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DEVELOPMENT OF INNOVATIVE FORMULATIONS FOR PAEDIATRIC USE

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SUMMARY

1. INTRODUCTION

There are several reasons for the lack of paediatric medicines worldwide. Economic factors certainly render paediatric research and development less attractive for pharmaceutical companies in term of achieving an adequate return on investment. Age-appropriate research makes the process more expensive and complex for organisations that are active in this sector (European Medicines Agency, 2013). In addition, undertaking clinical research in children presents unique challenges and the most obvious of these are the ethical ones. These enormous practical and ethical difficulties may have contributed to a dearth of high quality paediatric research (Smyth, 2001).

1.1. REGULATORY ASPECTS

Many drugs used in children are either not licensed (unlicensed) or are prescribed outside the terms of the product licence (off-label) (McIntyre et al., 2000), with the associated non-negligible risk of inefficiency and adverse reactions. Such a situation was contrary to the general goal to provide high-quality medicinal products to the entire paediatric population.

To address this problem, the establishment of a system of both obligations and rewards and incentives has proven necessary. The Paediatric Regulation came into force in the European Union on 26th January 2007 with the aim to improve the health by facilitating the development and the availability of medicines for children aged 0 to 18 years. The Regulation also aims at ensuring that medicinal products used to treat the paediatric population are subjected to ethical research of high quality and are appropriately authorised for use in children and at improving the information available for the use of medicinal products in the various paediatric populations (European Union (EU), 2006. REGULATION (EC) No 1901/2006). These objectives should be achieved without subjecting the paediatric population to unnecessary clinical trials.

The Regulation introduced sweeping changes, for instance the creation and operation of Paediatric Committee (PDCO), with expertise and competence in development and assessment of all aspects of medicinal products to treat the paediatric population. PDCO should be primarily responsible for the scientific assessment and agreement of the Paediatric Investigation Plans (PIPs) and for the system of deferral and waivers thereof.

Pharmaceutical industries have to submit early during the product development a PIP, a plan including details of the timing and measures proposed to demonstrate the quality, safety and efficacy of medicinal products in all subsets of paediatric population. It aims at ensuring that the necessary data are obtained through safe studies in children to support the authorisation to a paediatric medicine.

The paediatric investigation plan:

- includes a description of the measures to be carried out in children;
- describes the measures to adapt the medicine's formulation to make its use more acceptable to children;
- covers the needs of all age groups of children;
- defines the timing of measures in children compared to adults (http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000608.jsp&mid=WC0b01ac0580925b1b).

PDCO, in addition to assessing the PIPs, has to assess waivers and deferrals, compliance of the application for a marketing authorisation, and to formulate an opinion on the quality, safety or efficacy of the paediatric product. The Paediatric Committee has to assist scientifically in the elaboration of any documents related to the fulfilment of the objectives of this Regulation and to establish a specific inventory of paediatric medicinal products and update it.

Where the intention is to apply for a marketing authorisation, a PIP shall be drawn up and submitted to the Agency with a request for agreement. Within 30 days following the request, the Agency may verify the validity of the request and prepare a summary report for the PDCO. It shall adopt within 60 days an opinion as to whether or not the proposed studies will ensure the generation of the necessary data determining the conditions in which the medicinal product may be used to treat the paediatric population or subsets thereof, and as to whether or not the therapeutic benefits justify the studies proposed (European Union (EU), 2006. REGULATION (EC) No 1901/2006). Within the 60-days period, the PDCO can request the applicant to propose modifications to the plan.

Once the product concerned is authorised or the product information is amended, companies that have complied with the obligation of the Paediatric Regulation may benefit from a reward. The reward takes the form of a six-month extension of the supplementary protection certificate provided for the Regulation. In case of an orphan

medicinal product, the reward is an extension of the ten-year period of orphan market exclusivity to twelve years.

The '5-year Report to the European Commission', a general report on the experience acquired as a result of the application of the Paediatric Regulation, specified that the assessment of PIPs was evaluated for 682 medicines up to the end of 2011. Among the opinions adopted, 476 (70 %) were on the agreement of a PIP and 206 (30 %) of a full waiver.

Between 2008 and 2012, 10 new medicinal products (new active substances) were centrally authorised and received a paediatric indication (out of 113 new active substances in total), under the requirements of the Paediatric Regulation. For one of the 10 products, a Paediatric Use Marketing Authorisation (PUMA) had been requested and was granted.

For medicines already authorised centrally or nationally, 18 and 12 respectively received a new paediatric indication developed under the Paediatric Regulation between 2008 and 2012. This means that the implementation has already had a positive impact in keeping with the main objectives of the Paediatric Regulation, and that paediatric development is increasing (EMA/428172/2012). A systematic paediatric development is leading to age-appropriate medicines and increasing paediatric information. Achieving the objectives of the Paediatric Regulation is a realistic goal based on the experience gathered so far, but sufficient time is needed as medicines development spans decades. Meanwhile, opportunities for improvement of the process have been identified and are being addressed to increase the positive impact of the Paediatric Regulation and make medicines available with appropriate information to children.

1.2. AGE-CLASSIFICATION

Paediatric patients represent a changing and dynamic population due to the anatomic and physiological changes that occur during development (Abdel-Rahman et al., 2012).

As a starting point, the paediatric population can be divided in six age groups, in relation to developmental stages:

- preterm newborn infants;
- term newborn infants (0-27 days);
- infants and toddlers (1 month to 23 month);
- children (2 years to 11 years);

- adolescents (12 years to 18 years).

The first three groups are the most challenging, as regard the pharmacokinetic aspect, due to rapid developmental changes in adsorption, distribution, metabolism and extraction. It is important to consider that there may be large pharmacokinetic and pharmacodynamic differences among preterm and term infants and within infants over time, depending on their gestational or postconceptional age. However, it is advisable to consider factors such as gestational age, postnatal age, birth and body weight, renal function and the amount of serum albumin in pharmacokinetic studies within the paediatric population. Determining the protein binding of highly bound drugs and their active metabolites should be considered when studying newborns.

Children involves a large group of population, from 2 to 11 years, and the 2-4 years group is the least predictable as regard the pharmacokinetic studies. With increasing knowledge about liver maturation at the enzyme level, further extrapolations might be possible based on the relationship between liver and body weight, especially in the older children.

The pharmacokinetics in adolescents is often similar to the pharmacokinetics in adults (EMA/CHMP/QWP/805880/2012).

1.3. PHARMACOKINETIC DIFFERENCES BETWEEN CHILDREN AND ADULTS

Children cannot be considered ‘small adults’, since differences in pharmacokinetics and pharmacodynamics and in adverse reactions occur compared to adults. Gastrointestinal (GI) fluid composition, pH and volume differences at each age group were identified as critical for the development of paediatric formulation because these influence age-based biorilevant solubilities and dissolution rates from the dosage form (Abdel-Rahman et al., 2012).

As would be expected, saliva secretion increases with age, from 0.03 ml/min in neonates to 1.2 ml/min in adulthood, with a pH of about 7-7.4.

The pH of the various parts of the GI tract is important in oral drug delivery in terms of drug release, solubility and absorption (Kaye, 2011). It is established that neonatal and birth gastric pH are very close to neutral (6-8) with significant acid secretion during the first 48 hours, bringing the pH down into the more acid range (1-3). The gastric acid secretions stabilize for the next 10 days, after that pH, increases back to neutral, then it restarts to decrease toward the normal adult pH ranges at about 3 months of age.

However, it is believed that the pH levels do not fully reach adult levels until 2 years of ages. This difference is manifested in faster absorption of acid labile drugs in neonates and infants as compared to adult population. The absorption of weak acid drug is decreased whereas absorption of basic drugs is increased (Ali et al., 2014). Furthermore, food acts as a buffer, and thereby increases the gastric pH, which in turn would decrease the dissolution of weakly basic drugs and increase that of weakly acidic drugs (Kaye, 2011).

In addition, stomach capacity changes considerably with age, starting at 10-100 ml in neonates and reaching 3 litres in adults.

As already stated, the major site of drug absorption is the small intestine, and thus, gastric emptying into the small intestine can be the rate-controlling factor for drug absorption of oral dosage forms. There was evidence that the gastric emptying rate appears to be slower in preterm neonates and reaches adult values after six months. Because of this, drug absorption is slower in neonates and the time required to achieve maximum plasma levels is increased. For this reason, the European Medicines Agency (EMA) suggests giving drugs to children intravenously or rectally. The situation is made more complex when considering the fact that food can have various effects on gastric emptying. For instance, fat reduces gastric emptying, which can delay the onset of action of certain drugs. On the other hand, solid and liquid foods create stomach distension and peristaltic waves, increasing the rate of emptying. Liquids empty more rapidly than solids, and this may be one important criterion for selecting suspension dosage formulation over tablets dosage forms for children (Kaye, 2011).

The intestine is more alkaline than the stomach due to the neutralising of the gastric acid by sodium bicarbonate secreted by the pancreas into the duodenum, which neutralises the highly acidic chyme from the stomach. The pH gradually rises along the length of the small intestine, from 6 to 7.5. The colon has a lower pH (6-6.5) compared to that of the small intestine, because of the colonic bacteria breaking down undigested carbohydrates into short-chain fatty acids. The rectal pH of neonates and infants, partly depends upon the type of milk they are being fed. The pH differences between age groups have the potential to affect drug delivery from oral dosage forms.

The small intestine ranges from 275 cm at birth and continues to grow and mature into the adolescence, when it reaches the adult size of 575 cm. The growth rate and length of the small intestine increase most rapidly from the gestation until the 1 year of age, after which it grows in direct proportion to the body length into the adulthood. The length of

the small intestine directly affects small intestinal transit time; thus, variability is inherent based on the growth rate and stage of development of the child. The regional liquid GI transit time for a child were reported to be 4-7.5 and 17-34 hours for the small and large intestine, respectively. However, the intestinal motility is irregular in neonates and infants, leading to very variable transit time, which also contributes to the preference of parenteral or rectal routes. Children generally have faster transit times, respect to the other age groups, which may result in controlled release of the drug from the oral dosage forms (Kaye, 2011). GI motility is also a function of disease states, particularly in smaller children who are susceptible to GI conditions, such as diarrhoea. Furthermore, the surface area of the small intestine in infants is proportionately greater than in adults, and so may result in proportionally greater drug absorption.

Several age-dependent factors, such as body composition and plasma protein characteristics, can influence the distribution of the drug within the body. As a percentage of total body weight, the approximate total body water falls from 87 % and 75 % in the premature and full-term neonate, respectively, to 60 % at 1 year and to 55 % in adulthood. Extracellular water falls from 45 % of total body water in the full-term neonate to 20 % in the adult. The clinical implication of this shift in body composition have not been fully defined (McLeod et al., 1992).

Another factor which influences drug distribution in the young child is the amount of binding proteins. Albumin, globulins, alpha-1 acid glycoprotein, lipoproteins and other proteins bind drugs in plasma; in particular, albumin, the major drug binding protein, binds primarily acidic drugs, while basic drugs bind more avidly alpha-1 acid glycoprotein and lipoproteins. Many drugs have been found to be less bound to serum proteins in neonates leading to an increase of the unbound fraction and thus an increased pharmacologic or toxic response. Plasma proteins generally achieve adult value around 1 year of age. Many organs and tissues in the body, including blood, liver, lungs, kidney and gastrointestinal tract, are capable of metabolising drugs.

Although most hepatic enzymes are functional in human foetal and neonatal liver, the activity of some P-450 enzymes (phase I) is absent at birth. Because of this, newborn and neonates metabolize drugs at a rate several times lower than adults. For example, the oxidative metabolism of phenobarbital and phenytoin is severely impaired in infants. The glucuronidation pathway (phase II) is also relatively undeveloped in newborns. The insufficient metabolism of chloramphenicol by glucuronyl transferarses in the inactive glucuronide metabolite causes the famous Grey Baby Syndrome.

The kidney represents the primary route of elimination for many drugs. Renal elimination is dependent on glomerular filtration, tubular secretion processes and renal blood flow. There is evidence of morphological and functional tubular immaturity at birth; tubular function appears to be fully developed by 7 month of age and renal blood flow increases with age. The immature renal elimination system leads to accumulation of many drugs, such as aminoglycosides and penicillin, which necessitates less frequent dosing intervals. Therefore, as the kidney function matures, there may be a shift from potential drug over-dose to potential drug under-dose, for instance in the case of theophylline. Tables 1-3 summarize the differences of the physiological data within the different age groups of paediatric population.

Table 1. Summary GI tract pH data in patients of different age groups in the fasted state.

Organ	Neonates			Infants	Children	Adolescents	Adults
	Birth	24 hours	1-27 days	(1-23 months)	(2-11 years)	(12-18 years)	(>18 years)
Saliva	7	7	7	-	7.1	7.4	6-7.4
Stomach	6-8	1-3	6-8	1.4	1.5	1.5	1-2.5
Small intestine	-	-	-	-	6.4-7.4	6.4-7.4	6-7.5
Colon	-	-	-	-	5.9-6.5	5.9-6.5	7-7.5

Table 2. Summary of physiological data in patients of different age groups.

Parameter	Neonates			Infants	Children	Adolescents	Adults
	Birth	24 hours	1-27 days	(1-23 months)	(2-11 years)	(12-18 years)	(>18 years)
Saliva secretion (ml/min)	0.03-0.04	0.03-0.04	0.03-0.04	0.47	0.25-0.66	1.2	0.3-1.2
Stomach capacity (ml)	10-100	10-100	10-100	90-500	750-960	1.5	2-3
Small intestine lenght (cm)	275	275	275	380	450	-	575

Table 3. Summary of GI transit time data in patients of different age groups.

Organ	Neonates			Infants	Children	Adolescents	Adults
	Birth	24 hours	1-27 days	(1-23 months)	(2-11 years)	(12-18 years)	(>18 years)
Oesophagus	3-4 s	3-4 s	3-4 s	4-8 s	5-8 s	5-8 s	10-14 s
Stomach	54-82 min	54-82 min	54-82 min	12-70 min	12-70 min	12-138 min	5-120 min
Small intestine	4 h	4 h	4 h	3-7.5 h	3-7.5 h	3-4 h	6-7.5
Colon	28-96 h	28-96 h	28-96 h	32 h	17-34 h	17-34 h	2-48 h

1.4. PAEDIATRIC BIOPHARMACEUTICS CLASSIFICATION SYSTEM

The concept of Biopharmaceutical Classification, born in 1995, allows drugs to be classified because of their *in vitro* solubility and intestinal permeability. A molecule is considered highly permeable, according to the Biopharmaceutics Classification System (BCS), when more than 85-90 % of the administered drug is absorbed. Moreover, a molecule is considered highly soluble when the highest dose (dose unit) needed is soluble in 250 ml of aqueous liquid at a relevant pH range. The dose number is calculated as the maximum dose (M_0) divided by the saturated solubility (C_s), multiplied by the initial gastric volume (V_0).

$$D_0 = M_0/(C_s * V_0)$$

In adults a value of 250 mL is used for V_0 and for this reason, adult patients are recommended to assume the medicine with 250 ml of water (Batchelor, 2014).

However, the above mentioned differences in anatomic and physiologic properties of paediatric patients require adaptation of existing biopharmaceutical methods to ensure that *in vitro* predictions are relevant for this population. It is difficult to predict differences in permeability between adults and paediatric population, but it is generally recognized that the permeability in children above 2 years is equivalent to that of adults. As regard the *in vitro* solubility of a drug in paediatric population, the National Institute of Child Health and Human Development Paediatric Biopharmaceutics Classification System Working Group assumed an initial gastric volume of children, especially younger children, of 25 ml.

