/organic solvent solution or making an oil-in-oil (o/o) emulsion.

Co-solvents can be added to help the drug dissolve in the disperse phase (ethanol is a common choice due to its miscibility with water) or porosity generators such as hexane can be used to produce pores in the microspheres to increase drug release rate in the case of aspirin microcapsules containing aspirin (Yang et al., 2000). However increasing the porosity of the microspheres may also decrease encapsulation efficiency so this must be balanced with the requirements of the product. Porosity generators can also reduce diameter and lead to an irregular surface morphology (Yang et al., 2000). Reducing the water content of the first emulsion can also decrease the porosity as can using a more volatile solvent as can increasing the water content of the second emulsion (due to increased polymer precipitation) – up to a certain rate where the increased solvent removal will form pores as was seen in the case of somatostatin (Herrmann and Bodmeier, 1998).

Surfactants (amphiphilic molecules that consist of a hydrophobic and hydrophilic section) may be added to the continuous phase to ensure uniform sized microspheres with a small size distribution and predictable drug release. These may be anionic (-ve charge) like SDS, cationic, amphoteric (anionic in alkaline pH and cationic in acidic pH) or non-ionic like partially hydrolysed PVA: increasing concentrations create smaller microparticles by lowering the surface tension of the continuous phase until the CMC is met (Yang et al., 2001)

A number of particle parameters can be modified to achieve the size, morphology and drug encapsulation desired. The polymer concentration or molecular weight can be increased which will increase the viscosity of the disperse phase: this leads to a larger, smoother microsphere with increased

encapsulation efficiency and slower drug release (Alhnan et al., 2010). The volume of disperse phase relative to continuous phase can be increased which may decrease diameter or may have no impact. Increasing the quantity of drug in the dispersed phase can make the microsphere become more porous and have a more irregular shape; it may also reduce encapsulation efficiency due to the formation of pores if the quantity of drug contained is too high (Witschi and Doelker, 1998).

Operating conditions can be modified such as increasing the agitation rate to decrease the average size of microspheres (Freitas et al., 2005). The rate of solvent evaporation can be increased by either increasing the temperature or by reducing the pressure - However increasing the temperature also increases the particle size formed and makes the particles less uniform, decreases encapsulation and reduces particle recovery (Witschi and Doelker, 1998). The boiling point of the solvent and thermal stability of the drug must also be considered. Hence reducing the system pressure may be more beneficial especially since it can also improve the drug encapsulation efficiency and smooth surface/smaller surface area (thought to be due to the solvent removal being too rapid for the polymer to crystallise) (Alhnan and Basit, 2011). Care must be taken not to reduce the pressure too far as once it becomes lower than the saturated vapour pressure of the solvent, the solvent will start to boil and the bubbles produced will destroy any potential microspheres. Low temperatures have also been shown to increase the particle size due to an increased viscosity.

Particles have been produced by emulsification/solvent evaporation for use in suspensions as detailed in Section 1.4. with Eudragit® polymers and cellulose derivatives proving popular. In terms of taste masking, a range of drugs have been successively masked by this method (Gao et al., 2006, Hashimoto et al., 2002, Chiappetta et al., 2009).

Despite the promise of this emulsification approach for microparticle production, this technique was not chosen as to date there is a lack of information on performing this technique on an industrial scale, so does not

fit the industry requirement for a manufacturable platform formulation approach. In addition the need for specific solvents for each drug does not suit this methodology to providing a platform approach for multiple drugs.

1.4.3.2. Coacervation

Particles can be formed by precipitation which is where an insoluble solid is formed from a solution such as in a coacervation approach. There are three types of coacervation: simple, complex and salt. Simple coacervation is when a water-miscible non-solvent for example ethanol is added to an aqueous polymer solution which causes the formation of a polymer rich phase to form microparticles around a drug that may either remain in solution in the polymer or form insoluble particle. Complex coacervation depends on mutual neutralisation between two oppositely charged colloids (usually positively charged gelatin with a negatively charged component). Salt coacervation is where an electrolyte is added to an aqueous solution which results in the polymer separating from the aqueous solution.

Microcapsules of indomethacin were produced using gelatin-cellulose acetate phthalate by complex coacervation or cellulose acetate phthalate by simple coacervation prior to incorporation into slurry to make sustained-release tablets. The morphology was found to be temperature-dependent. For both polymers, the majority of the microcapsules were in the size range 15 to 60 μm. Although cellulose acetate phthalate appears to encompass the drug more effectively, the higher drug loadings of the complex gelatin-cellulose acetate phthalate may be a consequence of a higher proportion of drug being used in the preparation so may be a result of incomplete coacervation. Tablets prepared from these were physically and chemically stable with both showing sustained release and reducing stomach irritation in rats. However as the gelatin containing microsphere tablets were linked with formaldehyde and the other microspheres with the less toxic, acetic acid, and there is no significant difference in activity between the two it would make sense to choose the cellulose phthalate microsphere tablets (Lu et al., 2007).

Previously a number of approaches to make pH sensitive microparticles for site-specific delivery to the gastro-intestinal tract using Eudragit® polymers were investigated (Kendall, 2007). It was found that a simple, non-aqueous coacervation technique was successfully able to produce microparticles but it was not possible to harvest these due to agglomeration of the particles and phase-separation on removal of the solvent phase. It was also found that aqueous spray drying was unsuccessful, as collapsed microparticles were produced which were unable to control drug release over acidic pH values. The problems of these above techniques were overcome by producing microparticles through an emulsification/solvent evaporation process.

Co-acervation had also been used to make taste masked particles of mefloquine with Eudragit® E and clarithromycin with gelatin/various Eudragit® polymer coatings (Shah et al., 2008, Friend, 1992).

Again, coacervation was not considered a suitable approach for this project as; coacervation can be difficult to control in terms of size and agglomeration; the multiple steps and use of organic solvents render it difficult to scale up above lab scale and the requirement for specific solvent/antisolvent pairs for different drugs mean that these cannot be adapted into a platform approach for multiple drugs.

1.4.3.3.   Spray Drying

Due to its adaptability and industrial scale applicability spray drying suggests itself as the most likely approach to producing microparticles that could be of benefit in this application. Spray Drying is a process whereby a liquid feed is transformed into a dry particle by spraying the feed into hot gas. It can be seen to consist of 4 main steps:

1.   Atomisation of the Feed Spray
2.   Air-Fluid Contacts
3.   Particle Drying
4.   Separation of Spray Dried Particles from the Drying Air

The progress of spray drying of the solution or suspension feed can be seen visually in Figure 1-8 where the feed is pumped through the spray dryer until it is atomised at the nozzle and reaches the drying chamber before the particles are separated as they reach the collection container after passing through the cyclone.
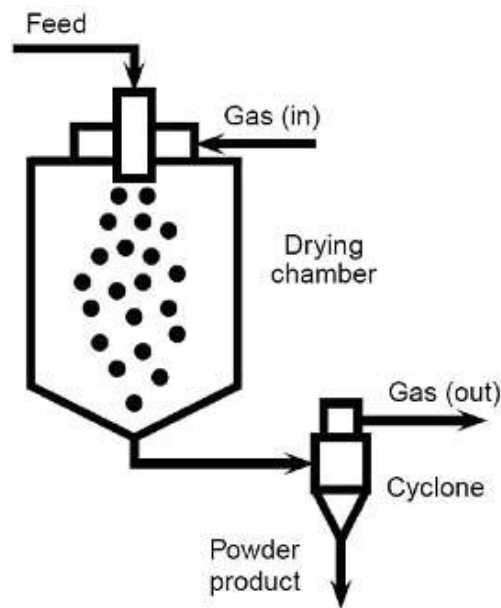
Figure 1-8: Diagram of Spray Drying Product Flow (CHEC Research Centre, 2007)

Spray Drying has been commonly used by the food, agricultural and pharmaceutical industries for a number of years. Spray drying is a frequently used technique as it is a one-stage technique to dry and embed the drug into a polymer network that can be relatively easily scaled up to an industrial scale. Its advantages include short duration, reproducible results, cost-effectiveness, good flow properties and with a good yield of production and encapsulating efficiency (Masters, 1991).

The properties of the spray-dried powders can be controlled by the polymer used, operating conditions and properties of the feed to be spray dried (Al-Zoubi et al., 2008a). Modifiable parameters include inlet and outlet temperatures, spray-rate and drug-polymer ratio of the feed as are shown in Table 1-7.

Table 1-7: Effect of Different Spray Drying Parameters on Product (Xu et al., 2008a, Rattes and Oliveira, 2007, Wan et al., 1992)

Parameter	Effect on Particle Characteristics
Solid concentration of feed	Increased solid concentration leads to increased particle size and density
Solvent	Choice of solvent affects the particle morphology and size
Atomising Pressure	Increased pressure leads to a smaller particle size
Feed Rate	Increased feed rate leads to larger particles with a higher residual moisture
Air Flow	Increased flow rate leads to decreased drying
Inlet Temperature	Increased temperature may reduce particle seize or make a porous product whereas too low a temperature may allow crystallisation due to slow evaporation of solvent
Outlet Temperature	A low outlet temperature can lead to increased particle wetness and crystallization (outlet temperature not directly controlled but is linked to many parameters including inlet temperature, spray rates and solvent)

In terms of spray drying, the use of no organic solvents would be desirable for reasons as detailed in Chapter 4 although the vast majority of research still involves organic solvents. Particles formed from various cellulose derivatives with different additives containing paracetamol produced by aqueous spray drying in drug to polymer ratios of 1:1 or 10:1 (Billon et al., 1999). The highest production yields were achieved with aqueous suspensions containing microcrystalline cellulose (MCC) or ethylcellulose (EC), both optimal at 1%, followed (in order) by hydroxyethylcellulose (HEC), sodium carboxymethylcellulose (NaCMC), hydroxypropyl methylcellulose (HPMC) and hydroxypropylcellulose (HPC) which were all optimal at 0.1 %. The availability of drug from multiparticulate systems depends on the hydrophilicity of the polymer. Hydrophilic polymers such as NaCMC and HEC gelled faster than the other polymers examined resulting in the fast formation of a viscous gel barrier and hence prolongation of drug release, with increasing polymer concentration

increasing the thickness of the layer and hence further slowing paracetamol release. Faster drug release was seen with higher drug: polymer ratios due to the lack of/too thin continuous gel layer.

Sustained release and enteric-coated tablets of theophylline have been prepared by compressing spray dried microspheres prepared by organic-solvent free spray-drying using Eudragit® L30D or L100-55 for enteric-coating and Eudragit® E30D for sustained release. Colloidal silica and talc were added to all three feeds to prevent adhering of the particles to the spray drier chamber walls with polyethylene glycol (PEG) 6000 as a plasticiser. Eudragit® L30D and E30D are aqueous dispersions so the drug is in a solution feed whilst the theophylline was suspended in the Eudragit® L100-55 feed. Ammonia 2% w/w (aq) was used as a solvent. Eudragit® L100-55 at a drug to polymer ratio of 1:1 or 1:3 formed particles in the range of 10-30µm with smooth surfaces with the larger particles due to agglomerated crystals in those particles with less polymer. The crystallinity of the drug in the particles decreased by increasing polymer concentration and was absent in those particles above drug to polymer ratios of 1:3. A similar decrease was seen with Eudragit® L30D but there are still some crystals due to drug undissolved in the fluid and also with Eudragit® E30D. Enteric behaviour was seen in tabletted microparticles of L100-55 and L30D at a drug to polymer ratio of 1:3. and sustained, pH independent theophylline release was seen with the tabletted Eudragit® E30D microspheres (Takeuchi et al.). Whether the microspheres would produce modified release whilst as discrete particles is unknown. Aqueous spray drying is discussed further in Section 4.

One of the newer options for taste-masking formulations for patients with swallowing difficulties is to produce particles coated with the polymer Eudragit® E PO. Eudragit® E PO has been used to produce famotidine microspheres by aqueous spray drying which were formed into an orally disintegrating tablet which disintegrates rapidly in the saliva without the need for water (Xu et al., 2008a). The polymer may also be extruded. Eudragit® E PO is a cationic copolymer of dimethylaminoethyl methacrylate and neutral methacrylic esters. This co-polymer dissolves below pH 5 so is soluble in the

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stomach so that the bioavailability of this medicine which is designed to act on the stomach is not effected. At the higher pH of the buccal cavity the particles remain intact. The tablets formed from microspheres were found to disintegrate within 30 s in the buccal cavity and were rated as having an acceptable taste by a human taste panel. Spray drying for taste masking is discussed further in Chapter 4.

In summary, spray drying was chosen as it is a microencapsulation method which can be performed without organic solvents which is able to be scaled-up to industrial scales. Due to the pH dependency of Eudragit® E retarding release in at salivary pH (and hence potentially being able to be administered in a pH controlled suspending vehicle) along with the ability to be aqueously spray dried. This polymer was chosen to try to produce a taste masked multiparticulate form as detailed in Chapter 4.

1.5.   Administration of Multiparticulates

1.5.1. Multiparticulates Commercially Available in the British National Formulary for Children

The British National Formulary for Children was first produced in 2005 and is updated annually. It is different to the British National Formulary in that it contains advice for patients up to 18 years of age – however it does not only list licensed medicines, merely those with paediatric use and hence some formulations are unlicenced completely in children with the associated risk of adverse effects. The Children's British National Formulary (2008) was searched and reported over 400 dosage forms of which a large percentage were monolithic dosage forms. The search terms used for finding commercially available multiparticulates: granules, bead, pellets and minitablets (beads are likely to be pellets). The resulting entries found were assessed for drug, therapeutic group, functionality, age licensed from and pharmaceutical form/administration with the aid of the Summary of Product Characteristics for each product with Figure 1-9 showing the types of multiparticulates and functionalisation retrieved.

- 40 multiparticulate formulations were available
- Granules were the most common type of multiparticulates (as shown in Figure 1-9)
- Most formulations were functionalised such as modified release or enteric coated (as shown in Figure 1-9)
- Over half (22 out of 40 formulations) were for delivery to the gastrointestinal tract e.g. by enteric coating
- Pancreatin was the drug with the most formulations (8 Multiparticulate Products)
- Most multiparticulates were available in capsules (which may have to be opened for younger children and not all manufacturers provide advice as to the feasibility of this)

- Three formulations were unlicensed in all ages of children (Cacit® D$_3$ *calcium carbonate/colecalciferol,* Motifene® *diclofenac sodium* and Coracten SR® *nifedipine*)
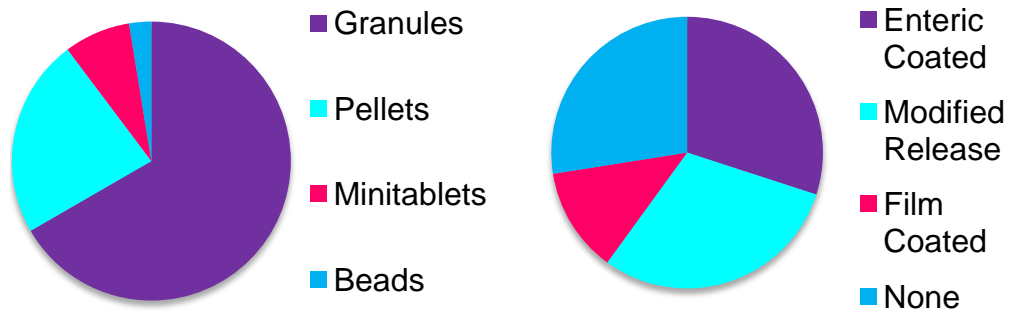
Figure 1-9: Multiparticulate Dosage Forms Available in the British National Formulary for Children 2008 in terms of Type of Multiparticulates and Functionality (n=40 for each as all multiparticulate formulations are accounted for in each graph)

When the search was updated in December 2012, five more formulations were found of which three are described as "granules for suspension" (with one being modified release, one enteric coated and one immediate release), one modified release pellet in a sachet and one enteric coated capsule for opening showing increased development in this area. Three dosage forms are licenced from birth (with the others from one year old and twelve years) and two are for gastro-intestinal therapeutic effects.

It can be seen that commercially available multiparticulates are mainly supplied for administration in capsules, sachets or multi-use containers. In terms of administering multiparticulates, these can be administered wet or dry as detailed below.

1.5.2. Dry Administration

By administering the multiparticulates dry, the multiparticulates will be from a dry dosage form such as a capsule, from a sachet/stickpack/bottle, from being compressed into tablets or via an administration device (Walsh et al., 2011).

As seen in Section 1.5.1, commercially marked multiparticulates are commonly administered sprinkled into a spoonful of food and the two most commonly recommended soft foods are yoghurt and apple sauce which were investigated in Chapter 2 but may have issues with food compatibilities and chewing. These dosage forms could also be administered directly into the mouth but may have problems with adverse mouth feel or grittiness as discussed in Chapter 3.

Multiparticulates can be processed into tablets such as orally disintegrating tablets (ODTs) which can help with taste masking due the lower surface area exposed to the mouth when compared to uncompressed microspheres and are easier to swallow than conventional tablets since they rapidly disintegrate and disperse in the saliva. This form is commonly used for the administration of taste masked multiparticulates in the literature (Khan et al., 2007, Anand et al., 2007a, Randale et al., 2010, Xu et al., 2008a) . While this appears to be a valid approach, the integrity of the polymer layer must be ensured so that taste masking occurs and younger children will probably still require a liquid dosage form.

1.5.2. Wet administration

Wet administration of a multiparticulate is being taken to be administering a multiparticulate in a suspension.

Based on the BNF for Children, there are five granules for suspension multiparticulate formulations available in the UK, namely (British Medical Association and the Royal Pharmaceutical Society, 2012, Electronic Medicines Compendium, n.d.):

- Klaricid® *Clarithromycin (containing carbomers and HPMCP)*
- MST Continus® *Morphine (containing Dowex 50WX8 100-200 mesh cationic exchange resin)*
- Carbomix® *Activated Charcoal (containing only acacia, glycerol and citric acid)*
- Modigraf® *Tacrolimus (containing HPMC and croscarmellose sodium)*
- Nexium® *Esomeprazole (containing HPC, HPMC and Methacrylic acid –ethyl acrylate copolymer (1:1) dispersion 30 %)*

In addition to those multiparticulates in suspension available in the BNF for Children, some modified release suspension dosage forms have been patented. LiquiXR® technology from Tris Pharma used various ion exchange resins (IER) but predominantly Amberlite IRP 69 to complex the drugs in this technology. These drug-IER complexes are then mixed with an aqueous polymeric dispersion (most commonly Kollicoat® SR 30D). This slurry is dried, milled and passed through a 40 micron mesh. An aqueous polymeric dispersion (usually Kollicoat® SR 30D) with triacetin is applied using a fluid bed processer to coat the drug-polymer-IER complexes before these are placed into a suspending base. The types of IERs, polymers and excipients of the suspending base differ depending on the active pharmaceutical ingredient as do the quantities of each used; hence the formulation is not a "uniform platform." Various drugs are named in the patent including cardiovascular drugs, ibuprofen and dextromethorphan.

In the literature, there are several papers as described below detailing the formulation of multiparticulates within a suspension to allow for functionality. These particles are usually formed by the complexation of a drug with an ion exchange resin or less commonly through coating or encapsulating the drug in a polymer. Where the drug is encompassed in a polymer, a variety of methods can be used including solvent evaporation/emulsification and co-acervation. However, none of these used an organic solvent free process, was scale-upable and or shown to encompass multiple drugs.

Some of the drugs with the most research into their functionalised suspensions are ibuprofen, theophylline, codeine, morphine and chlorpheniramine. Most are weak acids with codeine and chlorpheniramine being slightly soluble in water and morphine and ibuprofen (weak acid) being practically insoluble. Ibuprofen microparticles could be beneficial to provide enteric coating to protect the stomach from the gastro-intestinal side effects associated with local irritation while the other drugs would benefit from modified release to reduce the frequency of dosing.

Ion Exchange Resins

The loss of drug into the suspending medium is mainly due to the solubility of the drug in this medium; hence water-insoluble drugs can be suspended in aqueous media without significant leeching. One of the ways to overcome the leeching of water-soluble drugs into aqueous media is by binding the drug to an ion-exchange resin which will prevent the diffusion of drug when in non-ionic suspension: drug release occurs due to the ions of the GIT on oral administration as covered in the review by Anand et al., 2001. Ion exchange resins (IERs) are insoluble polymers that contain acidic or basic functional groups with different capacities and can exchange a range of counter ions with aqueous solutions. IERs combine with a drug to form a complex: this is known as a resinate which retains the drug and prevents dose dumping into the suspending media especially in the case of water soluble drugs. Resinates can be formed of different sizes and prepared as either a batch or continuous process.

Terbutaline was loaded onto an ion-exchange resin sulphonic acid cation-exchange resins in the H$^+$ form (Dowex® 50W-x4, 200–400 mesh) in Eudragit® RS/RL microparticles which were suspended in hydroxypropylmethylcellulose (HPMC) 0.75 % solution to achieve controlled release (Cuna et al., 2000). Those microcapsules made by an oil-in-water solvent evaporation showed good stability for 6 months whereas those made by an oil-in-oil method were not stable even after one week. This was thought to be due to rupture of the polymer coating due to swelling on contact with the aqueous suspending medium.

Codeine and chlorpheniramine (both soluble cationic drugs with short half lives) were also successfully loaded onto AMBERLITE® IRP 69 (45–125 µm) which is a cation exchange resin prepared as the sodium form of the sulfonated styrene divinylbenzene copolymer that is insoluble in water (Zeng et al., 2007). This resinate was encompassed in ethylcellulose particles and coated with PEG 4000 dispersed in an aqueous suspending medium containing xanthan gum 0.5 % and HPMC 0.5 % w/w for sustained release. While resins have been seen to control release in a multiparticulate suspension containing different drugs, the resin can only be bound to specific drugs dependent upon the drug and resin functionality e.g. metformin with activated Indion 234 or etoricoxib with Indion 234 resin and hence would not provide a uniform platform for a functionalized particle in suspension (Bhoyar and Amgaonkar, 2011, Singh et al., 2010, Roblegg et al., 2010).

Multiparticulates in Suspension

Relatively few suspensions of multiparticulates are available in the literature which may highlight the difficulties in formulation. A number of cellulose derivatives have been used as continuous phases for controlled-release microspheres/capsule suspensions. Both carboxymethylcellulose (CMC) and methylcellulose (MC) have successfully been used to form stable suspensions as have other suspending media like tragacanth and xanthan gum as seen in Table 1-8 and detailed below.

Table 1-8: Summary of Some Multiparticulate Suspensions in the Literature in terms of Polymers, Production Methods, Composition, Release and Stability (Kawashima et al., 1991, Dalal and Naruker, 1991, Morales et al., 2004, Morales et al., 2010, Emami et al., 2007)

Drug	Polymer	Production Method	Suspension Composition	Release	Stability
Ibuprofen	CAB and cellulose propionate	Emulsification/ Solvent Evaporation	Methylcellulose 0.5 %	Sustained blood levels in rats	Redispersability and dose uniformity up to one month
Ibuprofen	Eudragit® RS-PM (Contains 0.5 % talc)	Emulsification/ Solvent Evaporation	Acidic (< pH 2) solution of NaCMC 0.5% and D-sorbitol >28 %.	100 % release at eight hours	Resuspendable for six months and release profiles of microspheres identical
Morphine	EC	Emulsification/ Solvent evaporation	Not given	100 % release at 25 hours	No data
Tramadol	EC	Emulsification/ Solvent evaporation	Xanthan gum, carbopol or NaCMC 1%	46-55 % release at 8 hours	No data
Theophylline	EC and HPMCP	Organic Spray Drying	Simple Syrup, Sorbitol, Distilled water, Tragacanth, Tween 80, Methyl-/ Propylparaben	61-65 % dissolution after eight hours	Release similar after one day and one week of storage

The addition of D-sorbitol which acts by its dehydrating effect and pH below 3 increased the absorption of NaCMC to ibuprofen/Eudragit® RS-PM solvent evaporation prepared microspheres (Kawashima et al., 1991). Hydrogen bonds form amongst CMC molecules so the microspheres were embedded in a CMC network to aid physical stability. Microspheres of 105 μm average diameter remained suspended for more than six months. As ibuprofen has a low solubility (pka 5.2) in the acidic medium, it was unable to diffuse out. Following storage for two years, the sedimentation value was 0.7 and this suspension was easily redispersed on shaking. The suspension had highly desirable rheological properties, showing shear thinning. In addition the zeta potential impacted on the stability since as the ibuprofen-Eudragit® microspheres had a low zeta potential at high pH due to the slight ionisation of its quaternary ammonium groups which became more ionised as the pH decreases and hence become positively charged below 3.2.

Similarly, at pH 3.5 a suspension of cellulose acetate butyrate (CAB) or cellulose propionate (CP) ibuprofen microspheres with a drug: polymer ratio of 1:2 and 1:3 was prepared although the mouth feel of this acidic suspension is not known (Dalal and Naruker, 1991). The CAB microsphere suspension had pH independent release less than 5% after thirty days, whereas the CP microsphere suspension showed pH dependent release of 8% which may be due to the higher permeability of CPL. Both microsphere suspensions were easy to redisperse and showed uniformity of dose, even after storage for six months. The CAB microsphere suspension maintained blood levels for longer than the CP microspheres and produced the minimum gastric mucosal damage whereas the CPL suspension produced slightly more mucosal damage on increasing dose (thought to be due to dose dumping of ibuprofen crystals on the surface initially).

Two different ethylcellulose suspensions of morphine were produced: one where the morphine was incorporated into the microspheres during synthesis and the other where the drug was absorbing to the surface of the microparticle (Morales et al., 2004). As may be expected the suspension produced by the former was able to hold more morphine, 92 % was entrapped compared to 15 % absorbed to the surface in the later case. A pseudolatex was formed from ethylcellulose 30 % (a pseudolatex is similar to a latex but is formed from an already existing polymer as compared to a latex which is made by polymerisation of monomers). In a diffusion model, the suspension with entrapped drug transferred the drug over 24 hours whilst the adsorbed drug suspension released over 5 hours. A disadvantage of these suspensions was that they were produced using a solvent containing 85% benzene (a class 1 solvent meaning that there is evidence of this solvent being a carcinogen)! A suspension of tramadol was produced by a similar method by the same research group (Morales et al., 2010)

A theophylline microcapsule suspension was prepared by spray drying from ethyl cellulose and hydroxy propyl methyl cellulose phthalate (HPMCP) either as a solution or dispersion feed. The effect of different solvents (ethanol/water, acetone, ammonium hydroxide and methylene chloride),

polymer to drug ratios (1:1, 2:1 and 3:1) and two different continuous phase compositions on stability and drug release were investigated. Only HPMCP in acetone (a solution feed) and ethylcellulose in methylene chloride (a suspension feed) in all polymer-to-drug ratios produced spherical microcapsules capable of sustained drug release (Emami et al., 2007).

Administration technologies for multiparticulates are available such as straws (like the Clarosip® Straw containing clarithromycin [not longer marketed] which had multiparticulates encompassed within the straw) and, Parvulet® and Vismon® Technologies, both of which consist of commercial dry multiparticulates which become soft and semi-solid following the addition of water of unknown composition (Breitkreutz and Boos, 2007a, Egalet). There are however proprietary technologies with cost implications which may not offer significant advantages over other administration methods.

In summary, multiparticulates can be formulated which are stable in a suspension and which can provide taste masked or modified release in a liquid dosage form by either ion exchange resins or microencapsulation. By using a suspension form, we allow for swallowability and reduce the challenges of other multiparticulate administration methods such as food compatibility, choking or the use of expensive proprietary technologies. A suspension dosage form also offers the possibility of dosing by volume hence permitting the dosing flexibility that is often required by the diverse paediatric population. By using a microencapsulation approach, a uniform multiparticulate for suspension should be able to be achieved compared to drug specific ion exchange resins.

1.6. Multiparticulates and Gastro-Intestinal Transit

Functionalised multiparticulates are used in adults as they are thought to produce less drug variability due to delays in gastric emptying and intestinal transit and less local irritation/dose dumping, when compared to monolithic dosage forms. The knowledge of transit of multiparticulates in children is explored below. The general age differences in overall gastrointestinal factors relative to adult values (Bowles et al., 2010) are briefly summarised in Table 1-9.

Table 1-9: Age differences in gastrointestinal factors relative to adult values (Bowles et al., 2010, de Zwart et al., 2004, Alcorn and McNamara, 2003)

Physiological factors	New born (Full term)	Neonate (0-1month)	Infant (1month -2yo)
Volume stomach (fasted)	-	2.5ml	2.5ml
Acid /pepsin output	-	Relatively low	~ Adult (/BW)
Gastric pH	Neutral at birth then 1-3	>5	~ Adult
Gastric emptying time	Reduced (variable)	Reduced (variable)	Increased
Gastric Motility	Low in first days of life	Reduced	~Adult (6-8 Months)
Intestinal surface area	Reduced	Reduced	~ Adult
Intestinal transit time	Reduced	Immature	Increased
Pancreatic/biliary function	Very immature	Immature	~ Adult
Bacterial flora	Very immature	Immature	Immature
Enzymes/transporter activities	Very immature	Very immature	Approaching adult

The main areas of gastro-intestinal transit which are specifically important with regard to the gastro-intestinal transit of multiparticulate dosage forms are: gastric emptying, fluid volumes, intestinal transit times and pH values which are examined further.

1.6.1. Limitations of Available Data

Little is known about how any non-disintegrating formulation, let alone multi-particulates, would transit through the paediatric gastric-intestinal tract. There are a number of reasons for this gap in knowledge. Due to the radiation burden or invasive nature of diagnostic methods involved, the few available studies were generally carried out on paediatric patients already suffering from gastro-intestinal symptoms: hence most of the gastric emptying data available was from infants suffering from Gastro-Oesophageal Reflux Disease (GORD) and much of the intestinal transit information is from paediatric patients under investigation for constipation. Very little data is available from healthy or control patients as noted below.

Often trials have a large age range classification in order to recruit enough patients and many reported the results only as a mean and standard deviation without age stratification. The significance of these averaged values seems debatable especially when details on how physiological parameters change from year to year or even week to week in pre-term infants are not known but are likely to be significant (see Table 1-9). The conditions of testing also affect the results achieved. Much of the data for gastric emptying was in pre-term and term infants using liquids (milk), due to the obvious restrictions in administering solids in this age group. In older children, food was used for transit studies but different types of meals were used which gave different results making trial comparison difficult.

This is further complicated by the different methods used for assessing gastro-intestinal transit (commonly ultrasound measurements, scintigraphy, coloured/radiopaque makers and breath tests) and the variety of experimental protocols used covering parameters such as the patient's

posture on measurement, the time period of pre-fasting and the method of reporting results (Hiorns, 2011). However it is important to explore published data in order to see how dosage forms are likely to be handled compared to the adults and what, if any, impact that may have on dosage form development and clinical outcomes.

1.6.2. Gastric Emptying

Gastric emptying time depends on the dosage form and the fed or fasted state of the stomach. In the fasted state the migrating myoelectric complex governs the activity of the stomach and hence the passage of intact dosage forms. It comprises of four steps ranging from inactivity through increasing contractions to very powerful contractions known as a housekeeper wave at stage three before returning to its inactive state at phase 4 as seen in Figure 1-10. This process may take a couple of hours and is repeated until food is eaten. During the fed state, the stomach relaxes ready to receive food and peristalsis occurs. During this fed period liquids and small particles such as multiparticulates can pass though the pyloric sphincter and onto the small intestine whereas larger dosage forms may need to wait for the next peristaltic wave, which may take a while (Kendall, 2007).
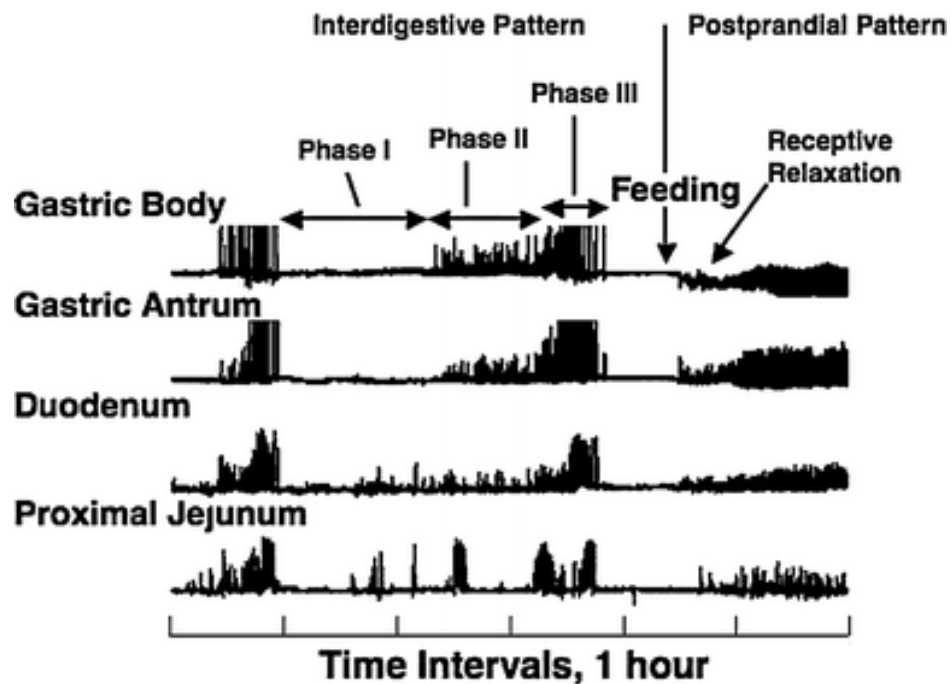
Figure 1-10: Representation of the Different Phases of the Migrating Myoelectric Complex (Kudoh et al., 2009)

Thus multiparticulates spread out more uniformly in the GIT, reducing irritation compared to monolithic dosage forms, leading to more uniform drug distribution and absorption. This emptying also depends upon the size, shape and density of dosage form and presence and type of food (Davis et al., 1986). Multiparticulates are less affected by the different states of the GIT, thus causing less intra- and inter-subject pharmacokinetic variability.

The structure of the stomach is largely developed by fourteen weeks of gestation and motility and secretion by around twenty weeks (Lu and Lebenthal, 1994). Gastric motility and emptying develop further when the infant swallows amniotic fluid from around 28 weeks of gestation (Carlos et al., 1997). The proximal stomach or fundus is responsible for the regulation of fluid emptying through a pressure gradient between the fundus and the duodenum. In contrast, the distal stomach is responsible for the grinding and propulsive motion required to empty solids (Grill et al., 1985). There are no contractions of the stomach to propel solids during the first few days of life (Heyman, 1998) and hence gastric emptying can be delayed immediately after birth in both term and preterm infants (McLeod et al., 1992). In adults, one of reasons for the use of multiparticulates is to circumvent the need for the MMC stage III (housekeeping wave) as shown in Figure 1-10 for emptying into the intestine. Information related to the limiting size of particles for pyloric passage in various ages of children would be of great interest to see if which sizes empty more like liquids in different aged children.

Scintigraphy showed that gastric emptying of milk was slower in premature infants born at a gestational age of less than thirty two weeks but in older preterm and term infants, the emptying time was the same. A similar pattern of reduced gastric emptying time was also seen using ultrasound in patients born at 26 weeks gestational age and followed through until 32 weeks. Gastric emptying time of milk further decreases until it reached adult values by around six to eight months of age as commonly reported in textbooks since the 1960s (Heimann, 1980).

1.6.3. Gastric pH

The pH in the gastro-intestinal tract varies from around 1 in the stomach to 7 or 8 in the large intestine. Environmental pH can influence the ionisation hence absorption of drugs and is also important for the passage of enteric coated dosage forms as the enteric-coating polymer (cellulose acid phthalate, ethylcellulose and some methacrylic acid co-polymers) must not be soluble in the acidic environment of the stomach, usually to protect either the stomach from the drug or vice versa, but must be soluble in the higher pH of the intestine to allow drug absorption.

A summary of the changes in gastric pH is shown in Figure 1-11. Generally, the gastric pH is rather neutral in neonates and then drops to acidic values over the first two years of life as acid secretion and feeding develop. For example gastric pH measured three to four minutes post birth ranged from 1.4 to 7.8 (with a pH above seven being observed in all patients born before 34 weeks) (Miclat et al., 1978). This alkaline pH is due to the presence of amniotic fluid (pH 6.9 – 7.9). It became acidic after removal of the gastric contents and drops to 2.2 (on average) 5 to 6 hours later (Ebers et al., 1956). There was a general trend of increasing pH from 1 to 3 hours, followed by an increase at 4 hours which is no longer seen by 24 hours of age (Avery et al., 1966). Even preterm infants from 24 weeks of gestation were able to maintain pH below 4 when measured during their first 6 to 12 hours of life although the proportion of time the gastric pH is below 4, 3 or 2 were all less than in adults and correlate with the post delivery age (Sandheimer et al., 1985) Lower gestational age premature infants were seen to have higher gastric acid values (especially within the first 3 days of life) (Kelly et al., 1993). These pH values were seen to slightly rise but then have decreased again by day 17, with all infants having a median pH between 1.3 and 2.3 by their third week of life.
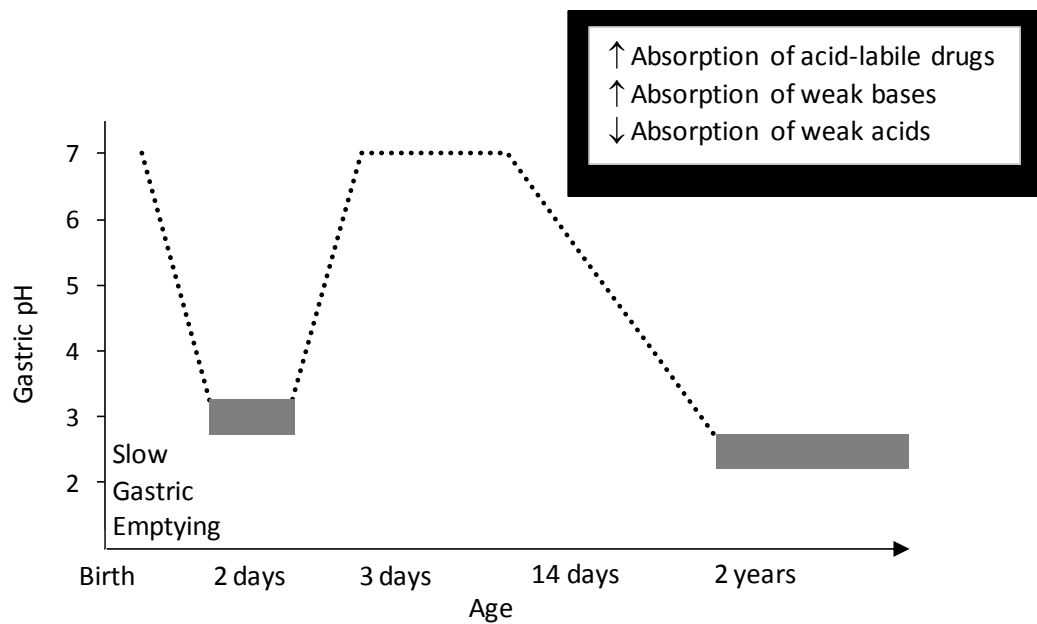
Figure 1-11: Fasted gastric pH changes with age and its effect on drug absorption (provided by S.D. Krämer, ETH Zurich, CH, adapted from Bartelink et al., 2006, Fackler et al., 2001) This graph shows the high pH at birth resulting from the alkaline amniotic fluid present in the neonates stomach at birth so the pH drops as this is removed – as milk feeding is established the Ph again increases due to the buffering effect of milk and by two years of age, acidic secretion is becoming more developed so is able to keep the gastric pH lower like in adults

In adults, average values are available for many gastro-intestinal variables including fasted free stomach volume (45±18 ml), acid output (6-40 mEq/hour), gastric pH (1.0-2.5) and small intestinal transit time (3-4 h) as discussed elsewhere (McConnell et al., 2008). The maximal acid output is similar to that of adults at 0.2 mEq/h/kg of body weight by 6 months of age hence is always quoted as the time at which gastric acid secretion reaches adult values (Boyle, 2003). This does not however mean that gastric pH profiles are the same. On continuous pH monitoring, adults maintain their gastric pH below two for around 65 % of time whereas for a group of children, a similar percentage of time below pH 2 was not achieved until around 14 years (Nagita et al., 1996). The gastric acidity profile hence changes rapidly through infancy to 3 years old and then more slowly until it reaches adult values around 13 to 14 years old. Variations in pH amongst children under 2

or 3 are especially relevant when developing pH-sensitive multi-particulate formulations. The effects of gastric pH are further pronounced when gastric residence time is prolonged and depending upon the characteristics of the drug e.g. the pKa, solubility profile etc.

1.6.4. Gastric Volume

Fasted gastric volume increases with age and is frequently reported in the units of ml/kg (Cook-Sather et al., 2003). Difficulties in determining the age at which it meets adult values stem from the different fasting and sampling conditions. From one of the trials, an interesting effect was seen with temazepam elixir as a premedication (Meakin et al., 1987) The elixir was seen to significantly increase both gastric volume and pH which was not seen with temazepam capsules although the age of the capsule group was higher with a mean age of 9.1 years versus 6.6 years in the elixir group due to swallowing issues in younger children. This increase in gastric volume and pH are thought to be due to the composition of the elixir vehicle (Ethanol 9 %, Sorbitol 45 % and Glycerol 50 %). Glycerol is an irritant which stimulates mucus secretion and both glycerol and sorbitol have osmotic properties which can cause the influx of water into the stomach. Both mechanisms dilute and increase the volume of the stomach contents. Due to the mechanism of action, these effects will also occur in adults or older children and it is simply the ratio of dose volume to stomach content volume that renders them more significant in young children. Hence it is not the excipients per se that are the issue, just their use level. The elixir is an adult formulation containing ethanol and this example serves as a reminder of the problems of using adult formulations in paediatrics without due thought.

1.6.5. Small Intestinal pH

A radio-transmitting pH capsule (24 mm by 7 mm) was used to determine the time taken to pass through the gut and pH at various points in fasting patients (Fallingborg et al., 1990). This technique gave useful information about the conditions throughout the gastrointestinal tract but due to the large size of

capsule could only be used in older children. Twelve healthy 8 – 14 year olds (median age 12 years) were found to have a mean gastric pH of 1.5. The pH became more alkaline (6.4) in the duodenum up to 7.4 in the distal region of the small intestine before reaching 5.9 in the caecum. These pH values were similar to those found in adults. Similar values of small intestinal pH determined by aspiration have also been seen in children ranging from neonates to adolescents (Mean pH 6-7.8) (Ellett, 2004).