Since paediatric population, and in particular children, is represented by a wide range of age, from 2 to 11, it is clear that this wide range corresponds to several physiological and anatomical differences. It is apparent that there is a need to establish an age specific biopharmaceutics classification system for children to ensure that development work is relevant in producing age appropriate medicines for children. In addition, standardisation in conducting bioequivalence studies for paediatric products both in adult and in paediatric populations would assist in understanding how medicines perform across wide age bands.

1.5. ROUTES OF ADMINISTRATION AND DOSAGE FORMS

Paediatric patients require different drug delivery systems than other subsets of the population because of their continuing development hence dosing and administration requirements (Lopez et al., 2015). Due to this extensive variability in children, there is also an obvious need for drug formulations tailored to children in all the target age groups (Ivanovska et al., 2014).

The criteria for choosing a specific paediatric dosage form and a particular route of administration should be discussed and justified for children in each of the target age-groups.

1.5.1. ORAL ADMINISTRATION

Oral administration is an extremely useful route for the administration in children, and several types of dosage forms can achieve it: solutions, syrups, suspensions, emulsions, powders, granules, tablets, effervescent tablets, orodispersible tablets, etc. In general, the main choice is between liquids and solid dosage forms (EMA/CHMP/QWP/805880/2012).

Liquid formulations

Oral liquid dosage forms are normally considered acceptable for children from full term birth and pre-term neonates who are able to swallow. Liquids are the best option in paediatric treatment: they permit dose flexibility, the dose is easy to adapt to the body weight and the body surface area and there are no problems with swallowing (Standing et al., 2005).

Liquid oral formulations contain drug either in solution or in suspension in the vehicle and may be supplied as solutions, syrups, suspensions and emulsions (Ali et al., 2014). The dose volume is a major consideration for the paediatric acceptability. Typical target dose volumes for liquid formulations are ≤ 5 ml for children under 5 years and ≤ 10 ml for children of 5 years and older. Water is the preferred vehicle for drug substances with high solubility and agreeable taste. A solubilized formulation is one of the most common type of paediatric formulations as at least 24 commercially available solutions or syrups were identified on European market. Oral solutions of very small volumes (i.e. oral drops or concentrates) can be developed and diluted in suitable food or beverages, as fruit juice or milk, to improve the palatability. Moreover, the volume of dilution should be minimised to avoid the incomplete ingestion and under-dosage

(EMEA/CHMP/PEG/194810/2005). Oral solution can be very simple formulations containing only one solvent and one buffer, flavour and preservative, or can also be quite complex with multiple solvents, solubilizing excipients, buffers, sweeteners, flavours and preservatives (Stryckley et al., 2008). Aqueous-based oral solution formulations are challenging for solubility, chemical stability, taste-masking and preservation. In general, they require flavours, antimicrobial agents, sweeteners and colours. Commercial examples of aqueous-based oral solution formulations include Emtriva® (emtricitabine), Ziagen® (abacavir sulfate), Epivir® (lamivudine). Water-soluble and chemically stable drugs, in which the taste can not be masked in an aqueous-based solution, can be often successfully formulated in an aqueous syrup formulation. A syrup is a viscus solution containing high concentration of sugar along with sweeteners or flavours which can mask the poor taste of several molecules since only a fraction of the drug makes contact with the taste buds. The low concentration of water makes the syrup resistant to the microbial growth and thus no preservative is required if the syrup is used immediately after the preparation, but in a multi-use product the antimicrobial preservation is needed. Examples of commercially available aqueous-based oral syrups formulations include Retrovir® (zidovudine), Zyrtec® (cetirizine) and Clarinex® (desloratadine).

Organic solvents are used in oral solutions and elixir formulations when the aqueous solubility of the active ingredient is low, such that an impractically high dose volume would be required to administer the intended dose. It is important to minimise the amount of organic solvent to achieve the necessary solubility, chemical stability, taste-masking and preservation. Many paediatric oral solutions contain small amount of ethanol, propylene glycol or PEGs, and four commercially available products include very large amount of organic solvents, for example Norvin® (ritonavir), Agenerase® (amprenavir) and Sustiva® (efavirenz). Usually, alcohols are avoided in paediatric formulations due to their toxicity. It is recommended an alcohol limit of 0.5 % and 5 % for patients under 6 years and between 6 and 12 years of age, respectively.

Suspension formulations should be considered when solubility cannot be modulated. They are biphasic formulations containing chemically stable but not water-soluble actives. The challenges associated with suspension formulations include physical-chemical characteristics such as viscosity, pH-dependent chemical stability, solubility, dose uniformity, potential for foaming, air entrapment, sedimentation, sticking of the active substances to the primary container, rheology, particle settling, taste-masking and preservation.

Suspensions may be very useful for formulation of substances with poor taste characteristics: by minimizing the amount of the drug in solution form, suspensions improve palatability and allow increased drug load in reduced dose volume. Moreover, taste-masking is normally accomplished by the use of sweeteners and flavours. When sedimentation cannot be avoided, more information about how to shake the product to ensure correct dosing is necessary, to avoid the risk of over or under dosing because of inadequate shaking. Six commercially available paediatric oral suspensions were found on European market, for example Zovirax® (acyclovir), Viramune® (nevirapine) and Viravan Suspension® (phenylephrine tannate, pyrilamine tannate and dextromethorphantannate).

Oral emulsions are liquid preparations for oral use containing one or more active ingredients. They are stabilized oil-in-water dispersions, either of both phases of which may contain dissolved solid; solids can also be suspended in oral emulsions. They can show evidences of phase separation, but in general, they are readily re-dispersed on shaking. A change in colour may indicate chemical degradation or microbial contamination.

Despite all these advantages, liquid dosage forms have several limitations: they are bulky and difficult to transport, require careful handling and have special storage requirements. In liquid state drug is more susceptible to degradation and has a lesser shelf life than in solid dosage forms. In addition, liquid dosage forms are prone to microbial growth; in addition to being a risk to the health, microbial contamination may cause changes in pH, appearance, odour, smell and palatability of the preparation. Therefore, preservatives are usually added. Drugs undergoing hydrolysis in a liquid dosage form can be stabilized by formulating them with buffers at pH of maximum stability; drugs undergoing to oxidative degradation may be stabilized by antioxidants, or stored in containers containing nitrogen or carbon dioxide.

Another limitation of liquid products about patient acceptability is the lack of controlled release formulations resulting in the need to administer multiple doses throughout the day. Several approaches have been investigated for the development of sustained release liquids, such as ion exchange resins, coated microparticles in suspension or drug microemulsions, among others (Lopez et al., 2015).

An interesting growing field related to liquid dosage forms is the development of administration devices such as baby bottles, coupled to syringes for aiding the administration of liquid formulations. Others include modified pacifiers and the dose

'sipping syringe'. The main potential limitation for their applicability is the overall cost of the product.

Solid formulations

Solid dosage forms represent a better choice for paediatric administration rather than liquid formulations because of the greater stability, the good dosage uniformity, options for different doses, good portability and low manufacturing costs. One of the key advantages of solid dosage forms is the possibility to develop taste-masking techniques and modified-released formulations, which are technically more challenging for liquid formulations.

Conventional and ready to use solid dosage forms, involve tablets, capsules, chewable tablets and orally disintegrated tablets. The age at which children can swallow intact tablets or capsules is highly dependent on the individual and the training they received. In general, children aged < 6 years do not accept them easily and can learn to swallow solid dosage forms, especially for chronic diseases (EMEA/CHMP/PEG/194810/2005). However, the size of tablets or capsules has to be kept as small as possible even for children > 6 years of age.

A drug molecule can be formulated as a tablet if the dose is low enough to make a small tablet that can be easily swallowed. If it is necessary to have a partial dose, tablets can be scored, or even double scored, or crushed and mixed with drink or food. Commercially available tablets are: Dextrostat ® (dextroamphetamine sulfate), Malarone (atovaquone and proguanil HCl) and Caduet® (amlodipine besylate and atorvastin calcium). Commerial examples of capsules are: Strattera® (atomoxetine) and Vyvanse® (lisdexamfetamine dimesylate).

Chewable tablets are also used to administer drugs to children of 2 years or older under elderly supervision to ensure tablets are chewed and not ingested. They are growing in popularity and the potential as drug delivery system is expanding thanks to the fact that they are safe, well tolerated, palatable, stable, portable, and do not need water for administration. Some of the problems related to this dosage forms include taste-masking, grittiness, tooth picking and manufacturing issues. Taste-masking can be achieved by using coated drug particles, or flavours and sweeteners; microcrystalline cellulose, along with mannitol enhance smooth feel, eliminate grittiness and tooth picking (Ali et al., 2014). Commercially available chewable tablets are Singulair® (montelukast sodium), Videx® (didanosine) and Tegretol® (carbamazepine).

However, conventional dosage forms are often not suitable for paediatric patients with difficulties of swallowing and for the fact that they often allow poor dose flexibility. Thus, tablet splitting has become usual daily practice to obtain various dose strengths, with all the safety issues related. For infants aged < 2 years, a new promising development is the orally disintegrated mini-tablets, which combine mini-tablets and fast dissolving dosage forms.

Orally disintegrated tablets (ODTs), also known as fast dissolving, mouth dissolving, orodisperse, fast melt, rapidly dissolving or disintegrating tablets rapidly disintegrate in the mouth in small particles in few seconds in contact with saliva, thus overcoming swallowing problems. They improve efficacy, safety and palatability respect to other dosage forms. Some of the challenges associated with this dosage forms include taste-masking, mechanical strength, fast disintegration, hygroscopicity, manufacturing, tablet compression and packaging. ODTs available in the market are: Prevacid (lansoprazole), Zofran (ondansetron) and Clarinex Redi-Tabs (desloratadine).

Dispersible tablets are another kind of dosage form suitable for children, because they disintegrate within 3 minutes in water in a uniform dispersion, before administration. They are manufactured with commonly available technology and packaging, and the taste should be adequate for acceptability. Dispersible tablets are sensitive to moisture, hence should be packed in stronger packaging.

Effervescent tablets have the same problems of moisture sensitivity, they have to be manufactured at low humidity (< 30 % RH) and temperature (< 25°C). They are dissolved or dispersed before administration, and contain acid and carbonates, which react in the presence of water to release carbon dioxide. Effervescence creates a palatable sparkling solution, which may enhance the drug permeability due to carbon dioxide bubbling effect on the intestinal epithelium.

Tables 4 and 5 summarize the commercially available oral liquid and solid formulations, respectively.

Table 4. List of commercially available paediatric oral liquid formulations.

Marketed name/Drug name	Marketed formulation	Active in formulation	Dose	Excipients
Emtriva/emtricitabine	Oral solution	10 mg/ml	6 mg/Kg up to 240 mg	Cotton candy flavor, EDTA, methylparaben, propylparaben, propylene glycol (2%), sodium phosphate, water, xylitol, HCl/NaOH to pH 7.2
Ziagen/abacavir sulfate	Oral solution	20 mg/ml	8 mg/Kg up to 300 mg b.i.d. 3 months to 16 years	Artificial strawberry and banana flavor, cytric acid, methylparaben, propylparaben, propylene glycol, saccharin sodium, sodium citrate, sorbitol solution, water
Epivir/lamivudine	Oral solution	10 mg/ml	4 mg/Kg up to 150 mg b.i.d. 3 months to 16 years	Artificial strawberry and banana flavor, cytric acid, methylparaben, propylparaben, propylene glycol, sodium citrate, sucrose (200 mg/ml), water
Retrovir/zidovudine	Syrup	10 mg/ml	160 mg/m^2 t.i.d. not to exceed 200 mg every 8 h, neonate: 2 mg/Kg every 6 h 6 weeks to 12 years	Sodium benzoate 0.2%, cytric acid, flavors, glycerin, liquid sucrose
Zyrtec/cetirizine	Syrup	1 mg/ml	6 months-5 years:2.5-5 mg per day, 6-11 years: 5-10 mg per day	Acetic acid, banana flavor, glycerin, grape flavor, methylparaben, propylparaben, propylene glycol, sodium acetate, sugar syrup, water (pH 4-5)
Clarinex/desloratadine	Syrup	0.5 mg/ml	6-11 months: 1.0 mg, 12 months-5 years: 1.25 mg, 6-11 years: 2.5 mg, > 12 years: 5 mg all q.d.	Propylene glycol, sorbitol solution, citric acid, sodium citrate, sodium benzoate, EDTA, water, sugar, bubble gum flavor
Norvir/ritonavir	Oral solution	80 mg/ml	<600 mg b.i.d. or >1 month: 350-400 mg/m^2 up to 600 mg b.i.d. (0.8-7.5 mL)	Ethanol 43%, water 15%, polyoxyl 35 castor oil, propylene glycol, citric acid, saccharin sodium, peppermint oil, creamy caramel flavor
Agenerase/amprenavir	Oral solution	15 mg/ml	4-12 years: 22.5 mg/Kg b.i.d., or 17 mg/Kg t.i.d. up to 2800 mg per day	Acesulfame potassium, artificial grape bubblegum flavor, citric acid, TPGS, menthol, natural peppermint flavor, PEG 400 (17%), propylene glycol (55%), saccharin sodium, sodium chloride, sodium citrate
Sustiva/efavirenz	Oral solution	30 mg/ml	270-600 mg	Medium-chain triglycerides, benzoic acid, strawberry/mint flavors
Zovirax/acyclovir	Suspension	40 mg/ml	< 40 Kg: 20 mg/Kg q.i.d., >40 Kg: 800 mg (20 mL) q.i.d.	Methylparaben, propylparaben, carboxylmethylcellulose sodium, banana flavor, glycerin, microcrystalline cellulose, sorbitol, water
Viramune/nevirapine	Suspension	10 mg/ml	4 mg/Kg q.d. for the first 14 days followed by 4-7 mg/Kg b.i.d. thereafter. Maximum daily dose 400 mg	Carbomer 934P, methylparaben, propylparaben, sorbitol, sucrose, polysorbate 80, NaOH, water
Viravan/phenylephrine tannate, pyrilamine tannate, dextromethorphane tannate	Suspension	2.5, 6, 5 mg/ml	2-6 years: 2.5 mL, 6-12 years: 5 mL, >12 years: 10 mL	Citric acid, glycerin, grape flavor, magnesium aluminium silicate, methylparaben, sucralose, ammonium glycyrrhizinate, sodium benzoate, sodium citrate, sucrose, xanthan gum, water

Table 5. List of commercially available paediatric oral solid formulations.

Marketed name/Drug name	Marketed formulation	Active in formulation	Dose	Excipients
Dextrostat/dextroamphetamine sulfate	Tablet scored and double scored	5 mg (scored), 10 mg (double scored)	3-5 years: 2.5 mg/day, >6 years: 5 mg/day	Acacia, corn starch, lactose, magnesium stearate, sucrose, the 10 mg tablet contains sodium starch glycolate
Malarone/atovaquone, proguanil HCl	Tablet	Fixed dose combination: 62.5 and 25 mg	1-3 tablets q.d.	Hydroxypropylcellulose, magnesium stearate, microcrystalline cellulose, poloxamer 188, povidone K30, sodium starch glycolate
Caduet/amlodipine besylate, atorvastin calcium	Tablet	Fixed dose combination: 2.5/10, 2.5/20, 2.5/40, 5/10, 5/20, 5/40, 5/80 mg/mg	6-17 years: 2.5-5 mg (amlodipine) q.d.	Calcium carbonate, croscarmellose sodium, microcrystalline cellulose, pregelattinized starch, polysorbate 80, HPC, water, colloidal silicon dioxide, magnesium stearate
Strattera/atomoxetine	Capsule-hard gelatin	10, 18, 25, 40 and 60 mg	0.5-1.2 mg/Kg up to 100 mg q.d.	Pregelatinized starch, dimethicone
Vyvanse/lisdexamfetamine dimesylate	Capsule	30, 50, 70 mg	20-70 mg/day q.d. in the morning	Microcrystalline cellulose, croscarmellose sodium, magnesium stearate
Singulair/montelukast sodium	Chewable tablet	4-5 mg	4 mg q.d.	Mannitol, microcrystalline cellulose, HPC, croscarmellose sodium, cherry flavor, aspartame, magnesium stearate
Videx/didanosine	Chewable tablet	25, 50, 100, 150, 200 mg	120 mg/m2 b.i.d.	Citric acid, sucrose, aspartame, calcium carbonate, microcrystalline cellulose, magnesium hydroxide, magnesium stearate, crospovidone, sorbitol, mandarin-orange flavor
Tegretol/carbamazepine	Chewable tablet-single scored	100 mg	<6 years: 10-35mg/kg/day q.i.d., 6-12 years: 50 mg q.i.d. up to 1000 mg daily	Silicon dioxide, flavors, gelatin, glycerol, magnesium stearate, sodium starch glycolate, starch, stearic acid, sucrose
Prevacid/lansoprazole	Delayed release orally disintegrating tablet	15 or 30 mg	<30 mg: 15 mg t.i.d., >30 mg: 30 mg t.i.d.	Lactose monohydrate, microcrystalline cellulose, magnesium carbonate, HPM, HPMC, titanium dioxide, talc, mannitol, methacrylic acid, polyacrilate, PEG, glyceryl monostearate, polysorbate 80, triethyl citrate, ferric oxide, citric acid, crospovidone, aspartame, artificial strawberry flavor, magnesium stearate
Zofran/ondansetron	Orally disintegrating tablet	4-8 mg	4-11 years: 4 mg t.i.d.	Aspartame, gelatin, mannitol, methylparaben sodium, propylparaben sodium, strawberry flavor
Clarinex Redi-Tabs/desloratadine	Orally disintegrating tablet	2.5-5 mg	6-11 years: 2.5 mg q.d., >12 years: 5 mg q.d.	Mannitol, microcrystalline cellulose, pregelatinized starch, sodium starch glycolate, magnesium stearate, butylated methacrilate copolymer, crospovidone, aspartame, citric acid, sodium bicarbonate, colloidal silicon dioxide, ferric oxide, flavors

1.5.2. ORAL TRANSMUCOSAL ADMINISTRATION

The oral transmucosal route offers many advantages over the oral route: the adsorption is rapid thanks to the rich vascular supply and the lack of the stratum corneum epidermidis. This allows a rapid increase in blood concentration and the achievement of the peak blood levels within 10-15 min (American Academy of Pediatrics, 1997). Oral transmucosal administration has the advantages of avoiding the enterohepatic circulation and the immediate destruction by gastric acid. For a significant drug adsorption, the dosage form, and thus the drug, must have a prolonged exposure to the mucosal surface. Drug adsorption is generally greater from the sublingual, buccal and oral mucosa, than from the tongue and the gingiva. The advantages of a rapid adsorption make it a

reasonable alternative to intravenous therapy. Important limitations associated with this route of administration are: lack of cooperation of children, difficulties in coordination, risk of choking and aspiration, and the accuracy of dosing because the dosage form can be swallowed or spat out prior to sufficient adsorption taking place.

Buccal tablets are designed to dissolve slowly and give a prolonged release of the drug in the mouth, while sublingual tablets are intended to a rapid release of the drug and permeation into the systemic circulation. Limitations include the small number of drug candidates, restrict buccal and sublingual area, the palatability and probable local irritation. For these reasons, buccal and sublingual tablets are not useful for very young children.

Mucoadhesive preparations are intended to be retained by adhesion to the mucosal epithelium in order to control the release and the permeation of the drug at the site of application. These dosage forms could be use in younger children.

Lozenges are hard solid preparation used for the release of active ingredients locally, in the oral cavity or through the systemic circulation. They are accepted also by older patients.

1.5.3. NASAL ADMINISTRATION

Intranasal delivery offers unique advantages in administration of paediatric actives. The highly vascularized nasal mucosa and the olfactory tissue in direct contact with the central nervous system, allow a rapid transport of the drug into the bloodstream and the brain, with an onset of action very close to the intravenous therapy. Therefore, the nasal delivery can represent a reasonable alternative to invasive intravenous administration. On the other hand, this route of administration could lead to unwanted side effects, irritation and pain on the mucosa, and inefficiency, due to abundant secretions. In the case of local action, minimum drug absorption and maximum residence time are necessary, whereas in the case of systemic action, an efficient absorption into the bloodstream is needed. Nasal dosage forms are solid, semi-solid or liquid preparations.

Nasal powders are intended for paediatric delivery of active ingredients, particularly stable in a dried and solid state (peptides and vaccines), by using special delivery devices.

Nasal sprays supply paediatric actives in the nasal cavity through squeeze bottles, mechanical dispersing systems or pressurizing containers. The applicability of squeeze bottles is restricted due to the dose variability, whereas pressurized devices are very

reproducible in dosing and droplet size distribution. In this case, the problem is the strong impact on the nasal mucosa, which may cause local irritation.

Nasal drops are intended for instillation into the nasal cavity and usually are supplied in multidose containers, with a suitable dosing device. From an anatomical point of view, nasal drops are preferred in infant treatment, because their nasal cavity is so small that one or two drops can cover the whole mucosa (EMEA/CHMP/PEG/194810/2005).

1.5.4. RECTAL ADMINISTRATION

Active ingredients may be administered in children by the rectal mucosal route for systemic effects, if other more preferable routes are not available (American Academy of Pediatrics, 1997). Rectal administration is useful if the oral route is contraindicated, the immediate systemic or the local effects are required, or if the oral dosage forms are rejected because of the palatability issues. The rectal route is use for either local (laxative, anti-inflammatory) or systemic (analgesic, antipyretic, anticonvulsive, sedative) actions; it allows rapid absorption of many drugs and may be a reasonable alternative to the intravenous route, having the advantage of being relatively painless. Many advantages are associated with this route, especially for the treatment of paediatric population; rectal dosage forms in fact avoid the problems of swallowing and taste-masking, along with the fact that they can also be administered in emergency to unconscious or vomiting patients (Jannin et al., 2014). Among all the marketed products available for rectal administration, suppositories are prevalent and they are versatile and present in a variety of strengths for various age groups. Alternative rectal dosage forms include gels, foams, creams, pastes, ointments, gelatine capsules and solutions. The choice of suitable excipients for each preparation has to consider the fact of avoiding any local irritation of the rectal mucosa of infants or children. Polyethylene glycol bases lead to irritation of the rectal mucosa due to their hygroscopic nature, which may be reduced by moistening the suppository with water prior to insertion (EMEA/CHMP/PEG/194810/2005).

Rectal route presents few disadvantages: the introduction of a solid unit in the rectum (leading to poor acceptability and compliance), the low absorption capacity of the lower rectum to some drugs, and the high inter-individual variability of the drug bioavailability, mainly depending on how the dosage form is inserted.

1.5.5. TOPICAL-TRANSDERMAL ADMINISTRATION

Topical and transdermal routes offer some clear and specific advantages for paediatric drug delivery (Delgado-Charro et al., 2014). Topical delivery permits the targeting of the drug to the local area, minimizing systemic exposure; topical formulations usually contain anti-inflammatory, anti-histaminic, antifungal, antiseptic and analgesic drugs incorporated into gel, creams, and ointments. In children < 2 years of age, the application area should be restricted; corticosteroids, in fact, present 2-10 folds systemic exposure. In addition, water-impermeable materials, as well as high lipophilic vehicles may increase the systemic exposure, while fever and external heat may increase the rate of permeation.

Transdermal drug delivery offers a non-invasive approach to avoid the first-pass effect; it is in general, well accepted, easy to apply and represents a valuable alternative when oral administration is difficult or may result in erratic absorption. Transdermal drug absorption depends on a variety of factors, as site of application, thickness and integrity of the stratum corneum, size of the molecule, state of skin hydration, pKa of the drug, etc. Moreover, important morphological and hence permeability differences occur between mature skin and that of neonates (Table 6). At birth, the dermis is only 60 % of its adult thickness and maturation takes 3-5 months after birth. Epidermis is perfused and hydrated to a greater degree than in adults, and infants have a reduced capacity for biotransformation and elimination of active substances, including those absorbed by the cutaneous route. This leads to a better permeation for neonates, infants and children. Thus, the potential for toxic effects, is particularly important for children, where the blood flow and the thickness of the skin vary with age; if in some cases it represents an advantage, in other case it causes important side effects.

Table 6. Correlation between surface area/body weight ratio vs age.

Age	Weight (kg)	Surface area (cm^2)	Ratio (surface/weight)	Comparison (adult = 1)
Newborn	3.4	2100	617.6	2.4
6 months	7.5	3500	466.7	1.8
1 year	9.3	4100	440.9	1.7
4 years	15.5	6500	419.4	1.6
10 years	30.5	10500	344.3	1.3
Adult	70	18100	258.6	1.0

1.5.6. PARENTERAL ADMINISTRATION

Parenteral administration is the most common route of administration for active substances for unconscious and uncooperative children and for clinically unstable term and preterm neonates. Medications administered via the parenteral route include intravenous, intramuscular and subcutaneous, intrathecal, intra-osseous, intra-cardiac and intra-arterial routes. There are suitable intravenous preparations available for children in which the dose administered is complete and accurate; the onset of therapeutic action is rapid, since the systemic access is direct, avoiding the first-pass metabolism and the bioavailability is predictable. One of the main disadvantages is that, once administered, the effect is not reversible. Moreover, the rate of administration should take into account the potential for toxicity and adverse reactions. Compatible infusion fluids include glucose 5 % and 10 %, sodium chloride 0.45 % and 0.9 % and combination of glucose and saline. It is also possible to accurately administer small doses of medications to children; however, adult preparations may require multiple dilutions, and this is critical because every dilution creates an opportunity for an error, in terms of contamination, safety and efficiency of the drug. Another important consideration about parenteral administration is about the volume of medication to be infused or injected; this is particular significant in neonatal population, who may only accept very small volumes (200-300 ml) of medicines in order to avoid volume overload and to allow sufficient room for essential fluid nutrition.

Even administrations through intramuscular and subcutaneous routes need particular attention about the volume injected; in the case of intramuscular administration, it is important to consider also the size/bulk of the muscle injected into.

Some actives, compatible with parenteral nutrition are ampicillin, clindamycin, fluconazole, insulin, metoclopramide; other actives are incompatible with this route and they cannot be given with a parenteral solution under any circumstances: acyclovir, cisplatin, mannitol and phenytoin. However, certain parenteral medications are known to have increased toxicity in children, compared to adults. These includes dopaminergic antagonist as metoclopramide or haloperidol, where younger children are at increased risk of acute dystonic reactions and seizures (Magrath et al., 2007).

1.5.7. PULMONARY ADMINISTRATION

Inhalation route can be considered a valid alternative to oral administration thanks to the capacity to avoid the hepatic first-pass metabolism and the possibility to reduce the dose needed. It may be preferred to the parenteral route, especially for the administration of peptides and protein, because it does not give pain during the application.

Medications delivered by inhalation include anaesthetic agents and those used for the treatment of asthma. In the case of anaesthetic medications, the dose required is proportional to the lung volume of the child, which is proportional to the weight.

However, the absorption of the drug into the systemic circulation is variable, depending on the capacity of the paediatric patients to use the specific device. Obviously, this is related to the age of the child.

Conventional devices include pressurized metered dose inhaler, dry powder inhaler and nebuliser. Pressurized metered dose inhalers are efficient but require good coordination between the actuation of the device and the inhalation action (press and breath), which preclude its use in young children. The use of spacer/holding chamber avoids the problem of coordination, and in particular the presence of a face mask allowing the utilization of this device even in young children. Dry powder inhalers, instead, require a sufficient inspiratory flow to permit the powder to achieve the nose, allowing the use of this inhaler only in old children.

1.6. MANIPULATION OF DOSAGE FORMS

Several paediatric formulations have to be manipulated in order to be administered to children. Examples of manipulated dosage forms are: drops for reconstitution to a suspension, solids to form a solution or suspension, effervescent tablets, dispersible and orally disintegrating tablets in water, and oral powders that are sprinkled into food or

drink prior to administration. In these kinds of dosage forms, it is important to consider the stability of both the active ingredients and the excipients, before and after manipulation, and in proper long-term storage (Stryckley et al., 2008).

1.6.1. SOLID FOR CONSTITUTION TO A SUSPENSION

These formulations include a solid phase as powders, tablets, granules, microcapsules, used for the preparation of suspension through the addition of water or other common beverage such as milk, fruit juices or specific liquids given in the package. It is a useful system for actives with a poor water solubility and bitter taste, and the chemical stability of the compounds has to be evaluated in the solid form and in the suspension. The formulation must also be physically stable as solid and as suspension with no foaming, minimal (slow) particle settling and easily re-dispersed if the solid particles do settle, and easy measurable. Four types of stability are at least necessary in solid for reconstitution to oral suspension:

- solid-state chemical stability (extended storage);
- solid-state physical stability (extended storage);
- suspension chemical stability (in-use phase);
- suspension physical stability (in-use phase).

Chemical stability is required not only for the active, but also for all the other ingredients, and special storage conditions are required in case of chemical instability, such as storage at 2-8 °C in a refrigerator, or desiccation of the solid. Physical stability of the solid state can be guaranteed by the addition of anticaking agents, such as starch, to avoid the agglomeration, whereas the physical stability of the suspension can be achieved by minimizing the foam with the addition of simethicone in the form of a powder.

When the solid phase is composed by powder or granules, other excipients, as sweeteners, flavours, preservatives and dispersible agents are added to the formulation.

Commercially available suspensions involve 15 products with powders, 6 with tablets, 1 with granules and 1 with microcapsules as solid phase.

1.6.2. SOLID FOR CONSTITUTION TO A SOLUTION

Three powders (two drugs) for constitution to a solution are on the market. The drug product is a powder formulation that had not been previously dissolved. The most important requisite for this kind of dosage form is the high water solubility of the active

ingredient that has to be able to dissolve in a relatively small volume of water. Sweeteners, flavours, antifoaming agents, and preservatives improve the properties of the dosage form.

1.6.3. CONCENTRATED SOLUTION DILUTED

Only two commercially available products are represented in this formulation. The manipulation involves the easy dilution of a small amount of the original product, by using a dropper, into a defined volume of a liquid (water or fruit juice) or in a semi-solid substance.

1.6.4. EFFERVESCENT TABLETS

Effervescence is the release of carbon dioxide when a solid containing sodium bicarbonate or sodium carbonate and an acidic excipient like fumaric acid or citric acid are added to water. For the administration of the entire dosage, a tablet has to be dissolved in a volume of water and the obtained solution is given by a dropper or a syringe or directly drunk.