1.6.6. Intestinal Transit

There is lack of information on the developmental aspects of intestinal transit which ideally would be needed in the development of age appropriate formulations to determine fully how the intestinal transit of dosage forms may differ at different ages. The rhythmic activity of the intestine increased with gestational age with disorganised activity from 25 to 33 weeks giving way to a propagating MMC and eventually mature interdigestive motility at full-term (Commare and Tappenden, 2007). The choice of test to determine intestinal transit can be dependent upon age: there is a risk of harm by younger infants or neonates attempting to swallow pellets and lactose 13C Ureide test is unsuitable in infants less than 8 months as they lack the enzyme required to metabolise it (Van Den Driessche et al., 2000). Hence most of the time carmine dye is used as it is easy to administer and appears to be well tolerated in all age groups but it only gives the time taken for the dye to transit from the mouth to excretion in the faeces.

Lactulose-Hydrogen breath tests which measure oral-to-caecal transit time and hence remove the long colonic transit phase were found to have a transit time of 80 to 90 minutes on average in patients aged from 1 to 5 years old (Myo-Khin et al., 1999). However, lactulose increased the intestinal motility through its osmotic effects as was seen by the greater time of 255 minutes using the lactose 13C Ureide test in children aged from 3 to 17 years (Van Den Driessche et al., 2000).

Pre-term infants generally have longer intestinal transit times than infants born at term. The intestinal transit time of a pre-term infant decreases with enteral feeding (milk) and on increasing gestational age (Berseth, 1990). Despite the fact the intestine grows, the issue of when intestinal transit time reaches adult values is less clear. No difference in intestinal transit time were found in children aged from 2 months to 3 years versus 3 to 12 years (with large pellets of 5 mm which were swallowed with milk having an average whole gut transit time of 23.7±3.08 hours and 25.4±3.7 hours respectively) nor when children grouped by year from age 1 to 5 years were investigated using a breath test.(Myo-Khin et al., 1999, Corazziari et al., 1985). A standard time of normal transit is often used in constipation studies. It is defined as when a radioactive tracer reaches the caecum within 6 hours and is largely excreted within 24 hours (Clarke et al., 2009). Other data seemed to also support a whole-gut-transit-time of carmine somewhere between 12 and 48 hours as normal in children aged 3 to 13 years (Dimson, 1970).

Pellets have the advantage that they can be detected throughout the child's stool so that they can be reported as a range unlike carmine which can only be reported as the first appearance of red stool. When children less than 3 years old were tested with cuboids pellets (2.7-3 mm) and carmine dye, the time to first red stool and first appearance of pellets were similar (17.5 hours versus 19.7 hours respectively) and in the majority of children occurred in the same stool. However this was not the case for patients suffering from diarrhoea where there appeared to be sequestering of pellets in the bowel and the carmine streaming into the liquid phase (Higgs et al., 1975). Similar mean transit times of pellets (diameter 5 mm) were seen in older children (up to 12 years old) (Corazziari et al., 1985). Transit of non disintegrating solids seems to be affected by size and smaller particles (3- 5 mm) seemed to behave more like liquids and semisolids than larger objects (20 mm). This pattern is similar in adults. The gastro-intestinal influences on multiparticulates is summarised in Figure 1-12. It can be summarised that there is still a lot that we don't know about the transit of multiparticulate dosage forms: this lack of knowledge would have become more important had the work progressed on from taste masking to modified release multiparticulates.

Swallowability
Semisolid food from 6 months

Oesophageal Transit
Liquid transit similar to adults

Gastric pH
- Neutral in neonates
- Drops to adult values by 2 years

Gastric Emptying
Milk emptying time decreased until adult values at 6-8 months

Small Intestinal pH
Similar to adults

Intestinal Transit
Transit time reduced amongst younger infants but total colonic transit time slightly slower amongst children

But:
Lack of research mainly done on sick patients using milk and with differing methodologies

Figure 1-12: Summary of Gastrointestinal Influences on Multiparticulates

To summarise, multiparticulates offer a range of advantages for a dosage form for children including ease of swallowing, dose flexibility, chemical stability and increased excipient tolerability and the potential for functionalised delivery such as taste masking or controlled release in a uniform platform formulation. Multiparticulates can be administered by compaction into tablets which removes their swallowability, as a sprinkle with food or in a suspension which was the chosen form. Spray drying without organic solvents is a production method that produces multiparticulates that have a small size to be able to suspend and is industrially scaleupable.

1.7.   AIMS AND OBJECTIVES

1.7.1. Aims

This project aims to prepare a universal platform formulation which contains functionalized microparticles in suspension, through aqueous spray-drying. Functionalised may be any functionality so includes taste masking as well as modified release. Table 1-10 proposes the product specification for the formulation from both the children and young people perspective and industry needs perspectives. It is proposed to develop this platform by aqueous spray drying of Eudragit® E PO with quinine and salt as model drugs.

Table 1-10: Product Specification

Consideration	Specifications	Minimum	Advantage	Disadvantage
Patient Needs	Stability	Stable in liquid for 7 days	Stable in Liquid for one month	Stable in liquid for 30 minutes (presented as a powder for dispersion)
	Particle Size	Acceptable grittiness	-	Slightly gritty if other specification met (washed down with water)
	Shape	Spherical	-	Spherical but big
	Drug Loading	Polymer: Drug 5:1	More drug than polymer	Polymer: Drug 10:1
	Density	Self-suspending and not floating on the surface	Disperses in any media	Sediments but redisperses easily
	Release Profile	Taste Masking	Controlled release for anything	-
Industry Needs	Yield	50%	100%	30% but all other specifications met
	Cost	Considered in all ingredients and techniques	-	-
	Solvents	Minimal Organic Solvents (Class I)	No organic solvents	Organic solvents of a high ICH value used
	Reproducibility	Results reproducible (n=3)	-	-
	Scale Up	Large Scale	-	-

1.7.2. Objectives

1. To investigate the influence of particle size and concentration characteristics in relation with suspending media viscosity on the suspendability of particles and grittiness/ acceptability of the resulting suspension

2. To produce functionalized multiparticulates and suspensions

3. To optimise the method of production by investigating the effect of different operating parameters/excipient levels on the multiparticulates characteristics and their behavior in suspension

2. SUSPENSION CHARACTERISATION

2.1. Background

2.1.1. Suspension Overview

Multiparticulate dosage forms can be administered in capsules, sprinkled onto food or in the form of a suspension as discussed in Section 1.5. As examined in Chapter 1, liquids remain one of the most popular dosage forms for younger children because they can cater for a large variability of doses required in a growing child without any problems in swallowing and require less manipulation which has been associated with medication errors and adverse events. As this research is attempting to make functionalised multiparticulates in a suspension platform, this section is about considerations in vehicle development, through examining some of those suspending vehicles commonly used in children at present, for the particles produced in Chapter 4.

A suspension is defined as a dispersion of finely divided, insoluble solid particles (the disperse phase) in a fluid (the dispersion media or continuous phase) (Billany, 2007). Suspensions are commonly used due to their ease of swallowing, for hydrophobic drugs and to attempt to reduce the bad taste of medicines by reducing contact with the taste buds as described in Chapter 1. Despite these benefits of suspensions, they are not without their technical challenges of chemical, physical and microbiological stability of the dosage form with the impact on the physical properties of the suspending vehicle the main focus of this section.

Suspension formulations often contain many different classes of excipients as summarised in Table 2-1 to try to overcome these various instabilities. As discussed in Chapter 1, liquid dosage forms require many different excipients and in higher levels compared to solid dosage forms: one of the reasons to use multiparticulate dosage forms to try to reduce this.

Table 2-1: Summary of Common Excipients Found in Suspensions and their Functions (Moreton, 2010, Billany, 2007)

Excipient	Function
Thickening/Suspending agent	Increases viscosity to increase physical stability e.g. tragacanth, alginates, xanthan gum, HPMC, MC, CMC, colloidal silica
Density Modifier	Reduces the difference in density between the particles and suspending media e.g. glycerol, sucrose, sorbitol
Flocculating agent	Causes flocculation of particles to increase physical stability e.g. surfactants, alginates, cellulosics, tragacanth, carbomers
Wetting agent	Helps disperse particles in the suspending media e.g. tweens, spans, cellulosics, xanthan, solvents
Buffer	Keeps the suspension at the desired pH range e.g. carbonates, citrates and phosphates
Osmotic agent	Keeps the suspension at the desired osmotic pressure (often maintain similar to biological fluid) e.g. dextrose, sodium chloride
Preservative	Prevents microbial instability e.g. benzoic/sorbic acids and their salts, hydroxybenzoates and derivatives
Antioxidant	Prevents oxidation and hence instability e.g. ascorbic acid, sodium metabisulphate
Flavour	Improves the taste of the suspension e.g. natural such as orange water or synthetic esters (cherry = ethyl aceto acetate)
Sweetener	Improves the taste of the suspension e.g. sucrose, sorbitol, syrup, aspartame
Colourant	Makes the suspension look elegant/aids in identification and can complement the flavour e.g. caramel, carmine, carrots (yellow)

The physical properties of a well-formulated suspension have been defined previously as (Marriott, 2007):

- The suspension must remain sufficiently homogenous for a suitable period of time (at least for the period between shaking the suspension and removing the required dose)
- Any sedimentation that occurs on storage must be easily resuspendable following moderate shaking of the container (the suspension may require controlled flocculation to achieve this)
- If the settling rate of the disperse phase needs to be reduced, the continuous phase can be thickened but must not become so viscous that removal from the container is difficult.

The determination of the flow or rheological characteristics of the suspending vehicle are hence very important to these physical properties of keeping the drug uniform in suspension and hence physical stability. Flow measurements are based on the principle that increasing the stress (σ) applied to a liquid will increase the flow of the liquid (γ) and that the proportionality between the two is the viscosity (η) as shown in Equation 2-1.

Equation 2-1: Viscosity

$$\eta = \sigma / \gamma$$

η = Viscosity (Pa.s)

σ = Shear Stress (Pa)

γ = Shear Rate (s^{-1})

Where the viscosity is constant over different shear rates, the liquid is said to be Newtonian. From the above desired suspension physical characteristics, it can be seen that it may be beneficial for the continuous phase to show thixotropic pseudoplastic (shear-thinning) behaviour as illustrated in Figure 2-1. Pseudoplastic liquids show their apparent viscosity decrease on application of increasing shear rate until the minimum apparent viscosity has been reached, where the viscosity does not decrease any further despite increasing the shear rate. A similar phenomenon happens in thixotropic

liquids but when the shear rate is no longer applied, there is a lag period before the viscosity increases again. In terms of suspensions this is desirable as the suspension will be thick whilst sitting, become thinner on shaking so that during the lag time this thinner suspension can be poured but then re-thicken again for physical stability during storage until the next time a dose is required.

This shear-thinning behaviour is a phenomenon of long, high molecular weight molecules such as polymers in solution (Florence and Attwood, 2006). At low shear stress, these molecules are entangled and contain entrapped solvent. As the shear rate increases these molecules become untangled and aligned in the direction of shear stress, releasing the entrapped solvent. This makes it easier for the molecules to move and hence reduce the apparent viscosity. The chains become entangled again following a reduction in shear rate. Materials which exhibit this type of flow include gums such as guar and xanthan, clays, tragacanth, methylcellulose, carmellose, PVP and polyacrylic acid (Marriott, 2007)
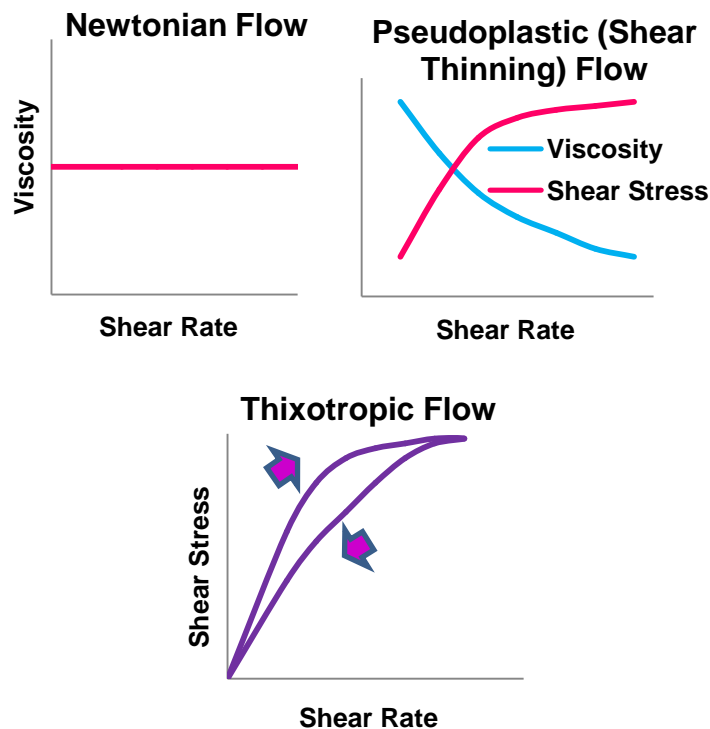
Figure 2-1: Examples of how Viscosity Differs with Shear Rate in Newtonian, Thixotropic and Pseudoplastic Flow

2.1.2. Suspension Stability

By virtue of existing of two phases (solid particles and liquid), suspensions can be inherently unstable with some of the types summarised in Table 2-2 and illustrated in Figure 2-2 If any of these types of instability occur, the child we are trying to formulate for will not get the uniform dose that they require which may lead to either under dosing or overdosing so it is important that these are minimized.

Table 2-2: Summary of Physical Instability

Types of Physical Instability	Description
Flocculation/Aggregation	Loose association of particles
Sedimentation	Particles settling to the bottom
Caking	Sedimentation where the particles cannot be redispersed easily
Particle Growth	Caused by dissolution and recrystallisation
Creaming	Floating of poorly wetted material on the surface of the suspension
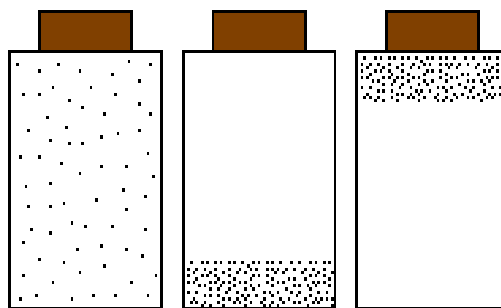
Figure 2-2: Diagram Illustrating (from left to right) a stable suspension, one which has sedimented and one where the particles have creamed

It can be seen that while the suspending media is important for stability the interplay with the particles in terms of their properties e.g. for sedimentation and wetting is equally important. Sedimentation of particles in a suspension is often described by Stokes Law in Equation 2-2**.**

Equation 2-2: Stokes Law

$$V = \frac{gr^2(\rho_1 - \rho_2)}{9\eta}$$

V = Rate of sedimentation (m/s)
g = Gravitational Acceleration (m/s^2)
r = Sphere radius (m)
ρ_1 = Density of continuous phase (kg/m^3)
ρ_2 = Density of disperse phase (kg/m^3)
η = Viscosity of continuous phase (Pa)

Stokes Law provides a simplistic assessment of sedimentation based on ideal, spherical, non interacting particles moving only in a laminar flow pattern which may not always be applicable to the real life situation of a pharmaceutical suspension. In spite of this it shows the importance of particle size and particle/suspending media density and viscosity on sedimentation with smaller, less dense disperse phase particles sedimenting more slowly especially in higher viscosities. However simplistic, the theory of Stokes Law may be important in that more viscous suspending agents or a denser suspending vehicle/ less dense particles may enable the suspension of larger multiparticulates.

The surface properties of the disperse phase should also be considered since good wettability, appropriate charge and a narrow particle size distribution are helpful for physical stability. Ostwald ripening is a potential cause of suspension instability where smaller particles dissolve and deposit on larger particles in suspension where there is a non uniform particle size leading to particle size growth and hence faster sedimentation. Ostwald ripening is less likely to be of concern where there is a narrow size range and slow dissolution rate, as is likely to be the case for the functionalized microparticles we aim to produce (Yao et al., 1993).

Formulating functionalized multi-particulates into a suspension may be challenging for a number of reasons. Diffusion/release of the drug into the suspending media and interactions between the continuous phase and microparticles must be avoided in order to maintain reproducible drug

release. Water-soluble polymers swell and gel in contact with water leading to the diffusion of drug through the gel whilst hydrophobic water insoluble polymers may allow drug release either through pores or by diffusion through the polymer. In addition to the issue of avoiding premature drug release, functionalised multiparticulates will be larger than non coated drug particles so may be difficult to disperse or suspend as shown in Figure 2-3 especially as there may be problems with wetting of the polymers or agglomeration due to 'sticky" polymers in contact with water.
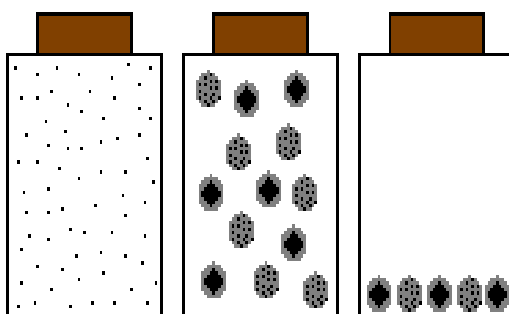
Figure 2-3: A diagram illustrating (from left to right) a stable suspension of drug particles, one with drug containing multiparticulates and the sedimentation likely to occur with multiparticulates which are larger than drug particles

Chemical stability problems in suspensions are often overcome by minimising the contact between the disperse and continuous phase by formulating a dry formulation for reconstitution which can be made up immediately prior to patient issue which has a shelf life of up to around seven to fourteen days in commercial products. Difficulties may arise in applying this approach to suspensions containing functionalised microparticles due to the necessity for the microparticles to remain completely unchanged on drying/further formulation and subsequent reconstitution. It is important in a suspension containing microparticles that the microparticles themselves remain unchanged otherwise this would remove the functionality (e.g. taste masking or modified release) intended to be achieved in an end product for administration. The functionalised multiparticulates by virtue of being hydrophobic are likely to be difficult to wet in aqueous formulation so wettability may be a problem.

The assessment of the stability of suspensions is complex. In a brief overview common methods are presented below (Kulshreshtha et al., 2010, Nielloud and Marti-Mestres, 2000, Streng, 1985)

1. Sedimentation volume is defined as the ratio of the equilibrium settled height to the original height

2. Redispersability measured as the number of rotations required to restore the suspension to homogeneity

3. Content uniformity of the drug/excipients

4. Viscosity/Flow Curves (Rheology)

5. Zeta Potential Determination

6. pH Testing

7. Microscopic Evaluation of Appearance

8. Degree of Flocculation

   The degree of flocculation is estimated by comparing the sedimentation volume of the flocculated suspension to that of the suspension when deflocculated

9. Stability Testing

   a. Freeze/Thaw Cycles

   Freeze/thaw cycles are where the physical and microscopic changes of suspensions which undergo a sudden temperature change are investigated. This can be achieved by keeping suspensions in a 40 °C oven for twenty four hours and then transferring them to a freezer at 0 °C for twenty four hours.

   b. Normal Temperature Fluctuation

   Normal Temperature Fluctuation is where the physical and microscopic changes of suspensions which undergo a gradual temperature change are investigated. Gradually decreasing the temperature from 40 to –5 °C and keeping the suspensions at each temperature for twenty-four hours can achieve this which could aid assessment for Ostwald ripening or polymer instability.

10. Microbial Stability

Preservatives may be required in multidose preparations where the drug itself does not have anti-microbial activity especially in those suspensions that contain aqueous phases although in terms of minimizing excipients in paediatrics, single unpreserved dose formulations may be preferable (Breitkreutz and Boos, 2007a). The efficacy of an antimicrobial preservative may be enhanced or diminished by chemical interaction with the drug, excipients or container. Test for efficacy of anti-microbial preservation is by challenging the formulation with a variety of test organisms, namely *Pseudomonas aeruginosa, Staphlyococcus aureus*, *Candida albicans* and *aspergillus niger*) (British Pharmacopeia, 2012). The inoculated suspension is then stored at 20 to 25 ^{o}C. For the preservation to be effective, there should be a log reduction of three for the number of viable bacteria and one for the number of viable fungi found in the oral preparation at day fourteen compared with a control. There should be no-increase at day twenty eight for either type of microorganism. Preparations for oral administration should not contain more than 10^3 bacteria or 10^2 fungi per gram or millilitre and should be absent of *Escherichia coli.*

In terms of experiments carried out in this chapter, macroscopic appearance as a measure of the content of larger particles, rheology, pH and density were characterised. Chemical and microbiological testing was not undertaken as no suitable formulation was achieved that would have required this.

2.1.3. Commonly Used Suspending Media

In order to administer a suspension/dispersion to a child, there can be largely thought of as three different types of suspending vehicles (Electronic Medicines Compendium, n.d.):

1. A prepared suspension or powders/granules for reconstitution produced and sold by pharmaceutical companies such as amoxicillin liquid for reconstitution or paracetamol suspension
2. An extemporaneous preparation at the pharmacy of a drug/crushed dosage form in a commercial vehicle or prepared media
3. Soft food recommended for dosage forms to be sprinkled on such as yoghurt or apple sauce such as pancreatin formulations or Epilim Chronospheres® (sodium valproate/valproic acid)

Relatively few oral ready to use suspensions and powder/granules for reconstitution exist – possibly due to the difficulties in formulating a stable suspension which is essentially for a reproducible dose. Those which do exist commonly use xanthan or guar gum, cellulosic derivatives, sugar/sugar substitute syrups, glycerol and colloidal silica (Electronic Medicines Compendium, n.d.).

Due to the historical lack of research into formulations for children as discussed in Chapter 1, extemporaneous dispensing still occurs despite the unlicensed status of the dosage form. While the onus is on the dispensing pharmacist to ensure that the dosage form is stable and fit for purpose, reports as to the quality and stability are variable ranging from no reported stability data through to validated formulations that are supported by industry such as the administration of Tamiflu® in Cherry Syrup, OraSweet® SF or Simple Syrup in an emergency (Genentech, n.d., Giam and McLachlan, 2008, Kairuz et al., 2007, Nahata and Allen, 2008).

CHAPTER 2: SUSPENSION CHARACTERISATION

An overview of suspending vehicles used in extemporaneous dispensing or compounding is provided in Table 2-3. As highlighted in bold in Table 2-3, many of the suspending vehicles are based on syrup just with different flavours. While having a sugary, flavoured vehicle may improve compliance through its pleasant and culturally acceptable taste, a vehicle containing predominantly sucrose is not without its issues including the potential for dental caries, calorific concerns, the requirement for a preservative and a high osmolality. Although syrup is used as a suspending vehicle, alone it may lack some of the properties of an ideal suspension as detailed above in that although it is a viscous vehicle of higher density, it has little/no shear thinning potential and thixotropic nature.

Table 2-3: Summary of the Suspending Vehicles used in Compounding/ Extemporaneous Dispensing with bold highlighting a syrup and red highlighting commercial suspending vehicles (Compounding Today, n.d.)

Suspending Vehicles Used in Compounding		
Acacia **Syrup**	Compound Benzaldehyde	Aromatic Eriodictyon
Cherry **Syrup**	Elixir NF	**Syrup**
Cocoa **Syrup**	Hydriodic Acid **Syrup**	Coca-Cola **Syrup**
Glycyrrhiza **Syrup**	Ora Blend® Flavored	Glycyrrhiza Elixir
Isoalcoholic Elixir, Low	Suspending Vehicle	Isoalcoholic Elixir, High
Ora-Plus® Oral	Ora-Sweet Sugar-Free	Ora-Blend Sugar-free
Suspending Vehicle	(SF) **Syrup** Vehicle®	(SF) Flavored
Orange Flower Water	Orange **Syrup** NF	Suspending Vehicle
Raspberry **Syrup**	Sarsaparilla Compound	Ora-Sweet **Syrup**
Sugar-free Suspension	**Syrup**	Vehicle®
Structured Vehicle	Suspension Structured	Peppermint Water NF
SyrSpend SF	Vehicle USP	Sorbitol Solution USP
Vehicle for Oral Solution	**Syrup NF**	Syrpalta
Wild Cherry **Syrup**	Vehicle for Oral Solution,	Tolu Balsam **Syrup** NF
Aromatic Elixir USP	Sugar Free NF	Vehicle for Oral
Citric Acid **Syrup**	Xanthan Gum Solution NF	Suspension NF

From Table 2-3, it can be seen that there are relatively few commercial vehicles but they are extensively used in the extemporaneous dispensing of medicines. Commercial vehicles are used due to their ready to use nature providing ease of use without individual decisions having to be made about

such criteria as microbial stability and combinations of sweeteners/flavours to use. In commercially used suspending vehicles, stability data may be available from the company about various drugs. Some of their drawbacks include availability outside the United States and cost. Some of the most commonly used commercially available suspension vehicles are described in Table 2-4. e.g.

- OraBlend® is a 50:50 mixture of the suspending agent (containing suspending polymers), OraPlus®, and the syrup vehicle, OraSweet®. Orablend® is marketed as the all in one flavouring and suspending agent (Paddock Laboratories Inc, n.d.)
- SyrSpend® SF is marketed as being free from the laxative effects associated with sorbitol and has a low osmolality (< 50 mOsmol) which may mean less gastro-intestinal upset (Fagron, n.d,)
- Versa Free® and Versa Plus® systems are marketed as unique in that they contain no parabens, dyes or sweeteners (Compounding Today, n.d.)

It can be seen from Table 2-4 that the suspending media all contain similar types of excipient such as a viscosity enhancer, sweeteners (with the exception of Versa plus), buffering agents and preservatives. This formulations are similar to commercially prepared suspensions in composition and largely marketed as shear thinning/thixotropic (no information found for Versa®)

The choice of suspending vehicle is important not only for ensuring physical stability but also as the taste may improve compliance, especially in bitter tasting medicines, and the texture should also feel pleasant, which may allow a larger volume of large particles in the disperse phase to be taken.

Table 2-4: Composition of Commercial Suspension Vehicles where red represents viscosity enhancers, blue sweeteners and flavouring agents, green buffers and purple preservatives. The concentration of each component is not publically available as these are commercial media

	Ora-Plus®	Ora-Sweet®	Ora-Sweet SF®	Ora-Blend®	Ora-Blend SF®	SyrSpend SF®	Versa Plus®	Versa Free®
Microcrystalline cellulose	+	-	-	+	+	-	+	-
Sodium CMC	+	-	-	+	+	-	+	-
Xanthan gum	+	-	+	+	+	-	+	+
Carrageenan	+	-	-	+	+	-	+	-
Modified Food Starch	-	-	-	-	-	+	-	-
Sucrose	-	+	-	+	-	-	-	-
Glycerol	-	+	+	+	+	-	-	+
Sorbitol	-	+	+	+	+	-	-	+
Sodium saccharin	-	-	+	-	+	-	-	-
Neotame	-	-	-	-	-	-	-	+
Sodium Benzoate	-	-	-	-	-	+	+	+
Sucralose	-	-	-	-	-	+	-	-
Malic acid	-	-	-	-	-	+	-	-
Flavouring agent	-	+	+	+	+	+/-	-	-
Citric acid	+	+	+	+	+	+	+	+
Sodium phosphate	+	+	-	+	+	-	+	-
Sodium citrate	-	-	+	-	+	+	-	+
Methylparaben	+	+	+	+	+	-	-	-
Propylparaben	-	-	+	-	+	-	-	-
Potassium sorbate	+	+	+	+	+	-	+	+
Simethicone	+	-	-	+	+	+	-	-
Purified water	+	+	+	+	+	+	+	+

Commonly used suspending media that were obtainable were assessed for their suitability as a vehicle for the taste masked particles discussed in Chapter 4. These included commercial media and others commonly used:

- the Ora- suspending vehicles are the most commonly used so OraPlus®, OraSweet®, OraSweet® SF, OraSweet®:OraPlus® blend 1:1 (similar to OraBlend®) and OraSweet SF®:OraPlus® blend 1:1 (similar to OraBlend® SF)
- SyrSpend®.
- Methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) obtained from a Paediatric Extemporaneous Formulary (Nahata and Hipple, 2003).
- Other known combinations e.g. syrup/MC and glycerol/MC

Sprinkling medicines onto food is a pragmatic approach to trying to administer larger than individual drug particles to children and is being more and more accepted even by regulatory authorities as was seen by the commercially available multiparticulates in Chapter 1. The most commonly recommended soft foods for administration of medicines are apple sauce and yoghurt therefore these foods were additionally included for characterisation (Electronic Medicines Compendium, n.d.).

In the suspendability experiments, microcrystalline cellulose pellets (Cellets®) were used as they are spherical inert particles available in a variety of narrow size distributions and as they contain no drug/only microcrystalline cellulose and water so could be administered as part of the grittiness trial in Chapter 3 safely. Cellets are a model hydrophobic larger particle so in a sense were useful to look at the suspendability of larger particles of narrow distributions but are likely to differ in hardness, morphology and from those particles produced by spray drying in Chapter 4 density (with the particles produced by spray drying being softer/more deformable, less dense and less spherical) - hence only general assumptions can be made in trying to make a uniform suspension of larger particles.

This chapter aimed to investigate commonly used suspending vehicles to find the most suitable vehicle for the suspendability and grittiness testing of particles. This investigation was largely in terms of rheology since the rheology of a vehicle will differ depending on the sample preparation/handling and parameters chosen for the test (e.g. type of rheology test, forces used, sample volume, geometry, type of rheometer/viscometer used) so assessing all the candidate suspending vehicles using the same methods a more robust comparison than trying to compare vehicles from the literature which have been assessed under different conditions.

2.2. Materials and Methods

2.2.1. Materials

Ora-Plus, Ora-Sweet and Ora-Sweet SF vehicles were obtained from Paddock Laboratories Inc, Mineapolis, USA and Syrup BP from William Ranson and Son plc, Hertfordshire, UK. SyrSpend SF was obtained from Gallipot Inc, Mineapolis, USA. Methylcellulose (400cP at 2% solution and 25C) with structure as shown in Figure 2-4 and Glycerol (99.5%) were obtained from Sigma Aldrich, Germany. Hydroxypropyl methylcellulose ((HPMC) 4000cP, substitution type 2906, grade 65SH-400) was from Shinetsu Chemical Company Ltd, Japan and Cellets® (Microcrystalline Cellulose Pellets) from Pharmatrans Sanaq, Switzerland.

Vanilla mullerlight yoghurt (Molkerei Alois Müller GmbH & Co. KG, Germany) and Apple sauce (Tesco Value, Tesco, Cheshunt) were used. These were included as they are the most commonly recommended soft foods for the administration of medicines (Electronic Medicines Compendium, n.d.). The apple sauce contained water, apples, sugar, modified maize starch, citric acid, antioxidant (ascorbic acid), preservative (sodium sorbate) and sweeteners (acesulfame K and aspartame). The yoghurt used contained "yoghurt", water, fructose, modified maize starch, gelatin, flavourings, stabiliser (pectins), colour (carotenes) and sweetener (aspartame)
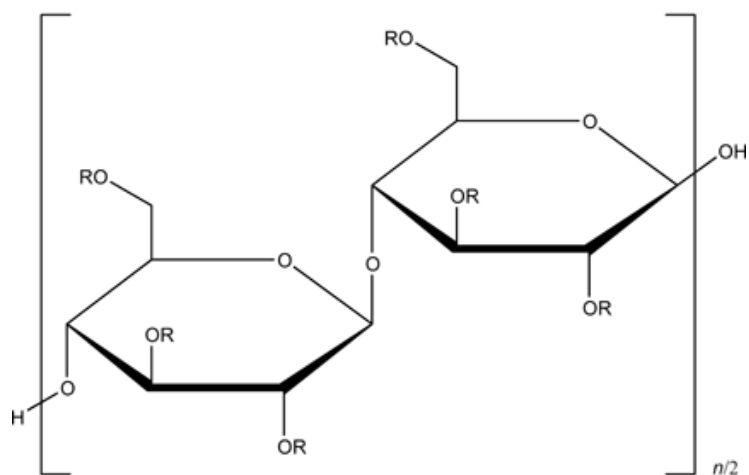
Figure 2-4: Structure of Cellulosic Polymers Where R is H, CH_3, or $CH_3CH(OH)CH_2$ for HPMC, H or CH_3 for Methylcellulose

2.2.2. Methods

2.2.2.1.   Media Preparation

The materials which were used for pH and rheology measurements without modification were Ora-Plus, Ora-Sweet, Ora-Sweet SF, Syrup BP, SyrSpend SF, glycerol and yoghurt. In addition, mixtures were prepared of Ora-Plus and Ora-Sweet or Ora-Sweet SF (1:1), Syrup BP: Methylcellulose 1 % (1:1) and Glycerol: Methylcellulose 1 % (2:5). All of these mixtures were made at least three times.

Aqueous solutions of methylcellulose and HPMC (0.1 to 10 %) were prepared. Methylcellulose solutions were made by adding methylcellulose to boiling distilled water, standing them on heat for fifteen minutes then making up to volume with cold water whilst stirring. Hydroxypropyl methylcellulose solutions were prepared by adding HPMC to boiling water with vigorous mixing until homogenous when iced water was added before the HPMC solutions were autoclaved at 121 °C for 20 minutes. The HPMC solutions were autoclaved as aqueous solutions of HPMC can be prone to microbial spoilage. A preservative could be added to the media if this was required.

2.2.2.2. pH

The pH of all suspending media was measured three times using a pH meter (Hanna Instruments pH 211 microprocessor) after calibration with buffers at pH 4 and pH 7.

2.2.2.3. Osmolality

Osmolality refers to the solute concentration and is measured in osmoles per litre. There are a number of techniques which can be used to assess osmolality: the technique used was measurement of freezing point depression as shown in Figure 2-5. Osmotically active compounds depress the freezing point of a solution so the aqueous solution is cooled below the freezing point of pure water and cooling needle applied in the supercooled state which causes ice to form and fuse. The temperature of the aqueous solution increases until a constant temperature and the difference between this temperature and the freezing point of water is the freezing point depression which is a measure of the osmotic concentration. Distilled water is used in calibration since it freezes at 0°C with an osmolality of 0 Osmol/L as is sodium chloride 0.9% w/v with an osmolality of 300mOsmol/L. The osmolality of the samples was measured three times using a standard Micro Osmometer (Type 5R, Roebling, Camlab).

Typical cooling curves of water and aqueous solutions
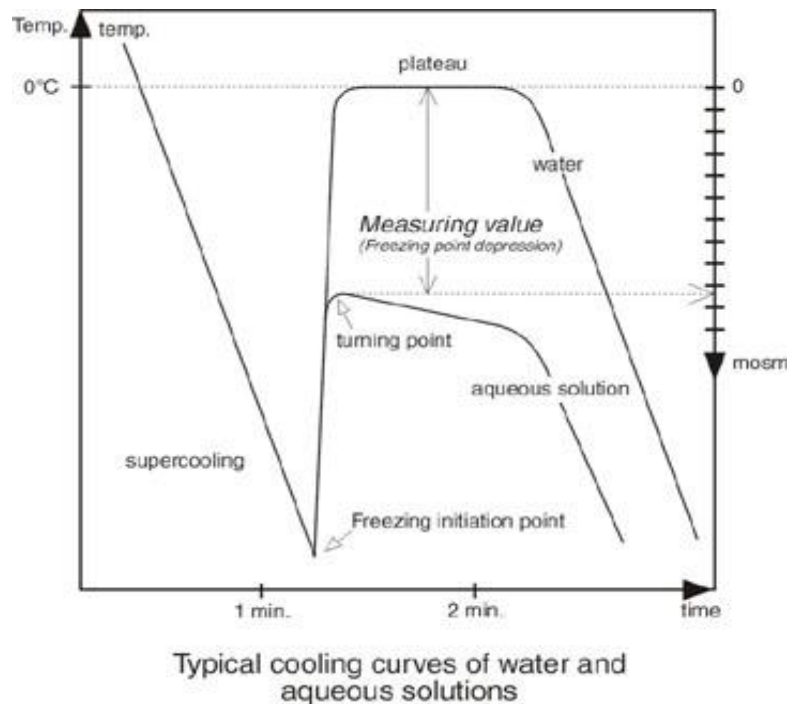
Figure 2-5: Representation of Freezing Point Depression Method of Assessing Osmolality

2.2.2.4. Density

The initial bulk and tapped density of microcrystalline cellulose pellets (Cellets®) were measured three times using a Copley Tap Density Volumeter as described in the Pharmacopeia. Briefly, around 100 g of the different sizes of Cellets® were poured into a 100 ml measuring cylinder at an angle of 45 ° with the initial volume and mass used noted. The cylinder was then tapped at intervals as defined below in Table 2-5 with the volume measured after each set of taps. If difference between the volume measured at 750 and 1250 taps was less than 2 %, the volume at 1250 taps was taken to be the tapped volume. If the difference was greater than 2 %, further intervals of 1250 taps were continued until a final, stable volume within 2 % difference of the last volume was achieved and noted as the tapped volume. This was repeated in triplicate and the results expressed as the mean and standard deviation of the initial and bulk density as defined by Equations 2-3 and 2-4 in g/ml.

Table 2-5: Tapping Intervals for Determining Tapped Density

Interval Number of Taps	Total Number of Taps Performed Overall
10	10
40	50
50	100
100	200
300	500
250	750
500	1250

Equation 2-3: Initial Bulk Density

$$\text{Initial Bulk Density} = M/V_0$$

Equation 2-4: Tapped Bulk Density

$$\text{Tapped Bulk Density} = M/V_t$$

Equation 2-5: Carrs Index

$$\text{Carrs Index} = (V_0/V_t)/V_0 \times 100$$

Equation 2-6: Hausner Ratio

$$\text{Hausner Ratio} = V_0/V_t$$

Where   M   =   Mass of sample (g)

             V_0 =   Poured volume (ml)

             Vt   =   Tapped volume (ml)

The Carrs index as given by Equation 2-5 is a way of evaluating and reporting the compressibility of a powder while the Hausner ratio shown in Equation 2-6 gives an indication of the flowability as shown in Table 2-6 were also calculated.

Table 2-6: Flow Character Nature as defined by Carrs Index and Hausner Ratio (Hausner, 1967, Carr, 1965)

Carrs Index (%)	Powder Character	Hausner Ratio
Up to 15	Good	< 1.25
16 – 20	Fair	
21 - 25	Passable	1.25 – 1.5
26 – 31	Poor	> 1.5
> 32	Very Poor	

The density of suspending media was assessed by weighing 20 ml of each media measured accurately in a measuring cylinder in triplicate.

2.2.2.5. *Rheology*

All rheology measurements repeated in at least triplicate on a Bohlin Gemini HR Nano Rheometer at 25 °C for all measurements. All samples were inverted thirty times prior to each measurement to ensure homogeneity in either the manufacturer's bottle or a 100 ml amber bottle.

2.2.2.5.1. Initial Measurements

A 2 °/55 mm cone and plate with a gap of 70 µm was used with around 2 ml of sample added before the excess was trimmed.

The tests performed were:

- Viscosity flow curve (with shear rates ranging from 0.9 to 200 s^{-1})
- Time to reformation/thixotropic step test (with a shear rate of 1 s^{-1} for 60 s followed by 1000 s^{-1} for 60 s then twenty minutes recovery time)
- Yield stress analysis (with a shear ramp from 0.33 to 38 Pa).

2.2.2.5.2. Effect of Shaking

20 ml HPMC solutions of differing concentrations (ranging from 0.1 to 2 %) were poured into sterile containers, one day prior to testing. After 24 hours, the viscosity of the undisturbed/sheared HPMC media was measured at a shear rate of 0.1 s^{-1}. The solutions were then inverted 2, 4, 8, 16 and 32 times and the viscosity again recorded at a shear rate of 0.1 s^{-1}.

2.2.2.5.3. Effect of Stirring

The viscosity of the HPMC solutions (0.1, 0.5, 1, and 2 %) were measured at a shear rate of 0.1 s^{-1} before being stirred on the magnetic stirrer (t = 0 min). The HPMC was stirred at a speed of 60 % on the magnetic stirring plate for one minute (mimicking the conditions during the suspendability experiment) before the viscosity was immediately measured (t = 1 min). The viscosity was measured in triplicate after 5, 10, 15, 20 and 180 minutes for all concentrations.

2.2.2.5.4. Oscillation

Flow curves tell us about a materials viscous properties i.e. how it resists flow. Therefore in order to characterise the viscous <u>and</u> elastic properties of a material, oscillation is used. A stress/strain is applied which is constantly changing and the delay of the resulting response is measured. An amplitude sweep is applied to determine the linear viscoelastic region then a frequency sweep to determine the material's response to different time scales by

examining response curves as shown in Figure 2-6. From the material's response, the complex modulus and the phase angle can be determined.

The complex modulus (G^*, measured in Pa) is a measure of the stiffness of a material so the higher the G^*, the tougher the material (e.g. the less it moves so the more stiff the material). It is described as a modulus as the shear stress/shear strain is constant in the Linear Viscoelastic Region (LVR) (above this, the structure breaks). This is a complex modulus as it is comprised of G' and G'' where G' is the Storage (elastic) modulus and G'' is the Loss (viscous) modulus: if G' > G'' then the material is solid-like and the reverse shows that a material is more liquid-like.

The phase angle occurs when there is a lag phase between the stress applied to the material and the resulting strain: For purely elastic materials, stress and strain are in phase (so phase angle is zero) and for purely viscous materials, stress and strain are ¼ cycle out of phase (angle 90 ^{o}); where the phase angle is 45° the material is as much as solid as liquid so is a gel. It therefore follows the lower the angle, the more elastic or solid like material and vice versa.
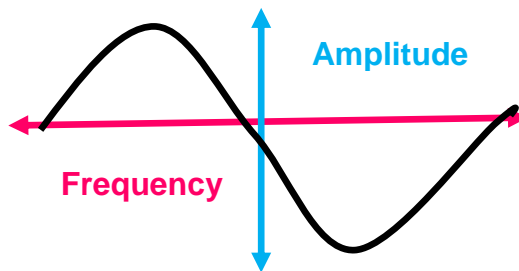
Figure 2-6: Figure Showing Amplitude and Frequency of a Wave as used in Oscillation Experiments

By looking at the G' or elastic/solid components over a range of different frequencies, this may help us find out which is the most stable suspending media e.g. if one is more solid at a low frequency indicating a long period of time required for sedimentation. This may be especially important in keeping our larger multiparticulates suspended and hence in providing a uniform suspension for patients.

An amplitude sweep was performed on all samples to determine the linear viscoelastic region (LVR) using a 40 mm parallel plate with a 500 µm gap and around 2 ml of sample with the excess trimmed. An auto stress of 1 Hz, strain units from 0.005-0.5 and an initial stress of 0.5 Pa were used. The stress and strain values 2/3 of the way along the LVR (which differed between samples) were used as the initial values in a frequency sweep run from 0.01 to 10 Hz to assess the storage and loss moduli over the different frequencies and timescales associated with them.

2.2.2.6. Suspendability

Cellets® (ranging from 100-1000 µm) at a concentration of 500 mg/5 ml were added to MC and HPMC (0.1, 1 or 3 %) solutions and stirred for one minute on a RCT Basic magnetic stirrer (Ika Labortechnik, Germany) on setting of 60 % after which the time taken for the pellets to completely settle was visually determined.

2.3. Results and Discussion

2.3.1. pH

All media should be compatible with the roughly physiologically neutral pH of the mouth which is to be expected. The pH is of critical importance in administering multiparticulates, as the drug release from multiparticulates may be pH dependent e.g. in the case of taste masking or for enteric coating. An incompatible suspending media or food pH for a sprinkle will lead to failure of the dosage form functionality hence may affect medication compliance. For example, a bitter tasting drug could be released into food if taste masked particles designed to release at acidic pH release in the administration media. The pH values for different media are shown in Table 2-7: those pH values which are acceptable are for administration of Eudragit® E which is soluble below pH 5.5 are highlighted in bold.

Table 2-7: pH Values (Mean ± SD) for Suspension Vehicles those pH values which are acceptable are for administration of Eudragit® E which is soluble below pH 5.5 are highlighted in bold

Vehicle	pH Value Mean ± SD
OraSweet®	3.79±0.01
OraPlus®: OraSweet® (1:1)	3.97±0.02
OraPlus®	4.19±0.01
OraPlus®: OraSweet SF® (1:1)	4.2±0.01
OraSweet SF®	4.28±0
Yoghurt	4.28±0.01
SyrSpend SF®	4.31±0.01
HPMC (Range)	4.31 – **6.46**
Glycerol	4.79±0.14
Glycerol: Methylcellulose 1% (20:50)	**5.84±0.10**
Syrup BP: Methylcellulose 1% (1:1) A	**6.31±0.05**
Methylcellulose (Range)	**6.13 - 7.25**
Syrup BP	**6.61±0.01**

It can be seen that the commercial suspending agents have an a slightly acidic pH thought to be largely due to being buffered to an acidic pH for the stability of the parabens preservative (with composition shown in Table 2-4) and flavouring/sweetening agents with all media within range of that expected by the manufacturers/Excipients Handbook. Given the criticality of pH in dosage form performance in this instance, it is likely that the suspending vehicle would need to be buffered to a neutral/alkali pH.

2.3.2. Osmolality

The osmolality of the gastro-intestinal secretions ranges from 127-357 mOsm/L from saliva to faeces (adult data, paediatric unknown). A high osmolality in a suspension may be associated with gastro-intestinal side effects yet when oral solutions and suspensions were assessed 54 of 58 had an osmolality ranging between 1050-10,950 mOsm/L which is largely in keeping with the experimentally observed values in Table 2-8 (Dickerson and Melnik, 1988)

Table 2-8: Osmolality Mean Values of Various Suspending Media

Media	Mean Osmolality ±SD (mOsm/L)
HPMC 0. 5%	0
HPMC 1 %	0
HPMC 2 %	17.3±0.6
HPMC 1 % (Flavoured & Sweetened)	28.0+3,5
MC 0.5 %	5.5+0.6
MC1 %	9.3+3.1
MC 3 %	12.5+0.6
OraPlus®	244.0+13.0
Yoghurt	481.7+105.1
OraSweet® SF:OraPlus® (1:1)	1308.0+21.7
Syrup BP	Higher than 1999 mOsm/L
Glycerol BP	Higher than 1999 mOsm/L
Methylcellulose: Syrup BP (1:1)	Higher than 1999 mOsm/L
Methylcellulose: Glycerol BP (5:2)	Higher than 1999 mOsm/L
OraSweet®	Higher than 1999 mOsm/L
OraSweet® SF	Higher than 1999 mOsm/L
OraSweet®:OraPlus® (1:1)	Higher than 1999 mOsm/L
Apple Sauce	Higher than 1999 mOsm/L

Many of the media, by virtue of containing glycerol and syrup, are over the maximum limit of the osmometer (1999 mOsm/L). Commercial vehicles osmolalities were in keeping with published values with the exception of OraBlend® SF (OraPlus: OraSweet SF 1:1 which is published at 1073 mOsm/L and measured as 1308 mOsm/L (Paddock Laboratories Inc, n.d.). This media made in and mixed in the lab by manual shaking, may not be as accurately made and as well mixed as that made commercially. It can be seen that HPMC and MC have desired osmolalities but also highlights that this will be changed by every addition e.g. in that the flavoured and sweetened HPMC with Orange PermaSeal Flavour and Sucralose has an increased value.

2.3.3. Density

The density of the Cellets® can be seen in Figure 2-7. It can be seen that the density of Cellets® increases slightly as the particle size range increases (as summarised in Table 2-9) which would be expected given that larger particles are less able to pack as closely and hence would have a higher volume and density. The density was found to be within range of that reported by the manufacturer.
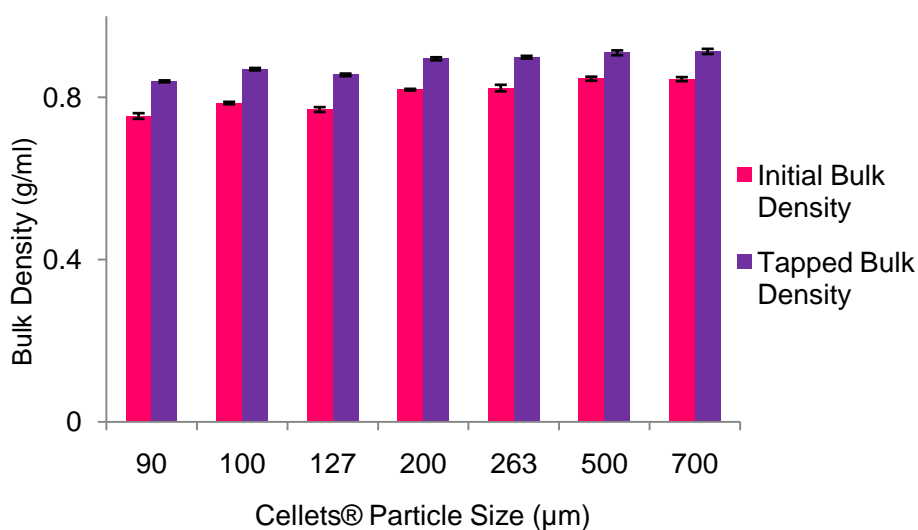
Figure 2-7: Mean Initial Bulk and Tapped Densities for Different Sizes of Cellets® Particles

Table 2-9: Particle Size and Bulk Density Ranges of Cellets® (PharmaTrans Sanaq AG Pharmaceuticals, n.d.)

Type	Particle Size range (µm)	Bulk Density (g/ml)
Cellets® 90	63-125	0.8±0.5 %
Cellets® 100	100-200	0.8±0.5 %
Cellets® 127	100-160	0.8±0.5 %
Cellets® 200	200-355	0.8±0.5 %
Cellets® 263	212-300	0.8±0.5 %
Cellets® 500	500-710	0.8±0.5 %
Cellets® 700	700-1000	0.8±0.5 %

The Carrs Index and Hausner Ratio for the Cellets were seen to decrease as the particle size increased. The Carrs Index was seen to range from a mean of 10.18 to 7.47 which, as <15, is seen to indicate excellent compressibility

which is not unexpected for a starter core particle which is usually coated and often compressed into tablets with a Hausner Ratio mean ranging from 1.11 to 1.08 which, as <1.25, can be seen to indicate good flow properties which are beneficial in terms of pharmaceutical processing

It could be seen that as all of the particle sizes had a bulk density of around 0.8 g/ml that they were all less dense than water which may be seen to suggest that suspending the particles uniformly would be difficult as they may float. The densities of some commonly used suspending vehicles are shown in Table 2-10, it can be seen that methylcellulose, HPMC and OraPlus were more similar to water and others were more dense. Again this compares with their sugar and glycerol content and may be useful in reducing the sedimentation of larger particles as per Stokes Law by reducing the difference in density between the particle and the media and hence forming a more stable suspension.

Table 2-10: Densities of Commonly Used Suspending Vehicles

Same Density as Water	Higher Density than Water (Results given as Mean±SD in g/ml)	
Methylcellulose 1 %	OraSweet® SF	1.04±0.01
HPMC 1 %	OraSweet® SF:OraPlus® (1:1)	1.05±0.00
OraPlus®	Yoghurt	1.10±0.00
	Apple Sauce	1.13±0.02
	OraSweet®:OraPlus® (1:1)	1.15±0.01
	Methylcellulose: Glycerol BP (5:2)	1.15±0.04
	Methylcellulose: Syrup BP (1:1)	1.22±0.00
	Glycerol BP	1.26±0.00
	OraSweet®	1.33±0.05
	Syrup BP	1.34±0.02

2.3.4. Rheology

2.3.4.1.   *Viscosity*

It can be difficult to compare rheology between different literature since everything a sample experiences   prior to measurement (the so called sample history) can change the rheology as can factors of the measurement itself including shear conditions, temperature and type of rheometer/viscometer/geometries. It can be seen from Figure 2-8 that the reproducibility of HPMC was better than that of MC at 1 %, this may be due to the difficulties in wetting MC and the effect that it foams more making accurate measurement less straight forward.
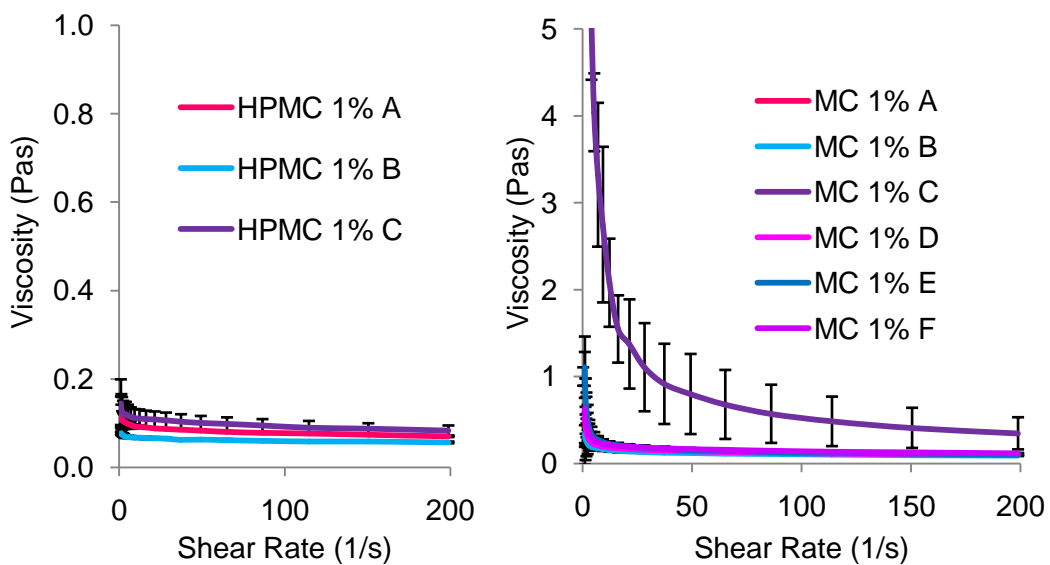
Figure 2-8: Shear Rate vs. Viscosity for Different Batches of HPMC and MC 1%

From Figure 2-9 it can be seen that as the concentration of MC or HPMC is increased, viscosity also increases as expected since the increased amount of polymer has more chains to intermingle and trap water. It can seen that MC has a higher viscosity than HPMC (despite both reported as 4000 cps) which may be due to its formation as a structured gel or may be that it is slightly more concentrated due to difficulties with it foaming in making to volume accurately.
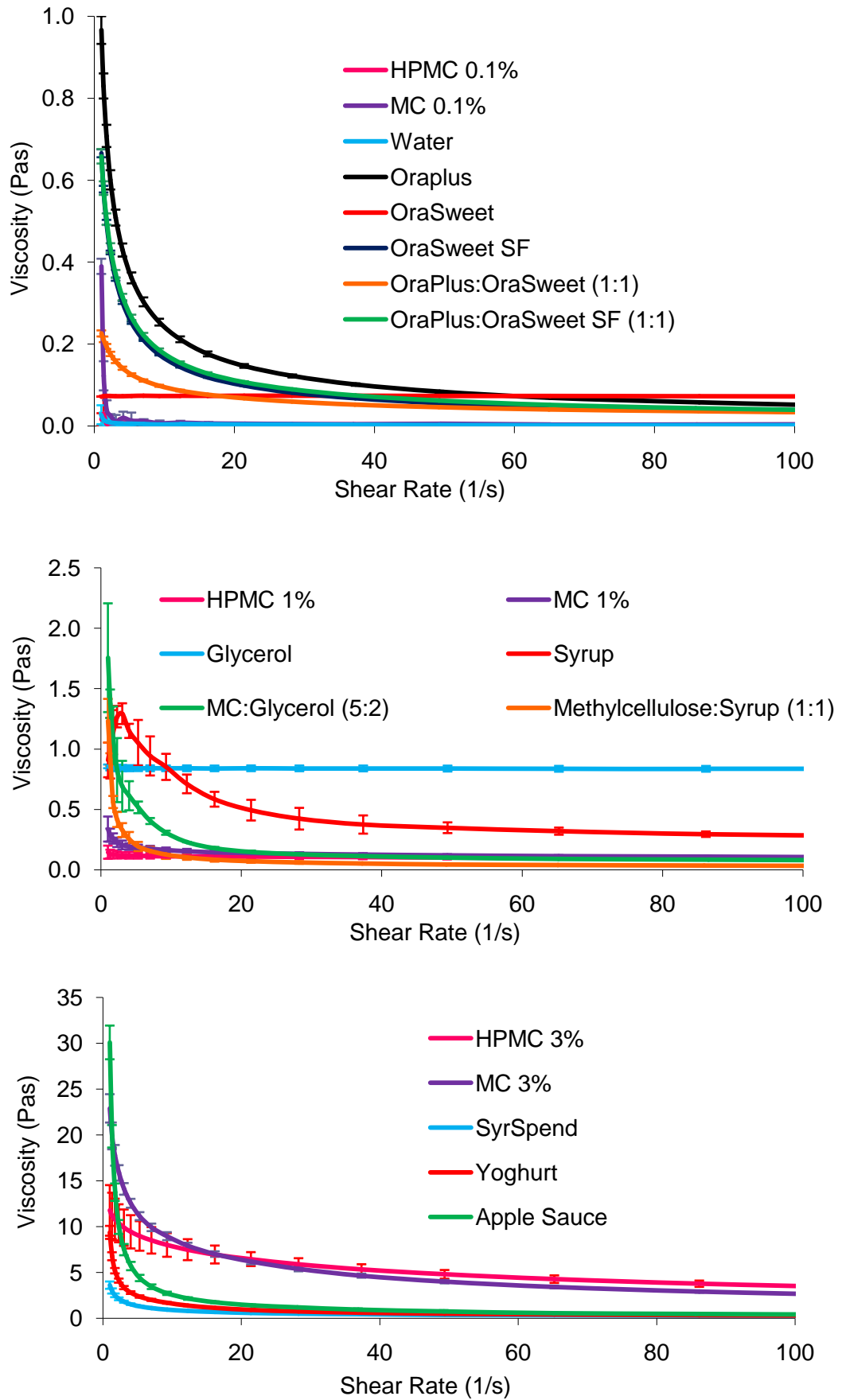
Figure 2-9: Mean Viscosities of Suspending Media against Ascending Shear Rate (a, b and c covering different viscosities of suspending media)

It can be seen that OraPlus® had a similar viscosity to the MC and HPMC 1 % solutions whereas the SyrSpend SF® is more viscous, but not as viscous as the MC or HPMC 3 % solutions which Is likely to be due to the compositions of their viscosity modifiers. The "Ora®" Suspending vehicles (composition given in Table 2-4) shown in Figure 2-9 have a viscosity in a similar range to MC and HPMC 0.1 % solutions with OraSweet® having a more Newtonian nature than the other media. Figure 2-9 shows that the viscosity of glycerol and syrup decreased through combination with MC 1 % aqueous solutions. Syrup was discovered to be very slightly shear thinning which is not expected but this may be an experimental artifact based on the stickiness causing some resistant to flow on initiation.

The viscosities of commonly used vehicles which are not pharmaceutical suspending agents can be seen to range from the less viscous water through to yoghurt (which can suspend fruit or flavours). The viscosity of water was seen to be similar to MC and HPMC 0.1 % solutions whereas yoghurt was seen to be similar to MC and HPMC 3 % solutions in Figure 2.9. In summary: water, commercial suspending vehicles and yoghurt where seen to have viscosities in a similar range of MC and HPMC 0.1, 1 and 3 % solutions respectively and to all be shear thinning. These suspending media provided a model suspending media to be taken forward in suspendability studies.

It can be seen from Figure 2-10 that autoclaving did not have an effect on the viscosity of HPMC as long as it was shaken after to ensuring mixing – this lack of effect of autoclaving has been reported by others.
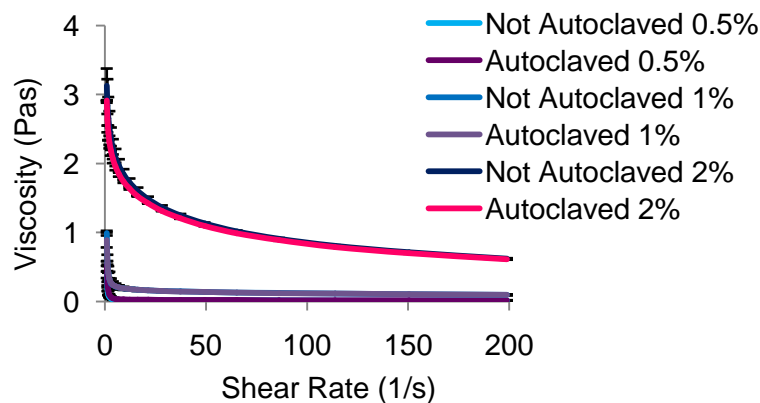
Figure 2-10: Effect of Autoclaving on the Viscosity of HPMC 0.5, 1 and 2% Solutions

2.3.4.2.   Time to reformation

The time a media takes to reform its internal structure after being sheared can be assessed by exposing a sample to a low, high and then low again shear rates as shown in Figure 2-11 by the pink line to see how long the suspending vehicle takes to return to the same viscosity again as shown by the blue line. In the case of this illustrated MC: glycerol 1:1 mixture, it can be seen that it rebuilt very quickly.
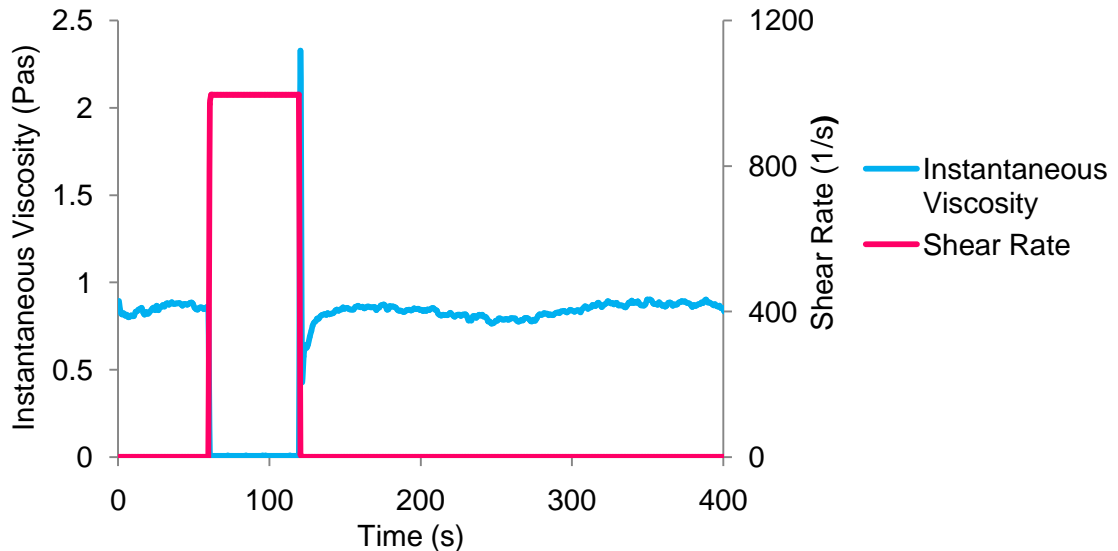
Figure 2-11: Step Test for Glycerol: Methylcellulose showing how Viscosity and Shear Rate changed with Time

The time taken to reform may be important as if the product reforms too quickly after shaking, the patient or carer may not be able to withdraw and administer the dose before the vehicle becomes more viscous again but if it takes too long to reform, drug or microparticles which are suspended in the media may sediment and cause problems with dose reproducibiltiy if they are not able to be easily resuspended.

From Figure 2-12, it can be see that media such as water and yoghurt reformed instantly whereas others took longer: those that were not shown had not reformed after twenty minutes. Although 100 % reformation has been looked at, assessing the time taken for the media to reform to certain percentages may give a more overall view and is a potential area for future work. It is possible that the structure of the media didn't reform after twenty minutes, that the high shear rate chosen damaged the structure (it was

deliberately chosen to be a high shear rate in excess of normal shaking to assess for how long reformation would take) so it was unable to reform or that the vehicle may have been shear thinning under the initial rate and hence carried on shear thinning. This could be better assessed in the future through shearing the sample without an initial stress and watching time to percentage reformation at different shear rates so as to ensure the structure is not broken or performing a thixotropic loop sweep.
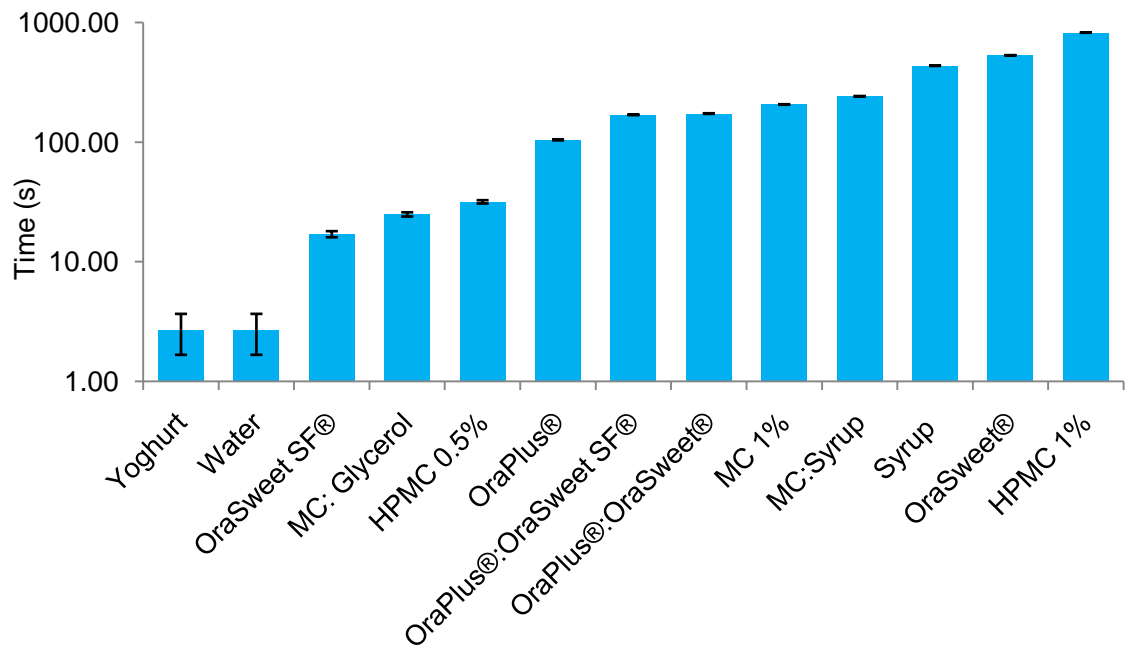
Figure 2-12: Mean Time Taken for 100% Reformation of Various Suspending Media

It can be seen from the data that was obtained we either have a time less than 100 sec or >20 minutes. Ideally a midpoint time would be acceptable for a multiparticulate suspension vehicle to allow pouring but also ensure reformation so future work would benefit from the improvements above.

2.3.4.3. *Yield Stress*

The yield stress is the maximum stress below which no flow will occur. Not all materials have one as can be seen from Figure 2-13:

- yoghurt has a clear peak and hence yield
- glycerol has a no clear peak
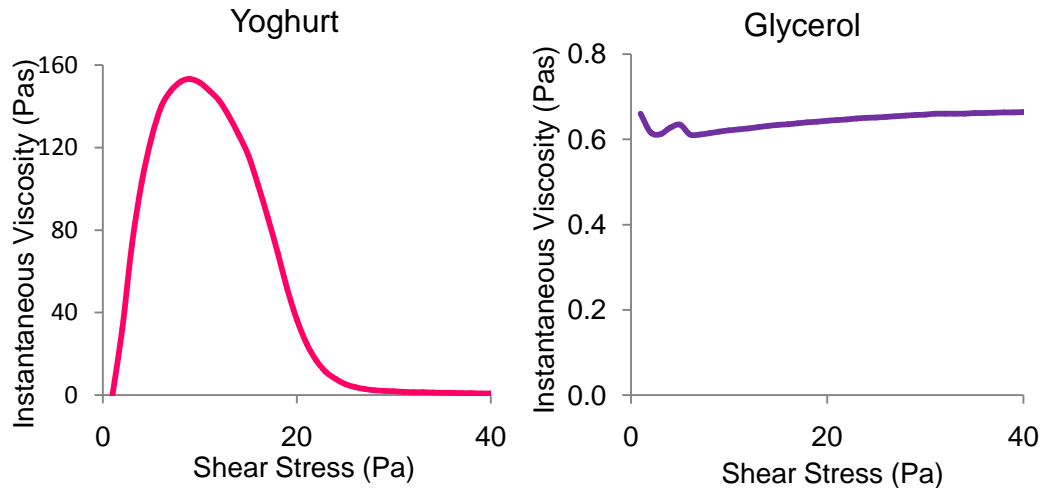
Figure 2-13: Mean Instantaneous Viscosity of Yoghurt and Glycerol against Shear Stress

In trying to keep microparticles suspended, a suspending vehicle with a higher yield stress may keep them suspended better since it would have a value of stress that must be overcome for the vehicle to start to flow: If this is the case it can be seen from Figure 2-14 that vehicles like the higher concentrations of MC and HPMC may be prefered although the shear rate of sedimentation is likely to be below the lowest stress used and shaking has not been well quantified.This experiment would benefit with being run at lower stresses to differentiate more between vehicles at the lower end which may still be able to provide protection against sedimentation and to directly quantify which lower materials have a yield stress.

Increasing Yield Stress

No Yield Stress/Less than 0.3 Pa
OraSweet, Syrup, Glycerol SyrSpend OraSweet: Plus, MC 0.1 to 1% and HPMC 0.1 to1 %

Yield Stress less than 1 Pa
OraSweet SF, OraPlus and OraSweet: OraPlus SF

Yield Stress less than 5 Pa
MC:Glycerol, MC:Syrup, MC 3 %, Yoghurt

Yield Stress less than 20 Pa
HPMC 3 and 5 %, Apple sauce

Yield Stress more than 38 Pa
MC 5 and 10 %
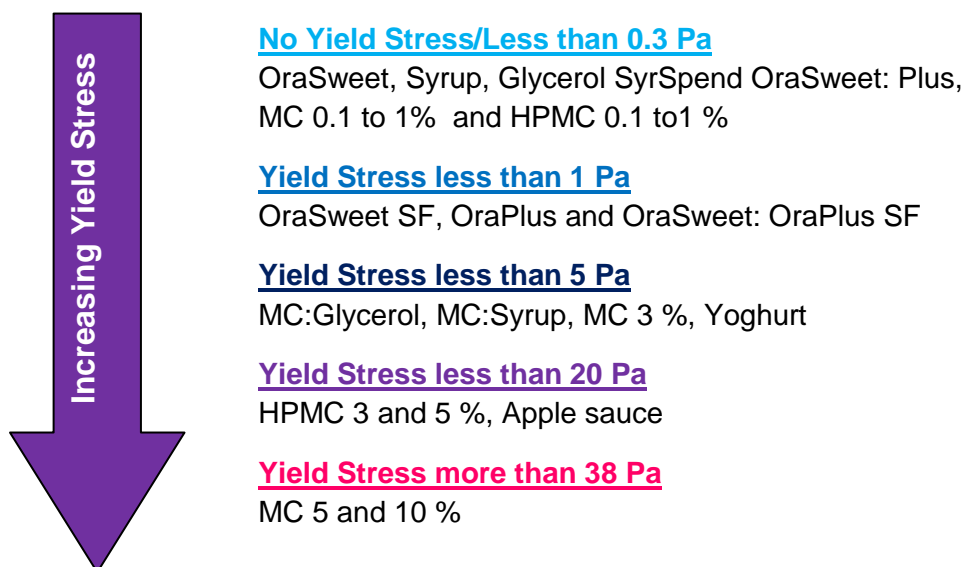
Figure 2-14: Yield Stresses of the Suspending Vehicles Tested

2.3.4.4. *Effect of Shaking and Stirring*

According to the British Pharmacopoeia, 30 inversions are recommended for the particles to disperse well in a suspension for homogeneity whereas a patient is unlikely to invert the bottle 30 times – hence the change in viscosity of different concentrations of HPMC (0.1-2%) was studied over a range of manual inversions ranging from 2 to 32. It can be seen from Figure 2-15 that there is no effect on number of inversions on the viscosity of the media which is highly variable as shown by the large standard deviations. This may however be useful to perform in more people in the future to see whether there is an effect of shaking on viscosity than in just one person and to try to define the shear rate of normal shaking which is not well defined.
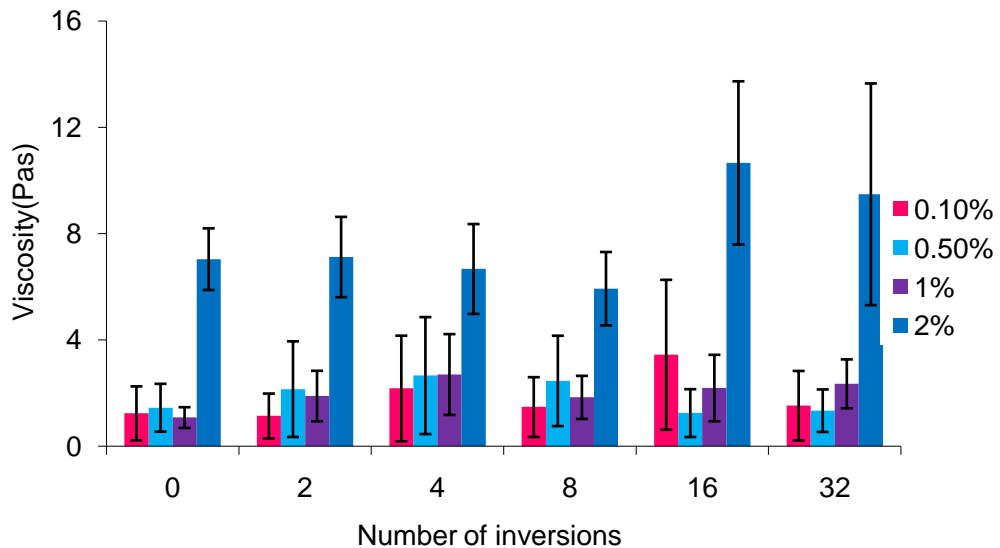
Figure 2-15: The Effect of Different Numbers of Inversions on the Viscosity of Different Concentrations of HPMC Solutions (0.1, 0.5, 1 and 2%)
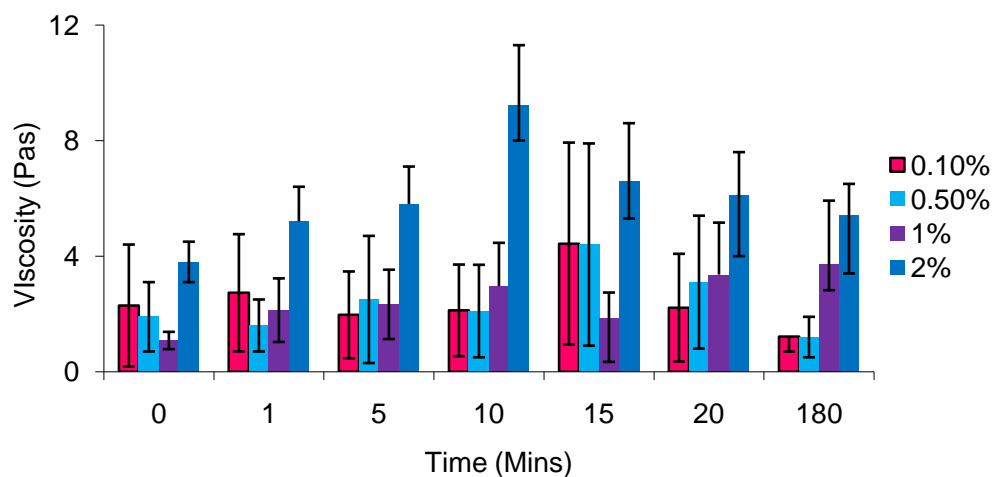
Figure 2-16: The Effect of Different Times of Stirring on the Viscosity of Different Concentrations of HPMC Solutions (0.1, 0.5, 1 and 2%)

It can be seen from Figure 2-16 that there is little clear effect on time after stirring on the viscosities of HPMC but that there seems to be a trend towards thickening after standing for 10-15 minutes in the most concentrated media which may be due to sedimentation of undispersed particles. This was undertaken to see the effect of stirring initially since it is recognized that many pharmacies in reconstituting/making a suspension will only be able to shake manually or have a stirrer for dispersion.

2.3.4.5. Oscillation

In order to assess the Linear Viscoelastic Region (LVR) for frequency assessments, an amplitude sweep was performed. It can be seen from the example in Figure 2-17 that OraPlus that as G' (the storage modulus shown by the blue line) is higher than G'' (the loss modulus shown by the red line) and the phase angel <45º (as seen by the orange line) that the OraPlus® can be seen to be solid-like under these conditions. The LVR (region where G' and G'' are parallel) is shown by the box and marked as two thirds of way along the LVR with an initial stress of 0.0073 Pa and Strain of 0.0051)
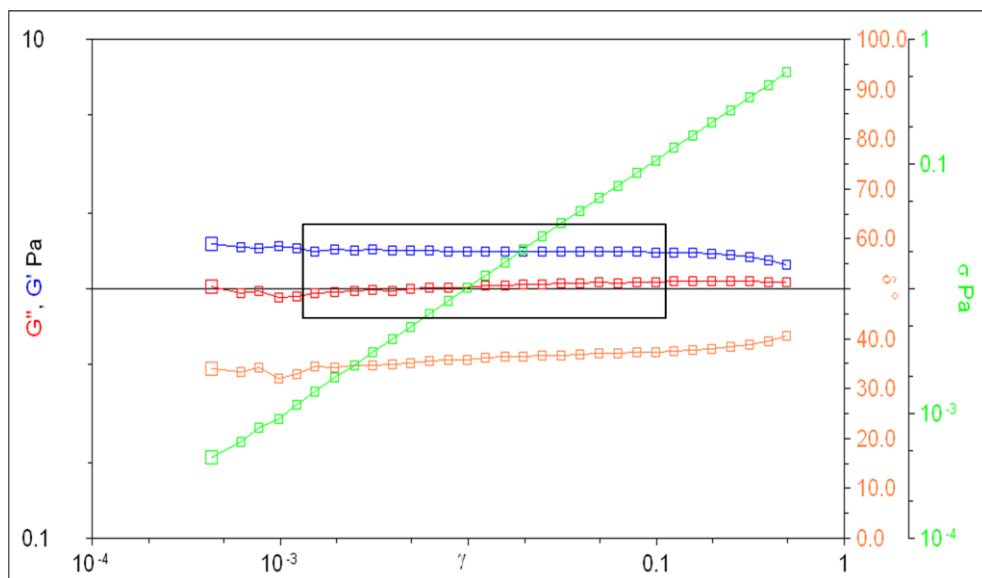
Figure 2-17: Amplitude Sweep on OraPlus ® (pp40, 1Hz auto stress, 0.005-0.5 strain units, 0.5Pa initial stress) with strain on the X axis against shear stress (green) G' or storage modulus (blue), G'' or loss modulus (red) and phase angle (orange) on the Y axis. The box illustrates the Linear Viscoelastic Region (e.g. the strain values over which the G' and G'' are parallel) within which region the Frequency Sweep should be undertaken. As the G' is higher than the G'', OraPlus® can be thought of as solid like

The raw data from a frequency sweep can be seen in Figure 2-18 from the example of Oral Sweet SF®: OraPlus® (1:1) where because at lower frequencies G' shown by the red line is lower than G'' shown by the blue line, the vehicle will be is liquid like at lower frequencies whereas as the G' and G'' lines cross it will be solid like at higher frequencies (shorter timescales). It may be desirable for stability to have a solid like structure over higher frequencies to prevent settling and liquid at lower to aid pouring.
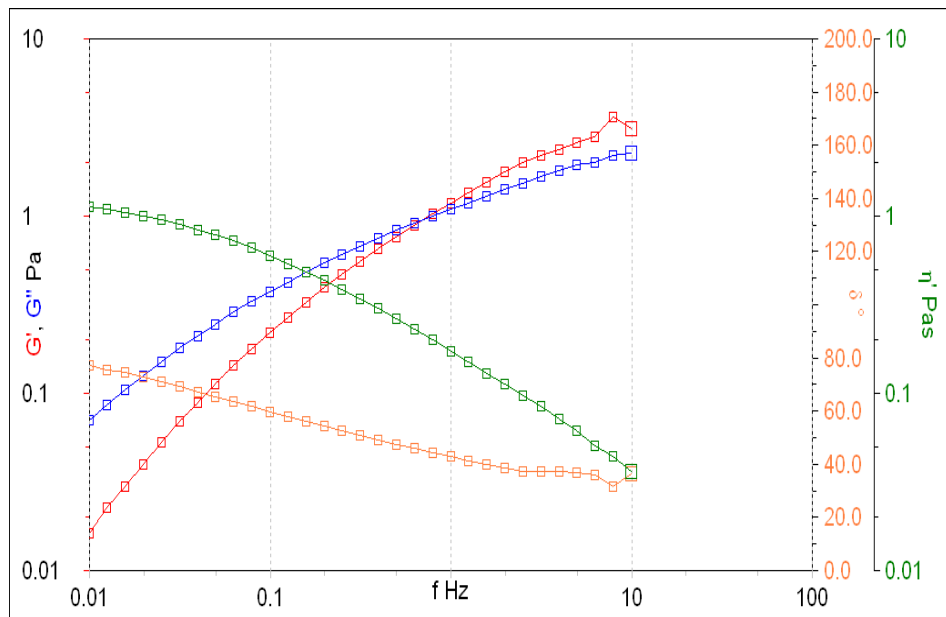
Figure 2-18: Frequency Sweep of Oral Sweet SF®: OraPlus® (1:1) (0.01-10 Hz, 31 samples, initial stress 0.0364 Pa/Strain 0.0314) with frequency on the X axis against instantaneous viscosity (green) G' or storage modulus (red), G'' or loss modulus (blue) and phase angle (orange) on the y axis. As the G'' is higher than the G' over low frequencies corresponding to longer timescales OraSweet SF®:OraPlus® can be thought of as liquid like whereas it changes to being more solid like at higher frequencies as shown by G' and G'' crossing over

From Table 2-11 , all of these all initially had their G' or G'' higher in the initial frequency (10 Hz) of the frequency sweep with the exception of OraPlus and OraSweet: OraPlus which initially had a higher G' – the cause for this is likely to be the very close magnitude between the viscous and elastic modulus (1.4 vs. 1.3) of OraPlus meaning that both components had virtually equal effect.

Table 2-11: Higher G' or G" in the Frequency Sweep

G' Higher	G" Higher
OraSweet SF	OraPlus
OraSweet SF: OraPlus	OraSweet
MC	OraSweet: OraPlus
MC:Syrup	Glycerol
MC:Glycerol	Syrup
	HPMC

From Table 2-12, glycerol and syrup were the only two media that showed little frequency dependent change in viscosity – this shows as the frequency sweep confirms by the constantly higher viscous modulus that these media are viscous liquids. Good storage stability e.g. in preventing sedimentation may be shown by being elastically dominated at lower frequencies corresponding to longer time scales so those with G' higher or transition to G' over lower frequencies would be preferred which corresponds to yield stress measurements. Hence in future, assessment of desired viscoelastic properties could be performed more quickly and easily by assessing the yield stress e.g. force required for the media to flow.