1.6.5. SPRINKLE SOLIDS

These formulations are represented by powders or granules with bad taste that cannot be masked; thus, the dosage form is not directly administered, but can be formulated as a oral powder/granule that is sprinkled onto the food immediately prior to administration. Packaging is a critical component of sprinkle solids and various configurations are used such as multi-use containers with bulk powder filled into a bottle and supplied with a scoop, unit-dose filled into a sachet, unit-dose filled into capsules that can be opened.
In table 7, it is possible to find some of the commercially available formulations-manipulation required, described above.

Table 7. Selected listing of commercially available paediatric oral formulations-manipulation required.

Marketed name/Drug name	Marketed formulation	Active in formulation	Dose	Excipients	Manipulation
Stavudine/Zerit	Powder for constitution to a solution	1 mg/ml	< 30 Kg: 1 mg/Kg >60 kg: 40 mg b.i.d	Methylparaben, propylparaben, sodium carboxymethylcellulose, sucrose, antifoaming and flavoring agents	Constitute with 202 ml of water
Cefadroxil/Cefzil	Powder for constitution to a sospension	25-50 mg/ml	7.5-15 mg/Kg b.i.d. up to 500 mg/day	Aspartame, cellulose, citric acid, colloidal silicon dioxide, flavors, glycine, polysorbate 80, simethicone, sodium benzoate, sodium chloride, sucrose	Constitute with water
Cefurexime axetil/Ceftin	Powder for constitution to a sospension	25-50 mg/ml	20-30 mg/Kg/day up to 500-1000 mg divided b.i.d.	Acesulfame potassium, aspartame, povidon K30, stearic acid, sucrose, flavors, xanthan gum	Constitute with water, must be administered with food
Clarithro-mycin/Biaxin	Powder for constitution to a sospension	25-50 mg/ml	7.5 mg/Kg b.i.d. for 10 days	Carbomer, castor oil, citric acid, hypromellose phthalate, maltodextrin, potassium sorbate, povidone, silicon dioxide, sucrose, xantham gum, titanium dioxide, fruit punch flavors	Constitute with 27-55 mL of water
Montelukast sodium/Singulair	Oral granules	0.8% w/w	4 mg q.d.	Mannitol, HPC, magnesium stearate	Directly in the mouth, dissolved in 5 mL baby formula or breast milk, mixed with spoonful of soft foodscarrots, rice or ice cream
Topiramate/Topamax	Sprinle capsule	15 and 30 mg	5-9 mg/Kg/day starting with 25 mg b.i.d.	Sugar spheres, povidone, cellulose acetate, gelatin, silicon dioxide, sodium lauryl sulfate	Swallowed whole or carefully open capsule then sprinkle the entire contents on a small amount of soft foods

1.7. RISK ASSOCIATED WITH MANIPULATION OF ADULT DOSAGE FORMS

Many medicinal products are authorised only for adult use and contain no validated provisions for paediatric use. In these cases, where the adult-only products may also be of benefit in the paediatric population, a number of practices have involved to manipulate these adults medicinal products in order to render them suitable for dosing to paediatric patients (EMEA/CHMP/PEG/194810/2005).

Sometimes there is little information about the bioavailability of the manipulated dosage forms; some points have to be noted:

1. only simple manipulations can be carried out at home by caregivers (breaking or crushing tablets, opening capsules);

2. sophisticated manipulations of adult medicinal products have to be carried out by healthcare professionals in a hospital or community pharmacy setting;
3. manipulation of adult medicinal products is always considered as a secondary option;
4. it is important to check the safety and security of the final paediatric formulations for the presence of excipients, especially in neonates patients;
5. manipulated formulations have to be as simple as possible, avoiding additional unnecessary excipients.

1.7.1. SPLITTING TABLETS INTO SEGMENTS

If we suppose that the active is uniformly distributed through the volume of the tablet, splitting tablets is a very simple manipulation, especially because, often tablets are score to facilitate this. Obviously, a possible error is more evident with small tablets and low dosage. Enteric-coated tablets, layered tablets, and many modified-release dosage forms should not be manipulated in this way. The manufacturer should provide some information about the issues related to cutting tablets into smaller segments.

1.7.2. CRUSHING TABLETS

This manipulation consists in reducing the monolithic tablet to a fine powder in which the drug is assumed to be uniformly distributed. This allows both the dose reduction and the mixing in food or drinks to facilitate the administration. Sometimes, a mortar and pestle may be sufficient; however, the division of the powder can be made by visual inspection or by weight; some issues can occur, such as the segregation of the active substance in the bulk powder, caused by a prolonged handling and vibration. The use of a hammer mill in a hospital pharmacy is suggested to avoid these problems although it can lead to changes in particle size, and thus in bioavailability, and temperature rises, and thus solid-state transitions or chemical degradation.

Another important manipulation consist in blending the powdered tablets with lactose diluent, and then filled into sachet or hard gelatine capsules. Primary amines cannot be handled in this way because of their well-known interaction and instability in the presence of such reducing sugars.

There is also the risk that modified-released tablet, once manipulated and crushed, may lose their advantages.

In this case, the manufacturer has to provide information about the suitability of tablets for crushing and suitable powder diluents, as well as information about the compatibility and stability of the crushed tablets with common foods and drinks.

1.7.3. OPENING CAPSULES

This manipulation is a refinement of the crushing tablets, and, as in that case, the division of the powder can be made by visual inspection or by weight, or dispersed into foods or drinks to facilitate the ingestion. In the presence of modified release preparations of coated particles, they may be dispersed in the same way in foods or drinks.

As described above, the manufacturer should give information about the compatibility of all the compounds with different foods and drinks, as well as all the information about the effects on the bioavailability.

1.7.4. DISPERSING TABLETS/CAPSULES AND TAKING PROPORTIONS

A further manipulation of the powder prepared from tablets or taken from inside capsules is to disperse it in a suitable suspending liquid, and achieve dose reduction in a volumetric way and not gravimetrically. Sedimentation and settling of the obtained dispersion lead to a risk of dosage errors, and this may be minimized by using a high viscosity suspending medium. In some cases, this error is reduced when the active substance is dissolved from the powdered matrix and taken into complete solution. Manufacturers may provide information about the suitability of tablet and capsule dispersions for the purpose of volumetric measurements to administer a proportion of the adult dosage form.

1.7.5. CUTTING/COVERING TRANSDERMAL PATCHES

Since the amount of drug delivered through the skin is proportional to the surface area of the transdermal patch in contact with it, it is possible to halve the patch to obtain half of the dose delivered. This is particularly useful for paediatric patients, where the surface area of the skin is obviously reduced. In general, caregivers cut the patch by using scissors, allowed some important errors:

- despite transdermal patches present a specific ‘release-controlling’ membrane, it is likely that the main rate-controlling factor is the skin itself, and thus the most important problem is the difference in the structure of the maturing skin in neonates, and in particular of the stratum corneum;

- it is difficult to decide what is exactly the area of the patch, especially in particular area of the body, such as the eyes;
- cutting exposes the patch to the atmosphere, and thus to possible mechanism of erosion and oxidative degradation;
- cutting is contraindicated in case of gel-filled patches.

Even in this case, manufacturers have to provide information about the possibility to cut or cover transdermal patches and about any modifications on the release of the active substances.

1.7.6. CUTTING SUPPOSITORIES

Adjustment of the dosage in the case of suppositories is difficult to achieve since very few suppositories are present in a convenient way to facilitate halving by simple visual inspection. By considering the active ingredient uniformly distributed in the whole dosage form, a possibility is to cut along a plane of symmetry, although the resulting shape may not be optional for rectal insertion.

Manufacturers may provide information on uniformity of dispersion of the drug in the suppositories

1.7.7. INJECTABLE SOLUTIONS ADMINISTERED BY OTHER ROUTES

Injections for oral administration are expensive but they allow a reduction of the dosage errors by using, if necessary, small syringe. They are aqueous and non-viscous solutions, often presenting an unpleasant taste. In the case of preserved or multi-dose products, they contain benzyl alcohol, propylene glycol, or have pH and osmolality potentially harmful for neonates and children. Injections have sometimes been given by pulmonary route, following nebulisation. Ignorance of the precise composition of the parenteral (adult) formulation could pose a significant safety risk in the case of injections stabilised with sulphite-based antioxidants, which may provoke bronchoconstriction.

Manufacturers may give information on the suitability of an injection solution for the administration by other routes.

Table 8 lists the most common manipulations related to the conventional dosage forms.

Table 8. List of manipulations of the conventional dosage form for adult administration.

Drug dosage form	Manipulation of dose accuracy
Tablet	a. split/broken/cut and a segment given b. crushed and a portion of the powder given c. dispersed in liquid and a portion of the liquid given
Capsule	a. opened, dispersed in liquid and a portion of the liquid given b. opened and a portion of the powder given
Sachet (powder)	a. opened, dispersed in liquid and a portion of the liquid given b. opened and a portion of the powder given
Oral liquid	diluted and a portion given (to make the measurement of a small volume dose easier)
Suppository	cut/split and a segment given
Nebuliser solution	a. portion given b. diluted and a portion given
Enema/bladder irrigation	a. portion of sachet/unit given b. portion of the content removed and the remainder given
Transdermal patch	a. patch cut and a portion applied b. portion of patch uncovered and applied
Intravenous injection	a. reconstituted or ready prepared solution, further diluted to allow a smaller dose to be measured b. volume of fluid removed from IV container, drug added (to obtain accurate concentration for infusion)

1.8. CHOICE OF EXCIPIENTS

Paediatric formulations are often more complex than adult formulations due to taste masking, dose volume, delivery and aesthetic requirements, which demand incorporation of a broad range of excipients (Ali et al., 2014). In fact, the organoleptic appeal of a paediatric formulation is usually improved by the addition of flavours, colorants and sweeteners. Besides enhancing organoleptic properties, excipients are used to allow physical/chemical stability, precision and accuracy of dosing, improve bioavailability, control release and aid in manufacturing. Since they do not present pharmacological activity, excipients have always been considered inert agents, and thus, their inertness and innocuity were taken for granted, and their importance has been largely underestimated (Salunke at al., 2012). However, it is commonly known that excipients used in adult medicines have been associated with elevated toxicological risk and safety issues in children (Salunke at al., 2012). There are several well-documented cases of adverse effects of pharmaceutical excipients in the paediatric population; in fact, the

toxicity may differ between adult and paediatric patients and across the paediatric sub-sets. These differences are due to changes in developing child: paediatric patients, and in particular, infants and neonates, are not able to metabolize or eliminate an ingredient in the same manner as adult because of the immaturity of the liver and the kidneys (Salunke at al., 2013). An example of toxicity caused by accumulation of excipients is represented by the administration of Kaletra oral solution to neonates, especially those born prematurely. Ethanol, when administered together with propylene glycol, inhibits its metabolism, leading to adverse effects associated with the accumulation of propylene glycol.

A general lack of knowledge of excipients has proved an effective barrier to the development of novel materials, and pharmaceutical industries have tended to opt for using well-known, but not necessarily safe, excipients. Important problems can occur when excipients approved for one route of administration are applied to another route with a different systemic exposure (Salunke at al., 2013). In other words, just because an excipient is approved in a specific paediatric formulation, may not automatically qualify its safe use in another paediatric formulation, route or age (Schmitt, 2015).

1.8.1. SOLVENTS

Water is the solvent mostly used as vehicle thanks to the lack of toxicity, the physiological compatibility and the good solubilizing power. However, water causes instability of hydrolytically instable drugs and it is a good vehicle for microbial growth. Water-miscible co-solvents, as ethanol, glycerol, and propylene glycol are often use to increase solubility, taste, anti-microbial effectiveness or stability, to reduce the dose volume, and to optimise insolubility (in the case of bad taste). Water-immiscible co-solvents are instead used to prepare emulsions or micro emulsions.

Ethanol

Ethanol is a common solvent in oral liquid dosage forms, found in many drug preparations for newborns and infants, as in iron supplementations and in furosemide, possibly exposing paediatric population to both chronic and acute toxicities. Acute intoxication is caused by accidental overdose, and it is characterized by coma, hypoglycaemia, and hypothermia, along with seizures, hypotonia, gastritis, gastrointestinal bleeding, and respiratory depression. In the serious cases, acute intoxication may cause acute hepatitis, pancreatitis, and cardiovascular toxicity. Chronic toxicity is associated with routine use of chronic medications and the effect of long-term

exposure to ethanol has never been studied in paediatric population. Studies and observations on FAS (foetal alcohol syndrome) and FAE (foetal alcohol effects) children, however, give direct evidence of the grave deleterious effects of chronic ethanol exposure, for example, on neurological and cognitive developmental processes. Moreover, ethanol has known to have synergic negative effects on central nervous system when associated with some active substances. In fact, in a recent case report, 4-months old infants suffered from acute life-threatening intoxication including altered mental status, associated with an overdose of an over-the-counter cough syrup, which has been attributed to the association of the synergic effect of the active ingredient, dextromethorphan, and ethanol. As explained above, the concomitantly administration of ethanol and propylene glycol, as in the case of Kaletra® oral solution, may cause adverse effects, due to the inhibition of the metabolism of propylene glycol by the presence of ethanol.

Propylene Glycol

Propylene glycol is a general solvent with antimicrobial activity, used in a wide range of pharmaceutical preparation: oral, topical, and injectable medications, as well as in foods and cosmetics. It is often useful for those substances with poor solubility in water, such as phenobarbital, phenytoin, diazepam and dexamethasone. High doses of propylene glycol have been associated with cardiovascular (hypotension, bradycardia, widening of QRS interval), hepatic and respiratory adverse effects with depression of the central nervous system, and nephrotoxicity (hyperosmolarity, osmol gap), especially in newborns and infants. The US Food and Drug Administration has identified propylene glycol as being 'generally recognized as safe' and the World Health Organization has established that 25 mg/Kg represents an acceptable intake limit for adults (Lau et al., 2012); however, this threshold is largely exceeded in preterm newborns, newborns and infants. In fact, paediatric patients below 4 years have a limited metabolic pathway, especially as regard the alcohol dehydrogenase, thus allowing an accumulation of propylene glycol in the body, and all the adverse effects mentioned above.

1.8.2. ANTIOXIDANTS AND PRESERVATIVES

Oxidative degradation can be accelerate by the presence of light and mineral or metallic impurities, due to the formation of free radicals. Antioxidants agents are important to reduce the oxidation of active substances and excipients in the medicinal preparation. They are divided in three categories: true antioxidants (butylated hydroxytoluene),

reducing agent (ascorbic acid) and antioxidants synergists (sodium edetate). Other antioxidants, like sulphites, are associated with bronchospasm in asthmatic children, and thus with symptoms like wheezing, dyspnoea and chest tightness.

Benzyl alcohol is often use in injectable medicinal preparations as a preservative agents. It should not be given to neonates due to their immature metabolism; it should be avoided, especially in children up to 3 years, for the potential risk of developing Gasping Syndrome in neonates and infants. The syndrome may include metabolic acidosis, seizure, bradycardia, gasping respiration and cardiovascular collapse. The minimum toxic level of benzyl alcohol, and therefore the safety of use of medicines containing benzyl alcohol in neonates has not been established (http://www.who.int/medicines/publications/newsletter/Newsletter_2_2012.pdf?ua=1).

In addition, benzyl alcohol, sodium benzoate and potassium benzoate when used in parenteral dosage forms may increase the risk of jaundice in neonates.

Benzalkonium chloride is found in beclomethasone and ipratropium bromide nebuliser solutions, and is associated with bronchospasm in asthmatic children after having inhaled their medications. It causes bronchoconstriction with a non-IgE-mediated mechanism. Another important source of benzalkonium chloride for children is represented by topical medications like nasal saline, nasal corticosteroids and nasal decongestant solutions.

1.8.3. SWEETENERS

The use of sweeteners is particularly important in developing paediatric formulations, because they are used to improve their organoleptic properties, such as smell and taste and to increase the compliance of children to therapy. Sweeteners commonly used in paediatric medications are divided in three main categories: natural origin sweeteners (sucrose and sorbitol), semi-synthetic sweeteners (aspartame) and synthetic sweeteners (saccharin).

Natural sweeteners

Sucrose is one of the most used sweetening agents; it is a disaccharide that is readily hydrolysed in the intestine in the absorbable glucose and fructose; thus, it should be avoided in patient suffering from hereditary fructose intolerance and diabetes (EMEA/CHMP/PEG/194810/2005). For preparations intended for long-term therapies, large amounts of sucrose have to be replaced by sugar-free preparations, because sucrose causes a decrease in dental plaque pH, dissolving tooth enamel, and promoting dental caries.

Sorbitol is a mono-saccharide, not readily absorbed by the gut, and thus, it is considered safe for patients suffering from diabetes. The medicinal intake of sorbitol in paediatric population has been associated with gastrointestinal disorders, such as diarrhoea and malabsorption (Fabiano et al., 2011). Since sorbitol is metabolised to fructose, it is contraindicated in patients suffering from hereditary fructose intolerance and hypoglycaemia. In severe cases, it causes liver damage and come, resulting in death (EMEA/CHMP/PEG/194810/2005).

Semi-synthetic sweeteners

Aspartame is widely used in both food products and pharmaceutical preparations as sweetening agent. It is a dipeptide of aspartic acid and a methyl ester of phenylalanine; since it is a source of phenylalanine, it is harmful in children affected by phenylketonuria and particularly in homozygotes patients on special phenylalanine-restricted diet, in which the use of aspartame-containing products may allow a significant increase of phenylalanine blood concentration. Lastly, aspartame has been associated with hyperactivity in children; however, by now this association has remained unproven. The US acceptable intake of aspartame is 50 mg/Kg/day.

Synthetic sweeteners

Saccharin is a synthetic sweetener, largely used for its sweetening power, 500 fold higher than other common sugar. It is not metabolized and thus it is not a source of calories, so it is particularly useful in hypocaloric food products. In some articles, the use of saccharin has been associated with an increased risk of developing cancer, especially bladder cancer. It has been indeed demonstrated the existence of cross-reactions between saccharin and sulphonamides. The most common adverse reactions are dermatological and include urticarial, pruritus, dermatitis and photosensitivity. Other systemic reactions involve insomnia, irritability and strabismus in children assuming saccharin-containing feed formulas.

1.8.4. FILLERS

Lactose is a disaccharide of glucose and galactose, and is absorbed after hydrolysis by intestinal lactase. It is widely present in infant feed formulas, and in pharmaceutical preparations is used as diluent in tablets and capsules and in lyophilised powders, as sweetener in liquid formulations. In infants and young children, lactose intolerance may be associated with prolonged diarrhoea, dehydration and metabolic acidosis.

1.8.5. COLOURING AGENTS

Colouring agents are commonly used in paediatric medications to improve the appeal of the dosage form and increase the compliance of paediatric population to therapies. Most colouring agents used in developing paediatric formulations belongs to one of the following groups: azo dyes (tartrazine, sunset yellow and coccine), quinoline dyes (quinoline yellow), triphenylmethane dyes (FD&C blue) and xanthene dyes (erythrosine). Several side effects associated with colouring agents have been reported in literature, especially hypersensitivity and allergic reactions. For this reason, they should be avoided unless necessary.

1.8.6. FLAVOURING AGENTS

Flavouring agents (flavours and aromatic substances) are both natural and/or synthetic products. Several pharmaceutical paediatric formulations, especially liquid formulations, contain flavouring agents for conferring an acceptable taste to the drug. They are particularly accepted by children because they are represented by sweet fruit taste. However, a number of allergic reactions have been possibly associated with flavouring agents.

1.9. STEP DATABASE

Currently, most of the existing database are focusing in providing safety and toxicity information related only to adults and animals. Therefore, there is no central repository in public domain to capture, achieve, validate, manage, maintain and provide access to safety, tolerability and toxicity data that have been generated for paediatric excipients in drug development. In order to address this need, the European (EU) and United States (US) Paediatric Formulation Initiatives (PFIs) are working together to create and maintain a database of Safety and Toxicity of Excipients for Paediatrics (STEP) (Salunke at al., 2012).

The purposes of the STEP database are:

- to serve as a freely accessible evidence base for safety and toxicity of excipients for the pharmaceutical industries, academics and pharmacists clinicians to make informed decisions;
- to identify the possible safety issues related to excipients at the initial stages of the developmental process, when excipients are screened and selected;

- to help highlight any relationship between exposure and toxicity in the paediatric subpopulations;
- to identify possible differences in toxicity between adult and paediatric populations that means a need for generating new data for paediatric medicines;
- to support pharmaceutical companies and academia with readily available information, for both the regulatory aspect and research activities (Salunke at al., 2012).

The potential users include professionals in paediatric drug development: formulation scientists at pharmaceutical companies and academic centres, excipients manufacturers, pharmacists, developmental toxicologists, paediatric toxicologists and pharmacologists. The search may occur by using two different modules: 'Search by excipients' and 'Search for excipients'. The first module provides navigation tools for selection of specific excipients and should be used when searching for safety and toxicity information of particular excipients. The 'Search for excipients' modules instead provides enhanced tools for complex queries allowing for searching of excipients associated with specific studies, effects or pharmacological functions (Salunke at al., 2013).

1.10. PALATABILITY: TASTE, SMELL AND TEXTURE

Palatability is one of the main (but not exclusive) pharmaceutical attribute of the dosage forms which affects children's acceptability of an oral medicinal product and it is crucial for compliance of their treatment. The non-acceptance of a medicine due to the bad taste can have detrimental consequences on the treatment outcome if the medicine is partially or not taken, leading to suboptimal therapeutic effect or no effect at all. Palatability is defined as the overall acceptance of a (often oral) medicine by organolepting properties such as vision (appearance), smell, taste, aftertaste and mouth feel (texture, cooling, heating, trigeminal response) and possibly also sound (auditory clues). It is determined by the characteristics of the active ingredients and excipients. Palatability is also relevant for other routes of administration such as buccal, nasal administration or inhalation use, and when the product may contact the taste receptors indirectly, for instance by deposition on the throat and post nasal run off.

Although solid dosage forms are usually accepted by older children and adolescents, and can be easily taste-masked by encapsulation and film coating techniques, younger children tend to prefer liquid formulations. However, in this case, compounds with high

solubility can be difficult to taste mask, as they often cannot be easily formulated as suspensions. Even suspensions of poorly soluble substances may exhibit poor palatability properties, if the mouth feel is compromised by the amount of the substance in suspension, or if the amount of the dissolved substance exceeds the human taste threshold. Also other kinds of dosage forms, such as chewable and orally disintegrated tablets may be challenging in terms of taste masking possibility (Cram et al., 2009).

In addition, there is a series of issues that should be overcome: in the era of age-appropriate formulations, covering all age ranges of the target population is necessary but also challenging. Different taste acuity and preferences occur between teenagers and infants, male and female, and healthy and sick children (Cram et al., 2009).

Apart from the taste and smell of a dosage form, there are other potentially important parameters, which determine if a preparation will be accepted by a child. Texture and appearance for example play an important role in patient's acceptability, but their effects have little attention because preventive measures are limited.

1.10.1. THE PHYSIOLOGY OF TASTE IN CHILDREN

Taste sensations arise from the stimulation of specialized cells grouped in small clusters called taste buds, which exist in bumps, all around the tongue: on the front of the tongue, in folds of the side of the tongue, and in circular grooves on the back of the tongue surface (EMEA/CHMP/PEG/194810/2005). Each taste bud contains 50-100 taste cells and the taste sensation is elicited when drugs or excipients dissolve in saliva and interact with taste receptors (surface proteins) or ion channels (pore like proteins). Sweet and bitter tastes interact with surface proteins whereas salt and sour tastes interact with ion channels; the taste receptor stimulation increases the concentration of positive ions. The varying ion concentration initiates electrical changes in the negatively charged taste cells causing generation of chemical signals. This process allows the release of neurotransmitters, which are perceived by brain as taste (Ali et al., 2014).

The human fetus seems to have specialized taste cells at about the 7^{th}-8^{th} weeks of gestation, with structurally mature taste buds around the 13^{th}-15^{th} weeks of gestation. This means that newborn presents all the capacities to perceive different taste, but as explained above, differences in acuity and preferences occur between newborns and infants, children and adults.

1.10.2. METHODS TO ASSESS THE TASTE OF MEDICATIONS

It has been reported that children have a greater difficulty in recognizing the taste of a formulation than adults, due to their limited analytical skills in perceptual tasks. Their ability increases in pre-school period, until adolescence. However, it is unknown whether children are able to analyze and recognize more than one flavour in a taste mixture and the concentration of each flavour can influence the child's assessment. It has been reported instead that, in the case of sweetness and saltiness, paediatric patients have the ability to recognize and analyse them from an early stage of life, and they are able to recognize them in a mixture, and estimate the strength and the degree of sweetness and saltiness. In general, children prefer more sweetness than adults do, and in particular, female of 4-12 ages are more sensitive to sweetness and saltiness than male.

In addition, cultural influences can have strong effects on children's attitude and preference toward even the basic tastes and flavors. Market research has revealed standard combinations of specific sweeteners with relevant flavours, which may vary by country and target market. National favourites include "bubble-gum" and "grape" in the United States, "citrus" and "red berries" in Europe and "liquorice" in Scandinavia. For example, a bubble-gum or cherry flavour in combination with a high intensity sweetener may suit the US paediatric market, while a less intense sweetness may be more appropriate for Japan.

Children may find unpleasant and reject irritating sensations in the mouth such as effervescence or peppermint. Peppermint may be described as "spicy" or "hot" and rejected in the same way as bitter tastes.

For selection of the most suitable flavour for a paediatric medication, the type of flavour (acid, alkaline, bitter, salty or sweet; see Table 9) as well the health condition of the target population have to be considered (Table 10).

Table 9. Flavour type.

Basic sensation	Flavours to cover this taste
Acid	Cherry, lemon, lime, mandarin, orange, strawberry
Alkaline	Banana, caramel, cherry, liquorice, passion fruit, peach
Bitter	Cherry, chocolate, grapefruit, liquorice, strawberry, peach, raspberry
Salty	Caramel, grapefruit, lemon, orange, vanilla
Sweet	Banana, caramel, cream, chocolate, grape, vanilla

Table 10. Flavour preference in Europe as a function of the disease of the target group.

Condition	Associated flavours
Pain, fever, allergy, infections	Cherry, strawberry, banana, caramel
Vitamin deficiency (Multivitamins)	Blackcurrant, lemon, lime, mandarin, orange
Indigestion (Antacids)	Lemon, lime, orange, peppermint

Quantitative evaluation of taste on the basis of analytical methods

The analytical method used is very similar to that used for the determination of drug release, and it is based on the detection of substances within a short period in aqueous medium. In general, it is used to measure the efficacy of coating and complexation within formulation. Taste masking is achieved when drug substance is not detected in 1-2 minutes or the detected amount is below the threshold for identify its bitter taste.

Quantitative evaluation of the taste using a taste sensor

The electronic taste sensor (electronic tongue) is able to detect taste in a manner similar to human gustatory sensation. Taste substances cause changes in electrical charge density of the lipid/polymer membrane surface and/or ion distribution near the surface of the membrane of the sensor. The total electric change is given as the response membrane electric potential for the substances tested. Each taste gives a different response in different membranes, which means that one membrane differs from the other one; the result is given as membrane potentials.

The assessment of the taste of a formulation depends on the evaluation of a standard (quinine hydrochloride or caffeine solution at different concentrations) or a reference (formulation containing only the active substances without any taste masking agents). This method is inexpensive and easy to conduct, useful of several formulations and dosage forms.

Qualitative evaluation of the taste by a taste panel

In order to conduct a suitable panel taste, the best population, pre-screened and considered the actual user has to be selected. Thus, children represent the best choice in the assessment of the taste of a paediatric formulation. In the case of selection of paediatric population, it is really important that the test is short, to match children's

attention span, and intrinsically motivating and fun to do. The procedure has to be easy to follow by children and the number of variants to be tasted should be limited at a maximum of four.

In general, children of 4 years and older are selected for the panel test, because younger children are often shy and reluctant. In addition, they can lose attention and they can have difficulties in concentrating during the entire period of the test, and then they are not able to express their feelings. It is appropriate to increase the attention and concentration of children, by using at first, high concentration of the taste agent, represented by common flavours, and then try to use uncommon flavours (for example from strawberry and cherry to passion fruit).

When a panel test is conducted, one of the most difficult thing to do is to determine the objective, what exactly should be determined. Thus, questions as 'which sample do you prefer?', or 'how much do you like it?' or 'what don't you like?' are often addressed to children. Ranking is a very useful method for the evaluation of preference and analytical assessment ('please rank samples in order of your personal preference', or 'please rank samples in increasing order of bitterness').

However, the questionnaire should be simple, intelligible and plain of all participants, and characterized by simple terms, common for each age groups, to describe the following properties:

- ✓ sweet, salty, sour and bitter characterizing the taste;
- ✓ thin, thick, viscous, gritty characterizing the tasting item;
- ✓ sweet, salty, sour, bitter but also numbness, astringent or freshness for aftertaste.

The evaluation of the taste of a medication is conducted by following two essentially different methods in the assessment of the children's preferences: the first is the spontaneous verbal judgement followed by scoring in a scale 1-5 (score 1 corresponds to very good, and score 5 corresponds to very bad). In the second method, the facial hedonic scale allows the expression of preference using the pictorial scale in Figure 1. The child is asked a standardised, specific question: 'which one of these figures do you think has tasted this medicine?', and is then asked to indicate the appropriate figure on the form (Sjovall et al., 1984).

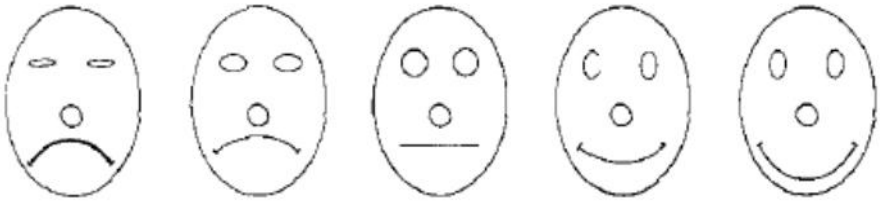

Figure 1. Hedonic scale used in a taste panel.