Table 2-12: Changes in G' and G" over Frequency

G' Higher at All Frequencies	G" Higher at All Frequencies
MC	Glycerol
MC: Syrup	Syrup
MC:Glycerol	
Apple Sauce	
Yoghurt	
Initially G' Higher but Crossover at:	Initially G" Higher but Crossover at:
OraPlus – 1Hz and again at 0.0126 Hz	HPMC - ~1 Hz
OraSweet: OraPlus: 3.981 Hz and 0.03981 Hz	OraSweet – 0.0126 Hz
OraSweet SF – 0.1995 Hz	
OraSweet SF:OraPlus – 0.631 Hz	

2.3.5. Suspendability

There is no official method for the assessment of suspendability. Initially the suspendability experiment was attempted in measuring cylinders to allow for the measuring of sedimentation volume. However, it was found that even with vigorous shaking it was not possible to disperse the Cellets® in the MC or HPMC solutions (the Cellets® aggregated either on top if they were added to the suspending media or at the bottom if the suspending media was added to the Cellets® probably due to their hydrophobicity) as shown in Figure 2-19
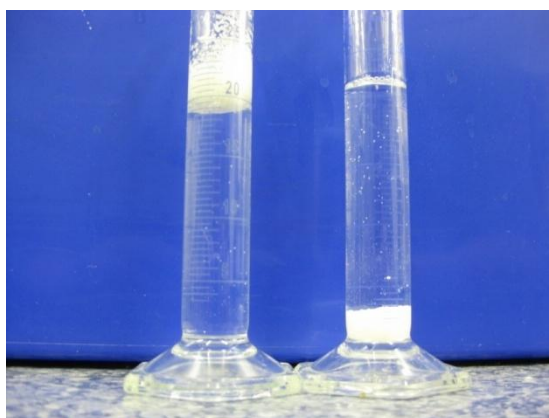
Figure 2-19: Poor Dispersion of Cellets in a Measuring Cylinder with Suspending Media added first (left) and second (right) after shaking

The experiment was then transferred to small beakers where the height of sediment was to be measured using a ruler and stirring with a magnetic stirrer on setting six for one minute to disperse the Cellets®. A similar aggregation of Cellets® occurred again with the thicker suspending media which was partially overcome by having the magnetic stirrer mixed the dry particles so that they were moving while the suspending media was poured on so hence less able to aggregate.

Some Cellets® did become suspended by this method as shown in Figure 2-20; however they were not uniformly suspended. The Cellets® closer to the magnetic stirrer were more likely to be suspended and at a higher height than those further away, hence it was not felt appropriate to assess by sedimentation height. Consequently, the time taken for all the suspended particles to sediment was visually measured but unfortunately this is a fairly subjective assessment with poor inter-observer reproducibility.

Figure 2-20: Dispersion of Cellets® (200 µm) after stirring in a Beaker in MC 1 % (left) and 3 % (right)

As shown in Figure 2-21 and expected from Stoke's Law, smaller particles took longer to sediment than larger particles and thicker suspending agents slowed sedimentation the most. MC 3 % solution was the best suspending agent at slowing sedimentation with results in excess of seven hours and water the worst lasting only seconds for the smallest Cellets®. However the dispersibility was worse in the thicker suspending agents. MC was found to be more difficult to disperse the particles in than HPMC due to the higher viscosity and so HPMC was chosen as the suspending media with which to perform the grittiness assessment in as shown in Figure 2-22.
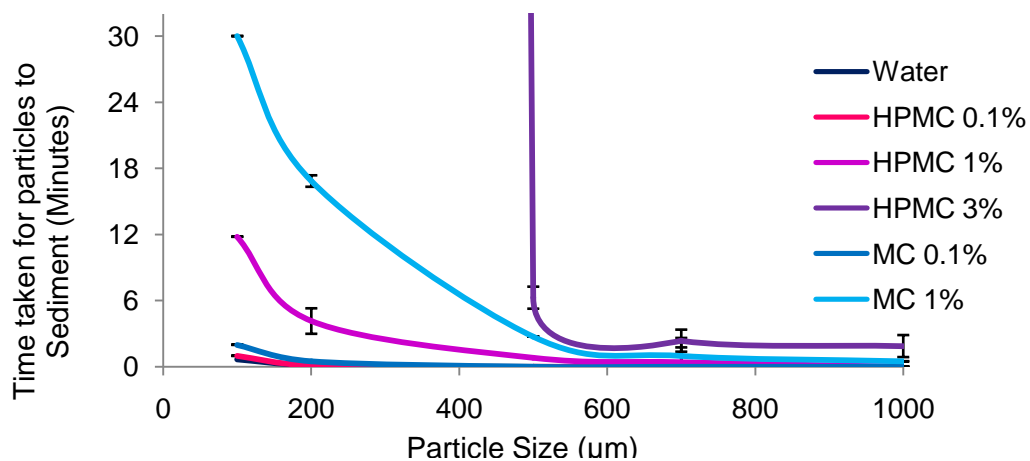
Figure 2-21: Particle Size versus Sedimentation Rate in Different Media as assessed by watching how long all the particles took to sediment (n=3)
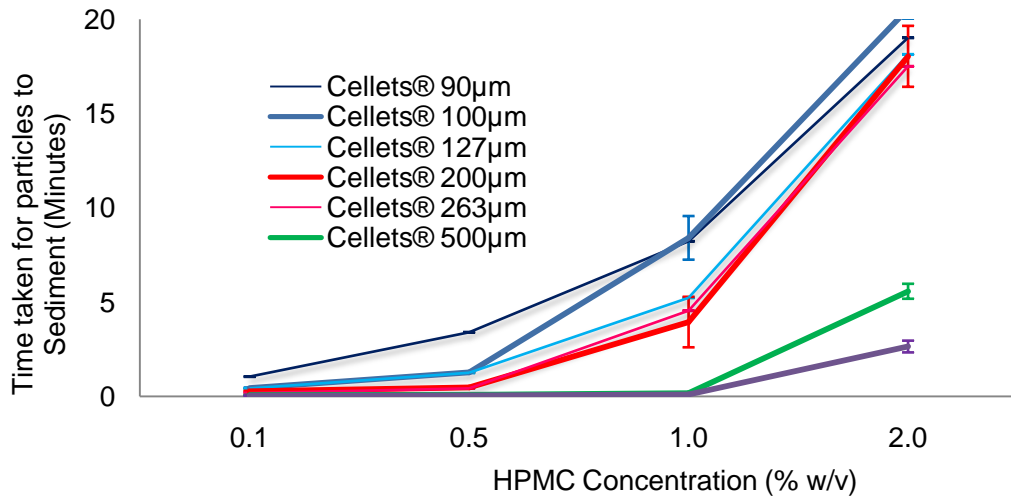
Figure 2-22: Time taken for Particles of Different Sizes of Cellets to Settle in Different Concentrations of HPMC as assessed by watching how long the particles took to sediment (n=3)

Once dispersed by mixture of stirring/shaking/mixing in plastic containers as seen on the top of Figure 2-23 which was not standardized and assessed by visual distribution of particles, a mechanical wheel as seen in Figure 2-23 was used to keep the suspensions for the grittiness tests on so as to keep the samples moving and to try to prevent sedimentation whilst the testing occurred and was removed just prior to administration to the volunteer for grittiness testing as described in Chapter 3.

Figure 2-23: Mechanical Wheel used to try to Keep Particles Suspended in Samples for Grittiness Trials (after the particles had been dispersed by stirring/mixing/shaking until just prior to administration)

As the problem with the Cellets® was more trying to suspend them rather than the sedimentation, further work would need to look at how to get the Cellets® to disperse better. This may include finding a better method of dispersion or adding a surfactant (*but would have to be balanced with the desire to minimise excipients in paediatric formulations*) which may also allow such good dispersibility/suspendability that longer term stability or resuspendability may be able to be assessed.

In the future, a better method of assessing dispersibility and sedimentation may be to look at percentages of particles dispersed/suspended (if particles are) such as by light scattering or image analysis and the time taken for certain numbers/percentages of particles to sediment in order to make a more objective measurement or using a sedimentation balance approach.

2.4. Conclusions

The choice of a suspending vehicle is always going to be a decision weighing up a variety of factors including stability and acceptability along with minimising numbers and levels of excipients, especially in children. Some suspending vehicles commonly used in children were characterised to determine a suitable vehicle for the administration of functionalised multiparticulates (which ideally could be the same vehicle for all particles made from the same polymer if the particles produced were robust enough).

As the pH of suspending media is of critical importance in the development of a functionalised suspension since if the pH is incompatible with the multiparticulates, the functionality is lost and hence we are left with a bitter tasting medicine with the potential that the child will not take it and so not be able to take advantage of the therapeutic benefit of the medicine. In this case only higher concentrations of HPMC and all methylcellulose concentrations including those in combination with glycerol and syrup along with syrup on its own are the preferred media of those tested although are likely to need to be buffered. Other suspending media not tested of desired pHs include NaCMC and xanthan/guar gums which should be assessed in any future work especially as they are easier to wet than HPMC and MC and can be used in a powder for reconstitution which in terms of particle stability, may be what is needed.

It was thought that rheological measurements would be the key to finding a vehicle that was able to produce a stable suspension of multiparticulates which would be larger than individual drug particles in most suspensions and hence more difficult to keep in a uniform suspension. There are a range of tests which can be used to investigate the rheology of suspensions and in this case a flow curve was used, the yield stress (that is stress that requires to be overcome before the vehicle can flow) determined and a thixotropic step test applied to see how quickly the suspending vehicle reforms after shearing.

No clear effect on viscosity on number of inversions to a mix a suspension or on the viscosity reformation post stirring was observed which would have been interesting in assessing the in use performance of suspensions (since in a pharmacy, a stirrer or shaking are likely to be the only two methods available to make suspensions where needed). Future work on the stresses exhibited on a formulation in terms of shaking would be interesting as this has not been well studied – it may be that by assessing the viscosity after shaking that population effects will be found if it is performed in more people.

It was found that all the suspending vehicles were shear thinning with the exception of OraSweet® and glycerol with apple sauce, yoghurt and higher concentrations of HPMC and MC (3 %+) having the highest viscosities under the conditions used. As HPMC and MC from 0.1 to 3 % were seen to cover the range of viscosities of suspending media from water to soft foods, these media were used in suspendability studies. The yield stress assessment did not differentiate well between those of lower, more in use yield stresses such as those associated with sedimentation and should ideally be rerun, similarly the thixotropic loop test shear rate may have been too high either in the initial or harsh shearing phase as many liquids did not completely reform within 20 minutes – this may be due to slow reformation, shear thinning during the initial period or structure breakdown by high shear rates .A better way to examine this in future would either be to have no initial shear rate phase and shear the sample under different shear rates and watch rebuilding time, to calculate percentages of reformation over time or to perform a thixotropic loop sweep. Oscillation experiments showed little structure not already determined by higher values of yield stress and hence in future, this would be a quicker way to assess for structural stability under sedimentation conditions.

By using only HPMC and MC solutions in suspendability testing it is acknowledged that only the effect of similar viscosities is assessed – different properties of vehicles due to excipients, wetting etc are not examined so an area for future work may be to look at suspendability compared to commercial suspending agents and with more comparable

particles than Cellets. In terms of suspendability, smaller particles and more viscous suspending media caused the particles to take longer to sediment as per stokes law however smaller particles however dispersibility (and hence potential dose uniformity) of the smaller pellets (still >100 µm) in the thickest solutions was difficult. Further work is needed to improve this dispersibility which is likely to be by the addition of "child-friendly" excipients (e.g. those with ideally a history of use in the food industry or long standing pharmaceutical use and tolerance) such as a surfactant or use of a commercial suspending media as the other main alternative of using a different mixer would be difficult for parents or pharmacies to implement as a way of producing suspensions. Methods of assessing dispersibility should also be improved by methods which are more reproducible and quantifiable such as the use of light scattering or image analysis or using a sedimentation balance

3.   GRITTINESS OF SUSPENSIONS

3.1.   Introduction

3.1.1. Acceptability

Medicine compliance is a problem in paediatric therapy with compliance rates ranging anywhere from 11-93 % depending on many factors including factors such as the frequency of therapy and taste being critical to compliance (Matsui, 2007). These reasons for the lack of compliance are important since they may be overcome through the use of age appropriate taste-masked or modified release formulations such as functionalised multiparticulates. By virtue of their larger size when compared with individual drug particle size, the suspendability as examined in Chapter 2 and grittiness of suspensions were thought to be key in the use of functionalised particles in medicines.

Acceptability has been defined by the European Medicines Agency as the "*overall ability of the patient and caregiver (defined as 'user') to use a medicinal product as intended*" (European Medicines Agency, 2011). It can be seen from this definition whether a medicine is accepted will depend on both the user and the medicine.

One aspect of acceptability is palatability which has been defined as "*the overall appreciation of an (often oral) medicine by organoleptic properties such as smell, taste, aftertaste and texture (i.e. mouth feeling), and possibly also vision and sound*" (European Medicines Agency, 2011). Palatability will depend upon the nature of the active ingredient, the excipients and how it has been formulated. As can be seen from palatability above, other sensory components rather than just taste, such as texture or mouth feel, are important. Mouthfeel and texture are often studied in the food industry where grittiness is undesirable. It is often assessed in the comparator testing of products such as chocolate and dairy products where the opposite property of creaminess is pleasurable for consumers or in consumer healthcare for products such as toothpaste where a gritty texture may adversely affect performance although these are at smaller sizes (<30 μm) compared to the

>100 µm multiparticulates that may be required to control release in the sense of taste masking or modified release.

If the suspended particles feel gritty in suspension (where grittiness can be thought of as the sensation of sand in the mouth), the paediatric patient may refuse to take their medicine with the potential for clinical deterioration. This may be of particular significance if the particles had been dispersed or suspended in a food or drink for administration leading the already ill child to refuse these. Grittiness is therefore of great importance in paediatric formulations. Yet there are no definitive answers of what particle size produces a gritty sensation with figures ranging from anywhere up to 1mm often quoted with no reference (Billany, 2007). This acceptable particle size is known to be dependent on the shape/hardness of the particle and viscosity of the suspending media it is given in. There are a limited number of formulations that contain large particles in suspension such as activated charcoal, though this is often used in emergency situations and little is reported about its acceptability (Cheng and Ratnapalan, 2007, Engelen et al., 2005b, Tyle, 1993, Imai et al., 1995, Engelen et al., 2005a, Tyle et al., 1990).

The majority of pharmaceutical sensory analysis in children involves assessing liquid medicines (drug solutions/suspensions) for taste, aftertaste and Mouthfeel (Cohen et al., 2009, Hames et al., 2008, Baguley et al., 2012). Very few have examined multiparticulates or the larger sizes of multiparticulates in suspension although a bead size of less than 1.5 mm-2mm has been recommended for a sprinkle (Nagavelli et al., 2010, FDA, 2011, Van de Vijver et al., 2011). Given that weaning is generally advised from four to six months of age it is expected from then that children are able to cope with semisolid foods, although it is unlikely that they have a texture like pharmaceutical particles in a suspension. Few studies have been undertaken on the acceptability and preference of different dosage forms in children and those which have employed various methodologies including different scales and caregiver assessments (Davies and Tuleu, 2008, Cram et al., 2009, Bays et al., 2010). Hence it may be interesting to assess the

grittiness and acceptability of larger particles in suspension in young adults to screen grittiness samples and then hopefully one day, to apply this to children to assess their views on acceptability. Adults have a number of benefits including ease of access and the ability to undertake a larger number of samples without getting bored but obviously are not children (Liem et al., 2004)!

3.1.2. Sensory methods

Ways of assessing for taste can include chemical analysis which often works by generating a fingerprint assessment (often potentiometrically by electronic tongue) of the dissolved organic and inorganic components in a sample and relating these chemometrically with the taste of a product (Alpha MOS, Anand et al., 2007b). As such it needs to be trained using data from human subjects. Time was not available for such a training exercise during the period of this project and hence this technique was not used. Measurements of texture or mouthfeel can be assessed in vitro through the use of a texture analyser instrument. Again these need to be correlated with human hedonic responses to provide fully reliable data.

As well as these instrumental measurements it is possible to standardize the assessment of formulations for organoleptic characteristics using the methodologies of sensory analysis and thus obtain reliable and objective data from otherwise subjective assessments (Meilgaard et al., 2007). The area of mouthfeel assessment is particularly difficult to assess by any other method and so sensory analysis was employed in this research. Details of the methodology employed are given later.

There are a number of different categories of sensory methods depending upon what the study aim is. For each category there are different types of tests which can be employed as summarised in Table 3-1 with scaling tests and affective tests chosen in this research to get an overview into how gritty suspensions are found to be and what level of grittiness is acceptable since

this has not been extensively covered in the literature for larger particles (<100μm). A visual analogue scale (shown later) was used to record this range of grittiness with controls given to be the most and least gritty (Lim, 2011).

Table 3-1: Different Types of Sensory Methods (Meilgaard et al., 2007)

Category	Description	Tests
Descriptive Testing	Differentiating between samples	Difference tests Ranking
Scaling Tests	Scoring a sample on an attribute	Scoring
Affective Tests	Measuring how much a product is liked or disliked	Preference Acceptability
Descriptive Methods	Objective Description	Flavour Profiling

Sensory testing often involves assessing a product in terms of its profile (e.g. assigning different intensities to qualities such as appearance, taste, aroma, flavour, texture, mouthfeel and aftertaste). Grittiness is classed as the amount of particulates perceived by the mouth which is sometimes included into the more generic criteria of texture or mouthfeel. Mouthfeel can also cover where the sample feels chalky, oily or astringent. The suspending media itself can also affect grittiness, texture (viscosity) or mouthfeel.

As grittiness is often dependent upon viscosity, the rheology of a range of commonly used suspending vehicles (Nahata and Hipple, 2003) were measured and compared in Chapter 2. The results of these experiments allowed a logical determination of the viscosities of suspending media to be used in an investigation of the influence of viscosity of suspending media, particle size and particle concentration on the sensation of grittiness here. HPMC was chosen from rheology experiments in Chapter 2 and its use/tolerability as a food additive. Microcrystalline cellulose pellets (Cellets®) were chosen as the placebo particles due to their non swelling, non-disintegrating form, range of sizes, narrow stated size distribution,

reproducibility, generally regarded as safe acceptability, acceptable hardness and the fact that they have been used as starter seeds for coating for formulations and therefore have a history of pharmaceutical use.

In this chapter, the aim was to use a human panel to assess various formulations for grittiness and to test the influence of particle sizes along with concentration of particles and viscosity of suspending media together on the sensation of grittiness. The data will then be used to inform the target size of spray dried particles in Section 4 that would be acceptable for the platform

3.2. Materials and Methods

3.2.1. Materials

Hydroxypropyl methylcellulose (4000 cP, substitution type 2906, grade 65SH-400) was obtained from Shinetsu Chemical Company Ltd, Japan and Sucralose Granular NF (Emprove, NF) from Merck KGaA, Germany. Cellets® (97 µm-1000 µm) (Microcrystalline Cellulose Pellets) were kindly received from Pharmatrans Sanaq and Orange Flavour Givarome Permaseal/Orange Flavour Permaseal both received from Givaudan, United Kingdom.

3.2.2. Methods

3.2.2.1.   *Initial Grittiness Trial*

Ethical Approval for the use of human volunteers in these grittiness trials were given by the University Of London School Of Pharmacy Research Ethics Committee (REC/A/09/01) with recruitment Information and consent forms in the Appendix. The sensory set up is shown in Figure 3-1. This set up was chosen as it was clean with no distracting noises or smells to intrude on the sensory test and not a chemical laboratory where placing food in the mouth would have been prohibited. The spoon shown in Figure 3-1 was chosen as it can hold the full 10ml of sample required for the test whereas

other spoons tried could only hold around 7 ml (the sample was removed from the sample tube and placed on the spoon immediately before giving to the volunteer). It was felt that trying to use two standard 5 ml medicine spoonfuls may cause variability in two spoonfuls had to be sampled for each formulation or may allow the volunteer an addition chance to see the particles which may modify their assessment of grittiness (e.g. if they saw more, larger particles – they may score the sample even higher than if they had not seen these particles).

Figure 3-1: Sample preparation area in dispensary and sensory station (top left and right) with spoon used (bottom)

Twenty subjects (aged between 18-24 years of age including an equal number of males: females) were recruited as these were the youngest age group that could easily be assessed for research. Each subject tested 27 samples overall and two controls in each session due to the grittiness trial taking place over two sessions to allow for subject comfort. Each sample had one of three levels of HPMC concentration (to give different viscosities), microcrystalline pellets (Cellets®) concentration and Cellets® particle size with all combinations of each sampled (as seen in Table 3-2) with sample order in Table 3-3

Table 3-2: Composition of Grittiness Samples and Controls

	HPMC Concentration (%)	Particle Size (µm)	Particle Concentration (mg/ 5ml)
	0.1	100	5
	1	200	100
	3	500	500
Negative Control	0.1	No Particles Added	
Positive Control	0.1	1000	500

All participants were given the samples in the same randomised order. Subjects rinsed 10ml of sample around their mouths for 15 s to cover all oral surfaces. The negative and positive controls were tested first and the participant told that these samples will be the smoothest/most gritty samples respectively that they will receive in order to rate the texture in relation to these benchmarks. Immediately upon spitting out the sample, participants rated the intensity of grittiness on a bipolar 100 mm Visual Analogue Scale (VAS) as shown in Figure 3-2.

VERY SMOOTH .... **VERY GRITTY**
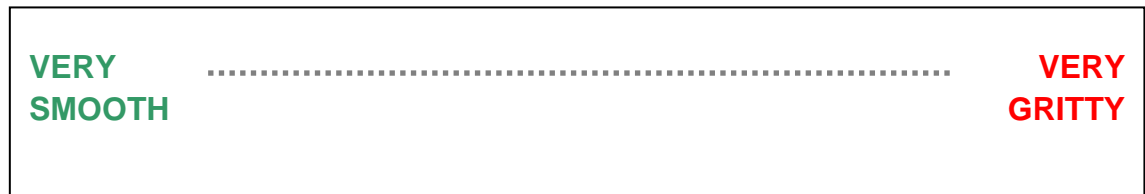
Figure 3-2: Example of the Visual Analogue Scale used to Assess Grittiness (100mm – not to scale) where the volunteer makes a mark on the scale to represent how smooth/gritty they feel the sample is which ranges from 0 for very smooth to 100 for very gritty when measured with a ruler from start of the very smooth line

Participants waited for a minute between experiments and rinsed their mouths with water before and after each sample. The subjects were also asked to record the two samples that they felt were the most pleasant to test in each session (from here on, known as the "most acceptable samples").

Table 3-3: Randomisation Order: Initial Trial showing the control samples composition and those samples assessed during each session

When Sample was Assessed	Sample	HPMC Concentration (%)	Particle Size (μm)	Particle Concentration (mg/5 ml)
Both sittings	Positive Control	0.1	1000	500
	Negative Control	0.1	-	-
Assessed On first sitting	1	1	500	5
	2	0.1	200	5
	3	1	500	100
	4	3	200	500
	5	0.1	500	5
	6	3	500	100
	7	0.1	100	100
	8	3	100	100
	9	3	200	5
	10	3	200	100
	11	1	200	5
	12	1	200	100
	13	0.1	500	100
	14	0.1	200	100
Assessed On second sitting	15	0.1	100	5
	16	1	200	500
	17	3	100	5
	18	0.1	200	500
	19	0.1	500	500
	20	1	100	100
	21	1	500	500
	22	0.1	100	500
	23	3	500	500
	24	1	100	5
	25	3	100	500
	26	3	500	5
	27	1	100	500

Basic descriptive statistics were calculated using Excel 2007 and repeated measures ANOVA (Within subjects factors) run using SPSS 17 (SPSS, Illinois). The "most acceptable" samples were determined from assessing

which five samples were chosen as most acceptable most often (e.g. as a frequency of the number of times chosen).

3.2.2.2. Refined Grittiness Trial

Based on results achieved from the initial trial, a number of modifications were made as shown in Table 3-4. Orange was chosen as a flavor as it is one of the most common flavours in the United Kingdom and sucralose due to its sweetening and excipient safety/tolerability profile.

Table 3-4: Modifications with Rationale to the Refined Grittiness Trial

Modification	Rationale
Sample Size increased to 30 (16 females: 14 males)	To try to reduce high standard deviation
Particle size range narrowed to: 90, 127 263 µm	To focus more on a more narrower size range
Particle concentration range narrowed to 125, 250 & 500 mg/5ml	To focus on a narrower particle concentration
HPMC concentration range narrowed to 0.5, 1, & 2 %.	To focus on easier to handle & better accepted concentrations
sweetener and flavouring agent included *	To reduce the mouth coating effect of HPMC alone
Individual randomisation & blinded controls added	To allow for order effects to be investigated with individual orders shown in the Appendix

Due to supply issues, the composition of flavouring agent used differed between the 2 batches used during the trial as shown in Table 3-5. The relative compositions used differed between the flavours but the composition's were chosen as they had a comparable flavour intensity according to the manufacturer and had a similar orange flavour as assessed by the researchers (n=2).

Table 3-5: Flavouring Composition of HPMC Batches used in the Refined Trial

Batch 1 (Given to volunteers 01-12)	Batch 2 (Given to volunteers 13-30)
Orange Flavour Permaseal® **0.5 % w/v** (Contains: Volatile oils 9-11 %, Water content 6 % Maltodextrin 87.5 % Modified Starch 3.5 % Ascorbic acid 0.6 %)	**Orange Flavour Permaseal®** **0.37 % w/v** (Contains: Volatile oils 9-11 %, Water content 6 % Maltodextrin 87.5 % Modified Starch 3.5 % Ascorbic acid 0.6 %) **Orange Flavour Givarome Permaseal® 0.044 %w/v** (Contains: Volatile oils 16-20 %, Maltodextrin 77.5 % Modified Starch 3.5 %)
Sucralose **0.1% w/v**	**Sucralose** **0.1% w/v**

The rheology of HPMC 0.5, 1 and 2 %, unflavoured was slightly lower than that of the flavoured and sweetened as expected by the increased solids concentration. Each of the two orange flavouring agents with sucralose (composition as detailed in Table 3-5) was investigated as detailed in Section 2.2.2.5.1. and the similarities between the two batches are illustrated in Table 3-6

Table 3-6: Comparison between the Viscosities of Different Batches of Flavoured and Sweetened HPMC

HPMC Concentration (%)	Mean±SD of Viscosity at 50 s^{-1} (mPas)		Mean±SD Yield Stress (Pa)	
	Batch 1	Batch 2	Batch 1	Batch 2
0.5	19.5 ± 0.4	24.7 ± 1.3	<0.3	
1	161.9 ± 0.5	169.3 ± 1.48	1.8 ± 0.4	2.2 ± 0.5

| 2 | 1632.5 ± 13.4 | 1625.5 ± 21.9 | 3.5 ± 1.1 | 4.0 ± 0.98 |

3.3. Results and Discussion

3.3.1. Initial Grittiness Trial

The sample with the highest grittiness score contained HPMC 0.1 % with particles of 500 μm at 500 mg/5 ml (which scored an average of 84 ± 16 mm) with all 500 mg/ 5ml samples scoring high as seen in Figure 3-3. The sample with the overall lowest grittiness score as well as the sample which was most commonly ranked the lowest grittiness sample by individuals contained HPMC 0.1 % and particles of 100 μm at 5 mg/ 5 ml (which scored 11 ± 11 mm), with all 5 mg/ 5ml samples scoring low.
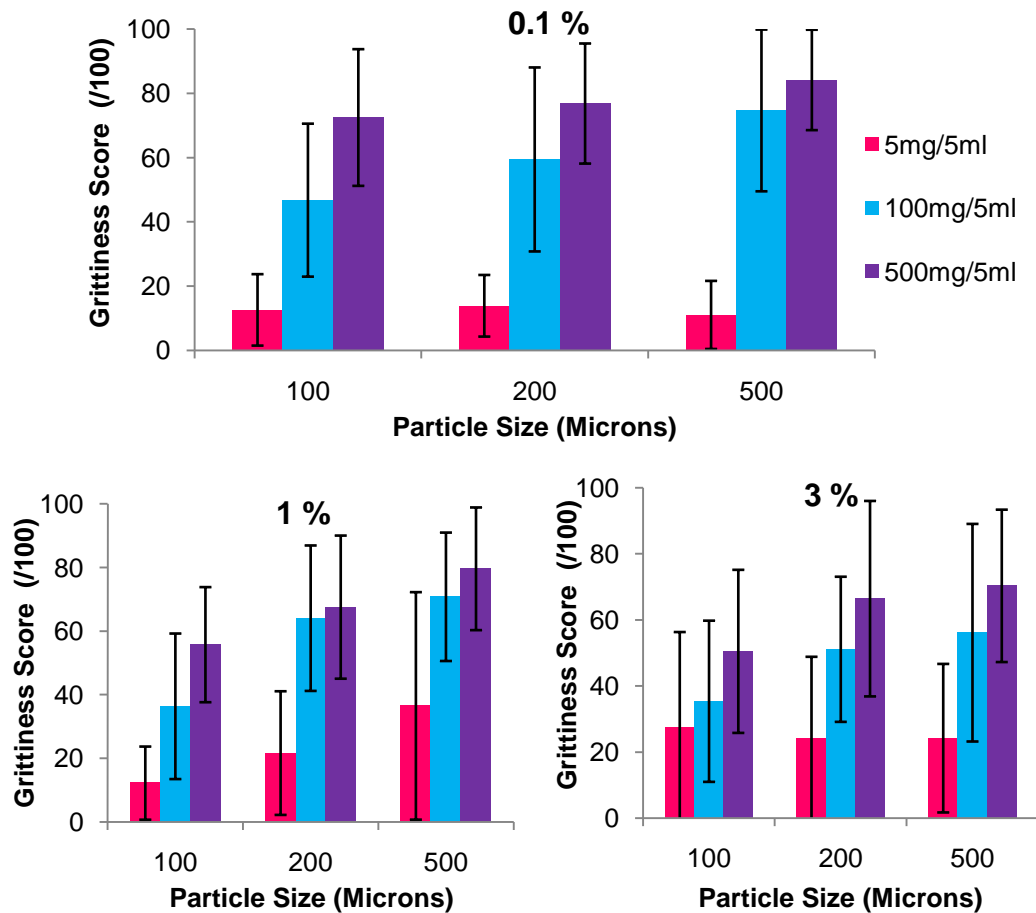
Figure 3-3: Mean Grittiness Scores (±SD) for all Samples containing HPMC 0.1 %, 1 % and 3 % solutions

Using a repeated measures ANOVA to assess the grittiness scores, particle size and particle concentration were found to have a significant effect on

grittiness ($p < 0.005$) whilst the effect of viscosity was not significant with the raw SPSS output shown in the Appendix (Field, 2009).

The reason for this lack of significance for viscosity may be due to the poorer suspending ability of the less viscous HPMC 0.1 % solutions and difficulty in dispersing the particles in the more viscous HPMC 3 % as seen in the suspendability assessment which may mean that the Cellets® separated out from the suspending media and hence felt gritty. The reason for this lack of significance may also be due to the unpleasant, mouth coating feel and taste of HPMC as reported orally by participants and shown through the lack of HPMC 3 % samples being rated as "most acceptable" in Table 3-7. It can be seen that the least gritty sample was not the most accepted and that the most important influence on acceptability was the particle concentration then viscosity as long as it didn't contain HPMC 3 %. Large ranges in grittiness scores were observed which are common in sensory research.

Table 3-7: Top Five "Most Acceptable" Samples for the Initial Trial showing the samples that were ranked as "most acceptable" with the highest frequency (out of the twenty volunteers along with the composition of the samples and the range of grittiness scores for each of the five most acceptable samples

Rank	Frequency (/20 volunteers)	HPMC Concentration (%)	Size (µm)	Particle Concentration (mg/5 ml)	Grittiness Score (mm) (Min - Max)
1	14	1	500	5	2 – 88
2	13	1	100	5	1 – 43
3	11	0.1	100	5	1 – 43
4	6	1	200	5	3 – 74
5	5	0.1	500	5	3 – 50

For females in the initial trial, concentration, size and viscosity were all significant effects as was [concentration* viscosity] and [concentration*size] whereas for the males there was a significant effect of concentration, size and [concentration*viscosity] only (overall: concentration, size, [concentration*viscosity], [concentration*size] and [viscosity*size] were seen to have a significant effect). The mean grittiness scores were similar

between males and females overall at 50 ± 33 mm and 46 ± 34 mm respectively. The Independent-Samples T Test procedure was used to test the significance of the difference between two sample means for the two sexes and as Levene statistic is greater than 0.1, the variances were not statistically significantly different.

3.3.2. Refined Grittiness Trial

The results of the refined grittiness trial can be seen in Figure 3-3. It can be seen that the grittiness scores appear to be similar for those 90 µm particles at 125 and 250 mg/ 5ml in all viscosities and that those suspensions containing 500 mg/5 ml at 263 µm have higher grittiness scores.
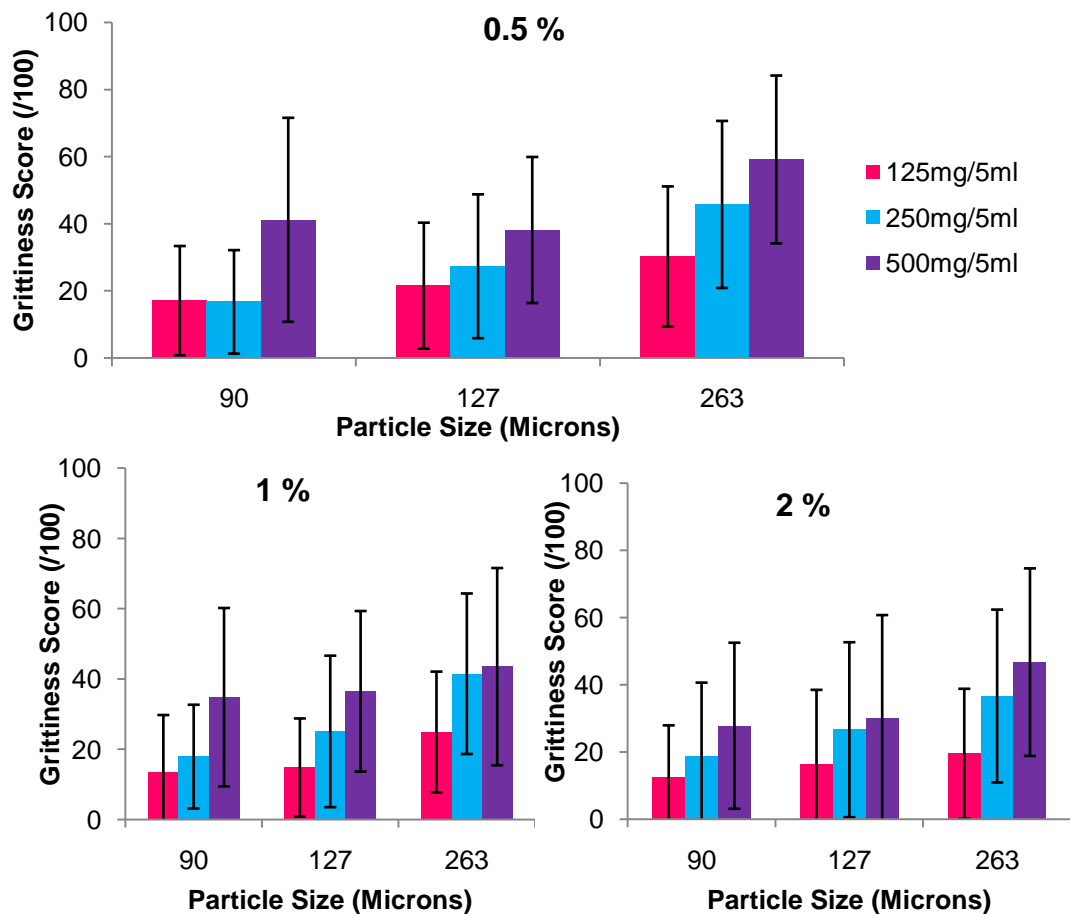
Figure 3-4: Mean Grittiness Scores (±SD) for all Samples Containing Hydroxypropyl methylcellulose 0.5 %, 1 % and 2 % Sweetened and Flavoured Solutions

From the ANOVA, it was be seen from the highlighted significance values that particle size, viscosity and size*viscosity have a significant effect on grittiness (p<0.005 with the raw SPSS output available in the Appendix) which suggests the acceptability of the HPMC was improved and narrowing the variables reduced grittiness.

In terms of acceptability in the refined trial, the most frequently rated as the most acceptable "sample" was the blinded negative control (containing HPMC 0.5 % with no particles) as expected. This sample was, unsurprisingly, the least gritty and only not reported as the least gritty on six occasions: when it was given. The most acceptable of the samples (not the controls) is shown in Table 3-8 where it can be seen that there seems to be little difference between particle sizes of around 100 µm and particle concentration of 125-250 mg/5 ml on acceptability scores. Again it is seen that the most acceptable score is not necessarily the least gritty and that there is a large spread as shown by the large range.

Table 3-8: Top Five "Most Acceptable" Samples for the Refined Trial showing the samples that were ranked as "most acceptable" with the highest frequency (out of the twenty volunteers along with the composition of the samples and the grittiness score for each of the five most acceptable samples to show that the most acceptable samples are not necessarily the least gritty

Rank	Frequency (/20 volunteers)	HPMC Concentration (%)	Size (µm)	Particle Concentration (mg/ 5ml)	Grittiness Score (mm) (Min – Max)
1	10	0.5	127	125	0 – 60
2	9	1	90	125	0 – 65
3	8	0.5	90	125	1 – 60
4	6	0.5	90	250	0 – 66
5	5	2	127	125	0 -86

Reproducibility of the grittiness scores was assessed by comparing the scores of the announced controls with the blinded controls. It was found that

the majority of times, the controls were within 5 mm of the announced negative controls and 10 mm of the announced positive controls, the slightly higher range of the positive control may be due to more varied suspendability/dispersibility of larger particles as seen in Chapter 2. Although the means were not always the same, the negative control was only not voted the least gritty 6 times (very low score so within 5 mm of the sample the participant scored the least gritty) showing good reproducibility. No effects on the order of sample presentation on grittiness scores were identified as samples of similar composition scored similarly irrespective of sample timing (e.g. those containing low concentrations of particles scored low grittiness scores wherever they came in the order of sampling). This study did not use trained volunteers so a degree of variability can be expected between results. The participants had not been pre-screened or trained as the aim of the study was not to be able to put samples into order of grittiness due to concentration etc, more to see what the average consumer found gritty.

3.3.3. Comparison between the Two Trials

In the initial trial, all individuals were aged between 18-24 years but individual data is not available whereas in the refined trial, the average age was 22.4±1.03 years (range: 20.6 – 25.3)

- Male and female groups average was similar but the standard deviation and range being higher in the male group due to the oldest male being 25.3 years compared with 23.9 years for females
- Grittiness scores had similar means, standard deviations and ranges for those aged 21, 22 and 23 years
- There were few 20 year olds, no 24 year olds and one 25 year old so the impact of age not be assessed

It is unsurprising given the narrow age range of healthy adults that no age related effects on grittiness were seen.

In summary, from comparing the two trials it can be seen, by narrowing the particle concentration, size and viscosity with improved mouthfeel, particle size and viscosity become significant impacts in grittiness unlike with the unacceptable HPMC of the first trial where particle concentration and size effect grittiness. Acceptability in the first trial largely depended upon particle concentration and rejected all more viscous HPMC concentrations whereas that of the refined trial found both lower particle concentrations and sizes to be acceptable along with all viscosities.

Particle concentration and size have both previously been seen to have a significant effect on grittiness whereas the effect of viscosity is less clear (Engelen et al., 2005b, Imai et al., 1995, Tyle, 1993). One study which looked at the significance of viscosity reported a difference and the other not: this may be due to the two viscosities of the group that did not find a difference being too close together for it to be noticeable but the lack of clear rheological testing complicates the issue (Engelen et al. 2005, Imai et al. 1995).

Although not strictly valid for assessing significance due to the difference in variables between the two trials, a t-test was used as a method of comparing the two trials. The most similar samples were compared:

- The HPMC 1 % solution with 100 µm Cellets® at 500 mg/ 5ml of the initial trial compared with the 127µm Cellets® at 500 mg/ 5ml in the refined trial
- The HPMC 1 % solution with the 200 µm Cellets® at 500 mg/5 ml of the initial trial compared with the 263 µm Cellets® at 500 mg/5 ml.

The newer samples scored lower (p values of 0.000922 for the 100 µm Cellets® and 0.000831 for the 200 µm), so it was concluded that narrowing the particle concentration, size range and viscosity along with flavouring and

sweetening the media reduced grittiness scores as a trend, as can be observed through looking at the raw data.

In both grittiness trials, participants were given announced positive and negative standards against which to base their scores which was given at the start only which may be a potential source of variation as individuals may be unable to remember accurately the grittiness of the controls but not having a freely available negative and positive control reduced the number of samples in a session which was already high. In this trial, a constant blinded control would be difficult to have freely available due to suspension stability as examined in Chapter 2 and the requirement for the samples to be kept rotating whilst waiting to be given to keep the particles suspended in the media which would not be achievable in real life but allowed for grittiness assessment. Despite the lack of constant controls, blinded controls were given in the refined trial which showed good reproducibility with the announced controls especially in the case of the negative control.

Magnitude estimation is a technique used in sensory analysis to attempt to account for the variety in a response between individuals. Through the use of this technique, responses received from participants can be normalised through comparison to the mean grittiness score of all samples for each participant to remove the effect of participants who rank all samples low or high to show a similar difference in score between samples hence the magnitude or gradient of the response is often similar. The samples are placed in an order since the technique of magnitude estimation depends on the gradient of the response e.g. the gradient of grittiness scores and hence cannot be calculated if the results are not in an increasing or decreasing order of magnitude. While it was found more people were able to score size and concentration into the expected corresponding order of grittiness than viscosity, less than half of the people could put the samples in the correct order. This may be due to the number of samples and their variables being tested being confusing or tiring for the volunteer (despite being within limits of other studies) or it could be that people perceive no real difference between these samples. It was seen that by increasing the number of

volunteers and narrowing the ranges, the number of people who could put the samples into the correct order increased but was still low meaning that magnitude estimation could not successfully be used due to the small numbers of volunteers' responses that could be used in determining similar gradients or magnitude of response for particle size, particle concentration or viscosity.

A Principal Component Analysis would be another way to analyse the data but again the variability and number of results for each sample which has three variables would make it unlikely a meaningful analysis and loadings plot could be obtained.

3.4.   Conclusions

The importance of a child not rejecting a medicine is critical to therapeutic outcomes so the impact of different sizes and concentrations of pellets in a commonly used suspending media (HPMC) at different concentrations (viscosities) was on grittiness and acceptability of the resulting suspensions in young adults aged 18-24 years of age as this was the lowest age group that the researchers had easy access to.

An initial trial found that grittiness of larger particles (>100 µm) depended upon particle concentration and size whereas viscosity showed no correlation. This trial highlighted the importance of acceptability of medicines in all ages given that the young adult participants did not find the thicker (3 %) HPMC to be acceptable due to its unpleasant mouth coating effect. Acceptability was seen to depend largely on particle concentration and any viscosity apart from the HPMC 3 % although large deviations of grittiness scores were seen for these samples.