Children below 5-6 years are excluded from the panel tests, because they are not able to express differences in taste perception. A reliable evaluation could be achieved by child's spontaneous verbal judgement, following a control question. The facial hedonic scale can not be used in younger children, as they may associate the figures with other feelings (for example, happy face: I will not stay longer in the hospital, sad face: pain and discomfort). In general, older children judge more critically than younger ones, so they use both the verbal judgement and the hedonic facial scale method to evaluate the taste of a medication.

In all the cases, independent from the age or the method used, it is useful to ask simple questions at the end of the test, like: 'which formulation is the best?', and 'which formulation tasted worst?'

1.10.3. GENERAL TASTE MASKING TECHNOLOGIES IN ORAL PHARMACEUTICALS

Considerable progress has been achieved in the last few years in the development of bitterness, tasteless and taste-masked formulations. Several approaches are used in masking the unpleasant taste of some active substances: the use of excipients like flavours, sweeteners and amino acids; taste masking by polymer coating; by common granulation, spray congealing with lipids, inclusion complex with cyclodextrins, freeze-drying process, multiple emulsions, ion-exchange resins,with gelatine, gelatinized starch, liposomes, lecithin or lecithin-like substances, surfactants, salts and polymeric membranes (Coupland and Hayes, 2014, Kaushik and Dureja, 2013).

✓ *Taste masking with flavours, sweeteners and amino acids*

Adding flavours, sweeteners and amino acids represents the simplest technique to obtain the reduction of unpleasant taste of medications. However, it is not particularly

successful for highly bitter and highly water soluble substances. Artificial sweeteners and flavours are used along with other techniques in order to achieve the same purpose. For example, in the case of dentifrices and mouthwashes, the active and unpleasant ingredients, such as eucalyptus oil and benzethonium chloride are masked with fenchone, borneol, isoborneol, the cooling effects of taste masking agents, menthol stevia-base sweetener and glycine, and imitation flavours like grape, maple, raspberry and wild cherry.

Zinc acetate dihydrate present in different lozenges, is covered by the use of saccharin, anethol-β-cyclodextrin complex, magnesium stearate, polyethylene glycol and fructose. Aspirin is prepared with anesthetizing agents such as sodium phenolate, to numb the taste buds sufficiently for 4-5 seconds, rendering the taste of the aspirin imperceptible.

Another conventional technique consists in combining citric acid, sodium bicarbonate and different flavours: orange and cream to mask chlorpheniramine and phenylpropalamine HCl, lemon to mask famotidine and cherry to mask acetaminophen. Starch, lactose and mannitol are always used to mask caffeine.

Aspartame is a common excipient useful as a prominent sweetener in providing bitterness reduction. 0.8 % of aspartame in the formulation is sufficient to mask 25 % of acetaminophen. Artificial sweeteners such as neohesperidine dihydrochalcone and hesperidine dihydrochalcone 4'-b-D glucoside have the ability to mask bitterness and saltiness by virtue of their lingering sweetness. A lingering sweetness provides taste masking, primarily because the taste profile of a bitter substance appears later in time than normal sugar sweetness generally lasts (Sohi, 2004).

✓ *Taste making with lipophilic vehicles*

Lipids, oils, surfactants and polyalcohols increase the viscosity in the mouth, and cover the taste buds, acting as taste masking agents. For example, the taste of acetaminophen is covered when the granules are sprayed with molten stearyl stereate, mixed with suitable tablet excipients, and incorporated into a taste-masked, chewable tablet formulation. The taste of guaifenesin is improved when mixed with carnauba wax and magnesium aluminium silicate. Cimetidine has improved taste when granulated with glyceryl monostearate and gabapentin taste is enhanced whit hydrogenated soybean and glyceryl monostearate.

Lecithin and lecithin-like substances are considered good ingredients in taste-masking. Talampicillin HCl is known to have bitter taste; it is dissolved in or dispersed into an

organic solvent such as chloroform. Magnesium aluminium silicate and lecithin are added into the solution or dispersion in order to cover the unpleasant taste of the drug.

✓ *Taste masking by inclusion complexation*

β - cyclodextrin is the most common complexing agent in the formation of inclusion complex. It is a sweet, non toxic, cyclic oligosaccharide obtained from starch. In general, the substance characterized by bad taste fits into the cavity of β – cyclodextrin, through different types of interactions, especially Van Der Waals forces. The taste is masked thanks to two different mechanisms: by decreasing the oral solubility of the substances on ingestion in the new system, and by reducing the direct interaction of the substance with the taste buds.

The bitter taste of ibuprofen solutions is masked by preparing 1:11 and 1:15 complexes of the drug and hydroxypropyl - β – cyclodextrin. The complex covers the bitter taste of the drug but creates a sore taste, easily masked with sweeteners.

✓ *Taste masking by ion-exchange resins*

Ion-exchange resins are polymers characterized by high molecular weight, cationic and anionic functional groups and a network based on the copolymer styrene and divinylbezene. The interaction between the drug and the resins can occur thanks to a repeated exposure of the drug to the resin in a chromatogram column, or thanks to the prolonged interaction of the resin with the drug solution. The interaction is based on weak ionic bound that do not break in the salivary environment, giving the possibility to mask the bitter taste of the drug. Drug release from the resin depends on the properties of the resin and the characteristics of the environments of the gastrointestinal tract. Ion-exchange resins can be divided in four groups:

- strong acid cation-exchange resins;
- weak acid cation-exchange resins work at pH values above 6;
- strong base anion-exchange resins work to the entire pH range and can be used for the taste masking of acidic drugs;
- weak base anion-exchange resins work well at pH values below 7.

✓ *Taste masking by coating with hydrophylic vehicles*

This is the most feasible, common and easy way for taste masking purpose. The unpleasant taste of a substance can be covered by creating a physical barrier and by reducing the direct interaction with the taste buds. A specialized technique is applied to powders, chewable tablets and liquid suspensions.

✓ *Taste masking by coating with carbohydrates*

The unpleasant taste of drugs in paediatric formulations can be masked by coating with carbohydrates. In some cases, for example, paracetamol, ranitidine HCl, doxycycline HCl, pseudoephedrine HCl, sodium naproxene, aspirin and theophylline, the core element of the drug is covered by water insoluble polymer, such as cellulose, in order to obtain taste masking and reduced dissolution profiles. Granules with bitter taste can be masked and coated with water-soluble polymers of hydroxypropyl methyl cellulose and sugars such as sucrose and lactose to reduce the bitter taste at the time of administration. The bitter taste of basic pharmaceutical salts can be reduced or masked with weakly alkaline compounds of good bioavailability. Table 11 illustrates some examples of hydrophilic vehicles used to mask active substances.

Table 11. Hydrophilic vehicles to mask active substances.

Active substance	Hydrophilic vehicle
Pinaverium bromide	Mixture of cellulose or shellac and a second film-forming polymer soluble at pH less than 5
Propantheline bromide	Hydroxypropyl cellulose and ethyl cellulose
Ibuprofen	Methacrylic acid copolymer
Clorpheniramine maleate	Avicel PH 101, xylitol
Triprolidine HCl	Hydroxypropyl methyl cellulose
Dimenhydrinate	Methacrylic acid copolymer or carboxymethyl cellulose or starch
Enoxacin	hydroxypropyl cellulose and hydroxypropyl methylcellulose and then coated with a mixture of ethyl cellulose and hydroxypropyl methylcellulose
Aspirin	Cellulose acetate latex and triacetin at not more than 1 % of the coated medicament
Amoxicillin trihydrate	Microcrystalline cellulose, hydroxypropil cellulose
Acetaminophen	Cellulose acetate, cellulose acetate butyrate, and hydroxypropyl cellulose, or cellulose acetate, methacrylic acid copolymer, polyvinyl pyrrolidone
Morphine hydrochloride	Cellulose, methacrilic acid copolymer, talc, avicel RC591NF, sucrose, D-sorbitol, sodium saccharin, methyl paraben, vanilla essence

✓ *Taste masking by coating with protein, gelatine and prolamines*

Gelatine, and a number of proteins, first of all prolamines, are often use for the taste masking of antibiotics, vitamins, analgesics, dietary fibres, enzymes and hormones. Although prolamine coatings do not affect the release of the drug, zein and gliadin in combination with a plasticizer are able to mask the bitter taste and to control the release of the drug from encapsulated particles. Hydrolized gelatine has been found to be effective in improving the palatability of magaldrate and calcium carbonate. For mint-flavoured oral pharmaceutical gums, incorporating a prolamine/cellulose ingredient of high pH can reduce bitterness of flavour.

✓ *Taste masking by effervescent agents*

Effervescent agents are employed to mask unpleasant taste of substances in dosage forms that are not dissolved in water before the oral administration. Recently, effervescent tablets of fentanyl and prochlorperazine were developed for the administration of the drug in the oral cavity, for buccal, sublingual and gingival absorption. The effervescent

ingredient promotes the absorption and the taste masking to improve the palatability. The bitter taste of caffeine may be masked by formulating it as a carbonated oral solid preparation using sodium bicarbonate, ascorbic acid, citric acid, and tartaric acid.

✓ *Taste masking by rheological modifications*

The increase of the viscosity of the preparation through the addition of modifiers such as xanthan gum or carbohydrate, reduce the diffusion of the bitter substances into the taste buds. Thus, acetaminophen suspension can be formulated to address this purpose with xanthan gum and microcrystalline cellulose. The bitter taste of tannic acid is masked by gelatine and flavouring agents or adding sodium alginate to the aqueous solution.

✓ *Taste masking by salt preparation*

Another useful approach to mask the unpleasant taste of a substance consists in preparing salts, as in the case of aqueous solution of ibuprofen salts. The addition of sodium bicarbonate allows the increase of the palatability of the formulation.

✓ *Taste masking by solid dispersion systems*

The solid dispersion is defined as a dispersion of one or more substances in an inert solid carrier. Taste masking of bitter ingredients can be achieved by the preparation of solid dispersions with the addition of polymers, sugars and other suitable excipients. This is the case of the dimenhydrinate whose bitter taste is masked by a solid dispersion, with the addition of polyvinyl acetate phthalate.

✓ *Taste masking by freeze-drying process*

The high porosity derived from the freeze-drying process allows the preparation of tablet-shape dosage forms able to dissolve in the mouth in few seconds (Zydis technology). Gelatine and mannitol are the most common excipients used in this technology, although other suitable excipients can be used, such as starches and gums. The palatability of the formulation is achieved by the use of artificial sweeteners (aspartame) and flavouring agents. A number of different actives, characterized by bitter taste are masked with this technology: lorazepam, piroxicam, loperamide, loratadine, ondansetron and selegiline.

1.10.4. RECENT APPROACHES AND DEVELOPMENTS IN TASTE MASKING

Spray congealing is one of the recent approaches to achieve the taste masking of substances characterized by unpleasant taste. The palatability of conventional granules of clarithromycin is improved by using this technology and glyceryl monostearate and aminoalkyl methacrylate copolymer E (AMCE) as the most suitable ingredients (Yajima, 1996).

It has been reported that the preparation of microspheres and the compression technology may improve the palatability of Cefuroxime axetil and Pirenzepine HCl and Oxybutynin HCl (Robson et al., 2000, Ishikawa et al., 1999).

Lunstroth et al. (1999) formulated a carboxymethyl cellulose gel with lemon flavour to improve the taste of gut layer solution. The gel was quickly mixed with the lavage solution and showed improved palatability.

The taste masking of Indeloxazine HCl was achieved by heat treatment of wax coated microparticles. The coating was composed of hydrogenated oil and surfactants in a fluidized bed (Sugao et al., 1998).

It was also developed a taste-masked microcapsule composition for oral administration of a drug. The composition comprised microcapsules of drug and a substantially water-insoluble polymeric material, typically a cellulosic polymer (ethyl cellulose). Taste masking was done by phase separation coacervation technique in which the drug was coated with relatively high levels of a polymeric material (Hu et al., 2012).

A method for the taste masking of Diclofenac Sodium was developed by microencapsulation without interfering with an adequate rate of drug release. Diclofenac sodium microcapsules were successfully prepared and masked by using a system of ethyl cellulose-toluene-petroleum ether (Al-Omran et al., 2002).

In conclusion, taste masking of the bitter drugs and the improvement in preparing palatable and acceptable dosage forms have significantly increased the quality of treatment provided to patients, especially children. In the last decades, several approaches and technologies have been developed with the aim to respond to the need of taste masking. However, the applicability of these methodologies depends on the single drug and dosage form needed and a huge research is needed when a new formulation is in development.

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2. AIM OF THE WORK

Children around the world are still routinely being treated with medicines that have not been designed or developed with their own specific physiologies and needs in mind, putting them at risk of inaccurate and suboptimal dosing and side effects from potentially toxic ingredients.

In the view of these considerations, the aim of my research project was the use of specific formulation strategies for the development of innovative paediatric dosage forms.

The research has been structured in three case studies, concerning different oral formulations: mucoadhesive buccal films, dispersible granules and liquid complex suspensions.

The first part of the research was focused on the preparation and evaluation of mucoadhesive polymeric films for transmucosal delivery of ondansetron hydrochloride with the purpose to improve the conventional dosage forms already on the market, in term of ease of administration, dosing frequency and dosage flexibility. Moreover, buccal films have the advantages of allowing the reduction of the dose needed to achieve the therapeutic effect, through the direct access of the drug into the systemic circulation, avoiding the hepatic first pass metabolism. The selection of suitable polymeric mixtures permits the modulation of the residence time of the dosage form on the application site, the release of the active substance and the permeation of the drug through the buccal mucosa.

In the second part of the work, dispersible granules were produced, as a flexible dosage form for paediatric administration of Praziquantel, an anthelminthic drug, widely used in developing countries for the treatment of Schistosome infections. The novel dosage form is intended as an alternative to the conventional tablets, already on the market and unsuitable for paediatric administration due to the size and inadequate dose flexibility. In addition, the manipulation of the dosage form with milk and fruit juices, allows the taste masking and the enhancement of the palatability, leading to an increase of paediatric patient's compliance.

The last part of the work has been carried out at the University of Birmingham, under the supervision of Dr. Hannah Batchelor, member of EuPFI, European Paediatric Formulation Initiative and faced one of the central challenge of administering medicines to children: the taste masking of molecules characterized by bitter taste. To achieve this

purpose, polyelectrolyte complexes, composed of whey protein and pectin, and able to encapsulate active substances were developed. Caffeine was selected as model drug for its particularly bitter taste. The potential of encapsulation to mask bitter taste was evaluated by quantitative analytical method, through dissolution studies in biorelevant medium, simulating the environment of the mouth.

3. CASE STUDY 1

DESIGN AND EVALUATION OF BUCCAL FILMS AS PAEDIATRIC DOSAGE FORM FOR TRANSMUCOSAL DELIVERY OF ONDANSETRON

3.0. PAEDIATRIC CHALLENGE: ADMINISTRATION ROUTE

3.0.1. TRANSMUCOSAL ROUTE

Traditionally, drugs are administrated in children by oral and parenteral routes. Although the oral route is preferred for the administration of drugs, particularly those required for chronic therapies, it is often not feasible. The low bioavailability of drugs administered through this route is largely due to a number of physical and physiological factors, such as chemical and enzymatic degradation in the gastrointestinal tract, low permeability across the gastrointestinal mucosa (Pauletti et al., 1997), and the hepatic first pass metabolism and clearance, predominantly by the liver but also by the gut mucosa. In addition, oral route is often not indicated in patients with nausea, sedated or unable to swallow. On the other hand, parenteral administration avoids the degradation of the active ingredients in the gastrointestinal environment, and the hepatic first pass metabolism, but it may be cause of pain and discomfort during the injection, leading to poor compliance by paediatric patients, especially if multiple daily injections are required (Hinchcliffe et al., 1999). Risk of infusion of air, microorganisms and pyrogens, together with the risk of sepsis and phlebitis are also associated with parenteral administration of drugs.

Consequently, other routes are being considered as alternatives to oral and parenteral ones and potential sites for drugs administration include the nasal, rectal, vaginal, ocular and oral mucosae (Patel et al., 2011). These transmucosal routes offer several advantages over the oral administration, such as the avoidance of the first pass metabolism and the pre-systemic elimination by the gastrointestinal tract.

In particular, drug delivery by buccal route has gained success compared to the parenteral administration, due to its potential for the high patient compliance. This is particularly desirable during an emergency, when a rapid therapeutic response is required, or in a case of patient's unconsciousness, when swallowing is impaired. Medications can be easily administered by parents and carers without special professional skills and techniques, although the cooperation of the child is often necessary (Lam et al., 2014).

Within the oral mucosal cavity, the delivery of drugs is classified in two main categories: (i) local delivery and (ii) systemic delivery by either buccal or sublingual mucosa (Patel et al., 2011). In the first case, the aim is to achieve a site-specific release of the drug on the mucosa, while the second case involves drug absorption through the mucosal barrier

to reach the systemic circulation (Rossi et al., 2005). Generally, the sublingual route is employed for high permeable drugs and used in the treatment of acute disorders, whereas the buccal route is generally used in the treatment of chronic disorders, when a controlled release is required (Sangeetha et al., 2010).

The main challenges associated with the administration of active substances through the buccal mucosa, are related to the limited absorption area and to the barrier properties of the mucosa. There are two different permeation pathways for passive drug transport across the oral mucosa: paracellular and transcellulare routes. Drugs may traverse these two pathways simultaneously, but in general, one pathway is preferred respect to the other, depending on the physicochemical properties of the active substance. Hydrophilic compounds are more soluble in the intercellular space, because it has a less lipophilic character, while the cell membrane is more lipophilic and thus, is more useful for the permeation of the lipophilic compounds (Sharma et al., 2013).

In this contest, buccal films represent the most recently developed dosage form for buccal administration. They have gained success as efficacious and novel drug delivery system, especially for the treatment of paediatric patients (Madhavi et al., 2013). Buccal films are dosage forms based on hydrophylic polymers and can be formulated to achieve both local and systemic action; in this latter case, the active substance loaded in this dosage form has direct access to the systemic circulation by the jugular vein, avoiding the hepatic first pass metabolism leading to high bioavailability.

Potential benefits of buccal films involve:

- ease of administration in paediatric patients: no need of chewing or swallowing, no risk of chocking;
- good mouth feel and possibility of taste masking;
- accurate dosing;
- increase of the systemic bioavailability of the drug, by avoiding the hepatic first pass metabolism and by extending the residence time of the dosage form at the site of absorption;
- rapid onset of action and minimum side effects.

3.1. INTRODUCTION

The extensive changes into the regulatory environment for paediatric medicines, designed to better protect the health of children, have stimulated the research into child-appropriate dosage forms. These dosage forms should satisfy important requisites: easy administration, possibility of weight-based dosing and dose titration, acceptability and palatability, and finally minimum dosing frequency. Moreover, excipients should be safe in the target age group (EMA/CHMP/QWP/805880/2012; Ernest et al., 2007; Strickley et al., 2008; World Health Organization, 2012).

One approach in the process of implementation and innovation of paediatric dosage forms for young children is represented by the use of buccal films for transmucosal administration of drug (Borges et al., 2015). Buccal films are relatively new dosage form intended to deliver drug substances through the oral mucosa directly onto the systemic circulation, avoiding the hepatic first pass metabolism and similarly, the drug degradation along the gastrointestinal tract, thus allowing the reduction of the dose necessary to achieve the therapeutic action. Compared to conventional buccal tablet formulation, they are thin, flexible and better adaptable to the mucosal surface, and therefore more acceptable to younger patients. Moreover, buccal films are safe and convenient unit dosage systems since they can be easily applied or removed from the application site, even during a state of patient unconsciousness or when swallowing is impaired (Dixit and Puthli, 2009; Lam et al., 2014; Patel et al., 2011).

From the technological point of view, buccal films are matrices fabricated using mucoadhesive and film forming polymers and loaded with the active ingredient(s). The use of mucoadhesive polymers is essential to maintain an intimate and prolonged contact of the formulation with the oral mucosa allowing a longer duration of absorption (Sudhakar et al., 2006). Polymers that are commonly used in the development of buccal films include cellulose derivatives, chitosan, gelatin, hyaluronic acid, carrageenan, pectin, sodium alginate and poly(acrilic acid)-based polymers (Salamat-Miller et al., 2005).

Effective design of such delivery system requires careful consideration of other relevant parameters, including the choice of the active substance (World Health Organization, 2010; World Health Organization, 2015). These involve good lipophilicity and water solubility at physiological pH, as well as high potency. Ondansetron (ODS), a selective inhibitor of serotonin (5-hydroxytryptamine) subtype 3 (5-HT_3) receptors indicated in

paediatrics for the prevention and treatment of nausea and vomiting caused by cytotoxic chemotherapy or radiotherapy and postoperatively, represent a suitable candidate for buccal delivery (octanol/water log P at pH 7.4: 2.4, water solubility at pH 7.4: 2.42 mg/ml, small molecular size: 365.9 Da) (Lam et al., 2014; Mashru et al., 2005; Patel et al., 2011). ODS is commercially available as injection, oral liquid and solid oral dosage form. All these formulations are indicated for administration in multiple daily dosing, potentially for a series of days (recommended oral maintenance dose for children of 4-11 years: 4 mg every 4-8 hours). This is due to the pharmacokinetic profile of ondansetron, which has a half-life of approximately 3-6 hours and with a time to peak plasma levels of approximately 2 hours. This profile is often associated with alternating periods of increased side effects and lacking efficacy and therefore, there is a need to develop sustained release formulations able to maintain a constant drug concentration for a specific period of time with minimum side effects (Koland et al., 2011; Kumria et al., 2013; Patil et al., 2015; Park 2012).

The objective of this study was to: (1) implement paediatric dosage forms for young children with buccal films intended for ODS systemic absorption through the buccal mucosa over a prolonged period of time; (2) prepare mucoadhesive films based on non-toxic, biocompatible and hydrophilic polymers as hydroxypropylmethylcellulose (HPMC), chitosan (CH), sodium hyaluronate (HA) and gelatin (GEL), and by using an easy and economic method as solvent casting method; (3) investigate the influence of preparative parameters on the physico-chemical properties of drug/dosage form; (4) study the influence of polymeric composition (different polymer blends and different weight ratio) on the drug loading, mucoadhesion potential, water uptake properties, and drug release and permeation ability.

3.2. MATERIALS AND METHODS

3.2.1. MATERIALS

Hydroxypropylmethylcellulose (MW 250 kDa, methoxyl content 19-24 %, hydroxypropyl content 7-12 %) was purchased from Eigenmann & Veronelli (Milan, Italy); chitosan (MW 150 kDa, deacetylation degree 97 %, pKa = 6.3) was commercially obtained from Fluka (Milan, Italy); sodium hyaluronate (MW 1800-2300 kDa, D-glucuronic acid > 42 %) was provided by ACEF (Piacenza, Italy); type B Gelatin from bovine skin (MW 50 kDa, isoelectric point in the range of pH = 4.7-5.2) and ondansetron hydrochloride (MW 365.85 g/mol) were commercially obtained from Sigma-Aldrich (USA). All other chemicals and solvents were of analytical grade and supplied by Carlo Erba (Milan, Italy). Release and permeation studies were conducted in NaCl solution (0.9 % w/v); mucoadhesion studies were carried out in aqueous buffer with the follow composition (g/L): 4.609 KH_2PO_4, 16.748 Na_2HPO_4 x $12H_2O$ adjusted with hydrochloric acid to pH = 6.8 (healthy saliva pH = 6.7 - 7.4).

3.2.2. METHODS

3.2.2.1. PREPARATION OF BUCCAL FILMS

Buccal films were prepared by casting-solvent evaporation method. An aqueous solution of GEL, an aqueous solution of HA and an acid solution (acetic acid 1 % v/v) of CH were separately added to an aqueous solution of HPMC at different weight ratios (10:0, 9:1, 7:3, 5:5, 0:10 HPMC:GEL or HPMC:HA or HPMC:CH), in order to obtain 1 % w/w polymeric mixtures. All mixtures were stirred at room temperature for 2 hours and allowed to stand overnight to eliminate the air bubbles. 15 g of each polymeric solution were spread on a Petri-dish (diameter = 5 cm) and oven-dried at 50 °C for 6 hours (heating oven FD series, Binder, Tuttlingen, Germany). Loaded films were prepared by the same procedure, adding to each mixture 17.45 mg of ODS. Circles of 1.3 cm in diameter (surface area = 1.33 cm^2) were cut to obtain a child-appropriate dosage form and were used in the following experiments. Each circle contains theoretically 1.18 mg of drug.

Different films were named in this work as follows: HPMC:CH(HA,GEL) 10:0, loaded film based on HPMC; HPMC:CH(HA,GEL) 0:10 loaded films based on CH(HA, GEL); HPMC:CH(HA,GEL) 9:1, HPMC:CH(HA,GEL) 7:3, HPMC:CH(HA,GEL) 5:5 loaded films based on HPMC:CH(HA,GEL) 9:1 (w/w), 7:3 (w/w), and 5:5 (w/w), respectively.

Table 12. Composition of the mixtures used for loaded film preparation (% w/w on wet basis).

FORMULATION	HPMC	CH	HA	GEL
HPMC:CH(HA,GEL) 10:0	1.0			
HPMC:CH 9:1	0.9	0.1		
HPMC:CH 7:3	0.7	0.3		
HPMC:CH 5:5	0.5	0.5		
HPMC:CH 0:10		1.0		
HPMC:HA 9:1	0.9		0.1	
HPMC:HA 7:3	0.7		0.3	
HPMC:HA 5:5	0.5		0.5	
HPMC:HA 0:10			1.0	
HPMC:GEL 9:1	0.9			0.1
HPMC:GEL 7:3	0.7			0.3
HPMC:GEL 5:5	0.5			0.5
HPMC:GEL 0:10				1.0

3.2.2.2. SOLUTION VISCOSITY

The viscosity of the polymeric solutions used for the preparation of buccal films was measured at room temperature with an Ubbelohde capillary viscometer equipped with an electronic time-measuring unit ViscoClock (capillary tubes I and II; Schott, Mainz, Germany) for CH and GEL solutions (1 % w/w) and with a rotational viscometer (spindle TR8-TR9, RPM 60-200; Visco Star, Fungilab S.A., Barcelona, Spain) for all the others.

3.2.2.3. CHARACTERIZATION OF BUCCAL FILMS

Thickness

Thickness of the polymeric loaded films was determined at three different positions of each film using a Mitutoyo pocket thickess gauge (Mitutoyo Mfc. Co. Ltd., Tokyo, Japan).

Scanning electron microscopy (SEM)

SEM analysis was performed to evaluate the morphologic characteristics. Films were cut with a razor blade, fixed on supports and coated with gold–palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with LEO 420 (LEO Electron Microscopy Ltd., Cambridge, UK) using secondary electron imaging at 15 kV in order to examine their surface morphology and their internal structure.

Drug content

The uniformity of the distribution of the drug in the film was ensured with a content uniformity test. Loaded buccal films have been dissolved in 20 ml of 0.9 % (w/v) NaCl solution. The system was stirred for 2 hours until complete release and the amount of drug in solution was evaluated. The results were expressed as milligrams of drug for square centimetre (mg/cm^2).

In these tests as well as in subsequent experiments the ODS concentration was determined by HPLC equipped with a UV detector. The HPLC system consisted of Shimadzu (Milan, Italy) LC-10ATVP chromatographic pump and a Shimadzu SPD-10AVP UV-Vis detector set at 310 nm. Separation was obtained at room temperature on a Phenomenex (Torrance, CA, USA) Sinergy Fusion-RP 80A (150 mm x 4.6 mm I.D., 5 µm) coupled with a Phenomenex (Torrance, CA, USA) SecurityGuard C18 guard cartridge (4 mm x 3.0 mm I.D., 5 µm). The mobile phase was prepared by mixing acetonitrile (33 % v/v) and 20 mM sodium hydrogen phosphate buffer pH = 4.0 (67 % v/v). The flow rate was 0.4 mL/min and manual injections were made using a Rheodyne 7125 injector with a 20 µL sample loop. Data processing was handled by means of a CromatoPlus computerised integration system (Shimadzu Italia, Milan, Italy). The calibration curve of concentration versus peak area was plotted at concentration range of 0.24 - 24 µg/mL; good linearity was found ($r^2 = 0.9997$).

Surface pH

The surface pH of loaded buccal films was determined in order to evaluate their compatibility with the pH of buccal mucosa. The films were left to swell on a sponge soaked with phosphate buffer (pH = 6.8) and the pH was measured after 3 hours by placing universal pH paper on the film surface (pH: 6.0-8.1; Carlo Erba, Milan, Italy).

3.2.2.4. PHYSICOCHEMICAL CHARACTERIZATION OF BUCCAL FILMS

Differential scanning calorimetry (DSC) and X-Ray Powder Diffraction (XRPD) experiments were performed on loaded polymeric films to identify the solid-state properties of the drug in the formulation and possible phase transitions of the drug during the film formulation process.

The DSC analysis were performed using a Perkin-Elmer DSC 6 (Waltham, USA). The experiments were conducted in non-hermetically sealed aluminum pans using nitrogen as purge gas at a flow rate of 20 ml/min. Samples of 8.0 ± 1.0 mg were heated from 30 to 220 °C at the heating rate of 10 °C/min.

The XRPD analysis was performed using a Panalytical X'Pert PRO Diffractometer (Almelo, The Netherlands). The voltage and current were 40 kV and 40 mA, respectively and the measurement were carried out in the angular scan rage from 3° to 40° (2θ).

3.2.2.5. *IN VITRO* WATER-UPTAKE STUDIES

Water uptake ability was studied to investigate the maximum time required for films to hydrate and the maximum capacity of swelling. A sponge (5 cm × 5 cm × 2 cm) fully soaked in the hydration medium (0.9 % NaCl solution) was placed in a glass container filled with the same solution to a height of 0.5 cm (Bertram and Bodmeier, 2012). Filter paper was also soaked in the hydration medium and positioned on the top of the sponge. The experimental set-up was equilibrated for 30 minutes. Accurately weighted films (unloaded samples) were then placed on the filter paper and the water-uptake ability was determined as weight increase of the film after 3 hours, according to the following equation:

$$\% \text{ Water Uptake (WU)} = (W_2 - W_1) \times 100 / W_1$$

where W_1 was the initial weight of dried film and W_2 is the weight of hydrated film.