The refined trial aimed to remove the lack of acceptability of HPMC by adding a popular flavour of orange and a sweetener, sucralose, and reducing the highest concentration which reduced the mouth coating effect. This trial made improvements on the methodological design by increasing participant numbers, randomising orders and checking reproducibility of results with "blinded" controls along with narrowing the range of particle sizes and concentrations since these were seen to have a significant effect in the initial trial so it was interesting to look at a narrower region to see if this significance would still hold.

The refined trial showed a significant impact of particle size and viscosity on grittiness, whereas particle concentration did not. From observation of the data, this appears to be due to similar grittiness scores of the 125 and 250 mg/ 5ml particle concentrations. In terms of acceptability, all HPMC concentrations were in the top five most acceptable rated formulations, with both of the lower particle sizes and particle concentrations. This suggests

that an acceptable formulation can be made with a particle size of around 100 µm (since 90 and 127 µm are similar) with a particle concentration of 250 mg/ 5ml (with up to 500 mg/ 5ml possibly not have unacceptable grittiness but not being rated as acceptable) and any viscosity from HPMC 0.5 - 2 % showing acceptability so more viscous media may be preferred in keeping a multiparticulate suspension stable and uniform over time/masking grittiness. It is however acknowledged that Cellets may have different densities, morphologies and particle sizes to those produced by spray drying so different results may occur and would be worthy of future study.

Future work to develop on this topic would be to try a smaller particle size to see if a region where particle size does not have a significant effect on grittiness could be assessed. It would be interesting to try a smaller range of grittiness samples in children to assess whether they find the same things gritty and acceptable as young adults. While it is believed that children will have similar orders to adults in sensory tests, the magnitudes of their responses may vary. The visual analogue scale scoring system used has been used in children before so a similar trial may be achievable in older children. Alternatively hedonic "smiley face" scales or care giver observations may be used, dependent upon the age of the child.

Taken together the work reported above on particle suspendability (Chapter 2) and grittiness/mouthfeel (Chapter 3) provides targets for the particles that need to be produced in Chapter 4 and the suspending vehicle that any successful particles could be suspended in for paediatric dosing. The major factor that can be controlled by varying the spray drying parameters is the particle size hence a particle size of ca 100µm or less was targeted in the work described below.

4. PRODUCTION OF MULTIPARTICULATES

4.1. Background

Multiparticulates offer one attractive route to generating a platform (e.g. non drug containing base) formulation approach addressing the issues associated with dosing of API's to paediatric patients of a wide range of ages as discussed in Chapter 1. The overall aim of this project was to produce coated particles that could be suspended in a suitable vehicle as discussed in Sections 2 and 3 to produce a stable, liquid dosage form that would allow range of doses to be administered with acceptable grittiness. Hence this section discusses the production of taste masked multiparticulate formulations of insoluble and soluble model drugs (Quinine base and Quinine hydrochloride respectively) as an example of functionalised multiparticulates. It can be envisaged that other modified release profiles could be generated in a similar manner using alternative polymers as the coating agents although time constraints mean that this has not been demonstrated yet.

4.1.1. Bitter Tasting Drugs

As mentioned in Chapter 1, many drugs suffer from a bitter taste which can adversely affect compliance. Drug families which suffer from a bitter taste include a wide range of drugs required commonly in paediatrics including:

- Anti-Malarials (Shah and Mashru, 2009, Shah et al., 2008, Shah and Mashru, 2008b, Shah and Mashru, 2008a)
- Antibiotics (Sollohub et al., 2011, Hu et al., 2009, Ishizaka et al., 2007)
- Anti-HIV medicines (Chiappetta et al., 2009)
- Corticosteriods (Orlu-Gul et al., 2012, Hames et al., 2008)
- Gastro-intestinal medicines (Bora et al., 2008, Xu et al., 2008b, Khan et al., 2007)
- Analgesics (Guhmann et al., 2012, Hejaz et al., 2012)

Quinine was chosen as a model drug due to its extreme bitterness as it is used in the gustatory response scale where bitterness is equated to different molarities of quinine solution (British Pharmacopeia Online, 2012). Quinine is an anti-malarial currently only available in the United Kingdom as tablets due to its bitter taste or an injection for those children who cannot swallow (British Medical Association and the Royal Pharmaceutical Society, 2012). A variety of approaches have been tried to mask the taste of the medicine (Kayumba et al., 2007, Kayitare et al., 2010, Woertz et al., 2010). Quinine exists both in a water insoluble basic form and a variety of very soluble salt forms including the hydrochloride salt as illustrated in Figure 4-1 which enabled the effect of different solubilities to be examined. Quinine possesses a chromophore so is easy to detect and quantify by ultraviolet spectrophotometry.
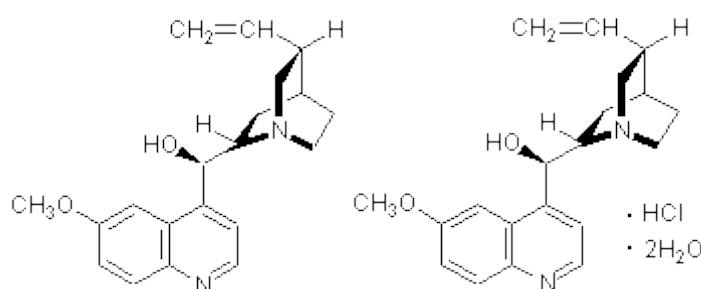
Form	Base	Salt
Aqueous Solubility	1 g in 1900 ml	1 g in 16 ml

Figure 4-1: Structure and Properties of Quinine and Quinine Hydrochloride dihydrate (Merck Index, 2006)

4.1.2. Taste Masking by Spray Drying

As discussed previously in Section 1 there are two general methods of overcoming the problem of objectionable taste: either to mask it by the addition of excipients such as flavours, sweeteners or taste blockers or to prevent the drug coming into contact with the taste buds e.g. by coating or microencapsulation. This work examines an example of this latter approach utilizing a pH sensitive polymer to form microparticles that are resistant to

releasing drug in the conditions found in the mouth but which would be expected to rapidly dissolve in gastric acid to allow the compound to be released for absorption with minimum adverse effect on the pharmacokinetic profile compared with uncoated drug particles.

There are relatively few reports of microspheres production for taste masking by spray drying with most preparing orally disintegrating tablets (ODTs) as a way of administering the prepared particles with individual microsphere release not always characterized (Xu et al., 2008b). Most reports of spray drying for taste masking use drugs which are less soluble than the quinine hydrochloride salt used for much of this research and have a higher bitterness threshold than quinine (Bora et al., 2008, Xu et al., 2008b, Yan et al., 2010). The Eudragit® Polymers (especially L30D55 and E PO) along with cellulosics such as HPMC are the most commonly used polymers for preparation (Xu et al., 2008b, Janczyk et al., 2010). In terms of using hydrophobic polymers such as the Eudragits®, organic solvents have generally been used to solubilise the polymer which allows a reduction in spray drying temperatures compared to those of aqueous processes as discussed later (Bora et al., 2008, Shishu et al., 2010). An interesting approach using food industry components of sodium caseinate and lecithin exhibited some degree of release retardation of paracetamol (Hoang Thi et al., 2012).

Eudragit® E is the one of the most commonly spray dried polymers for taste masking. Eudragit® E PO is a cationic copolymer of dimethylaminoethyl methacrylate and neutral methacrylic esters as shown in Figure 4-2. This co-polymer dissolves below pH 5 so is soluble in the stomach so that the bioavailability of this medicine is not affected, but it remains intact in the adult buccal cavity (pH 5.8-7.4).
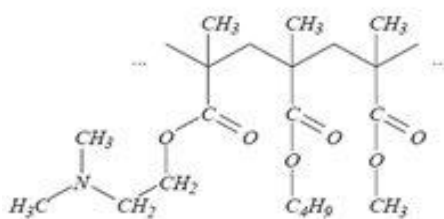
Figure 4-2: Structure of Eudragit® E PO

Eudragit® E has been used for film coating for protecting medicines from the moisture in the atmosphere in addition to taste masking due to its properties such as low vapour transmission as detailed in Table 4-1

Table 4-1: Properties of Eudragit® E (Evonik Industries, n.d.)

Eudragit® E	
Molecular Weight (g/mol)	47 000
Glass Transition Temperature (°C)	~ 48
Water Vapour Transmission Rate with stearic acid (g/m^2.d)	~ 100
Elongation at Break with 10%SDS+15% stearic acid (%)	~ 60
Thermal Stability Maximum Temperature for 1% damage (°C)	~ 210

While Eudragit® E has not had extensive use within paediatrics, it was chosen due to its pH profile (Evonik Industries, n.d.). Eudragit® E is biocompatible meaning that the body should tolerate the polymer and that it should not cause any adverse events, but it is not biodegradable so is not broken down by the body. Company data suggests a maximum of 20 mg/kg/day based on rat and dog toxicity studies with the highest amount in a dosage form of 566 mg) (Evonik Industries, n.d.). It can be seen from Table 4-2 that Eudragit E has precedence of use in the United Kingdom including in older children although the levels used are unknown (Electronic Medicines Compendium, n.d.).

Table 4-2: Oral Formulations Available containing Eudragit® E (basic butylated methacrylate copolymer) (Electronic Medicines Compendium, n.d.)

Formulation	Age Licenced From	Form Used
Amisulpride tablets 400 mg *Sandoz Limited*	<15 years	E100
Calpol 6+ Fastmelt Orodispersible Tablets 250 mg (Paracetamol) *McNeill Products Ltd*	< 6 years	E100
Liskonum Tablets 450 mg (Lithium Carbonate) *GlaxoSmithKline UK*	< 12 years	E12.5
Salofalk Gastro-resistant Tablets 500 mg (Mesalazine) *Dr Falk Pharma UK Ltd*	< 6 years	-
Risperidone Orodispersible Tablets 0.5, 1 and 2 mg *Sandoz Limited*	< 5 years	-
Paroxetine Film Coated Tablet 10 mg *Actavis UK Ltd*	< 18 years	-
Siklos Film Coated Tablets 100 mg (Hydroxycarbamide) *Nordic Pharma Limited*	< 2 years	-
Venaxx XL Tablets 75 and 150 mg (Venlafaxine hydrochloride) *Mercury Pharma Group*	< 18 years	E12.5
Zispin SolTab Orodispersible Tablets 15, 30 and 45 mg (Mirtazepine) *Merck Sharp and Dohme Limited*	< 18 years	-

Despite Eudragit® E PO having been sprayed from organic solvents and exhibiting retarded release as microparticles no reports were found of aqueous spray drying with release controlled by microparticles (only one which tabletted the microspheres without assessing release) (Xu et al., 2008b).

Two other reverse enteric polymers were found in the literature. Methyl methacrylate – diethylaminoethyl methacrylate copolymer (6:4) (commercially known as Kollicoat Smartseal® 30D) only become available

after much of this research had been undertaken but looks to be an interesting prospect due to its low water vapour transmission (BASF, Chivate et al., 2012) Another polymer polyvinylacetal diethylaminoacetate appeared interesting but no source could be obtained (Hashimoto et al., 2002). Hence Eudragit® E was used to try to taste mask.

4.1.3. Spray Drying

4.1.3.1.   Overview

An introduction to spray drying was given in Chapter 1. Briefly it involves the atomisation and drying of a feed to form a dry product. Spray drying was chosen as the technique to attempt to develop taste masked multiparticulates due to its scaleupability and desired product characteristics. The spray dried formulation will depend on a variety of variables including those of the drug, excipients and processing parameters.

Different types of spray drier, although largely working on the same principles, can have different atomisers (such as two fluid, pressure and ultrasonic nozzles or a rotating disk) and different patterns of drying media flow (e.g. co-current where the feed is sprayed in the same direction as the hot air flow, counter current where product and drying air flow in opposite directions or in a disk atomiser in the same direction as drying air). Both machines used in this research have a two fluid where the spray is due to the air and feed combining and co-current flow.

In terms of producing product by spray drying, the evaporation of the solvent from the droplet involves coupled heat and mass transport. The spray-drying process is driven by the vapour pressure of the solvents and their partial pressure in the gas phase (Vehring, 2008). The rate of evaporation depends on the vaporisation energy of the solvent and the energy that is available at the surface of the droplet (Handscomb et al., 2009). This process is shown in Figure 4-3
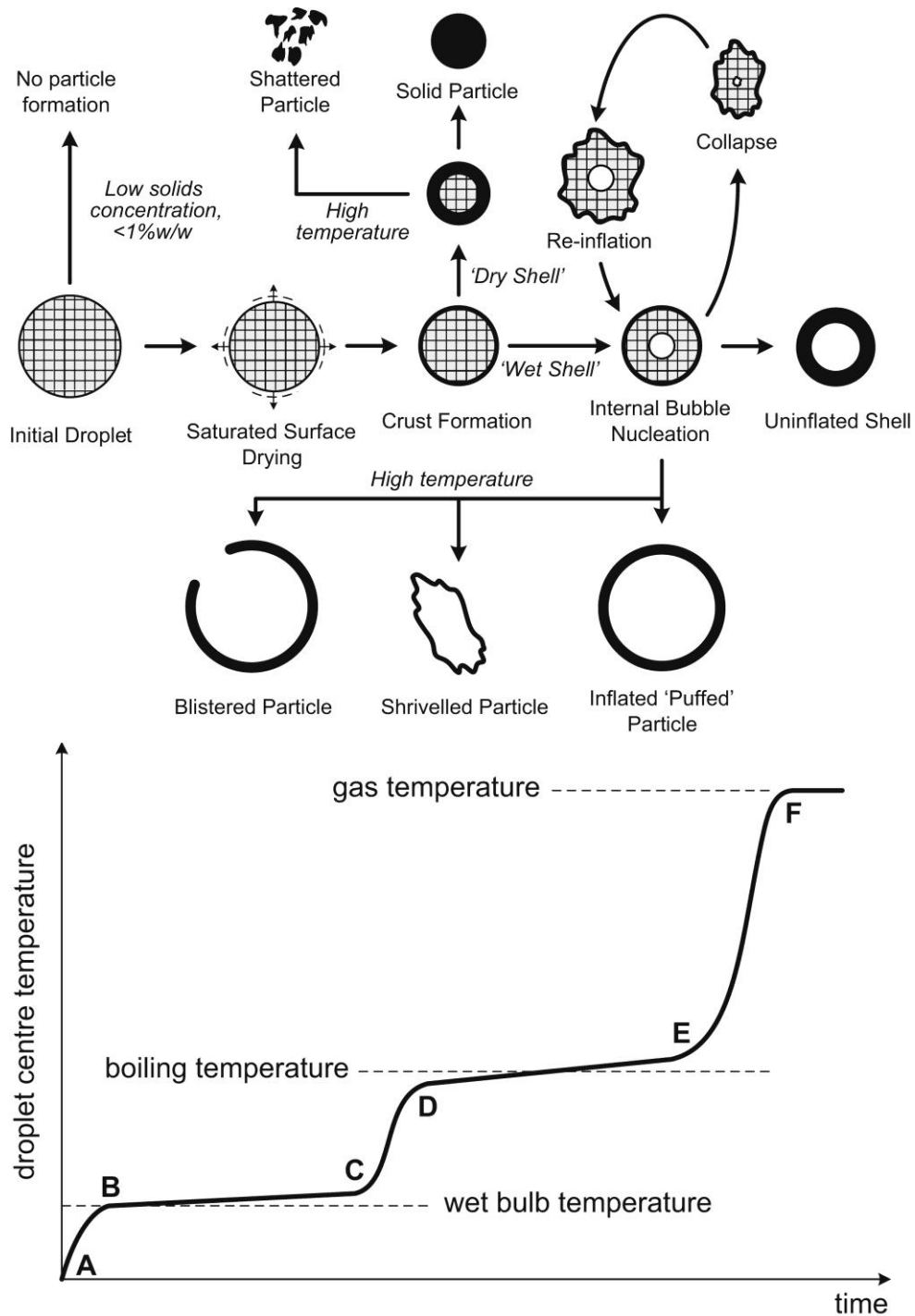
Figure 4-3: Schematic showing Different Particle Morphologies that can occur on Spray Drying along with a Temperature Profile for a Particle (where AB is where the particle is rapidly heated to the wet bulb temperature, followed by constant drying as the surface is still saturated with water shown by BC then CD when the moisture on the surface can no-longer be retained and a small rise shown by DE when the moisture boils off before all free moisture has been removed and EF is the temperature of the air) (Handscomb et al., 2009)

Microparticles produced by spray-drying possess a large surface area and hence can be thermodynamically unstable. These microparticles may have not reached equilibrium due to the short drying times employed and so may crystallise, coalesce or undergo polymorphic transformation – all of which could lead to the failure of the microparticles desired drug release profile.

The glass transition temperature of a material is important in spray drying. This is where the material goes from a brittle to a rubbery state as the molecules become more mobile and occurs with change in heat capacity. Below the glass transition temperature (T_g), the polymer will be more hard and rigid whereas above the T_g, the polymer is more soft and sticky as shown in Figure 4-4. Plasticisers, solvents and residual solvents can act as plasticisers and hence reduce the glass transition temperature and increase the elasticity and permeability, hence it is has been recommended to keep Eudragit E at 40 °C for at least 2 hours after coating to prevent this (Evonik Industries, n.d.).
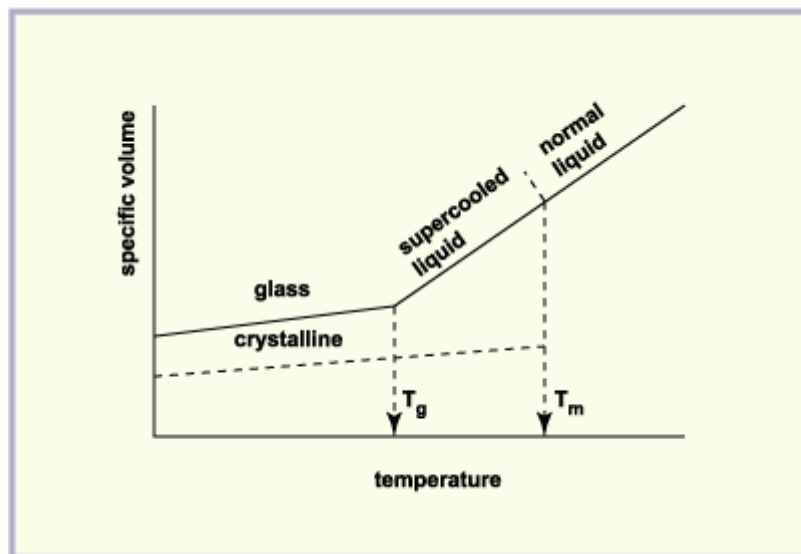
Figure 4-4: A Representation of Glass Transition Temperature and Physical State (University of Bolton, n.d.)

4.1.3.2.   Excipients

Different excipients are required in a spray drying feed in order to produce a product with acceptable properties. The most obvious excipient as covered in the previous section is the polymer which will be responsible for the functionalised release and the solvent as discussed in the next section. Other excipients are largely required to ensure optimum conditions for this polymer. Exact composition depends on the polymer and drug whether the feed is a suspension, solution or emulsion feed.

Components can be added to solubilise the other constituents such as pH modifiers e.g. stearic acid is used as a salt former with the Eudragit® E polymer. Stearic acid also reduces the water transmission rate of Eudragit E when compared with that of the organic formulation (~350 g/m^2.d)

Anti-tacking or anti-adherent agents (also known as glidants) are used to reduce the tendency of the product to stick to the spray drier and hence can increase yield. Common examples include talc, glyceryl monostearate and colloidal silica. The use of anti-adherents can increase permeability by decreasing effective concentration of polymer for film formation and hence solubility retardation or by providing a 'wick' to draw solvent inside the coated particle. Magnesium stearate is less commonly used and additionally lowers permeability possibly via generating small discontinuities, or holes in the coat.

Components such as Polyethylene glycol can act as permeability enhancers where required as well as a plasticiser and stabiliser. Plasticisers may be used to reduce the temperature needed to form the film especially where organic solvents are used. Other components used to stabilise the formulation include low viscosity NaCMC or SDS to solubilise/suspend/disperse polymers and antifoaming agents may be required.

4.1.3.3.   Solvents

The desirable attributes of a solvent for microencapsulation have been defined as (Li et al., 2008):

1. Be able to solubilise the required polymer to ensure encapsulation
2. Have a high volatility and low boiling point to ensure the solvent evaporates
3. Have a low toxicity

Unfortunately no individual solvent seems to achieve all of these criteria and hence decisions have to be made into which solvent to use.

The use of organic solvents can be problematic, especially in the scale-up of pharmaceutical production due to the potentially toxic hazards of both the fumes and residual solvents and flammability risks. It is for these reasons that no organic solvents will be used in the production of the microparticles – only aqueous methods.

The international conference on harmonisation of technical requirements for registration of pharmaceutical use (ICH) has a guideline which sets limits for the residual solvent that may remain in products after processing (European Pharmacopeia, 2008). The ICH guidelines categorise solvents into one of three classes depending upon their toxicity: Class 1 are solvents to be avoided such as known or suspected carcinogens or environmental hazards, Class 2 are solvents to be limited as they may be animal carcinogens or cause other irreversible toxicity and Class 3 are solvents with low toxic potential so have no health-based safety limit and a permitted daily exposure of 50mg/day or more. Ethanol is increasingly being used as a class 3 solvent or non-organic solvent containing methods are being developed

The ICH expects that testing for solvents should be carried out when production or purification processes result in the presence of solvents: only those solvents known to have been used or made have to be tested for.

Individual components can be tested or the complete medicinal product can be tested for impurities. The limits can be given as a concentration limit (ppm) or permitted daily exposure (mg/day). Residual solvents are usually determined by chromatography, for example by static headspace capillary gas chromatography. Obviously when we are trying to minimize chemicals in children's formulations as discussed in Chapter 1, having no residual solvents of toxicological concern would be an advantage.

It is reported that storing Eudragit® RS/RL microspheres of ketoprofen in sealed containers after storing in a dessicator caused them to lose their spherical shape and form clusters. This is thought to due to residual solvent acting as a plasticiser since those microparticles stored in open containers did not exhibit this behaviour so this is may be an additional reason that organic solvents should be minimised.

Aqueous spray-drying is desirable but due to the higher temperatures required to remove the water and lack of solubilising ability for hydrophobic polymers, it has still not been completely conquered with microparticles formed often being less spherical and more aggregated than those formed during organic solvent spray drying (Kendall, 2007). Drug loading may also be reduced as more excipients need to be added to solubilise/suspend the polymer and drug which is important as the lower the drug loading, the higher the mass of multiparticulates we would have to give to the child which may impact compliance.

The rate at which the solvent is removed is dependent on temperature, pressure and amount of water in the process. High temperatures can harden the microsphere whereas very high temperatures may damage them due to the very sudden evaporation of solvent. The thermal stability of the encompassed drug must also be taken into account.

In order to optimise both the efficiency of the spray drier and product production, the feed properties must be looked at. When organic solvents are used to solubilise the polymer and drug a solution feed is formed versus a

suspension feed when the drug is not solubilised (Emami et al., 2007). The different types of feeds can form different particles: from a solution feed the products formed may be polymer or drug spray-dried individually without any coating, the spray dried drug within a polymeric film or on the surface of it, whereas a suspension feed mainly produces microencapsulated drug with smoother surfaces than those formed from a solution feed (Rattes and Oliveira, 2007). Increasing the polymer to drug ratio further decreases dissolution rate compared to lower ratios, probably due to increased polymer coating thickness (Emami et al., 2007).

It is known to be difficult to produce smooth, spherical particles by aqueous spray drying due to insufficient forces being present to prevent the formation of fibres. A number of the papers on spray-drying report that their spray-drying apparatus is not equipped with a trap and hence smaller and lighter particles are lost in the exhaust gas. Adhesion of the powder to be spray dried to the walls of the drying chamber and cyclone collector are also another common limitation which can be reduced by the addition of an anti-sticking agent such as colloidal silica or talc to the spray drying feed which is of relevance as it reduces drug loading.

Aqueous dispersions contain polymer latex particles rather than the dissolved individual polymer molecules which require the evaporation of water to move closer and form a film dependent upon the particles elasticity and surface tension are a potential alternative approach (McGinty and Felton, 2008).

Aqueous dispersions of water-insoluble polymers have traditionally been used for the coating of tablets and pellets due to their lack of organic solvents: some examples are:

- Eudragit® RS 30D contains 30 % w/w co-polymer of ethyl-acrylate, methyl-methacrylate and trimethyl-ammonioethyl-methacrylate chloride in the ratio of 1:2:0.1 and 0.25 % sorbic acid (Rassu et al., 2008)
- Kollicoat® SR 30D contains polyvinyl acetate 27 %, polyvinylpyrrolidone 2.7 % and sodium lauryl sulphate 0. 3% (Al-Zoubi et al., 2008a)
- Surelease® is an aqueous dispersion of ethylcellulose, dibutyl sebacate as a plasticizer and ammonium oleate as a stabilizer (Rattes and Oliveira, 2007)

These dispersions are beneficial for medicines that are highly water soluble but poorly soluble in organic solvents such as buspirone hydrochloride (Al-Zoubi et al., 2008a). Spray dried microspheres of buspirone with Eudragit® RS 30D or Kollicoat® SR 30D. Microspheres with high (1:1) drug: polymer ratios with large agglomerates are formed due to coalescence caused by the crystallisation of the buspirone with those formed Kollicoat® being more spherical than those formed by Eudragit®. The yield was low (7.2 – 31 %) but this has been seen before during aqueous spray-drying as shown in Table 4-3: the yield was higher for Eudragit® which is thought to be due to the presence of PVP (a known binder) in Kollicoat® causing increased adherence of droplets to the internal surfaces of the spray dryer. The presence of PVP is also thought to be the reason for the slower drug release from tabletted microspheres made with Kollicoat® compared to that of microspheres made with Eudragit®.

Surelease® and Eudragit® RS 30D were used to produce diclofenac particles by aqueous spray-drying with a drug-to-polymer ratio of 1:1. Both were able to sustain drug release for several hours at pH 6.8 and provide

less than 10% release at acidic pH time (Rattes and Oliveira, 2007). Similar results were provided by both polymeric dispersions (Zeng et al., 2007).

The ethylcellulose aqueous dispersions, Surelease® and Aquacoat®, are stabilised by anionic surfactants and thus solutions/suspensions containing cationic drugs such as chlorpheniramine maleate, pseudoephedrine or propranolol hydrochloride may be unstable whereas Eudragit® RS and RL 30D are stabilised by quaternary ammonium groups and should be compatible with a wider range of drugs. (Zeng et al., 2007)

To summarise, quinine hydrochloride and base were chosen as model drugs for taste masking and Eudragit® E PO for the encompassing polymer along with water as a solvent for spray drying.

Table 4-3: Summary of Some Aqueous Spray Drying Papers (key: -: designated information not given):

Drug	Polymer	Drug: Polymer Ratio	Additional Chemicals	Operating Parameters	Size/ Morphology	Yield (%)	Encapsulation Efficiency (%)
Theophylline (Takeuchi et al.)	Eudragit® L30D, L100-55 and E30D	8:3 -1:3	PEG 6000 and colloidal silica or ammonia water	Inlet temp: 150-170 ^{o}C, Outlet temp: 105-110 ^{o}C, Solution flow rate: 1000 ml/h Rotation speed of atomiser 16500 rev/min	10-30 μm Agglomerated/ rough surface at high drug: polymer ratios	-	-
Buspirone (Al-Zoubi et al., 2008b)	Eudragit® RS 30D and Kollicoat® SR 30D	1:1 -1:9	-	Nozzle: 406 μm Inlet air temp: 133-136 ^{o}C, Outlet air temp: 70-80 ^{o}C, Spray air pressure: 1 kg/cm^{3}, Feed rate: 6 ml/min	- Agglomerated and not spherical	7.2-31	98-104
Paracetamol (Billon et al., 1999)	NaCMC, HPMC, HEC, HPC, MCC and EC	1:1 – 10:1	PVP with MCC, DBS with EC and PEG 6000 and succinic/phthalic/oxalic/tartaric/citric acid with NaCMC	Nozzle: 500 μm Inlet temp: 140 °C or 160 °C Spray flow: 700 NL/hr; Atomizing air pressure: 1 kg/cm^{2} Feed Flow: 4 ml/min.	- Agglomerated, rough particles	17-70	-
						Depending on polymer, plasticiser and inlet temperature	
Diclofenac (Rattes and Oliveira, 2007)	Suralease® and Eudragit® RS 30D	1 :1	Propylene glycol, talc, colloidal silica and titanium dioxide	Feed flow rate : 3-6 g/min Inlet temp : 100-150 ^{o}C Atomising gas pressure: 1 bar Air flow rate: 60 m^{3}/h	9.1+/-6.2 – 24.5+/- 15.1 μm Agglomerated but smooth particles	-	63.9 -97.9 (Higher for Suralease®)
Famotidine (Xu et al., 2008b)	Eudragit® E PO	1:2	SLS, Stearic acid, PEG 400 and colloidal silica	Nozzle Size: 700 μm Inlet Temp: 110 °C Air Flow Setting: 600 NL/h	<10 um Rough particles	33.25-41.23	37.59-61.56
Theophylline (Wan et al., 1992)	HPMCAS, HPMC, MC, NaCMC	1:5 - 5.5:1	Triethylcitrate and citric acid monohydrate	Inlet temp: 140 ^{o}C Feed rate: 9 ml/min Air Flow rate: 0.5 m^{3}/min Atomising air pressure: 1 kgf/cm^{2}	- Very aggregated, non-spherical particles	-	-

4.2. Materials and Methods

4.2.1. Materials

Eudragit® EPO and colloidal silica (Aerosil® 200) were obtained from Evonik Industries, Germany. Stearic acid and polyethylene glycol 400 were obtained from Fluka Analytical, Germany. Sodium dodecyl sulphate, sodium chloride and di-sodium hydrogen ortho phosphate were obtained from BDH Chemicals Ltd, Poole. Quinine (99 % anhydrous) and phosphoric acid from Acros Organics, Belgium. Quinine hydrochloride dihydrate and Acetonitrile Chromasolv® (Gradient Grade for HPLC) from Sigma Aldrich, Germany. Trifluoroacetic acid (HPLC grade), potassium dihydrogen phosphate, sodium hydroxide and hydrochloric acid (5 M volumetric solution) were obtained from Fisher Scientific UK Ltd, Loughborough.

4.2.2. Methods

4.2.2.1.   Feed Preparation

Initially a method was used as previously described (Xu et al., 2008b). An aqueous dispersion of Eudragit® E PO (15% w/v) was prepared by dispersing the Eudragit® in 100 ml of distilled water with Sodium dodecyl sulphate 1.5 g and stearic acid 2.25 g using a high shear rate homogenizer (Silverson 44RTs, USA) at 100 RPM for 30 minutes. Quinine or quinine hydrochloride dihydrate 7.5 g to assess for the impact of drug (or no drug) and colloidal silica 7.5 g were added to distilled water. The Eudragit® E PO dispersion was added to this drug solution/suspension along with Polyethylene glycol 400.

4.2.2.2. Experimental Parameters

The formulation was spray dried using a Niro SD Micro® spray dryer (Niro, Denmark) as shown in Table 4-4. All experiments were repeated in triplicate.

Table 4-4: Initial Spray Drying Parameters

Initial Spray Drying Parameters Used	
Atomising gas flow	2.5 kg/h
Chamber inlet flow	25 kg/h
Inlet Temperature	110 ºC
Feed concentration	13 mg/ml

Following initial settings, future experiments were modified in light of results as discussed throughout 4.3.1. by:

- Changing homogenization settings/conditions to 10 minutes each for the polymer and drug dispersions and then 10 minutes after both were mixed at 1000 RPM (after 5 sec at 3000 RPM for both of the dispersions)
- Changing the atomizing gas flow, chamber inlet flow and inlet temperature to 3 kg/h, 30 kg/h and 140 ºC respectively.
- Changing the spray drier to a Büchi Mini Spray B191 (Büchi, Switzerland) with inlet temperature 140 ºC, aspirator setting 90 % and pump setting 20-35 %.

4.2.2.3. Design of Experiments

A design of experiments approach was employed due to the fact that there are a large number of variables to be considered within the spray drying feed and particles so some method of handling this number of experiments was required. The advantages of an experimental design approach compared to a "one factor at a time" method of changing variables to assess for the effect of variables are shown in Table 4-5

Table 4-5: Comparison between One factor at a Time and Factorial Designs

One Factor at a Time	Factorial Design
Vary one factor at a time	Varies multiple factors
Estimates effects at Set conditions	Estimates effects at different conditions
No interaction effects	Able to estimate interactions
Averages by replication	Averages throughout
Lots of runs	Fewer runs
Design space not covered well	Design space well covered

There are many different types of design of experiments, each with their pros/ cons. An example of a full fractional design is shown in Figure 4-5: in this type of design all three factors are investigated at 2 levels (as given by -1, +1).
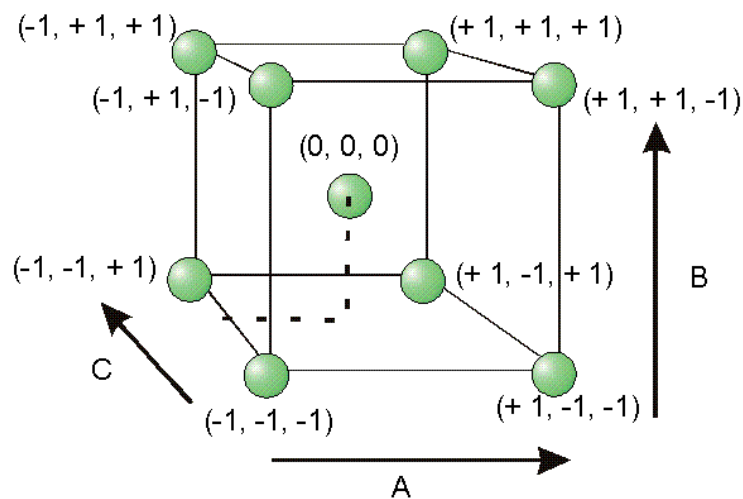
Figure 4-5: Full Fractional Design with Three Factors, Two Levels and a Midpoint (Cecchi et al., n.d.)

For a design with three factors, the number of experiment required for a full factorial design is $2^3 = 8$. It can be seen from this that as the number of variables to be assessed increases; the number of experiments required will increase exponentially: in part due to this reason fractional factorial designs can be used to reduce the number of experiments whilst still assessing a design area. As the spray drying formulation chosen had 5 components, 2^5 would make 32 experiments (without midpoints) to screen the effect of different levels of the five excipients. For this reason a fractional factorial design was run which took 16 runs (without midpoints), whilst this reduces

time and money spent experimenting, a reduction in the level of interaction effects which can be assessed additionally occurs.

A fractional factorial design with three midpoints was run as described in Table 4-6 (Design Expert 8) to screen for the effect of different excipient levels on the particles formed. The factors are the independent variables which in this case were the excipients with levels as shown and the response/dependent variables were:

- Yield (%)
- Encapsulation Efficiency (%)
- Drug release at pH 6.8 in 1min (%)

- Particle Size (% of fines)
- Particle Density (g/ml)

Table 4-6: Output showing Experimental Design Runs from Design Expert. (Red illustrates high levels of excipient, yellow middle levels and green low levels)

Std	Run	Eudragit® ®	SDS	SA	SiO2	PEG400
		(x Drug)	(% Polymer)	(% Polymer)	(% Polymer)	(% Polymer)
18	1	3.75	30	32.5	75	30
17	2	3.75	30	32.5	75	30
14	3	5	10	50	100	10
9	4	2	10	50	100	10
15	5	2	50	50	100	10
13	6	2	10	50	100	50
11	7	2	50	15	100	50
1	8	2	10	15	50	10
8	9	5	50	50	50	50
16	10	5	50	50	100	50
3	11	2	50	15	50	50
12	12	5	50	15	100	50
5	13	2	10	50	50	10
10	14	5	10	15	100	10
6	15	5	10	50	50	10
7	16	2	50	50	50	50
4	17	5	50	15	50	50
2	18	5	10	15	50	10
19	19	3.75	30	32.5	75	30

Basic descriptive statistics were determined using Microsoft Excel 2007 and analysis with Design-Expert Version 8.0.7.1 (Stat-Ease Inc, Minneopolis).

Levels chosen for SDS, Eudragit and colloidal silica were based on values found from the literature as to ratios/percentages of these excipients used in spray drying. For stearic acid and PEG 400, no relevant information was found so the arbitrary but realistic levels were chosen based on discussion with colleagues.

All formulations were spray dried on Büchi Mini Spray B191 (Büchi, Switzerland) and the feeds prepared in a similar way to the initial feed. All formulations contained quinine hydrochloride dihydrate (since this is likely to be the more difficult one to taste mask due to its aqueous solubility) and all had a total solids content of 50 g sprayed. Scoping work was initially undertaken to try to enhance the solids concentration of the feed and increase the spray rate by modifying homogenization and spray parameters as described in Section 4.3.3.

4.2.2.4. *Analytical Method Development for Testing Microparticles*

4.2.2.4.1. HPLC

A HPLC method for the determination of quinine hydrochloride dihydrate was developed to enable the quantification of drug both encapsulated in the microspheres and released by them to be determined. All method development used an Agilent Technologies 1200 HPLC with UV detection and a Phenomenex Kinetex 2.6μ C18 100 x 30 mm column at 40 °C and solvent flow rate of 0.5 ml/min. The solvents used were Acetonitrile + Trifluroracetic acid (TFA) 0.05 % w/v and TFA 0.05% v/v (aq) in differing proportions and under different wavelengths of detection. Standards solutions were prepared from a stock of 100 μg/ml in a range of 5-50 μg/ml by dilution in both acetonitrile 40 % +TFA 0.04 % v/v (aq) and distilled water in order to meet ICH criteria as detailed below with results and final conditions found in the HPLC Development Results in Section 4.3.2.1.

Specificity: is the ability to analyse the drug in the presence of other components. This was assessed by:

- Running standard solutions to find relevant wavelengths to use for HPLC and absorbance peaks

- Changing HPLC conditions to ensure peak resolution of multiple drug peaks

- Running all excipients in the microparticles individually to check whether they interfered with drug retention time and absorbance

- Deliberately subjecting standard solutions to extremes of temperature and light exposure to see whether degradation products interfere (stress test of the quinine salt 200 μg/ml solution diluted 1:1 in acidic (0.2 M HCl), basic (0.2 M NaOH) and neutral conditions (distilled water) at 75 °C at periods of 30, 60 and 180 minutes and overnight were undertaken as well as photo degradation using a Suntest CPS+ for periods of 30, 60 and 120 minutes and overnight at a setting of 500 W/m^2

Linearity   is the ability for the absorbance to be proportional to the concentration of drug. This was assessed by running standard solutions and plotting standard curves with a regression line with linearity found from the 5-100 μg/ml tested.

Sensitivity   involves determining the detection and quantification limits of the drug which are given as three times and ten times baseline noise as seen in equations 4.1 and 4.2 respectively

Equation 4-1: Limit of Detection

$$\text{Limit of Detection} = \frac{3\,S_{y/x}}{b}$$

Equation 4-2: Limit of Quantification

$$\text{Limit of Quantification} \quad = \quad \frac{10\,S_{y/x}}{b}$$

Where b =   Slope of the linearity correlation line

$S_{y/x}$ = $\sqrt{\Sigma(y_i - y)^2/n}$ -2

N   =   number of data points

Accuracy   refers to the closeness of reported measurements to the actual content and was assessed by running standards of known concentration at least three times. Accuracy of the method was demonstrated.

Precision   refers to the closeness between repeated measurements and was assessed by running multiple standards on different days over at least a week at least three times although no other analyst ran samples (all experiments using HPLC were undertaken by the same individual)

4.2.2.4.2.   Filter Compatibility and Filtration Optimisation

Filter compatibility was assessed for by drawing two standards of known concentrations of quinine hydrochloride dihydrate through the syringe attached filters which were available in the lab (filter units as detailed in Table 4-7) and assessing the quantity which passes through the filter by HPLC as detailed in Section 4.3.2.1. Filtering was used to ensure that only dissolved drug was sampled compared to that contained within microparticles which may release after sampling but prior to analysis and provide a source of variation. This is important since only dissolved and not encapsulated drug is free to interact with the taste buds and taste bad.

Table 4-7: Details of Syringe filter units used (from http://www.millipore.com)

Name	Pore Size (µm)	Diameter Size (mm)	Membrane Material
Millex® GP	0.22	25	Polyethersulfone
Millex® MP	0.22	33	Hydrophilic Polyethersulfone
Medical Millex® HA	0.45	33	Mixed Cellulose Esters

The effect of filtering microsphere samples was assessed by weighing around 20 mg of particles containing formulation F1 which as the first midpoint, contained all excipients at the mid level, into a vial with 20ml of water which was shaken for 30 inversions before being filtered and analysed.

As the particles would be expected to release their entire content in an acidic environment initially samples assessed for acid release had not been filtered due to operator error. To assess for the effect of filtering and location of sample removal on dissolution, three different conditions were investigated on dissolution testing in 900 ml 0.1 N HCl at 37 °C in a paddle apparatus over 45 minutes:

1. Samples removed using a syringe from the same location using as a sampling location an indwelling tube before being filtered using a 0.22 µm filter as chosen above
2. Samples removed using a syringe from the same location using an indwelling tube but without being filtered
3. Samples removed using a syringe randomly anywhere from the dissolution vessel with no regard for location without filtering

The effect of these conditions was investigated by HPLC analysis using different sizes of particles. To represent the range of particle sizes seen throughout the spray drying runs F6 and F16 particles were assessed. F6 has a particle size of largely less than 25 µm and F16 has a particle size of over 1 mm where filtration was seen to effect results.

4.2.2.4.3.   Washing Microparticles

In order to remove any drug attached to the surface of formed microparticles it is necessary to wash them. Different centrifuge speeds, times and centrifuges were tried in an attempt to optimise washing to remove unencapsulated drug from the surface of the formed microparticles since unencapsulated drug would be free to interact with the taste buds and produce a bitter taste. Initially around 200 mg of formulation F1 in 35 ml of deionised water was used in a centrifuge (Sigma 3k30) and around 200 mg in 1ml using a Heraeus Microcentrifuge from Thermoscientific. Visual observations were made and samples taken and filtered for HPLC analysis.

4.2.2.4.4.   Content

Eudragit® E PO (1 g) is reported as soluble in 7 g of methanol, ethanol, isopropyl alcohol, acetone, ethyl acetate, methylene chloride and hydrochloric acid (1 N). As on method development of HPLC in section 4.3.2.1, three peaks were seen using acetonitrile as a solvent compared to two with deionised water; 5 ml of ethanol, methanol, acetonitrile and hydrochloric acid 1 N were added to microparticles (100 mg) and inverted 30 times before being left for 5 minutes. Particles were also dissolved in hydrochloric acid 0.1 M by diluting 5 ml of 1 N to 50 ml. These samples were then filtered and analysed for content by HPLC in triplicate which enables the drug loading and encapsulation efficiency to be determined as shown in Equations 4-3 and 4-4 respectively. A high encapsulation efficiency means that drug is not left unencapsulated and able to interact with the taste buds or that drug is not being lost and drug loading is important since the higher the drug loading, there will be a lower excipient to drug ratio which is beneficial in minimizing excipients in children, a higher drug loading will also mean a lower mass of particles which will have to be administered to provide any given dose and hence a lower concentration of particles in suspension which may affect grittiness as detailed in Chapter 3.

Equation 4-3: Drug Loading

Drug Loading = <u>Total mass of drug – mass of drug unencapsulated</u> x 100
                              Mass of particles

Equation 4-4: Encapsulation Efficiency

Encapsulation Efficiency = <u>Total mass of drug – mass of drug unencapsulated</u> x 100
                                            Mass of drug expected

4.2.2.4.5.   Drug Release

It is important to quantify the release of drug from the formulation at pH 1.2 so as to be a immediate release dosage form and without intentionally changing the drug release and hence potentially pharmacokinetics, 80 % must be released within 40 minutes, It is also important to quantify the release at pH 6.8 as this is around salivary pH. The higher the concentration of drug released from the microparticles, the worse the bitter taste of the dissolved drug interacting with taste receptors and hence low drug release at pH 6.8 is desired. Ideally the release would have been tested in food or a suspending media which the particles would be given as these could extract drugs or impede release rate however suitable foods and media were not found in Chapter 2 (the foods tried were acidic which would have caused dissolution of the Eudragit® E PO and hence drug release).

For release at pH 1.2, washed formulations underwent testing in 900 ml 0.1 N HCl at 37 °C in a Pharmatest dissolution bath with paddle apparatus with manual sampling of 1 ml every 5 minutes for 45 minutes, the volume was kept constant by replacing the sample volume with further 0.1 N HCl and all samples filtered and analysed by HPLC in triplicate.

To evaluate release in water/buffers simulated saliva was employed, the composition of which is shown in Table 4-8. The methods used to assess release were either manual inversions in a 50 ml standard flask or by using a

Ika Vibramax at 200 RPM to shake 20 ml vials of the media, with around 200 mg of sample accurately weighed for up to 30 minutes in an attempt to provide a lower volume dissolution vessel where the flask could also represent a suspension bottle. In both cases, sampling was at 1 minute and then 5 minute intervals for 30 minutes with 1 ml of media being withdrawn and replaced with fresh media before samples were filtered through a 0.22 μm filter and analysed by HPLC. After 30 minutes, 1 ml of 1 N HCl acid was added which reduced the pH to less than 2 before an additional sample was removed after 5 minutes.

Table 4-8: Composition of Release Media Used to Assess for Taste Masking (Marques et al., 2011)

Simulated Salivary Fluid	Phosphate Buffer
Disodium hydrogen phosphate 23.8 g	Disodium hydrogen phosphate 35.3 g
Potassium dihydrogen phosphate . 9 g	Potassium dihydrogen phosphate 34 g
Sodium Chloride 8 g	-
(Phosphoric acid or Sodium hydroxide to pH 6.8)	(Phosphoric acid or Sodium hydroxide to pH 6.8)
Deionised water to 10 L	Deionised water to 10 L

4.2.2.5. *Product Characterisation*

4.2.2.5.1. Yield

The yield is a measure of the mass of particles recovered compared to that sprayed – this is very important as product which is not recovered because it has been removed as fines or coated the spray dryer is unable to be used and hence a waste of product and money especially in terms of trying to make an industrially viable formulation. The yield was calculated after measuring the weight of particles recovered using Equation 4.5

Equation 4-5: Yield

$$\text{Yield} = \frac{\underline{\text{Weight of particles recovered}}}{\text{Total Weight of Solids Sprayed}} \times 100$$

4.2.2.5.2. Size and Morphology

Particles produced from the initial spray drying were sized using a Sympatec Helos Particle Size Analyser with RODOS/M dry dispersing unit (Sympatec, Germany) and 0.5-350 µm lens using the Fraunhofer diffraction method. Each measurement was performed on around 100 mg (around ¾ of a dispersing bottle) in triplicate and the mean/standard deviation reported of the X_{10}, X_{50} and X_{90} which are the sizes that 10, 50 and 90 % of particles are below respectively. The sizes reported by this laser diffraction method were confirmed by microscopy.

Particles produced in the experimental design were produced in a different size and so underwent sieve stack analysis instead. Around 10 g accurately weighed was used where possible and initially a set of sieves (125, 180, 250, 355, 500, 710 and 1000 µm) were used in a Sonic Sifter (Endcotts, UK). Where greater than 50 % of the particles were found to be less than 125 µm on the initial sieve stack analysis, these particles were subjected to another analysis using a set of smaller set of sieves (25, 38, 53, 75, 106 and 150 µm) with results reported as size and/or percentage of fines less than 25 µm due to the limited range of sieve stacks available not allowing for full size determination. This test was only undertaken once due to the quantity of material required compared to that obtained since re-assessing the same sample would have given different results due to product breakdown or agglomeration.

The morphology was assessed using a scanning electron microscope (Philips XL30 TMP).

4.2.2.5.3. Density

Density of the particles was assessed since it will be important in suspending the particles to make a uniform suspension and it also allows assessment of how well the product which is important for processing powders on an industrial scale and compactability potential which may allow the microspheres to be administered as a orally disintegrating dosage form if required due to stability or requirements of other ages of patients. The initial bulk and tapped density of the particles were measured three times using a Copley Tap Density Volumeter as described in the Section 2.4. Briefly, around 10 g of the different particles were poured into a 100 ml measuring cylinder at an angle with the initial volume and mass used noted. The cylinder was then tapped at defined intervals with the volume measured after each set of taps until a final, stable volume within 2 % difference of the last volume was achieved and noted as the tapped volume. This was repeated in triplicate with the bulk and tapped density calculated and reported as mean/standard deviation and Carrs Index and Hausner Ratio calculated as previously reported.

4.2.2.5.4. X-Ray Diffraction

X ray powder diffraction can be used to assess the degree of crystallinity/ amorphism of powders. The diffractometer which is used to assess the powder works by applying an x-ray to the sample and has a detector which measures the intensity of the diffracted x-ray beam as shown in Figure 4-6 as determined by Bragg's Law as shown below in Equation 4-6
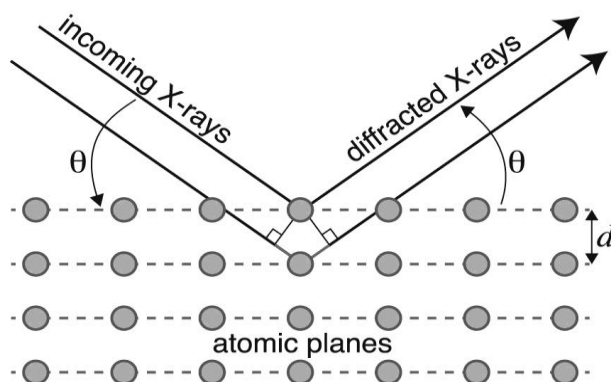
Figure 4-6: Representation of Braggs Law (Bertranda et al., 2012)

Equation 4-6: Bragg's Law

$$n\lambda = 2d \sin \theta$$

Where   n   =   Order of diffraction

              λ =   Wavelength

              d   =   Inter-planar spacing in the crystal

              θ =   Angle between the incoming rays and powder plane

A PW3710 Scanning X-Ray Diffractometer (Philips, Cambridge UK) was used to characterise the particles. Particles were compressed into a sample holder to provide a smooth surface and scanned at 0.02 °/sec from 6 ° to 35 ° with a voltage and current of 45 KV and 30 mA respectively with data shown using X'Pert High Score software (Version 2.0a).

4.2.2.5.5. Differential Scanning Calorimetry

A DSC 7 Differential Scanning Calorimeter (Perkin Elmer Instruments, UK) was calibrated with indium. Quinine hydrochloride dihydrate or microparticles (around 3 mg accurately weighed) were placed in an aluminium pan and sealed. The samples were heated from 10 to 300 °C at a rate of 10 °C/min with data recorded using Pyris Thermal Analysis software.

4.2.2.5.6. Suspendability

Suspendability of the Formulations was assessed similarly to that described in Chapter 3. Concentrations of particles tried ranged from 1 g/10 ml, 1 g/ 20ml and 1 g/ 40 ml was tried in HPMC 1 % media with particles stirred for 1 minute on a 60 % setting of a magnetic stirrer then dispersibility/suspendability assessed visually.

4.2.2.5.7.   Feed Characterisation

The formulation showing the greatest degree of release retardation at pH 6.8, F16 containing high levels of SDS, stearic acid and PEG, underwent characterisation of its feed properties following initial (low) conditions of homogenisation and increased homogenisation conditions. The samples under increased homogenisation were assessed by removing 20 ml both initially and after standing on a magnetic stirrer whilst the feed is being spray dried for two hours. The properties of: pH, particle size, zeta potential, viscosity and homogeneity of content were attempted in triplicate. A placebo (blank) formulation of F16 which had underwent increased homogenisation was also assessed before and after standing.

A Zetasizer ZS (Malvern) was used to determine the size and zeta potential of the spray drying feed at 25 °C with three repeats of 100 runs. The zeta potential was calculated automatically using Henry's Equation.

4.3.   Results and Discussion

4.3.1. Initial Spray Drying Experiments

4.3.1.1.   Initial Conditions

Using the initial conditions, the spray dryer clogged many times. As every time the spray dryer clogs, it must be turned off so that the tubes and nozzle can be cleaned; and the spray dryer takes several hours to reach the desired inlet temperature when restarted; much time and product was lost. An example of the type of "particles" initially formed is shown in Figure 4-7: it could be seen that there are particles of different sizes which may be an indication of poor droplet size control or that each component has dried separately and clumped together.
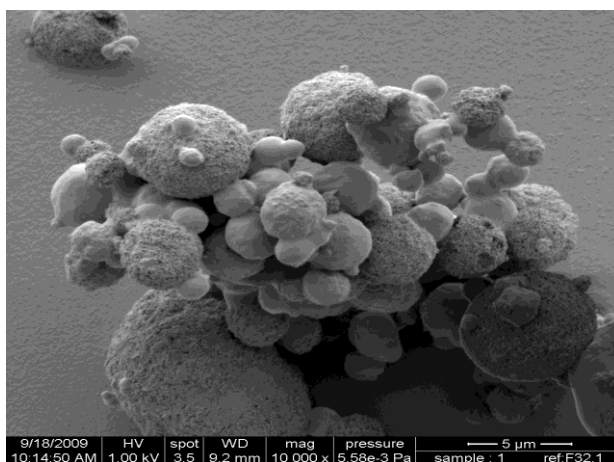
Figure 4-7: SEM Image of Spray Dried Eudragit® EPO: Quinine Base (2:1) at 10000X Magnification (Initial Conditions) showing aggregated particles

The extent of clumping can also be seen from looking at the size distribution as summarized in Figure 4-8 and Figure 4-9 . Some of the clumps were larger than 350 µm and the size profile saw multiple peaks showing different sizes of aggregates which is likely due to the stickiness of the polymer resulting from its low glass transition temperature (T_g).
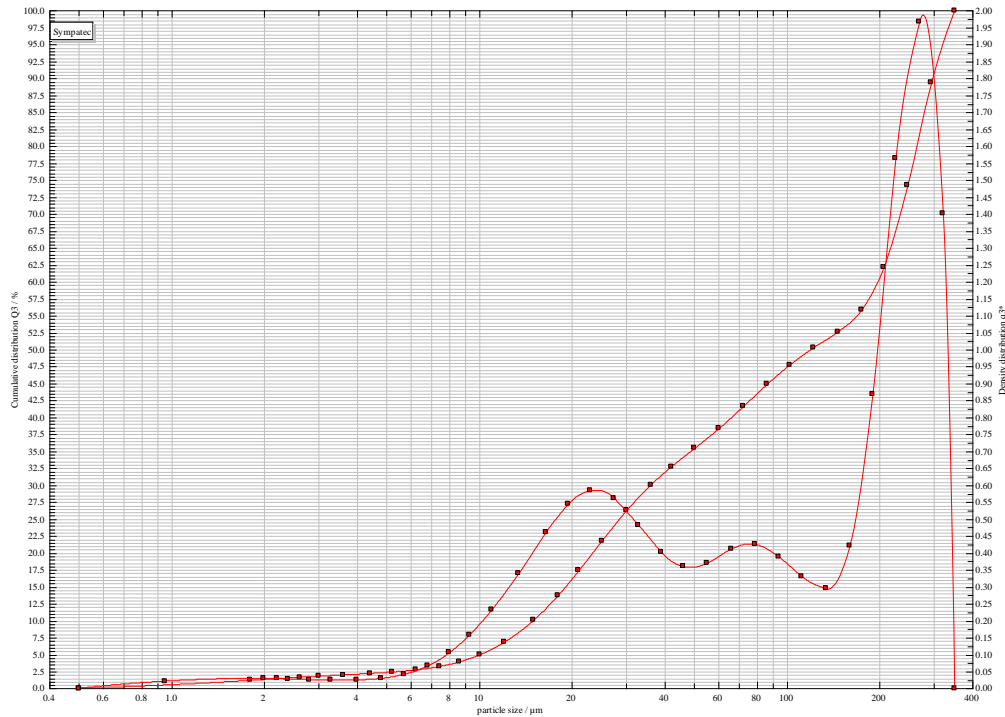
Figure 4-8: Laser Diffraction Particle Size Profile of Spray Dried Eudragit® EPO: Quinine hydrochloride dihydrate (2:1) under Initial Conditions showing aggregated particles of drug loading 20%
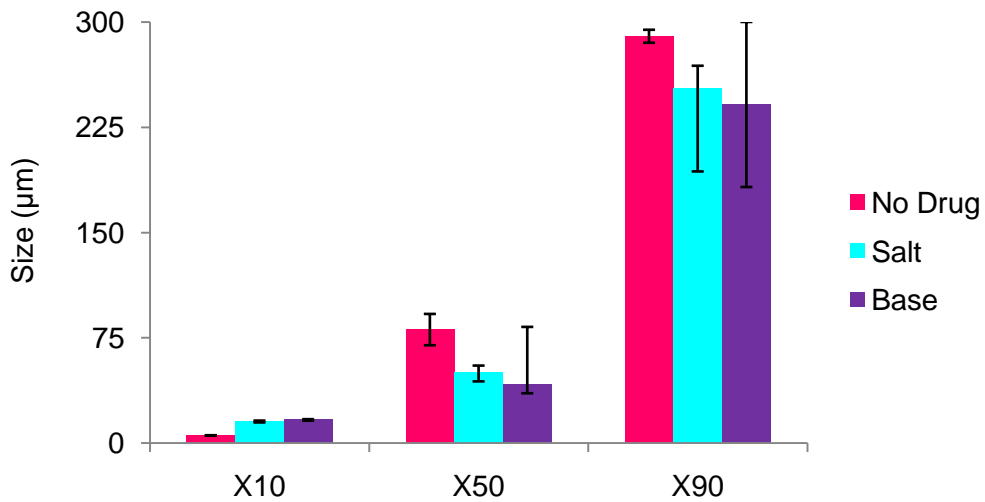
Figure 4-9: Size Distribution of all Spray-Dried Particles using Initial Conditions showing aggregated particles of drug loading 20 %

Several unsuccessful approaches were attempted to overcome the problem of the blockages which included decreasing the solids concentration of the feed (by increasing the volume of water) and increasing pumps rates. From sieving the feed, it became clear that there were lumps of solid in the feed which were blocking the tubes and nozzle so increased mixing was

attempted but still the lumps persisted. Blockages occurred even after sieving suggesting the blockages were due to particles aggregating or sedimenting whilst waiting prior to reaching the nozzle, despite the feed being stirred constantly. As mixing did not remove the lumps which occurred not only in the drug containing dispersions but also in the drug free feed, it was concluded that the suspension preparation required optimisation and the homogenisation conditions were investigated.

4.3.1.2.   Increased Homogenisation Conditions

Initially the polymer dispersion was homogenised for 30 minutes as per the reference method as described in Section 4.2.2.1. and as no rate was stated, 100 RPM was used. It became evident that the addition of the drug solution/suspension to the polymeric dispersion could also cause visible lumps so the drug dispersion also needed to be homogenised (the lumps were expectedly worse with the quinine base but also present on occasions with the quinine salt). The time and conditions used for homogenization of the dispersions needed to be a balance between minimizing the shear rates and homogenization time the formulation was exposed to due to the potential for damaging the polymer but also ensuring the feed did not have lumps.

Higher shear rates (e.g. around 1000 RPM) removed most of the lumps. However any particles at the top of the beaker (such as quinine base, which is hydrophobic and hence difficult to wet) remained there with the potential to block the spray dryer. Higher shear rates (e.g. around 3000 RPM) were able to provide enough energy to move the top of the dispersion and hence bring the particles into the bulk where they could be incorporated into the suspension but also obviously placed more stress on the formulation as well as seen by foaming.

A compromise was made whereby the drug and polymer dispersions were each homogenised for ten minutes and then for a further ten minutes once they were mixed together so as to reduce the time of homogenisation and to

homogenise each for 5 seconds at 3000 RPM (to enable surface particles to be brought into the bulk) and the rest of the time at 1000 RPM.

During the first drying run following this optimized preparation procedure, a whole batch of non-drug, quinine salt and base containing feeds were able to be successfully spray dried as seen by the particles in Figure 4-10. These particles are not perfectly spherical which is a common feature of spray drying without organic solvents and the morphology is generally poorer for the quinine containing feeds due to its hydrophobic nature so this was seen as something which needed to be developed by examining different processing conditions and excipient levels.
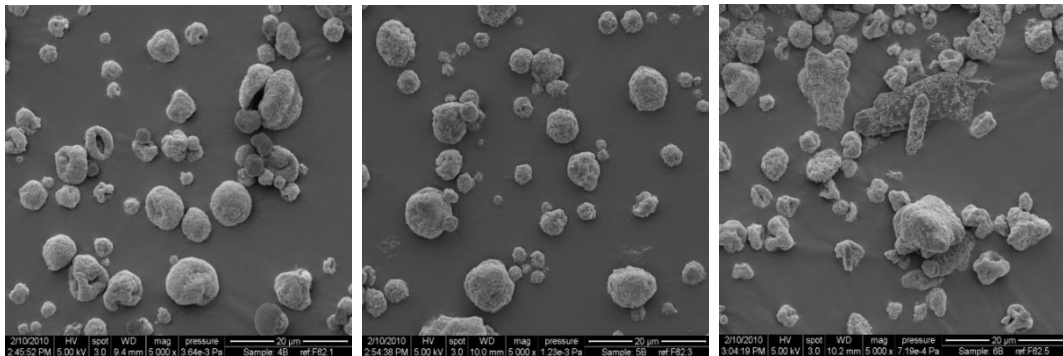
Figure 4-10: SEM Images of Spray Dried Eudragit® ® EPO with (From left to Right) No Drug, quinine hydrochloride dihydrate and quinine all at 5000X Magnification made using increased homogenisation showing particles of drug loading 20 %

The size distribution of the particles produced under the increased homogenisation is further proof of the reduction in clumping caused by the increase in homogenisation as can be highlighted by ninety percent of all particles being less than around 27 µm as seen in Figure 4-11 and Figure 4-12 compared to the 200-300 µm values seen for particles produced under the initial conditions seen in Figure 4-9.
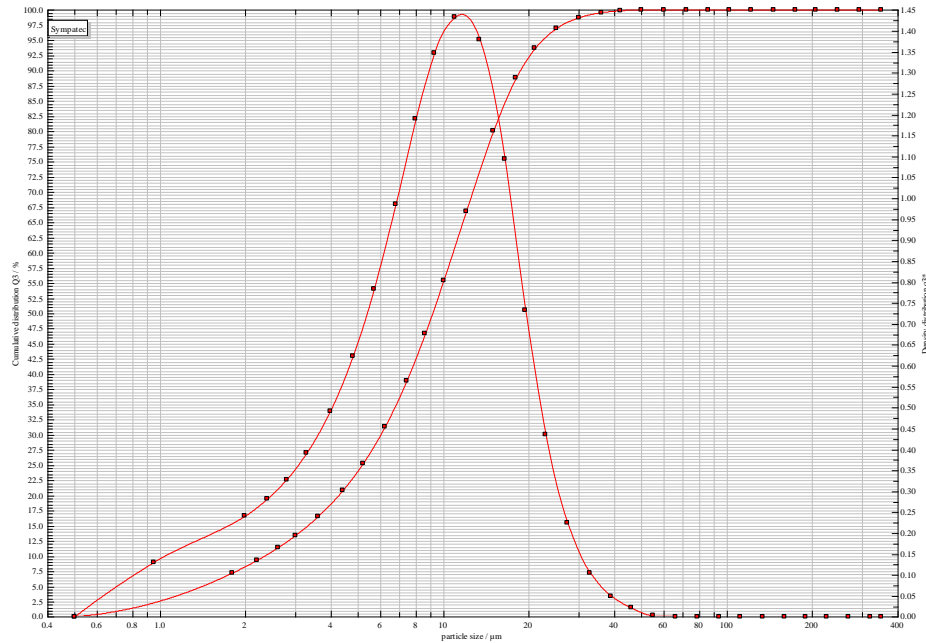
Figure 4-11: Laser Diffraction Particle Size Distribution of Spray Dried Eudragit® EPO: Quinine Hydrochloride (2:1) under Increased Homogenisation showing particles of drug loading 20 %
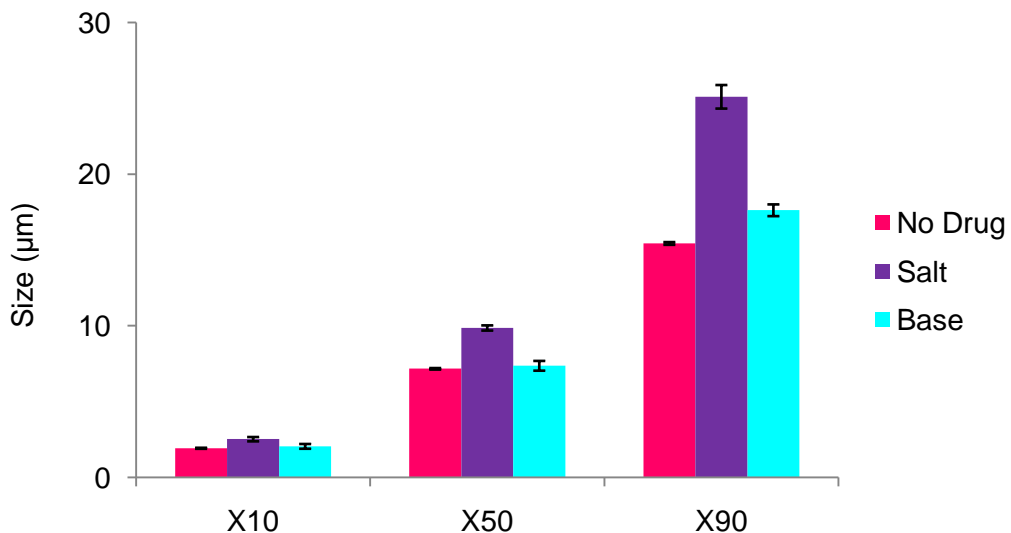
Figure 4-12: Size Distribution of Spray-Dried Particles made with Increased Homogenisation showing particles of drug loading 20 %

The higher purple line in Figure 4-12 is thought to be due to the soluble salt being encompassed into the particles more effectively than the base, which appears to have spray dried separately from the polymer as suggested by the morphology on SEM. This increased encompassment of the soluble salt compared to the base is further suggested by the smoother line of quinine

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salt particles compared with that of the base shown in Figure 4-13 and Figure 4-14 showing less crystalline drug in the salt form. This shows that the drug is in the amorphous form which is common post spray drying but can be detrimental to dosage form development as this form is unstable and hence it would be difficult to control the stability and release rate of the formulation as these can change e.g. over time.
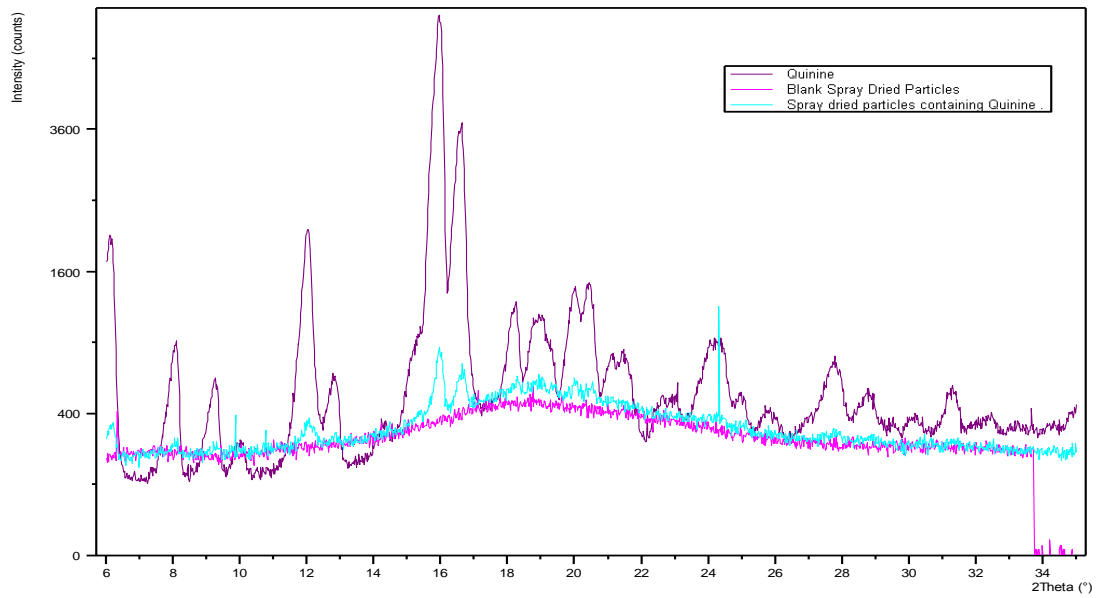
Figure 4-13: X-ray Powder Diffraction of Spray Dried Eudragit® E PO: Quinine Base (2:1) under Increased Homogenisation for particles of drug loading 20 %
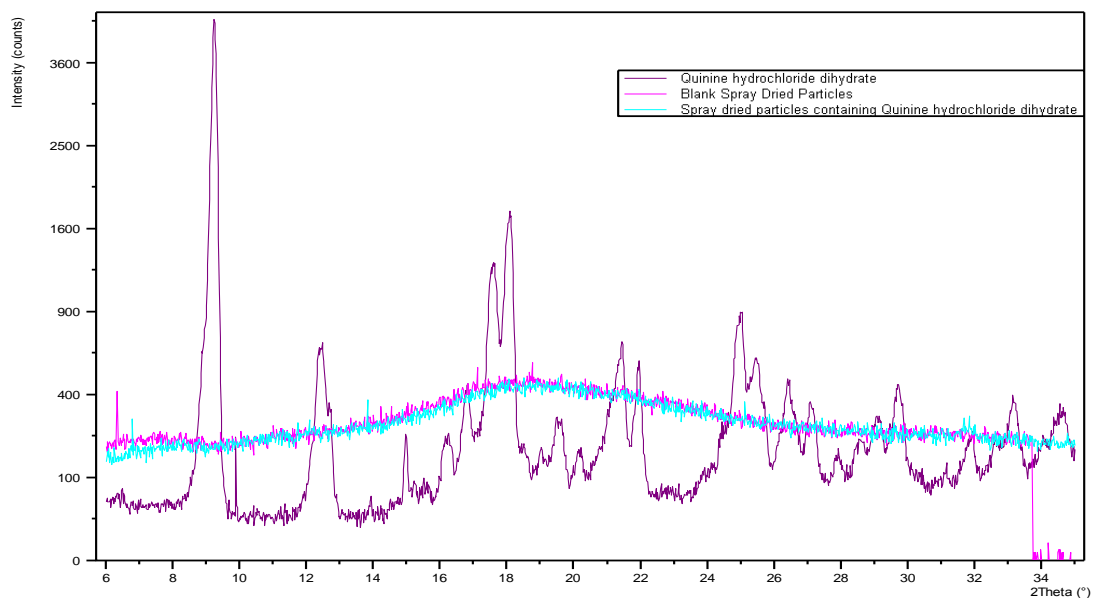
Figure 4-14:X-ray Powder Diffraction of Spray Dried Eudragit® EPO: Quinine Hydrochloride dihydrate (2:1) under Increased Homogenisation for particles of drug loading 20 %

4.3.1.3.   Increased Conditions of Temperature/Flow Rate

In an attempt to improve the particles and yield; the inlet temperature was increased to 140 ºC, the atomizing gas flow set at 3 kg/h and chamber inlet flow set to 30 kg/h. When the size distribution of the particles was compared with those made at lower temperatures and flows, it was seen that the quinine salt particles produced by the increased temperature/flow conditions were smaller than those produced by the initial conditions as shown in Figure 4-15. This is thought to be due to the increased atomizing pressure generated by the higher atomizing gas flow reducing the size of the spray dried droplet and hence particle size produced. For the base at initial conditions, the particle sizes were smaller than the salt particles which may be due to increased encapsulation of the soluble salt whereas as the settings were increased, the particle sizes increased which could be due increased stickiness caused by the higher temperature or gas flow.
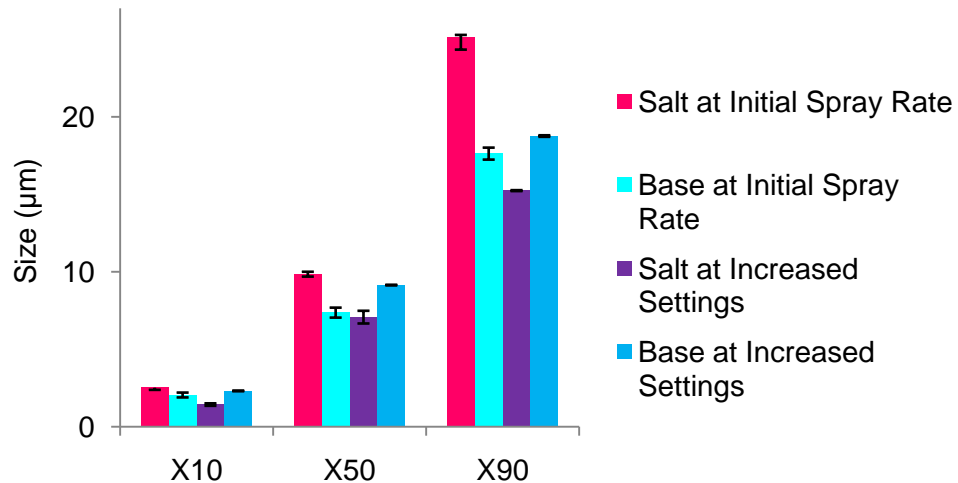
Figure 4-15: Size Distribution of Spray-Dried Particles made with Different Settings at drug loading 20 %

The morphology of the particles by SEM was similar to those seen in Figure 4-10 and increase in yield was observed as shown in Figure 4-16. It can be seen that under the initial conditions the yield was the worst with that of quinine base being poorest, most likely due to having a less uniform feed

suspension compared to the blank and salt which seems to be supported by the way the yield increased under increased homogenisation conditions although the yield was still better for all conditions tested in the salt which would have been more in solution versus the suspension of the quinine.
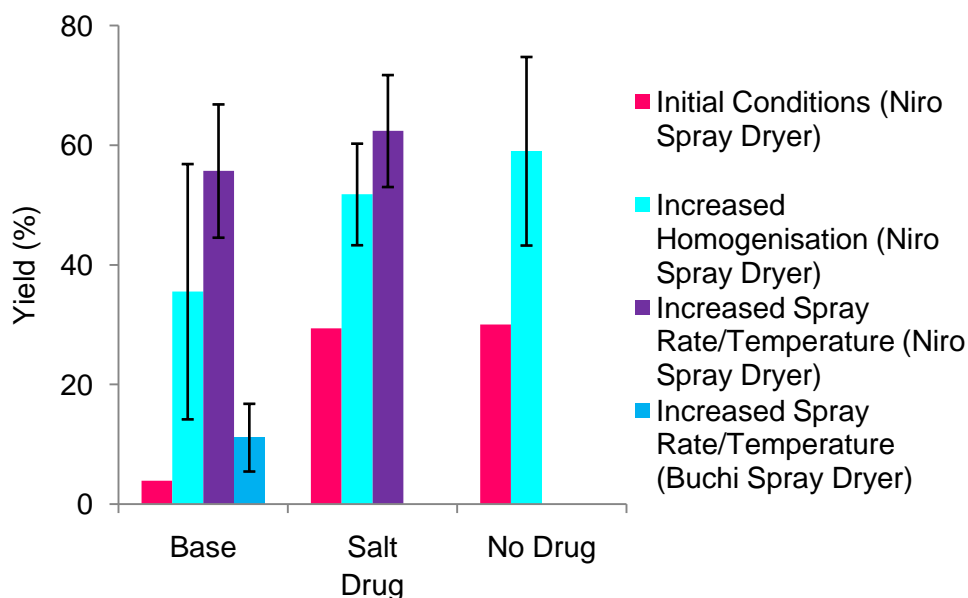
Figure 4-16: Yields of Spray-Dried Particles made under Different Conditions (All yields are n=3 except for initial conditions where the highest yield obtained was reported and drug loading 20 %)

4.3.1.4. *Büchi Compared with Niro Spray Dryer*

The effect of using a different spray drier (Büchi Mini Spray B191) on particle characteristics and blocking tendency was investigated Examples of the Büchi and Niro spray dryers are shown in Figure 4-17. Both machines exhibit co-current flow with two fluid nozzles but it can be seen by visual inspection that the sizes and set up differ. The Niro spray dryer allows organic solvents to be spray dried safely by virtue of a nitrogen atmosphere and is marketed as being the smallest spray drier with the same fluid dynamics as larger, industrial models for scale up (GEA Process Engineering).

Figure 4-17: Büchi Spray Dryer (Left) compared to the Niro Spray Dryer Used (Right)

The spray drying conditions used were picked to be similar to the increased temperature/spray rate conditions used in the Niro but only using the quinine base as this had previously been the more difficult to spray dry. When these conditions were used on the Büchi, the nozzle and tubes clogged repeatedly (despite using the same homogenization conditions as used successfully with the Niro spray dryer) and what little particles were produced were destroyed by water managing to reach the collecting vessel. When the spray pump was reduced to 20 % (from 30 %), the spray dryer was still repeatedly clogged and very little product was produced with most of the product that did manage to be sprayed coating the spray dryer. Scanning electron microscopy images of the particles were of clumps and this is further suggested by the size distribution as shown by Figure 4-18.
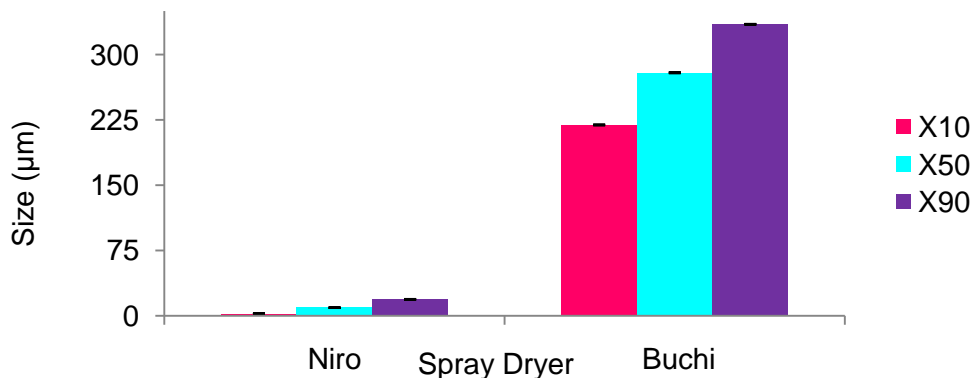
Figure 4-18: Size Distribution of Eudragit and Quinine Base (2:1) Spray-Dried Particles made with Different Spray Dryers

From observation, the nozzle of the Büchi spray dryer is different in design to that of the Niro spray dryer in that particles seem to be able to accumulate in the nozzle until they cause a blockage, unlike in the Niro where a lump causes an outright blockage which can be seen in Figure 4-19. This serves to highlight that although the Niro and Büchi are similar designs of spray dryer, formulations and parameters cannot be used interchangeably. Alternative atomization techniques such as a rotating disk may improve this. In order to try to improve particles, it was decided to look at the effect of excipients by experimental design by screening levels of excipients as reported in Table 4.6 above.

Figure 4-19: Büchi Spray Dryer Nozzle compared to the Niro Spray Dryer Nozzle Used

The data from this section demonstrates the importance of formulation and preparation of the spray drying dispersion. It may be that the current formulation is not optimal for spray drying hence the effect of different levels of excipients will be examined using an experimental design.

4.3.2. Analytical Method Development

4.3.2.1.   HPLC

An HPLC method was developed to quantify drug release/content from the particles after excipient interference on UV spectrometry. Initial HPLC parameters used a UV detection wavelength of 250 nm with the mobile phase changing from 5 – 95 % v/v Acetonitrile/0.05 % v/v TFA over 10 minutes for each sample with a washout period between samples of three minutes. A wavelength of 215 nm was tried for detection to see whether this gave an improved spectrum but the 215 nm spectra had a more uneven baseline. A triplet was observed as seen in Figure 4-20 which was attributed to the drug since the size of it increased as the drug concentration of the samples increased. The triplet was analysed as its three component peaks which gave a large relative standard deviation (throughout to be due to the autointegration setting the peak boundaries slightly differently with each sample). When the area under the whole triplet was manually calculated, the relative standard deviation was vastly decreased thus supporting this idea.
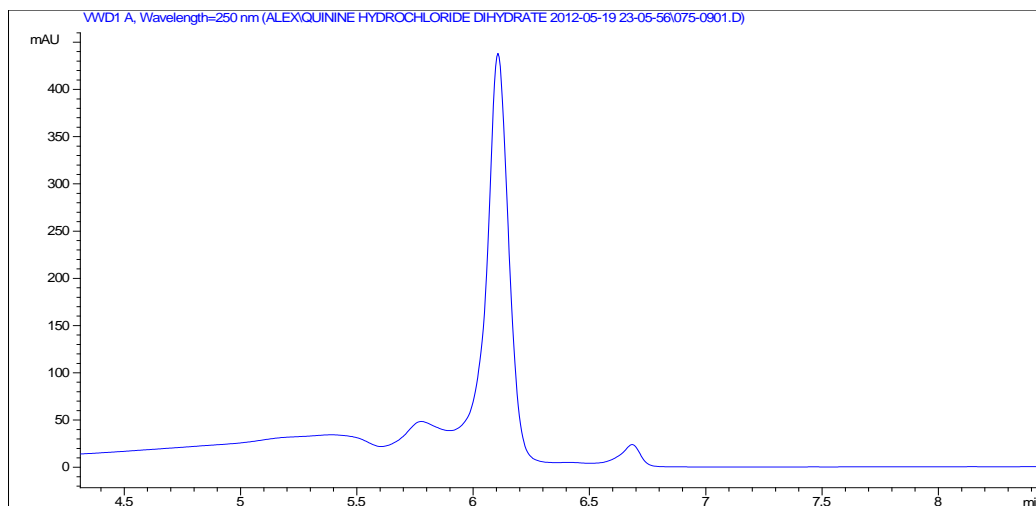
Figure 4-20: An Example of Incompletely Resolved Peaks of Quinine Hydrochloride dihydrate standard in Acetonitrile (100 µg/ml)

Further work was undertaken on trying to separate out the triplet so that the area of one peak could be analysed: 45 %:55 % of A: B as detailed in Table 4-9 was tried as this corresponded to the parameters at the 4 minutes elution time of the triplet. As this did not allow complete separation of the peaks (e.g.

there was still a raised baseline) 35 %:65 % A: B was then tried which also did not allow separation of the peaks so a new, slower method was started with 10 % organic solvent increasing to 40 %.

This new method was found to separate out the peaks and allow peaks to be analysed from baseline to baseline although– coming off at around 90 s with the solvent front at about 60 s. 93 % A vs. 7 % B was tried and was successful as shown by the final conditions in Table 4-9 .

Table 4-9: Final HPLC Conditions Chosen for Analysis

Final HPLC Conditions	
Column	Phenomenex Kinetex 2.6µ C18 100 x 30 mm
Mobile Phase	A: TFA 0.05% v/v (aq)
	B: Acetonitrile + TFA 0.05%v/v
Gradient	7 to 40 % B over 10 minutes (3 minutes recovery)
Flow	0.5 ml/min
Temperature	40 °C
Detection	UV at 250 nm

No interference around the 6 minute mark where quinine elutes was observed from any of the additional excipients in the microspheres with the chromatogram for SDS (typical of all excipients under these conditions) shown below in Figure 4-21.
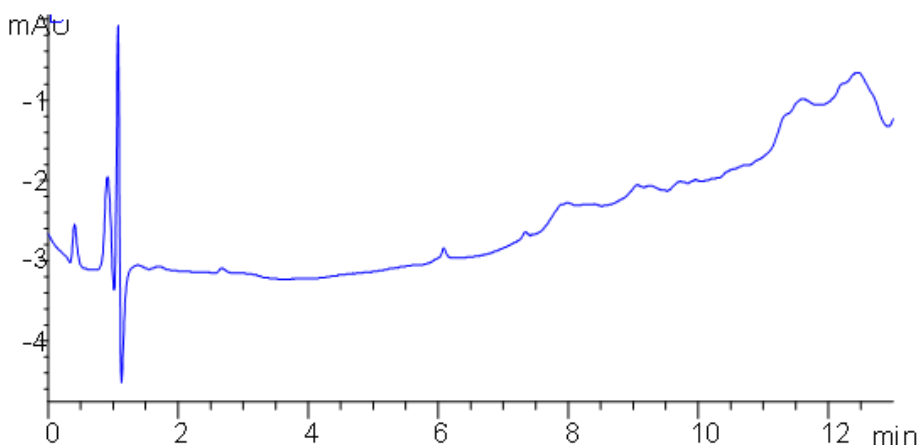
Figure 4-21: Example of a HPLC Spectra using Final Conditions for SDS 0.5mg/ml (no drug) showing a lack of interference of this excipient in the region of 6 minutes where quinine elutes

In terms of stability, three peaks were found in the standard (20 µg/ml solution in 40 % MeCN/0.04 % TFA) and all the photo degradation samples whereas two peaks with some smaller peaks (noise) were found in all the pH conditions. All of the photo degradation samples had around 48 % of the peak areas in the first peak and the highest peak height was reduced which fits with the literature as quinine is photo unstable. It was not the purpose of this work to fully investigate the degradation profile of quinine so further analytical development was not undertaken. The analytical conditions used are adequate for the purposes of this work.

When the DOE samples were analysed, it was found that the samples in water appeared to have a higher release than those in acetonitrile/TFA. Further analysis found that the acetonitrile TFA samples had an additional peak. In order to try to determine what this peak was, standards of quinine were made in acetonitrile, in TFA (aq) and in water. The acetonitrile samples both showed the additional peak hence water was used for standard preparation in all subsequent experiments.

The linearity of the standard curve was around 100 % as shown by Figure 4-22 and it was found that 100 µg/ml gave an absorbance of around 1000 mAU as shown by Figure 4-23 so this concentration level could be increased if required.

The graph shows Peak Area (y-axis, 0 to 3000000) versus Concentration (µg/ml) (x-axis, 0 to 30) with the equation:

$$y = 90206x - 9619.$$
$$R^2 = 0.999$$
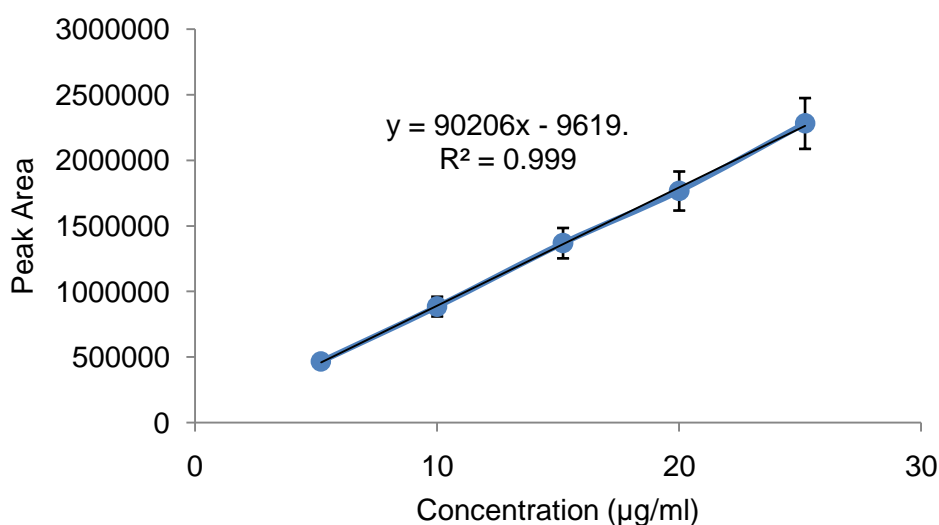
Figure 4-22: Quinine hydrochloride dihydrate HPLC Calibration Curve in Water (n=3)
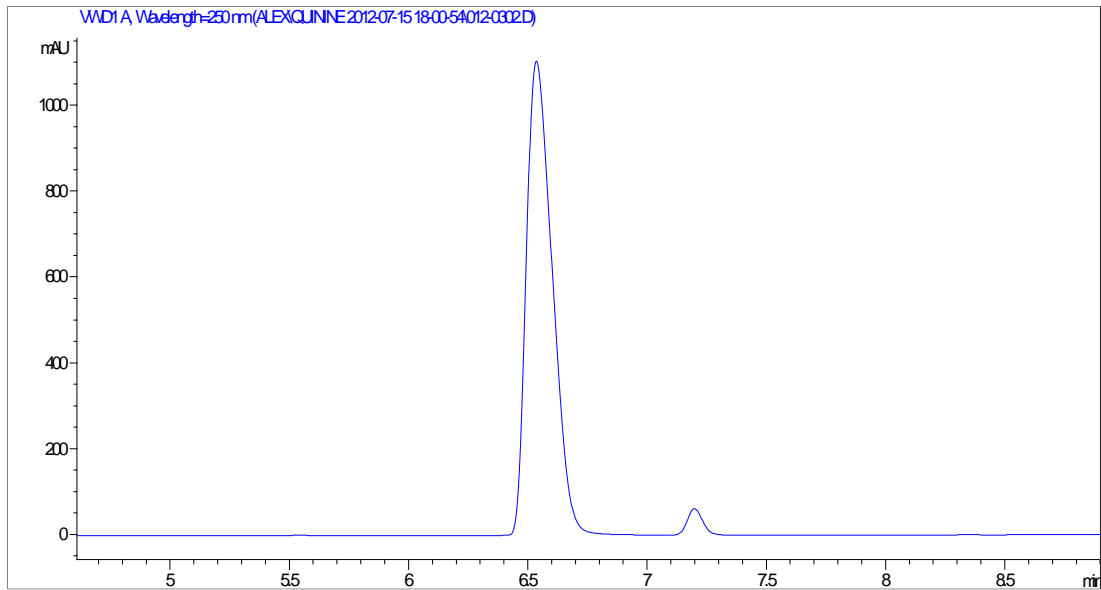
Figure 4-23: Example of a HPLC Spectra using Final Conditions for a Standard Solution of Quinine hydrochloride dihydrate 100mcg/ml

4.3.2.2.   Filter Compatibility/Filtration

Based on the results shown in Figure 4-24 , the Millex® MP filter unit was chosen. This was due to the low retention of both strengths of standard as well as a low standard deviation.
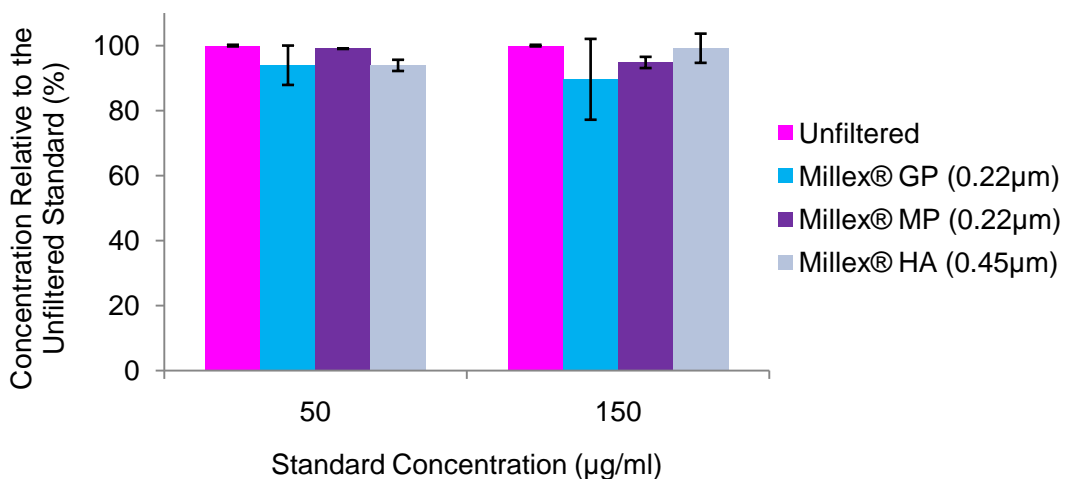
Figure 4-24: Percentage of Quinine hydrochloride dihydrate in water recovered after filtration through various filters

It can be seen from Figure 4-25 that filtering resulted in a lower drug concentration by only considering unencapsulated drug which is important since in taste masking, it is important to know exactly how much drug has been released since this is the drug which is available to interact with the taste buds.
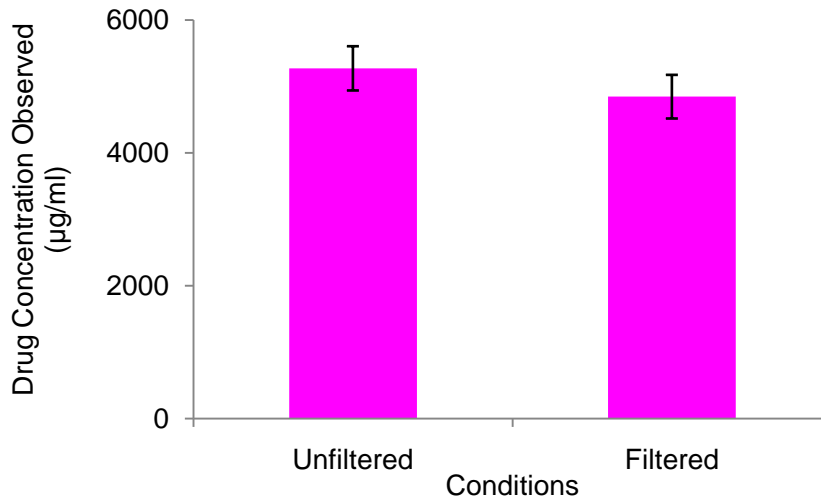
Figure 4-25: Effect of Filtering on F1

It can be seen that filtering has an effect especially on the initial time points up to 15 minutes when less of the drug might be released compared to that still contained within the particles due to the time taken for the polymer to dissolve. As a result of this, when the two formulations were filtered, more of a gradual increase in drug was seen compared to the other non filtered conditions. It is unsurprising that F16 which contained high levels of SDS, SA and PEG exhibited slower release of the drug as seen by comparison with F6 following filtered release due to the larger particle size of F16 possessing a smaller surface area and hence prolonged dissolution time. This prolonged dissolution time may also be why the filtered F16 has a larger variability as seen by the standard deviation compared to that of F6. When the samples are not filtered but removed from the same location, it can be seen that flatter lines representing more constant "drug release" are obtained which may correspond to the similar range of particles being in the same location, this is also seen when F6 is not filtered or sampled from the same location due to the small particle size being easier to disperse and having the drug contained throughout more, smaller, particles which is the opposite of

that of F16 where the location and hence number of larger, drug containing particles can vary widely as shown by the pale green line in Figure 4-26. meaning that formulations must be filtered.
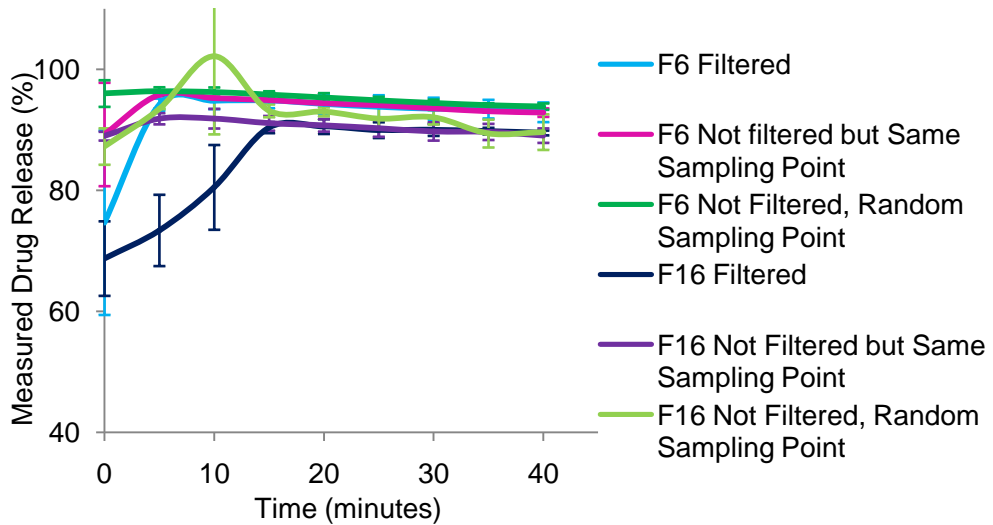
Figure 4-26: Effect of Different Filtering Conditions on Drug Release from F6 & F16

4.3.2.3.   Washing Microparticles

In order to try to remove any unencapsulated drug which would be free to interact with the taste buds and taste bitter, different centrifuge speeds, times and centrifuges were tried in an attempt to optimise washing. A Sigma 3k30 centrifuge with 35ml tubes was used to assess how much drug was removed as shown in

Figure 4-27.
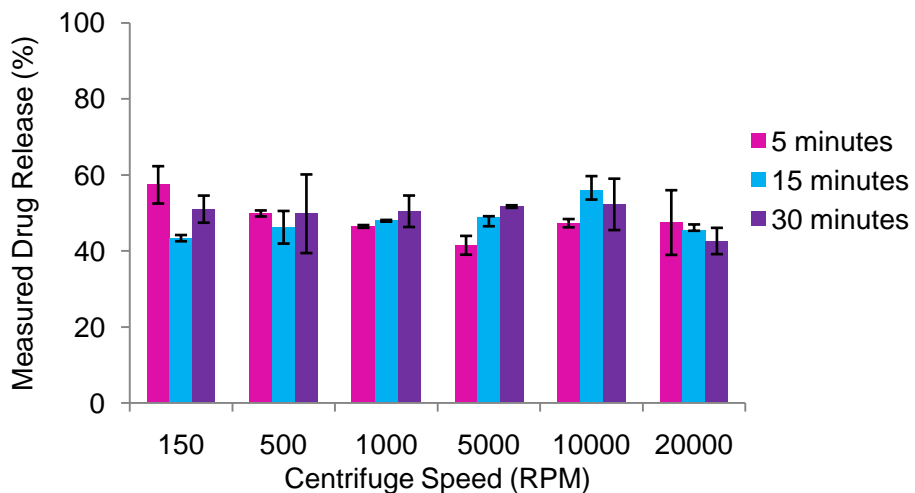
Figure 4-27: Effects of Different Centrifuging Conditions on F1 which was the first midpoint containing mid levels of all excipients

Similar results were obtained for lower times and centrifuge speeds (100 and 150 RPM for 1 minute gave a drug release of 48.5±2.8 % of the expected drug content and 49.8±0.8 % respectively) as well as for a longer time at a higher speed (at the centrifuge's maximum of 21,000 RPM for 120 minutes, 43.1±1.9 %). Similar release results also occurred from washing the particles using filter paper to trap the washed particles, but the filter paper method of washing was time consuming as it did not allow for washing multiple samples concurrently and it was difficult to remove particles from the paper. When all different formulations were centrifuged, it became very difficult to rewash them to remove all remaining drug or to obtain the microparticles as a "pellet" which was not removed by removing the supernatant. A pellet only occurred with formulations F9, 10, 11 and 12 and even then this was partially dispersible on movement. A Heraeus Microcentrifuge from Thermoscientific which can only take eppendorf tubes of 1-1.5 ml appeared to perform better as the particles had the base of the eppendorf to settle into. Moreover it was easier to gently remove 1 ml than 35 ml of supernatant. This washing was then repeated 5 times using similar relative centrifugal forces and durations as with the 3K30 centrifuge before drying at 40 °C until constant weight (within 24 hours).

4.3.2.4.   *Content and Release*

Methods of assessing encapsulation efficiency (defined as the percentage of expected drug encompassed in particles) were assessed as Eudragit® E is soluble in selected organic solvents or 1 N HCl. When organic solvents such as ethanol, methanol and acetonitrile were used to dissolve the microparticles, a triplet was seen on the HPLC spectra compared to the usual duplet. When 1 N HCl was used, less drug was measured on HPLC analysis. A compromise was reached by adding 5 ml of 1 N HCl to the particles which was then diluted in distilled water to 50 ml after shaking to 0.1 N HCl.

As media and techniques for assessing taste masking in vitro are not rigidly set in stone, two different media were compared to that of deionised water for release and in two different methods. The release was not tested in food or a suspending media as suitable foods and media were not found in Chapter 2.

From the results below in Figure 4-28, water appeared to show the lowest release but was close to the others. Increased release in buffer may be due to the higher pH of the unbuffered water when particles are present.

Simulated salivary fluid was chosen for further release experiments due to the presence of chloride ions making the media more physiological relevant and because it is buffered. The vial method described was chosen as its lower volume is more physiologically similar to the low volumes of saliva in the mouth although still higher than the salivary volume. This volume (20ml) allowed for withdrawing and replacement of 1 ml of media from 1 minute then every 5 minutes for 30 minutes. This work also suggests even the buffered media doesn't appear to ensure retardation of drug release for a prolonged so a sachet formulation may be needed to permit dosing. In this case the short residence time in the mouth may allow sufficient taste masking to be achieved.
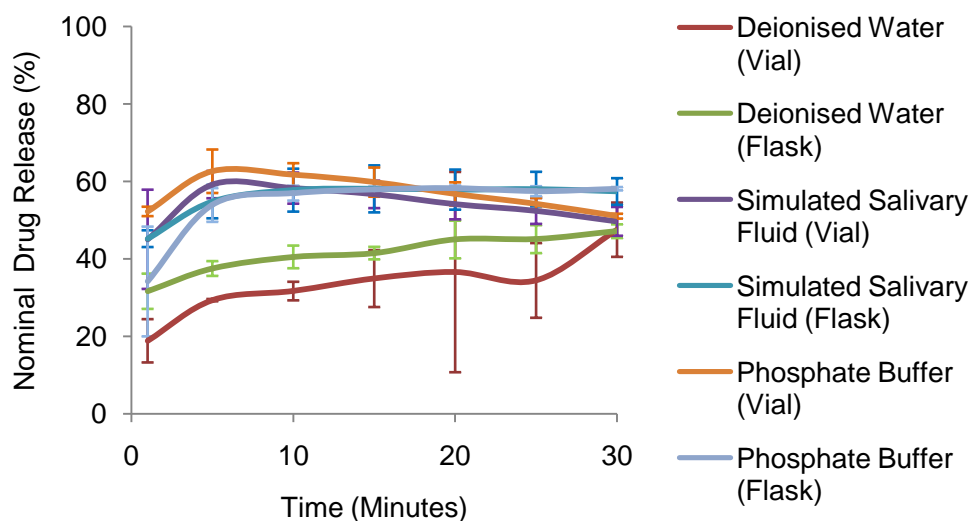
Figure 4-28: Drug Release from F1 under Different Conditions for Assessing Taste Masking

In summary, an HPLC method was developed and a compatible filter and the importance of filtering samples found. A method was optimized to wash the particles by centrifugation and a method of assessing release and content chosen for the experimental section below.

4.3.3. Experimental Design

4.3.3.1. *Scoping Work*

It was attempted to increase the solids content from the 4 % w/v tried in the initial work to a more industrially viable 20-40 % w/v. However at 40 % w/v, it was not possible to make a suspension, merely a semisolid mass of lumps. At 20 % w/v, initially a stick style of homogenizer (Ultra Thurax®) was used to form a sort of visibly lumpy suspension which blocked the spray dryer so another homogeniser (Silverson® with an emulsifying head was used) The balance between the time of homogenising for a suspension with no visible lumps versus the suspension becoming too foamy was seen to be using the homogeniser at 3000 RPM for 30 minutes overall due to the higher solids content. However, the 20 % w/v suspension blocked the spray dryer constantly despite attempts to try to overcome this by changing spray dryer settings including increasing/decreasing temperature (in the range 110-160 °C) and increasing/decreasing spray rate (from 5-20 %). The little amount of 20 % w/v feed which was sprayed prior to blocking produced large aggregated particles of several mm as shown by Figure 4-29.

Figure 4-29: The large size of F1 Particles Produced at Solids Concentration 20 % w/v when compared to a coin

When the solid content was decreased to 10 % w/v, the suspension intermittently blocked, hence the experimental design was restarted at 140 °C/20 % pump setting and 5 % solids concentration. Although this is not very industrially viable, it was attempted to see whether satisfactory particles could be made to then try to improve solids concentration on that feed.

A higher spray rate of 20 % of pump capacity was tried to attempt to make the process faster which would also minimize the time the product was exposed to the high temperatures required for particle drying. Using the 20 % pump setting, the initial midpoint scoping batch yield was less than 10 % so eventually a spray rate of 5 ml/min (corresponding to a pump setting of 7 %) was set with the best conditions able to be achieved summarized in Table 4-10

Table 4-10: Optimum Spray Drying Conditions Achieved

Parameter	Value
Inlet Temperature	140 °C
Air Flow Rate	600 L/hr
Aspirator Setting	90 %
Spray Rate	5 ml/min
Solids Concentration	5 % w/v

4.3.3.2. *Yield*

No significant effect was found of any excipient on yield. The maximum yield achieved was 39.2% for Formulation 9 as shown in Figure 4-30 . This formulation, as a result of the higher levels of both polymer and plasticizer, may have been thought to be likely to stick to the chamber of the spray dryer and hence reduce the yield. However this highest yield might be due to its high stearic acid and SDS levels. However this means for all formulations, more "product" is being lost than is recovered which obviously has a financial impact on the process.
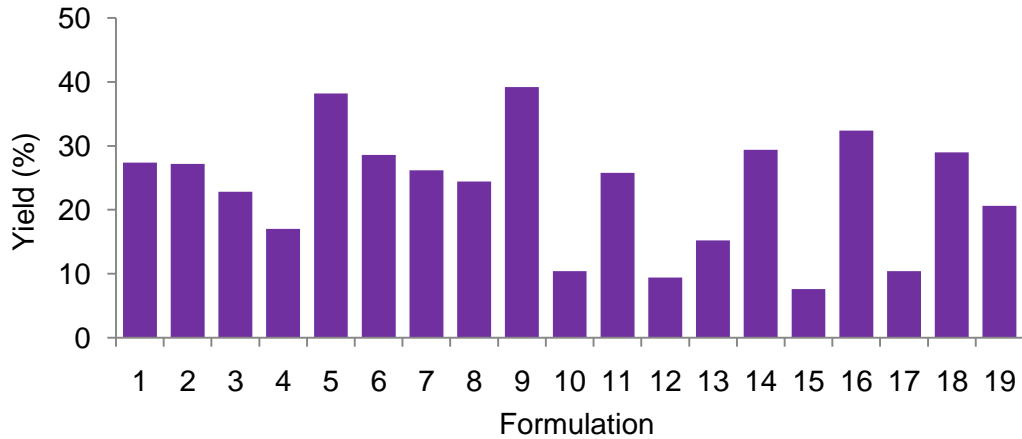
Figure 4-30: Yields for All Experimental Design Formulations

4.3.3.3. Size, Morphology and Density

Particle size for the majority of Formulations (F) were >500 µm or < 25µm and most particles showing some degree of aggregation on SEM as shown in Figure 4-31 and particle size analysis (given by percentage of fines less than 25 µm) in Figure 4-32. The percentage of fines is not a commonly used way to report size but was used due to the equipment limitation of only having to sieve stacks which did not adequately characterise the formulations in terms of size whereas the percentage of fines corresponded to the particle sizes seen visually and on SEM (e.g. formulations with a large particle size had low percentage of fine particles less than 25 µm). Complex effects on size were seen. Increased size can be seen to be due to increased aggregation on SEM and as expected this can be correlated with increasing polymer or plasticiser levels which would be expected to make the particles more "sticky" as shown by F9 on the right.

Fines (<25µm)   Mid-Size Range   Large Sized (>1mm)
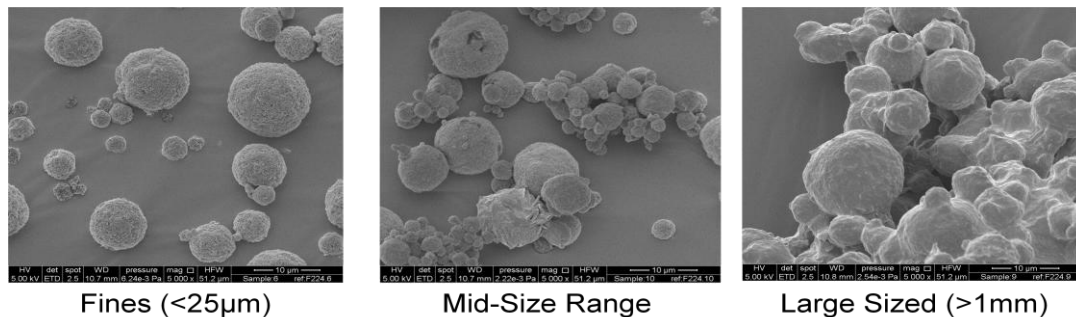
Figure 4-31: Morphologies and Size Ranges of Particles (F6, 10 and 9 from left)

190
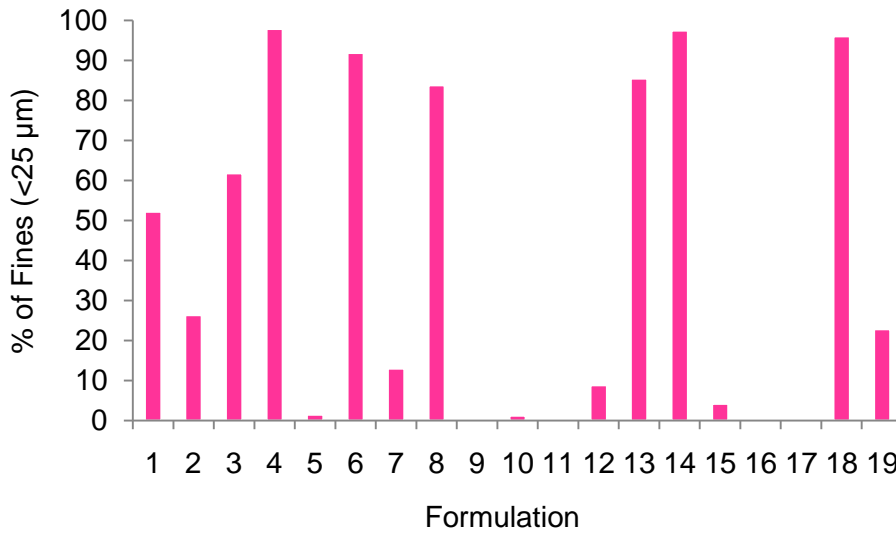
Figure 4-32: Percentage of Fine Particles (<25 μm) for Each Formulation

The density of the particles ranged from 0.14-0.31 g/ml as seen in Figure 4-33. This is fairly light compared to a drug and is likely to be due to the presence of colloidal silica: this low density would make the particles difficult to disperse and suspend, making them likely to float on top of the suspending media and the particles stick to the side of the container causing problems with dose uniformity as seen with Cellets in Chapter 2 and discussed later.
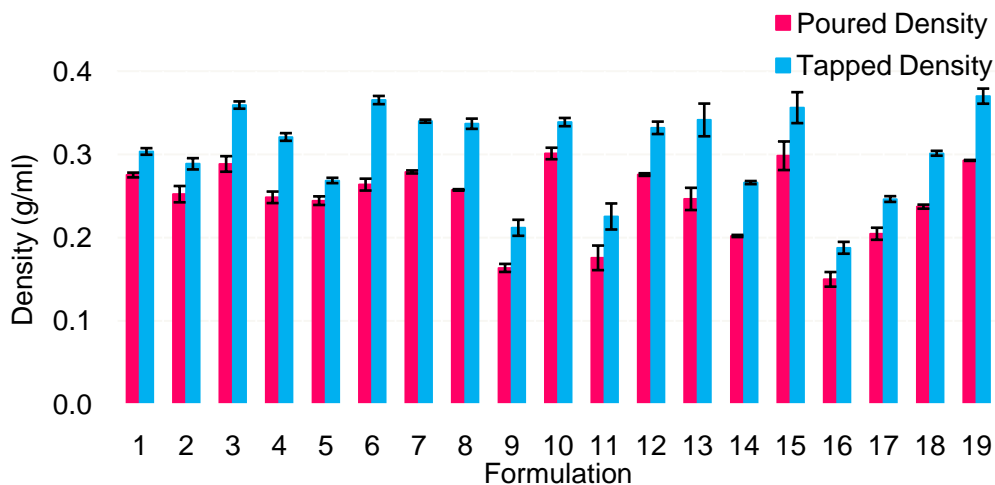
Figure 4-33: Poured and Tapped Density Mean ± SD of All Formulations

From Figure 4-34, it can be seen that colloidal silica and SDS have an effect on density.
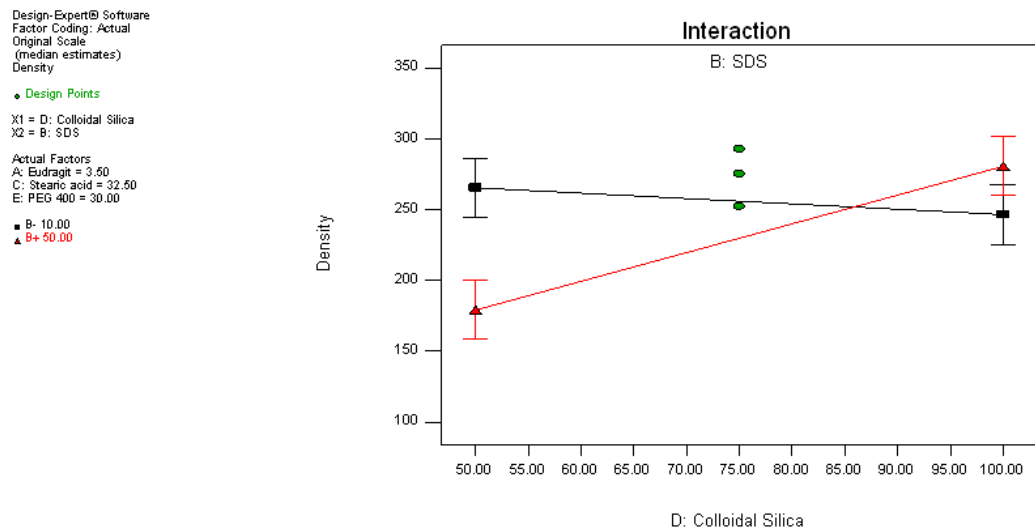
Figure 4-34: Interaction of Colloidal Silica and Sodium dodecyl sulphate on Percentage of Fine Particles with other Excipients at Midpoint levels

From Equations 2-5 and 2-6, the Compaction Compressability and Hausner Ratio were calculated and the results shown in Table 4-11. It can be seen that most particles had at least acceptable flow properties and compaction characteristics except F6 and F13. Both contained low polymer and SDS levels along with high stearic acid concentrations so may lack as uniform a coat/particle size as those formulations with less hydrophobic components.

Table 4-11 hence shows that if desired e.g. for a tablet or orally disintegrating tablet formulation, the majority of formulations have acceptable compactability and all formulations have acceptable flow which can be an advantage for pharmaceutical processing.

Table 4-11: Compaction Index and Hausner Ratio for all Formulations (where for Carrs Index good < 15 %, fair 16-20 %, passable 21-25 % and poor >26 % while for Hausner Ratio good/fair < 1.25, passable 1.25-2.5 and poor >1.5)

Formulation	Compaction Index	Hausner Ratio
1	Good	Good
2	Good	Good
3	Fair	Passable
4	Passable	Passable
5	Good	Good
6	Poor	Passable
7	Fair	Good
8	Passable	Passable
9	Passable	Passable
10	Good	Good
11	Passable	Passable
12	Fair	Good
13	Poor	Passable
14	Passable	Passable
15	Fair	Passable
16	Passable	Passable
17	Fair	Good
18	Passable	Passable
19	Passable	Passable

4.3.3.4.   *Release*

It can be seen in Figure 4-35 that all of the samples meet the requirement for immediate release dosage forms to release at least 80 % of their content within 45 minutes in the hydrochloric acid 0.1 M with the majority formulations releasing greater than 80% within five minutes (with the rest, formulations 7-12, releasing by ten minutes). This is desired so that the pharmacokinetic profile of the medicine we are trying to taste mask is not altered.
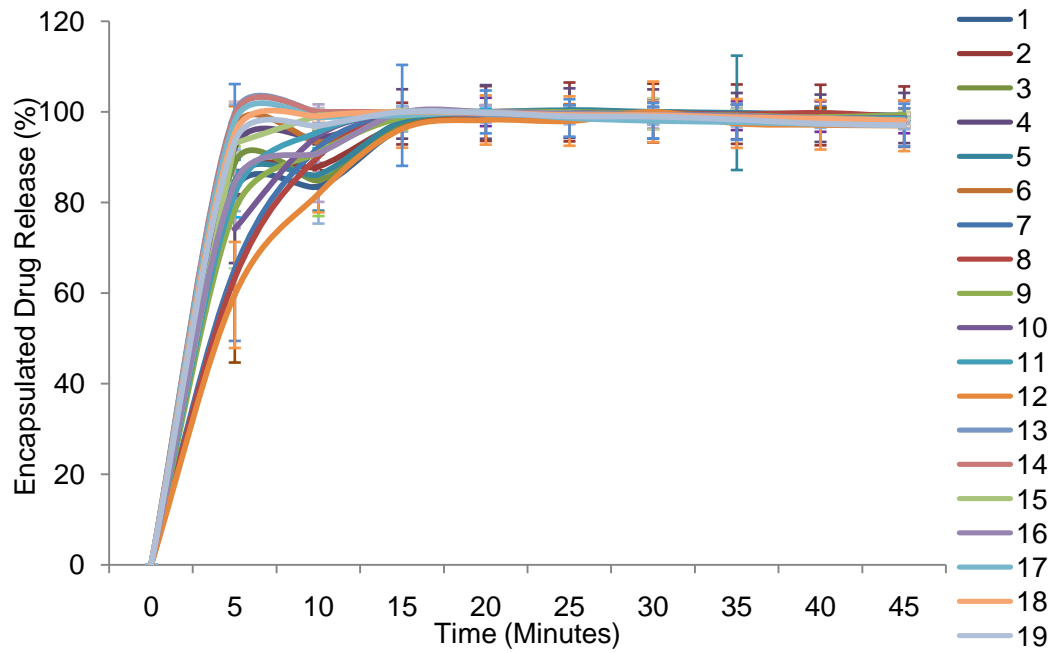
Figure 4-35: Release in Acidic Media of all DOE Formulations (n=3)

Taste masking was approximated by the mass of drug released in 1 minute in simulated salivary media compared to that of the quinine bitterness threshold of 0.000008M. It can be seen be seen from Figure 4-36 that drug release was highly variable as shown by the large standard deviations of many of the samples. This suggests that a uniform product was not made which may be due to aggregation of particles or may be due to individual components of the formulation separating out. It can be seen that all formulations released the drug even at the short time point of one minute. F9 and F16 both exhibited the most release retardation under the pH conditions designed to be similar to that of the mouth. However both formulations were over 1 mm in size and seen to be highly aggregated: even these formulations did not adequately retard release so as to have release below the bitterness threshold of quinine. A low colloidal silica concentration was seen to reduce drug release which may be due to less anti-adherent causing larger aggregates as suggested by the morphology as seen by SEM. The reduced drug release may also be due to an improved polymeric film retarding release or due to a reduction in the hydrophilic component retarding release possibly via reduction in their solvent wicking effect.
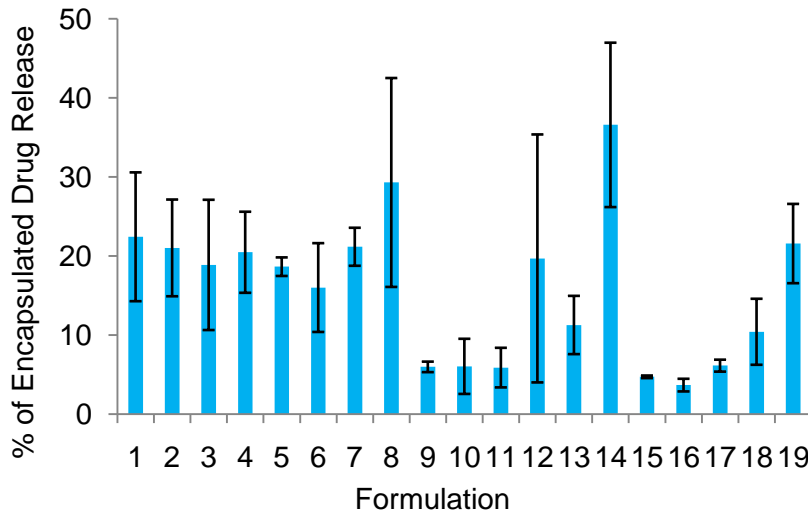
Figure 4-36: Encapsulated Drug Released at 1 minute at pH 6.8 for All DOE Formulations (n=3)

4.3.3.5. Content

Many particles exhibited low encapsulation efficiencies and drug loadings as shown by most particles in Figure 4-37. Increasing the encapsulation efficiency is desirable as it can prevent the loss of medicine and extend the duration of action and dose of the medication. No excipient was found to have an effect on drug loading and encapsulation efficiency of the formulations (which were calculated as described by Equations 4-3 and 4-4 respectively) when assessed using Design Expert®. The large standard deviations of the individual formulations show that the particles produced by spray drying were not uniform and hence some have more drug than others/are more aggregated than others as shown by the varied size distribution. The large variability between formulations in terms of drug loading and encapsulation efficiency is the result of the drug composition of the spray drying feed. The low drug loading is due to the high proportion of excipients required to retard drug release in the simulated salivary fluid. The drug composition varied as shown in Table 4-6 where the proportion of drug is set as 1, the proportion of polymer related to that of drug and the other components present as a function of the percentage of polymer present. Therefore the drug loading can be seen to be dependent on the proportions

of other components as to what proportion of drug was present in the feed and hence there is a variability of drug content in the particles. Those with encapsulation efficiencies higher than 100% may have had some unencapsulated drug present or may be due to the loss of fine particles of colloidal silica from the spray dryer as reported by others (Xu et al., 2008a).
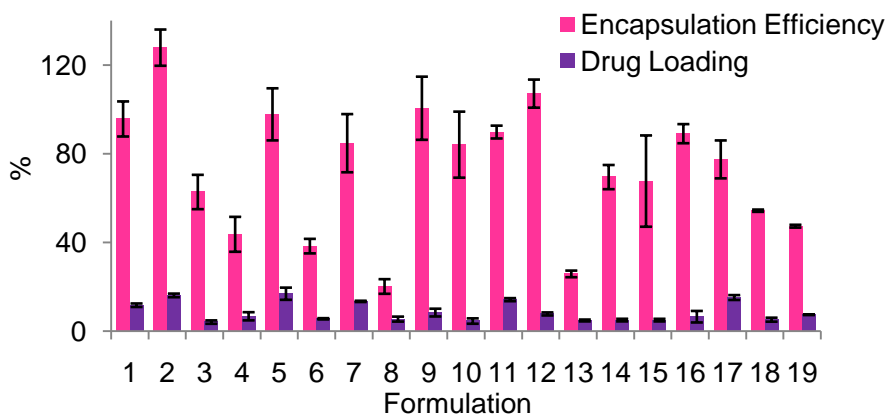
Figure 4-37: Encapsulation Efficiency and Drug Loading for All DOE Formulations (n=3)

4.3.3.6. Suspendability

The recommended dose of quinine salt (being hydrochloride, dihydrochloride or sulphate) for children is 10 mg/kg (up to a maximum of 600 mg) every 8 hours (British Medical Association and the Royal Pharmaceutical Society of Great Britain, 2008b). Using a mean weight value of 20 kg to correspond to that of a 6 year old (since children may not need liquids after this age) and a maximum concentration of 500 mg/5 ml, it can be seen that the volume the 6 year old would be required to take ranges from 11.9-49.1 ml which at the very lower end may be acceptable as it is around 10 ml.

Table 4-12: Mass of DOE Formulations and Volumes required for the Lowest and Highest Drug Loading DOE Formulations to provide Therapeutic Doses of Quinine hydrochloride dihydrate

	Lowest Drug Loading (F3)	Highest Drug Loading (F5)
Mass of Quinine hydrochloride dihydrate (QHD) in 100mg of particles	4.08mg	16.87mg
Mass of particles containing 1mg of QHD	24.51mg	5.93mg
For 20kg child (~6years) requiring 200mg - Mass of MP required - Dose volume if particle concentration of 500mg/5ml	4,902mg 49.1ml!	1,186mg 11.9ml

The suspendability of the particles was determined as reported in Chapter 2. Briefly a mass of particles as described below was mixed in 10 ml of suspending media using a magnetic stirrer on 60 % for one minute and the extent of dispersion of particles/time for particles to sediment assessed visually. The suspending media used was a HPMC 1 % solution (pH value of the solution used was determined as 6.4±0.1). HPMC 1 % was used as a suspending agent as it had been reported to be acceptable by both taste trials. The unflavoured and unsweetened HPMC 1 % was used, as the flavoured and sweetened HPMC 1% was found to be too acidic (pH value of 4.2+0.1) which would be incompatible with the Eudragit E Polymer which would be soluble at that pH. The unflavoured and unsweetened HPMC was found to be acceptable in the first trial.

All suspendability testing was attempted using a concentration of 1 g of particles/10 ml of media but: due to the low density of the spray dried particles, this was a large volume of particles and hence it was impossible to make a suspension within 10 ml. When this was reduced to 1 g/ 20ml, more of a semisolid was made. A more fluid suspension was produced by mixing 1g particles in 40 ml of vehicle. However as with the Cellets in Chapter 2, it was very difficult to disperse the particles due to their poor wettability as seen in Figure 4-38. The formulations with smaller particle sizes gave a

powder that stuck to the bottom/sides of the vessel and was difficult to disperse – what did disperse could be seen as aggregated (as shown to the right of Figure 4-38. For the large particle sized formulations, they were difficult to uniformly disperse, again a lot like Cellets as shown to the left of Figure 4-38. Hence dispersibility was difficult and so a uniform suspension was not achieved with any formulation so the homogeneity of content was not assessed. It can therefore be seen that the wettability and mixing of these particles would need to be improved but a sachet/powder for reconstitution is likely to be more achievable given the large size/mass and low release retardation as shown to the left of Figure 4-38.

Figure 4-38: An assessment of the Suspendability of Larger Particles (<1 mm) of F9 (left) and Smaller Particles (>25 µm) of F18 (right) at 1g of particles/40 ml of media. It can be seen that the larger particles are difficult to suspend and sediment to the bottom whereas the lighter particles appear to be compacted by the addition of the media and sticks to the bottom/sides of the beaker

4.3.3.7. Characterisation

As F16 was one of the formulations which exhibited the highest degree of release retardation, e.g. a low percentage of drug released in simulated salivary media used to assess for taste masking, had its feed characterised retrospectively as shown Table 4-13. This looked at how the viscosity, particle size and zeta potential of the F16 differed depending on whether it contained drug or not and the effect of standing and homogenisation on these characteristics in an attempt to relate this to the spray drying feed stability (in terms reducing lumps which formed throughout the process).

Table 4-13: Spray Drying Feed Characterisation of F16 formulations (both those containing drug and not) on the effect of drug, homogenisation and standing of the spray drying suspensions. It can be seen that the addition of drug decreases the pH of the feed and reduces the zeta potential also that the particle size and viscosity decrease with increasing homogenisation

Sample	Characteristic					
	Mean pH (SD = 0 for all)	Viscosity at $1s^{-1}$ (Mean±SD)	Size		Zeta Potential (mV) (Mean± SD)	Appearance
			Z-average (d.nm) (Mean±SD)	Mean Poly-dispersity index		
F16 (Initial Homogenisation)	8.8	2.93±1.44	7708.0±101.8	1	-10.74±2.90	White semisolid
F16 (Increased Homogenisation, before standing)	8.7	0.02±0.01	4894.0±11.3	1	-12.62±4.03	Milky white liquid
F16 (Increased Homogenisation, after standing)	8.8	0.04±0.01	6020.0±527.0	1	-11.65±5.54	
Placebo (before standing)	9.4	0.15±0.05	573.9±12.1	0.54	-57.05±2.34	
Placebo (after standing)	9.4	0.14±0.05	517.8±20.1	0.35	-54.48±1.38	

The differences between the placebo and drug suspension can be observed with the addition of the quinine hydrochloride drug salt decreasing pH and reducing the zeta potential (and hence stability). The viscosity of the suspensions highlights the importance of the increased homogenization in reducing particle size and reducing viscosity as was also confirmed by appearance. The large polydispersity index suggests that the particles were sedimenting or aggregating and that a Zetasizer with a larger particle size range should be used. The homogeneity of content was unable to be assessed on this occasion due to the suspensions blocking the filters used to try to filter the feed prior to HPLC analysis and so may need dilution in future.

4.3.3.8. Overview

Yield, encapsulation efficiency, drug release in 1 minute in simulated salivary fluid, particle size (assessed by percentage of fine particles <25 µm) and density were all assessed as separate responses to try to more completely understand the particle's characteristics but the primary response was drug release in 1 minute in simulated salivary fluid which we wanted to be as low as possible as this will depend whether the bitter drug would be tasted or not.

As there was not prior knowledge about the effect of different levels of the five excipients in the spray drying feed on various microparticle characteristics a screening experimental design using a fractional factorial design was used in an attempt to assess and whittle down the number of factors that have any effect. To have undertaken an initial response surface design, i.e. ignoring the screening step, for all five factors would have required 47 runs here! A very high-risk version of this design would have required 26 runs – but this wasn't recommended as there was not adequate knowledge of the interactions between the factors. The initial plan was run the screening design then after analysing the data, append this design with a response surface design (a central composite) to would hopefully give an idea of the optimum region. However, as the only factor found to have an effect on drug release 1 minute in simulated salivary fluid was colloidal silica (with decreasing levels causing decreased drug release) also was associated with particles <1 mm and hence too large for us to suspend, the response surface design was not carried out.

F9 and F16 were seen to have the highest degree of release retardation (e.g. the lowest percentages of drug released in 1 minute in simulated salivary fluid). The drug was dispersed as seen in Figure 4-39 which shows an amorphous nature which may cause problems in stability of the particles. Both F9 and F16 had aggregates >1mm as seen in Figure 4-40. Comparing the two, F9 had a slightly higher yield and encapsulation efficiency (EE), F16 was more reproducible in terms of EE (n=3) and had a higher drug loading (DL). F16 was hence classed as the "best" formulation and characterised as

above in Section 4.3.3.8. Low levels of colloidal silica had a significant effect on reducing release. These formulations which did retard release, by being larger, were very difficult to disperse/suspend and would require a large mass of particles for a therapeutic dose – hence they do not produce uniform particles. A lack of feed suspension variability may be present as shown by the variability between the repeated midpoints of F1, F2 and F19 throughout Chapter 4.
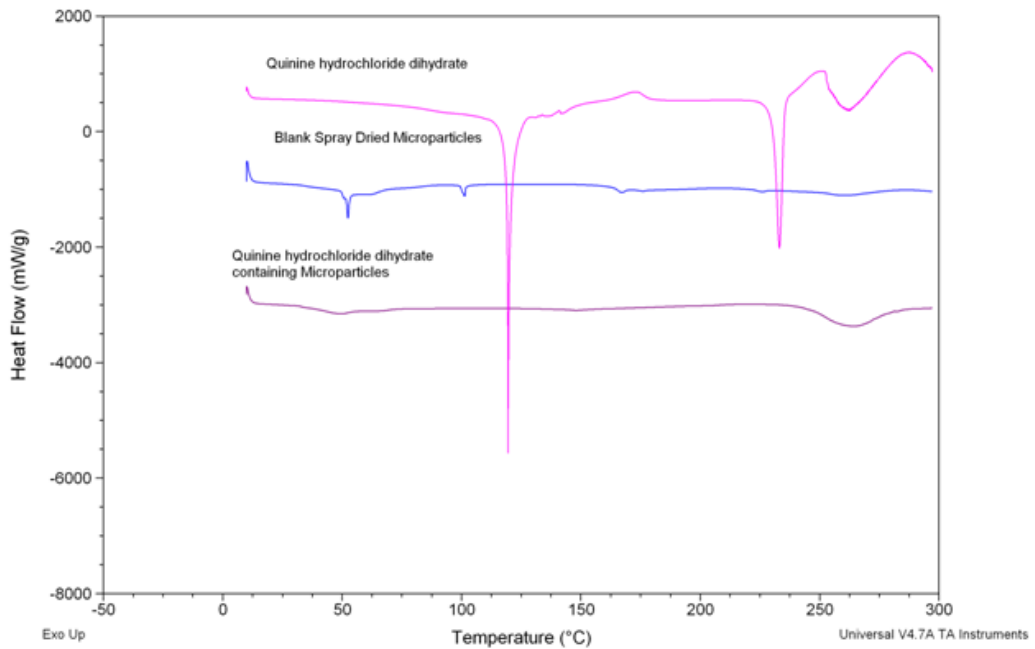
Figure 4-39: DSC Trace of F16
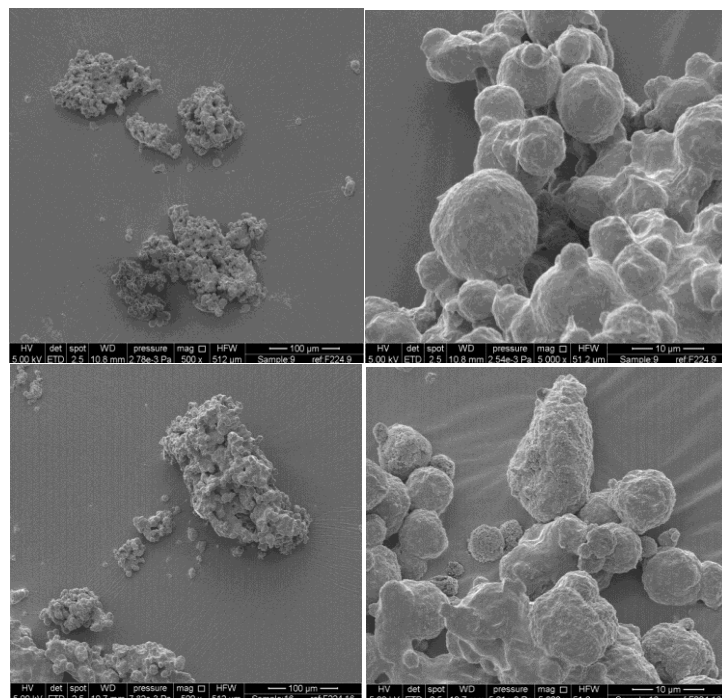
Figure 4-40: SEM images showing aggregates of F9 (top) and F16 (bottom)

4.4. Conclusions

The production of taste masked formulations is critically important to children's taking their medicine as covered in Chapter 1; however this work did not produce such a taste masked formulation as the particles did not adequately retard release so as to have release after 1 minute in simulated salivary fluid below the bitterness threshold of quinine. The aqueous spray drying process with the excipients and levels chosen did not make for an ideal microparticle production method due to a low solids content of the spray drying solution requiring high temperatures and spray rates which meant the outlet temperature went above the glass transition temperature of the polymer and hence sticky, aggregated particles occurred. As a result, low yields occurred which made for a process that would not be industrially viable.

Drug loading of particles was generally low due to the high levels of excipients being used in an attempt to gain release retardation which would lead to large particle masses/concentration required. Few formulations showed any degree of release retardation or "taste masking". Formulations which produced larger sized particles were more likely to retard release which were difficult to disperse and suspend. Low levels of colloidal silica were found to reduce drug release; this may be due to increased aggregation due to less anti-adherent effect or due to an improved polymeric film or less of a hydrophilic excipient.

Quinine hydrochloride is a very soluble drug with a low bitterness threshold and mid-level dose so not well suited to the current technique. Others in the literature have used less soluble, lower dosed drugs with lower bitterness thresholds e.g. medicines such as famotidine: drugs such as this may be suited to the current process. However it is clear that the process would not make a platform formulation. Uses for particles produced may include compressing them into ODTs or using them as agglomerate or sprinkle.

It is possible that taste masked individual particles produced by aqueous spray drying of Eudragit E cannot be produced. Particles produced by others using aqueous spray drying have suffered in terms of low yields, spray rates and aggregation. Within this current work the practical importance of suspension properties has been seen with the extensive blockage of spray dryers occurring. In future, any formulation should be characterized as a minimum in terms of particle size and viscosity prior to the attempted spray drying to avoid this.

As different spray drying parameters were tried during the scoping work, this method may be difficult to develop further unless a different spray dryer was used such as one which could contain a different type of nozzle like a rotating disk which cannot block. It is likely that future research will require the use of different excipients. Only low colloidal silica was seen to impact release with large particles so different anti-adherents should be tried such as talc or Glyceryl Monostearate and removing the plasticizer may reduce stickiness. The problem of the low glass transition of Eudragit® E is difficult to overcome and may need combination with other polymers such as alginates or cellulosics or to use an emulsion or organic solvent of minimum toxicity such as ethanol. If an alternative method was required, solvent evaporation/emulsification has produced taste masked particles but suffers from a lack of scale up information. It may also be worth considering a method based on pH changes since it may be possible to solubilise the Eudragit® polymer at pH below 5 and then reform it as a film by increasing the pH above 5. The industrial practicality of such an approach would need to be evaluated.

5. GENERAL DISCUSSION AND FURTHER WORK

5.1. General Discussion

The aims of this project were achieving a multiparticulate-based platform for delivering functionalized capability as an oral liquid dosage form. Although these very specific aims have not been met, a number of lessons have been learnt along the way that can inform future work.

From Chapter 1, the challenges in providing medicines for children were identified and it was seen that children have a number of additional needs to those of adults which have to be considered, such as swallowability, a lower tolerance of poor/taste acceptability, the need for different doses as they develop and the difficulties of requiring medicines throughout the school day. It was seen that a multiparticulate dosage form would be ideal as particles could provide a taste-masked or modified release functionality to meet these needs which many currently available medicines cannot. Formulating the particles in a suspension provides a way to administer these particles whilst keeping their dose adaptability and swallowability unlike compressing them into tablets or presenting them in a stickpack. Developing a platform formulation would ensure that time and money is not consumed in bespoke pharmaceutical development whilst children wait for much needed medicines.

Given that functionalized multiparticulates are, by virtue of containing drug and polymer, larger than individual drug particles, it was unknown what the limit of particle sizes of these larger particles would be in terms of suspendability and grittiness which were explored in Chapter 2 and 3 respectively. Microcrystalline cellulose starter cores (Cellets®) were used as model particles for the suspendability and grittiness studies because they come in a range of sizes with narrow size distributions, are inert and accepted as safe to be administered orally. Despite all the advantages of the Cellets®, they differ in some particle properties compared to those produced

by spray drying in Chapter 4 including density, size and morphology. Nevertheless the small amount of work undertaken on the suspendability of the microparticles described in Chapter 4 suggests that the general conclusions drawn from this work with Cellets® are likely to be valid.

As the suspendability of particles and grittiness of a suspension may depend upon the properties of the suspending media, some commonly used suspending media and soft foods often used for the administration of sprinkles were characterized, largely in terms of rheology. As methylcellulose and hydroxylpropyl methylcellulose solutions ranging from 0.1, 1 and 3 % w/v were seen to "mimic" the range of viscosities from water to commercial suspending media and had a largely acceptable pH range so that the Eudragit® E polymer to be used to try to produce taste masked particles in Chapter 4 would not be soluble, these vehicles are considered worthy of further evaluation. However it was found to be difficult to produce a uniform and physically stable suspension with any vehicle tested.

Grittiness trials were carried out in young adults due to the logistical difficulties with completing the research on children. However children and adults can both rank samples in the same order, just the hedonics are different. It was seen in the initial grittiness trial looking at the effect of particle size, concentration and viscosity on the grittiness of suspensions in Chapter 3 that particle size and concentration had a significant effect on grittiness whereas viscosity did not. The importance of palatability in all age groups was highlighted through complaints received from some participants about the "slimy" or mouth coating effect of the HPMC, especially at the 3 % w/v concentration. It is thought that this was why viscosity was not seen to have a significant effect on grittiness and it was notable that no 3 % w/v solutions were rated as the participants "most acceptable" formulations. This mouth coating effect may be due to the higher yield stress as covered in Chapter 2 of the more concentrated HPMC not being overcome by the mouth. The refined (second) grittiness trial in Chapter 3 made methodological improvements compared to the initial trial, with an increased sample size and narrower particle size/concentration and viscosity ranges.

Acting upon this feedback, the unpleasant mouth coating was overcome by the formation of a pleasant orange flavoured solution sweetened with sucralose. Thereafter viscosity and particle size were seen to have an effect but not particle concentration (likely due to the similar scores of the microcrystalline cellulose particle (Cellets®) concentrations of 125 mg/5 ml and 250 mg/ 5ml. The results from Chapter 3 suggest that viscosity can reduce grittiness and that particle sizes should be minimized where possible.

Aqueous spray drying as described in Chapter 4 was desired due to spray drying being a multiparticulate production technique which is industrially scale-upable and the absence of organic solvents removes risks and disadvantages associated with them. Spraying Eudragit® E was difficult with much time and product wasted through the continual blockages of both types of spray dryer nozzles until the extent of homogenization was increased and the solids content of the feed decreased to 4-5% w/v indicating in hindsight the criticality of feed properties on this process as assessed in Section 4.3.3.7. The low solids content along with the high temperature (140 °C) and low flow rate of required to successfully evaporate the aqueous solvent were not industrially viable and lead to an outlet temperature higher than the (low) Tg of Eudragit® E. In turn a low yield was achieved due to loss of product by sticking to the sides of the spray dryer.

A screening experimental design was undertaken to assess the effect of different levels of excipients. The particles produced by spray drying fell largely into two groups: those with particle sizes less than 25 µm or those greater than 1mm. The morphology of the particles showed those with larger particle sizes to be large aggregates with the largest aggregates showing the highest degree of release retardation. The lowest release at 1 minute was seen with low levels of colloidal silica seeming to suggest that the release retardation was largely due to reduced surface area suggesting that release cannot be adequately controlled by this method in forming unaggregated particles. Others in the literature who have taste masked with Eudragit® E have used microparticles produced to manufacture tablets such as ODTs without assessing whether the microparticles can control release

uncompressed (Xu et al., 2008a, Yan et al., 2010). The one paper which taste masked as individual particles used organic spray drying (Bora et al., 2008). Different excipients such as removing the plasticizer, altering the pH of the feed or using different anti-adherents may help improve an aqueous process in future experiments.

From both the grittiness trials in Chapter 3, it was seen that smallest particles (those around ~100 μm) are less gritty and more accepted than the larger particles for all viscosities. However in Chapter 4 only the larger particles (>1 mm) obtained best release retardation but particles produced by spray drying had a low density and the larger particles were aggregates which were deformable on pressing between fingers (therefore softer than Cellets®). Hence it is not known what the mouthfeel of these aggregates breaking down or their grittiness would be. From the initial grittiness trial in Chapter 3, it was found that low concentration significantly reduces grittiness but this would be unlikely in this process due to low drug loading thanks to high levels of excipients being required for retardation. High levels of excipients are questionable especially those such as Eudragit E as it is non-biodegradable and with limited paediatric toxicity data.

The refined grittiness trial in Chapter 3 did not see a significant effect of concentration – this is likely to be due the similar grittiness scores for the particles around 100 μm at concentrations of both 125 and 250 mg/5 ml. With this in mind, the suspendability of the particles produced in Chapter 4 was assessed at the maximum particle concentration for grittiness of 500 mg/5 ml in a 10 ml dose (to correspond to the mass of particles required for a quinine dose for a 6 year old). The most acceptable sample in both grittiness trials in Chapter 3 contained HPMC 1 % w/v along with the lowest particle sizes and concentrations in each trial. Hence this media was used to test suspendability as in Chapter 2. The unflavoured/sweetened HPMC 1 % was used due to the incompatible pH of the orange flavoring agents used in the grittiness trial with the Eudragit®. At a concentration of 500 mg/5 ml of spray dried particles, a suspension was not formed only a semisolid mush for all formulations due to high proportion of solids due to the low density of all

spray dried material. At 250 mg/5 ml, dispersibility was difficult and similar to that of the Cellets® in Chapter 2 in that smaller particles were more packed and stuck to the bottom of the preparation vessel but those that worked free were suspended vs. larger particles which dispersed less well throughout. With 1 g of particles in 40 ml of vehicle, followed by manual stirring, some form of suspensions were produced but these all had levels of creaming, aggregation and sedimentation suggesting more work is required on the addition of excipients to the suspending media to improve uniformity.

Only some limited degree of release retardation was achieved but this was not complete, so these particles would not be stable in a suspension for 30 minutes. This may require the use of an individual powder for reconstitution for the short period of administration time. This would have the advantage of requiring less excipients such as preservatives than a suspension formulation which are required to ensure stability for a longer period. Suspensions would have to be optimized especially for pH, dispersibility and taste. However, given the large size but low density and softness of the aggregates they may be more effectively administered as a sprinkle onto a more viscous food media like those assessed in Chapter 2. However, the commonly recommended apple sauce and yoghurt are incompatible due to their pH so a different food would have to be assessed such as rice. This approach does however suffer due to the large masses of particles required, the possibility of chewing and the criticality of drug administration which may be compromised if the food is not completely eaten or rejected as unacceptable.

5.2. Future Work

Functionalised Platform Formation

Since the overall aim of this research was not met, different modifications should be looked at as to how a uniform platform could be achieved which would be in terms of improving the process (by excipients or other solvents), using different polymers or other processes.

A formulation which could be produced by spray drying would still be the aim. In terms of spray drying, it may be that using Eudragit® E as a blend with a different polymer such as an alginate or a cellulosic derivative or removing the "aqueous" criteria and using a lower toxicity solvent such as ethanol may also be an option and remove the issue of the low Tg of Eudragit® E. Other modifications to a spray drying process which may improve the process include modifying the feed by using talc or a hydrophobic colloidal silica as an anti-adherent/hydrophobic component, or the pH to ensure the Eudragit® is in solution or seeing whether fitting a filter to the process helps. A different polymer such as using a blend of HPMC with Eudragit® E, Kollicoat Smartseal® 30D or investigating encapsulation in the food industry may offer enhanced performance. Different spray dryer components may be used such as a rotating disc atomizer to remove the nozzle which blocks and causes problems with increasing solids content or a different spray dryer such as that of the Buchi's nano spray dryer B90.

Although it does not provide a universal platform technology, it may be that quinine hydrochloride dihydrate as a soluble salt with a dose of 20 mg/kg of quinine base and a low bitterness threshold is not suited to the current approach but other less soluble, lower dosed, less bitter drugs would be.

Any future spray drying feeds should be characterized so that time and money is not wasted on formulations that do not work. Formulations should be assessed at least in terms of pH, viscosity and particle size, with other

factors such as zeta potential, surface tension, drug or solids uniformity as required.

If spray drying is not possible, small microparticles with controllable release have been produced by a variety of methods notably by emulsification/solvent evaporation or co-acervation although these suffer from poorer scale up potential and often organic components. Other non-universal approaches may include complexing drugs if charged to IER then coat if required.

<u>Suspensions</u>

Poor dispersibility of the Cellets® and spray dried particles of all sizes produced suggests that more work is required on additions to HPMC or MC to improve dispersibility. This may include the use of surfactant or different methods of dispersion such as using mechanical mixers although it is more likely, given the short period of taste mask control that a powder for reconstitution would need to be used. Having a more uniform dispersion would make assessing suspendability easier although assessing the uniformity of large particles is still difficult and the methods used were subject to interobserver variability. It is likely that the best method of assessing uniformity is by dose uniformity in terms of counted drug particles (this may also work for placebo particles if a large enough syringe was available and particles counted). The use of a Texture Analyser may be an objective way to assess the texture/suspendability of suspensions and if so, may also be relatable to grittiness to reduce the need for resource intensive sensory trials.

An attempt was made to assess the effect of shaking on suspending media to see if this could be linked to a shear rate for different durations of shaking of suspensions. It may be that this could be assessed through the population effect of having more participants shake suspending media and looking for an overall effect.

In terms of assessing for thixotropy, it is likely that determining the magnitude of hysteresis loop is likely to be more reproducible than the thixotropic step test used here. Or if the step test is used in the future, it should have a longer initial settling period at a lower shear rate with the time taken for different percentages of rebuild evaluated. Along with this, the time for structure rebuild could be related to particle settling by applying a stress to a sample e.g. by shaking then allowing different time periods before adding particles and timing how long they take to settle. To improve reproducibility a model 'particle' such as a ball bearing of known size and weight could be used. This would be analogous to the falling sphere viscometer. Other suspension characteristics which could be assessed include the surface tension, specific gravity, zeta potential and wettability of particles.

Grittiness:

All of the excipients used in the grittiness trial (HPMC, MCC, sucralose and Orange Flavour (Givarome) Permaseal®) are used in the food and Pharma industry hence it should be possible to perform a grittiness trial in children. The logistics of the trial may be challenging since the grittiness trials performed required lots of preparation and were fairly long and laborious. With this in mind, fewer samples should be given to ensure that the child does not become bored of the trial. As there are no formalized medicine acceptability tests for children, depending on the age of the child, the assessment of grittiness/acceptability may be based on a caregiver's perspective (e.g. ease of administration) and/or a hedonic scale ("smiley faces"), rank order or visual analogue scale as used in this research. A grittiness trial undertaken in children could also look at the acceptability in terms of the prior medication experience of the children (e.g. comparing those acutely unwell to those chronically unwell and those who are healthy school pupils).

Given the large size of particles produced, it would be interesting to look at the grittiness of softer particles like these deformable aggregates. This is likely to have to be as a sprinkle in a semisolid food so this would link with

compatibility in food to find a pH appropriate (e.g. not acidic) soft food type which may be something like rice or rice/milk pudding.

Gastrointestinal Considerations

In the introduction, it was highlighted how little we know about the paediatric gastro-intestinal tract in terms of drug delivery. By virtue of the difficulties of research in children, little is known about the characteristics of gastro-intestinal fluids such as osmolality, viscosity, surface tension and ionic composition and how these differ compared to adults. It would therefore be interesting to be able to obtain fluid samples to characterise them, although this is expected to be difficult as it is invasive so is only likely to be achievable in sick children. Even this knowledge may be used to assess whether an age appropriate biorevelant dissolution media is needed for the assessment of formulations for children.

A non-invasive and simple start to this research could be the assessment of the saliva of different aged children to see if/how their saliva differs compared to adults. Increased research is also required into how (in terms of media volume and composition, equipment and forces applied) taste masked particles should be assessed as there are a vast range of methods used in the literature.

Whilst the term 'functionalised' in this research was used to mean taste masking, other forms of functionalized particle such as modified release may be impacted by the gastro-intestinal transit of multiparticulates and pH profile throughout the length of the gastrointestinal tract of various ages of children. Again, very little is known about even healthy children, let alone the effect of various disease states. It may be that this data could be obtained through the use of models and simulations.

TNO's TIM is a multi-compartment, computer controlled, gastro-intestinal model system designed to simulate digestion in the upper and lower gastro-intestinal tract through controlling parameters such as flow, composition and

temperature. The model is advertised as being able to be used in paediatrics. As such, the TNO TIM was identified as a possible method of generating data about the transit of multiparticulates in paediatrics e.g. either for evaluating the effect of different particle sizes, concentration and viscosities of multiparticulates in suspensions on transit, or to compare to real pharmacokinetic data by trying to see what combination of settings would give comparable data to that of known in vivo performance. However, it was determined that no paediatric settings were recommended by the company, limited components could be modified to make the system "paediatric" and in work which had previously been tried with multiparticulates, the particles became stuck in connections and points which do not exist physiologically (Naylor, 2011). However it may be possible to modify this and such work would be valuable for future research.

In terms of obtaining gastro-intestinal transit data in children to supplement formulation decisions in the future, it may be possible to use radiation free technologies such as magnetic marker imaging to visualise dosage forms and interest was even found from paediatrician's who are academics and clinicians into using radiolabelled Cellets® as a link between the liquid and food they currently monitor at different ages, with a low radiation burden. However the process of radiolabellling and proving stability, along with ethical approval would have been outside the time course of this research.

As a final light hearted thought of how far still has to be travelled in the formulation development process for children, when study participants (n=55, aged 6-19 years at 2 months to 2 years post study) were asked on their views on drug development their answers included (Abdel-Rahman, 2011):

"There would be no more pills to swallow"

"All medicine would taste good"

"The study diet should consist of pizza, french fries and chocolate pudding"

Although such an idyllic world is never likely to be realized significant improvements in providing age appropriate formulations for children are possible and should be pursued.

LIST OF PUBLICATIONS AND PRESENTATIONS

<u>Publication</u>

<u>Bowles A</u>, Keane J, Ernest T, Clapham D and Tuleu C. (2010) Specific Aspects of Gastro-intestinal Transit in Children for Drug Delivery Design. International Journal of Pharmaceutics, 395 (1-2), 37-43; 2.

<u>Oral Presentations</u>

<u>Bowles A</u> (2011). Application of Multiparticulates for Children's Medicines, 8th Scientific & Technical Forum: Innovative Drug Delivery Systems, May 12 & 13 2011, Basel (Switzerland)

<u>Bowles A</u> (2010). Application of Multiparticulates for Children's Medicines, 2nd Workshop on Multiparticulate Dosage Forms and Controlled Release Formulations, October 19 & 20, 2010, Binzen (Germany)

<u>Poster Presentations</u>

<u>Bowles A</u>, Al-Haddad I, Manghani S, Ernest T, Clapham D and Tuleu C (2011). Overview: Development of a Multiparticulate-based Formulation Platform for Delivering Functionalised Capability as an Oral Liquid Dosage Form, EuPFI 3rd Annual Conference 'Formulating Better Medicines for Children', 21& 22 September 2011, Strasbourg (France)

<u>Bowles A</u>, Ernest T, Clapham D, Tuleu C. (2010) Evaluation of the Rheological and Suspending Properties of Commonly Used Oral Suspending Vehicles. FIP Pharmaceutical Sciences World Congress/Annual meeting AAPS, 14 – 18 November 2010, New Orleans, LA (USA)

<u>Bowles A</u>, Al-Hadad I, Ernest T, Clapham D, Tuleu C. (2010) Influence of Viscosity, Particle Size and Particle Concentration of Suspensions on Oral Grittiness and Acceptability in Young Adults. FIP Pharmaceutical Sciences World Congress/Annual meeting AAPS, 14 – 18 November 2010, New Orleans, LA (USA)

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APPENDICES

Grittiness Trial Recruiting Poster

Young Adult Volunteers Wanted!

We are carrying out research to evaluate the grittiness of a variety of placebo suspensions and are looking for young adult volunteers to help.

If you are between 18 and 28 years old, it could be you that we are looking for.

The study will involve tasting a range of suspensions and evaluating their grittiness over two sessions of 1 hour, commencing as soon as possible

Department of Pharmaceutics, The School of Pharmacy, University of London
to receive a participant information sheet

This study has been approved by The School of Pharmacy ethics committee

Information Sheet for Participants in Research Studies

You will be given a copy of this information sheet.

Title of Project: **Influence of Viscosity, Particle Size and Particle Concentration of Placebo Suspensions on Oral Grittiness**

This study has been approved by The School of Pharmacy Research
Ethics Committee [REC/A/09/01]

Name, Address and Contact   **Shivani Manghani, Alexandra Bowles & Dr Catherine Tuleu,**
Details of Investigators: **Department of Pharmaceutics,**
The School of Pharmacy, University of London
29/39 Brunswick square,
London WC1N 1AX

We would like to invite you to participate in this research project. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or you would like more information.

Details of Study. The main research objective is to investigate the influence of viscosity, particle size and particle concentration on the grittiness sensation of suspensions in order to try to eventually help to make more acceptable liquid medicines for children.

Your role in the study will be to taste but not to swallow various formulations and to rank them according to a given scale, with regards to their grittiness as well as pick the two samples that you find the most acceptable each day. The suspending media and particles you will be in contact with are well known (hydroxypropyl methycellulose and microcrystalline cellulose respectively). The study will take place over 2 sessions on 2 different days and will involve tasting a total number of 34 samples. During the 1st day, you will be asked to taste 17 formulations of varying grittiness, which will last a maximum of an hour. On day two, you will taste another 17 formulations over a maximum of an hour.

If the suspensions you taste are very gritty, there is a potential to suffer from temporary oral discomfort. Some, sensitive, participants, may gag in response to the suspensions and vomit. Nevertheless, the time of rinsing has been minimised to 15 seconds which minimizes the potential for adverse effects, risks or hazards and a delay of 1 minute will be respected between each tested solution. Subjects have to rinse their mouth with water before and after each test.

We will make sure that you know the outcomes of the study. If the study is published or presented to a wider audience, your anonymity will be respected through anonymisation procedures. All data will be collected and stored in accordance with the Data Protection Act 1998.
No payment for time or such as travel expenses, child-care expenses, demonstrable loss of earnings etc will be reimbursed.

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason and without any penalty at any stage.

Informed Consent Form for Participants in Research Studies
(This form is to be completed independently by the participant after reading the Information Sheet and/or having listened to an explanation about the research.)

Title of Project:	**Influence of Viscosity, Particle size and Particle Concentration of Placebo Suspensions on Oral Grittiness**

This study has been approved by The School of Pharmacy Research Ethics Committee [REC/A/09/01]

Participant's Statement

I .... agree that I have:

- Read the information sheet and/or the project has been explained to me orally;

- Had the opportunity to ask questions and discuss the study;

- Received satisfactory answers to all my questions about the research and my rights as a participant and whom to contact in the event of a research-related injury

- Understood that I must not take part if I have any sensory disorders affecting my mouth or have had local anaesthetics (pain-killing injections) into my mouth within 24 hours of the study

- Understood that the data produced will be published but that confidentiality and anonymity will be maintained and it will not be possible to identify me from any publications.

I understand that I am free to withdraw from the study without penalty if I so wish and I consent to the processing of my personal information for the purposes of this study only and that it will not be used for any other purpose. I understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 1998.

Signed: Date:

Investigator's Statement

I ………………………………………………………………….…
confirm that I have carefully explained the purpose of the study to the participant and outlined any reasonably foreseeable risks or benefits (where applicable).

Signed: Date:

Randomised Order: Refined Trial
Sample Composition

Sample Code	Internal Code	Sample Composition			
		[HPMC] (%)	Particle Size (µm)	[Particle] (mg/5ml)	
6394	1	0.5	90	125	
3353	2	0.5	90	250	
6530	3	0.5	90	500	
9306	4	1	90	125	
8062	5	1	90	250	
3161	6	1	90	500	
1444	7	2	90	125	
6690	8	2	90	250	
7918	9	2	90	500	
8580	10	0.5	127	125	
3446	11	0.5	127	250	
7040	12	0.5	127	500	
6790	13	1	127	125	
3416	14	1	127	250	
4146	15	1	127	500	
8416	16	2	127	125	
3435	17	2	127	250	
4857	18	2	127	500	
9722	19	0.5	263	125	
5219	20	0.5	263	250	
9621	21	0.5	263	500	
9207	22	1	263	125	
2230	23	1	263	250	
2430	24	1	263	500	
1437	25	2	263	125	
1547	26	2	263	250	
2337	27	2	263	500	
2576	28	0.5	500	500	Blinded +ve control
6906	29	0.5	0	0	Blinded -ve control
8754	30a	0.5	500	500	Blinded +ve control
1463	30b	0.5	0	0	Blinded -ve control

First 15 Volunteers Randomisation

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	12	27	13	17	14	2	6	26	24	16	1	26	9	20	8
	28	21	26	9	28	27	9	3	30a	13	3	16	8	8	7
	8	4	11	4	24	1	30a	30a	8	22	27	20	22	9	4
	6	8	7	15	23	8	24	16	2	26	7	10	25	2	28
	22	10	20	18	3	22	11	8	27	3	15	5	24	15	5
	1	25	22	14	17	3	8	20	18	28	2	27	10	30b	24
	17	14	30b	21	8	25	4	15	12	20	25	30a	2	14	26
	11	28	24	26	6	9	17	18	15	10	6	24	12	18	11
	19	12	3	3	19	15	23	7	11	27	14	22	28	3	16
	21	26	28	19	7	26	14	19	6	18	9	8	1	23	6
	23	3	23	22	13	12	15	1	3	6	22	15	20	27	10
	27	13	18	13	1	28	1	10	10	1	30b	25	27	28	20
	13	23	17	5	29	14	18	29	14	8	20	29	21	7	12
	26	29	12	16	26	30b	29	21	22	17	11	7	29	13	9
	15	6	15	30b	21	21	2	13	13	23	4	11	14	4	1
2	9	16	19	25	2	19	7	24	4	5	13	21	6	19	14
	3	17	2	24	22	23	27	9	21	15	18	2	18	26	15
	18	24	16	1	18	6	22	28	23	12	21	23	11	29	2
	4	9	10	23	25	29	26	22	5	11	24	13	3	24	19
	7	19	27	2	12	4	13	14	7	24	28	4	30b	11	30a
	2	5	25	8	16	5	3	17	20	25	5	12	19	22	18
	30a	30a	8	11	5	18	5	11	25	29	16	28	17	17	23
	5	2	29	12	11	17	20	2	28	19	19	9	23	5	21
	24	1	9	7	9	11	21	12	29	14	17	17	13	25	27
	16	15	14	20	30a	20	19	23	17	21	23	18	26	1	25
	10	20	6	29	20	10	25	5	9	4	8	3	7	10	3
	25	7	5	6	4	13	28	25	1	30a	29	1	16	12	22
	29	22	1	28	27	16	10	27	26	2	26	14	4	21	13
	14	18	4	10	15	7	16	6	19	7	12	19	5	16	29
	20	11	21	27	10	24	12	4	16	9	10	6	15	6	17

Second 15 Volunteers Randomisation

Day	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	22	11	14	7	12	2	7	17	23	14	22	16	6	21	13
	10	10	24	21	1	23	3	12	26	12	23	9	4	3	30b
	24	14	18	15	10	5	5	22	27	18	27	7	26	24	18
	2	27	29	27	8	14	27	15	10	22	15	1	22	8	28
	3	24	15	18	20	7	16	23	17	13	28	25	27	14	16
	27	9	5	1	14	9	18	9	25	28	24	10	17	6	21
	12	8	27	12	15	15	21	30b	21	23	14	15	19	25	6
	25	12	10	23	23	10	2	8	6	24	26	26	18	23	15
	19	19	6	16	16	18	12	6	29	4	12	28	29	17	9
	8	17	23	30b	4	6	15	10	12	10	13	29	2	20	19
	13	2	12	6	19	12	25	19	9	27	19	13	12	9	17
	7	25	25	28	29	27	4	7	15	3	10	17	16	12	20
	16	30b	20	14	11	3	10	28	14	15	25	21	3	7	22
	28	5	1	10	25	8	17	3	3	21	29	11	20	5	27
	14	22	17	24	2	28	28	18	30a	7	2	14	21	29	26
2	26	21	22	17	18	20	20	20	18	8	20	23	1	16	4
	15	23	3	20	30b	30a	13	29	19	25	8	18	25	2	1
	30a	29	19	19	21	25	9	26	24	20	30b	24	15	26	29
	5	13	2	2	26	29	19	11	22	9	18	30a	9	19	25
	18	16	26	26	13	11	8	14	4	6	16	6	23	4	11
	20	7	28	25	24	13	30a	27	28	1	11	27	30b	27	12
	1	1	9	4	5	17	1	4	8	19	7	12	28	28	10
	11	15	7	22	6	21	29	5	20	16	5	5	5	11	5
	6	6	8	5	28	19	6	16	11	5	17	8	24	13	7
	21	18	4	8	17	1	14	13	2	17	6	3	10	1	14
	4	28	13	29	27	16	23	25	5	2	9	19	8	22	2
	17	20	21	3	7	26	11	21	13	30a	3	20	11	30b	8
	29	3	11	13	3	4	22	24	1	26	21	4	7	18	24
	9	4	30b	9	9	24	24	1	7	29	1	2	13	10	3
	23	26	16	11	22	22	26	2	16	11	4	22	14	15	23

SPSS Output of the Initial Trial (Significant differences in Bold)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Concentration	Greenhouse-Geisser	227246.103	1.986	114415.642	124.66	**0.000**
	Huynh-Feldt	227246.103	2	113623.051	124.66	**0.000**
Error (Concentration)	Greenhouse-Geisser	34635.675	37.737	917.823		
	Huynh-Feldt	34635.675	38	911.465		
Viscosity	Greenhouse-Geisser	2750.108	1.474	1865.704	1.055	0.342
	Huynh-Feldt	2750.108	1.568	1753.9	1.055	0.345
Error (Viscosity)	Greenhouse-Geisser	49538.392	28.007	1768.81		
	Huynh-Feldt	49538.392	29.792	1662.812		
Size	Greenhouse-Geisser	28134.633	1.881	14960.26	27.878	**0.000**
	Huynh-Feldt	28134.633	2	14067.317	27.878	**0.000**
Error(Size)	Greenhouse-Geisser	19174.756	35.732	536.629		
	Huynh-Feldt	19174.756	38	504.599		
Size * Viscosity	Greenhouse-Geisser	15656.031	2.807	5577.159	10.551	**0.000**
	Huynh-Feldt	15656.031	3.344	4682.297	10.551	**0.000**
Error (Size*Viscosity)	Greenhouse-Geisser	28192.247	53.336	528.576		
	Huynh-Feldt	28192.247	63.53	443.765		
Concentration * viscosity	Greenhouse-Geisser	7419.306	3.474	2135.922	4.274	**0.006**
	Huynh-Feldt	7419.306	4	1854.826	4.274	**0.004**
Error (Concentration* Viscosity)	Greenhouse-Geisser	32986.083	65.998	499.804		
	Huynh-Feldt	32986.083	76	434.027		
Size * Concentration	Greenhouse-Geisser	4745.267	3.375	1405.802	3.959	**0.009**
	Huynh-Feldt	4745.267	4	1186.317	3.959	**0.006**
Error (Size* Concentration)	Greenhouse-Geisser	22774.9	64.134	355.113		
	Huynh-Feldt	22774.9	76	299.67		
Size* Concentration * Viscosity	Greenhouse-Geisser	3238.436	4.93	656.831	1.138	0.346
	Huynh-Feldt	3238.436	6.866	471.633	1.138	0.344
Error (Size* Concentration *Viscosity)	Greenhouse-Geisser	54090.953	93.678	577.417		
	Huynh-Feldt	54090.953	130.462	414.61		

SPSS Output of the Refined Trial (Significant differences in Bold)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Size	Greenhouse-Geisser	39418.749	1.629	24199.421	39.736	**.000**
	Huynh-Feldt	39418.749	1.712	23020.998	39.736	**.000**
Error(Size)	Greenhouse-Geisser	28768.802	47.238	609.012		
	Huynh-Feldt	28768.802	49.657	579.355		
Concentration	Greenhouse-Geisser	6769.766	1.351	5010.446	3.200	.069
	Huynh-Feldt	6769.766	1.394	4857.445	3.200	.068
Error (Concentration)	Greenhouse-Geisser	61354.059	39.183	1565.842		
	Huynh-Feldt	61354.059	40.417	1518.027		
Viscosity	Greenhouse-Geisser	58479.679	1.584	36916.236	68.743	**.000**
	Huynh-Feldt	58479.679	1.660	35218.523	68.743	**.000**
Error (Viscosity)	Greenhouse-Geisser	24670.243	45.939	537.017		
	Huynh-Feldt	24670.243	48.154	512.320		
Size * Concentration	Greenhouse-Geisser	1252.322	3.343	374.592	1.092	.360
	Huynh-Feldt	1252.322	3.831	326.879	1.092	.363
Error (Size* Concentration)	Greenhouse-Geisser	33247.961	96.952	342.933		
	Huynh-Feldt	33247.961	111.103	299.253		
Size * Viscosity	Greenhouse-Geisser	4813.773	2.975	1617.824	4.726	**.004**
	Huynh-Feldt	4813.773	3.353	1435.603	4.726	**.003**
Error (Size*Viscosity)	Greenhouse-Geisser	29536.705	86.288	342.302		
	Huynh-Feldt	29536.705	97.241	303.748		
Concentration * viscosity	Greenhouse-Geisser	1825.763	3.454	528.608	1.562	.197
	Huynh-Feldt	1825.763	3.978	458.988	1.562	.189
Error (Concentration* Viscosity)	Greenhouse-Geisser	33894.088	100.163	338.388		
	Huynh-Feldt	33894.088	115.356	293.821		
Size* Concentration * Viscosity	Greenhouse-Geisser	2204.177	5.586	394.567	1.326	.251
	Huynh-Feldt	2204.177	7.072	311.662	1.326	.239
Error (Size* Concentration *Viscosity)	Greenhouse-Geisser	48199.766	162.003	297.523		
	Huynh-Feldt	48199.766	205.098	235.009		