3.2.2.6. *IN VITRO* RESIDENCE TIME

Mucoadhesion properties of unloaded buccal films were determined in terms of residence time of films on a freshly excised mucosa. The porcine buccal mucosa, procured from a local slaughter house and used as a biological membrane due to its similarity to the human buccal tissue, was cut to an appropriate size (surface area = 1.54 cm^2), wetted with few drops of aqueous mucin solution (0.05 % w/v) and fixed on a microscope slide with cyanoacrylate adhesive. The films were then attached to the porcine buccal mucosa

by applying a light pressure for 2 min. The microscope slide was then placed in a beaker filled with 40 ml of phosphate buffer pH = 6.8 and slowly stirred to mimic the physiological conditions. The time taken by the films to completely detach from the mucosa was considered as the residence time (Nair et al., 2013).

3.2.2.7. *IN VITRO* RELEASE STUDIES

In vitro release studies were performed in order to evaluate the drug amount released from films over the time. Loaded films were attached on the internal side of a beaker containing 40 ml of 0.9 % (w/v) NaCl solution. The system was stirred at 50 rpm and maintained at 37 °C to simulate the physiological conditions. Samples of 500 µl were withdrawn at predetermined time intervals and replaced by fresh medium. The experiment were conducted for 5 hours and all samples were analized by HPLC analysis. The results of the release experiments are shown as cumulative drug amount released (expressed as fractional amount) plotted as a function of time.

3.2.2.8. *IN VITRO* PERMEATION STUDIES

In vitro permeation studies were performed in order to evaluate transmucosal absorption of drug from buccal films. These studies were made through a buccal porcine mucosa using Franz-type static glass diffusion cells (15 mm jacketed cell with a flat ground joint and clear glass with a 12 mL receptor volume, diffusion surface area: 1.77 cm^2) and equipped with a VSA stirrer (PermeGear Inc., Hellertown, Pennsylvania, USA). The buccal mucosa was excised using a surgical blade and immediately located in the donor chamber. Loaded films were placed on the top of the porcine mucosa. The receptor compartment was filled with 12 ml of 0.9 % (w/v) NaCl solution maintained at 37 °C by means of a surrounding jacket and has been continuously stirred. Samples of 100 µl were withdrawn from the receptor compartment at predetermined time intervals and replaced by fresh medium. Sink conditions were maintained at any time. The experiment was conducted for 6 hours and all samples were analyzed by HPLC analysis. The results of permeation studies are shown as cumulative drug amount permeated (expressed as fractional amount) versus time.

3.2.2.9. STATISTICAL ANALYSIS

All experiments were done in triplicate, while transport experiments were done with five replicas. Results are expressed as mean ± SD. Anova and t-test were used to determine

statistical significance of studies. The criterion for statistical significance was $p < 0.05$.

3.3. RESULTS AND DISCUSSION

The development of a suitable dosage form for paediatric patients still remains a challenge. An ideal paediatric formulation must allow accurate dose administration and be in a dosage form that can be safely handled by the target age group. Polymeric buccal films offer an exact and flexible dose and ease of handling; they also allow the direct access of the active into the systemic circulation avoiding the first-pass metabolism and thus reducing the dose needed.

3.3.1. SOLUTION VISCOSITY

Casting-solvent evaporation method was employed to prepare buccal films, using non-toxic and non-irritant polymers, such as HPMC, CH, HA and GEL, thus suitable for the administration in children.

This method is based on the dissolution of the polymers in appropriate solvents (distilled water or acetic acid 1% v/v), and on the subsequent mixture of polymer solutions in order to obtain the desired polymer weight ratio. All the final solutions had the same total polymeric concentration (1% w/w), but they showed different viscosities. As reported in Figure 1, single polymer solutions had viscosities of 250 ± 18, 38.77 ± 1.60, 1150 ± 101 and 3.20 ± 0.08 mPa × sec for HPMC, CH, HA and GEL, respectively. Furthermore, as regards the mixtures, the addition of increasing amount of HA to the HPMC solution, proportionally increased the solution viscosity, while increasing amount of CH and GEL decreased the viscosity of the HPMC solution. This behaviour is chiefly related to the different molecular weight of the polymers used for the preparation of the films. In fact, HA shows the highest molecular weight with respect to HPMC, CH and GEL. The presence of the active into the solutions did not affect the solution viscosity (data not shown).

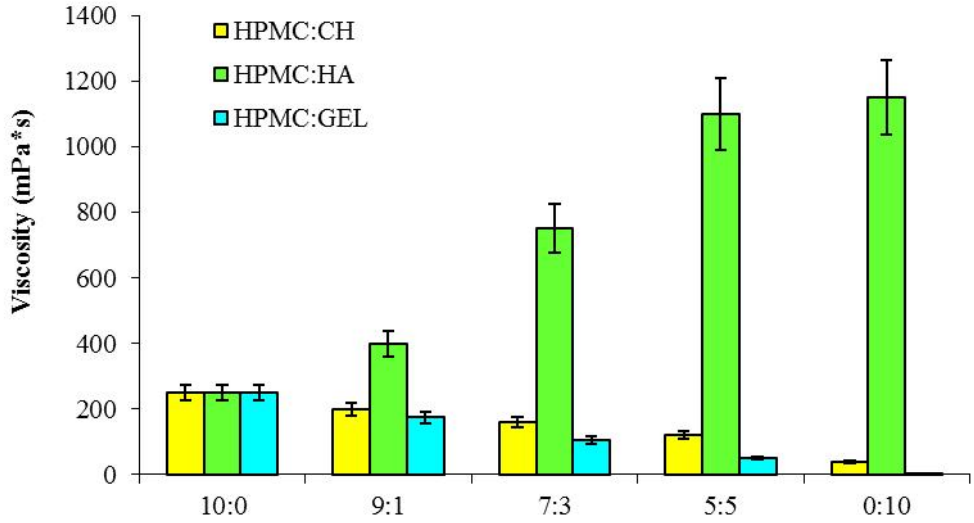


Figure 2. Viscosity of the solutions used for the preparation of unloaded buccal films.

3.3.2. CHARACTERIZATION OF BUCCAL FILMS

SEM analysis in Figure 2 showed that HPMC:CH 5:5 and HPMC:GEL 5:5 exhibited a dense and compact cross-section, while HPMC:HA had a heterogeneous structure characterized by flakes.

The results related to the measurement of thickness and drug content of buccal films are reported in Table 1. Film thickness is directly related to the accuracy of dose and the very low standard deviations suggested a uniform thickness all around the dosage form. HPMC:CH(HA,GEL) 10:0 demonstrated the maximum thickness, while the other films had a different thickness related to the different composition. In addition, the measurement of ondansetron hydrochloride content in the dosage form showed that the drug was uniformly distributed inside the films; the experimental drug content was very close to the theoretical one (0.9 mg/cm^2) for each formulation, indicating that casting-solvent evaporation method is a suitable technique to produce polymeric buccal films containing ondansetron.

The film surface pH was measured to investigate the possibility of any side effects due to acidic or alkaline pH of films that could hurt the buccal mucosa leading to patient discomfort (Nair et al., 2013). The surface pH of all prepared films was found near the neutral pH indicating its compatibility with buccal pH, causing no irritation to the mucosa and achieving patient compliance.

Table 13. Characterisation of buccal films: film thickness (µm), drug content (mg/cm^2) and water uptake ability (%).

FORMULATION	Film Tickness (µm)	Drug content (mg/cm^2)	Maximum WU (%)
HPMC:CH(HA,GEL) 10:0	107 ± 6	1.03 ± 0.21	1246.46 ± 38.23
HPMC:CH 9:1	63 ± 6	1.05 ± 0.13	1862.66 ± 60.50
HPMC:CH 7:3	57 ± 6	1.02 ± 0.11	2000.02 ± 110.55
HPMC:CH 5:5	53 ± 12	1.05 ± 0.14	1934.25 ± 60.20
HPMC:CH 0:10	63 ± 1	1.15 ± 0.20	2767.78 ± 90.54
HPMC:HA 9:1	73 ± 12	0.98 ± 0.10	1713.68 ± 88.57
HPMC:HA 7:3	67 ± 6	0.91 ± 0.15	5001.29 ± 210.43
HPMC:HA 5:5	69 ± 6	0.95 ± 0.08	4933.55 ± 180.40
HPMC:HA 0:10	44 ± 6	0.82 ± 0.15	5208.63 ± 225.34
HPMC:GEL 9:1	93 ± 2	1.03 ± 0.01	1305.12 ± 42.20
HPMC:GEL 7:3	96 ± 3	0.87 ± 0.15	1249.03 ± 79.92
HPMC:GEL 5:5	101 ± 3	0.90 ± 0.01	1182.97 ± 67.91
HPMC:GEL 0:10	76 ± 2	0.98 ± 0.05	1371.65 ± 84.86

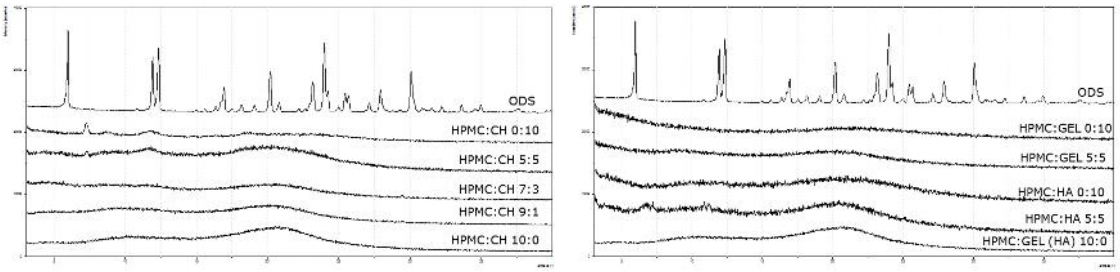
3.3.3. PHYSICOCHEMICAL CHARACTERIZATION OF BUCCAL FILMS

In order to evaluate possible phase transitions of the active during the film formulation process, differential scanning calorimetry and X-ray powder diffraction were used.

The DSC profiles in Figure 3 showed a single endothermic peak at 187.54 °C, in agreement with the melting point of ondansetron hydrochloride raw material and a large dehydration process between 50-120 °C. The thermograms of all films presented a large endothermic profile around 60-120 °C related to the dehydration of polymers. Conversely, the melting peak of the active was absent in the DSC profiles of all loaded films, except for HPMC:CH 0:10 and HPMC:CH 5:5. This means that in almost all the cases casting-solvent evaporation method induced the amorphization of the active, while in HPMC:CH 0:10 and HPMC:CH 5:5 part of it remained as crystalline material.

The same results were confirmed by the XRPD analysis. The diffractrograms of the loaded films did not report the characteristic peaks of ondansetron hydrochloride raw material (2θ values of 8.26°, 13.28°, 16.84°, 20.20°, 23.96°, 24.36°, 25.72°, 27.88°, 30.84°) (Pattnaik et al., 2011), indicating an amorphous profile of all the films, except for HPMC:CH 0:10 and HPMC:CH 5:5. In fact, these films exhibited XRPD patterns characterized by a peak of low intensity at about 7 °2θ, probably related to a crystalline form of the active.

(a)



(b)

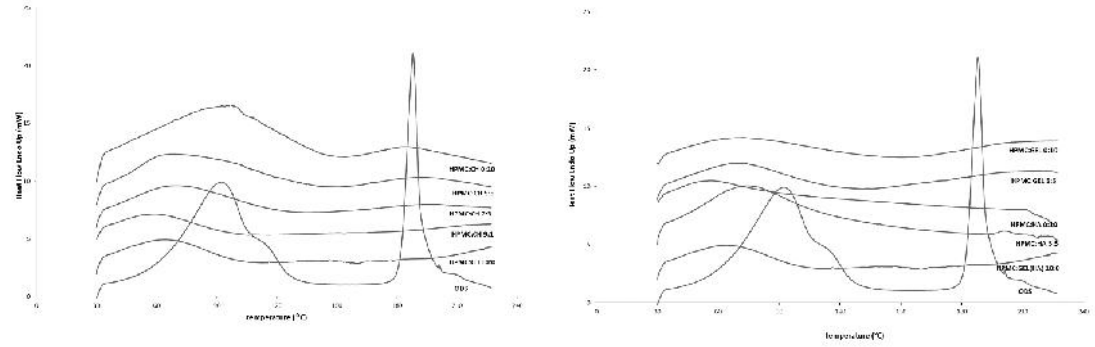


Figure 3. Physicochemical characterization of loaded buccal films: (a) XRPD patterns of HPMC:CH (all the mixtures) and HPMC:HA(GEL) (all the most significant mixtures), respect to pure ODS; (b) DSC profiles of HPMC:CH (all the mixtures) and HPMC:HA(GEL) (all the most significant mixtures), respect to pure ODS.

3.3.4. *IN VITRO* WATER UPTAKE STUDIES

In vitro water-uptake values after 80 min are reported in Table 1. The presence of HA and CH in the polymeric mixtures increased the water-uptake ability of unloaded HPMC:CH(HA,GEL)10:0. In particular, the increase of the hydration capacity was more evident for unloaded HPMC:HA, respect to unloaded HPMC:CH. When GEL was introduced in the polymeric mixtures, instead, it did not affect the hydration ability of unloaded HPMC:CH(HA,GEL) 10:0. This behaviour can be mostly related to the different polymeric charge density. In fact, in our operative conditions (0.9 % w/v sodium chloride solution at pH = 6.3) HPMC resulted completely neutral, HA (pKa = 2.9) resulted negatively charged with all its carboxylic group deprotonated, CH (pKa = 6.3) showed positive charge with 50 % of neutral amine groups and 50 % of protonated amine

groups and GEL (isoelectric point in the range of 4.7-5.2) was slightly negatively charged. In particular, the highest charge density (HA in our case) allows the highest entrance of water in the system and the highest hydration of the film, thus permitting the formation of gels with different viscosities.

3.3.5. *IN VITRO* RESIDENCE TIME

Once administered into the oral cavity, the films have to hydrate, adhering to the buccal mucosa, and forming a gel in order to allow an extended drug delivery. As shown in Figure 4, unloaded HPMC:CH(GEL,HA) 10:0 demonstrated the highest residence time (1320 min). HPMC is a long chained, non-ionic polymer and its mucoadhesion ability is chiefly attributable to the interpenetration and entanglement of polymer chains into the mucus layer. Furthermore, it possesses a large number of hydrophilic groups that are able to form hydrogen bonds between the hydrophilic groups of mucus (Bertram and Bodmeier, 2006). It has been reported that a minimum polymer molecular weight of 100 kDa is required for mucoadhesion (Lee et al., 2000). In our studies GEL has the minimum molecular weight (50 kDa), and unloaded HPMC:GEL 5:5 and unloaded HPMC:GEL 0:10 demonstrated the minimum residence time (120 and 28 min, respectively). All the other polymers have molecular weights higher than 100 kDa, even if the adding of CH and HA to unloaded HPMC:CH(GEL,HA) 10:0 did not increase its mucoadhesion properties. Unloaded HPMC:HA 5:5 and HPMC:HA 0:10 showed a residence time of 760 and 360 min respectively, a period of time sufficient to allow a prolonged delivery of ODS. The high molecular weight of HA (1800-2300 kDa) and its charge density at pH = 6.3 permit to attract and absorb water, associated with a consequent high hydration, a formation of a viscous gel and an intimate contact with the layer of the mucus. HA also presents numerous hydrophilic groups able to form hydrogen bonding with the mucus glycoproteins. Unless CH has positively charged amino groups that can electrostatically interact with the negatively charged sialic acid of mucin, it has less charged groups at pH = 6.3 than HA, allowing a lower hydration and thus residence time (420 and 37 min for unloaded HPMC:CH 5:5 and HPMC:CH 0:10, respectively).

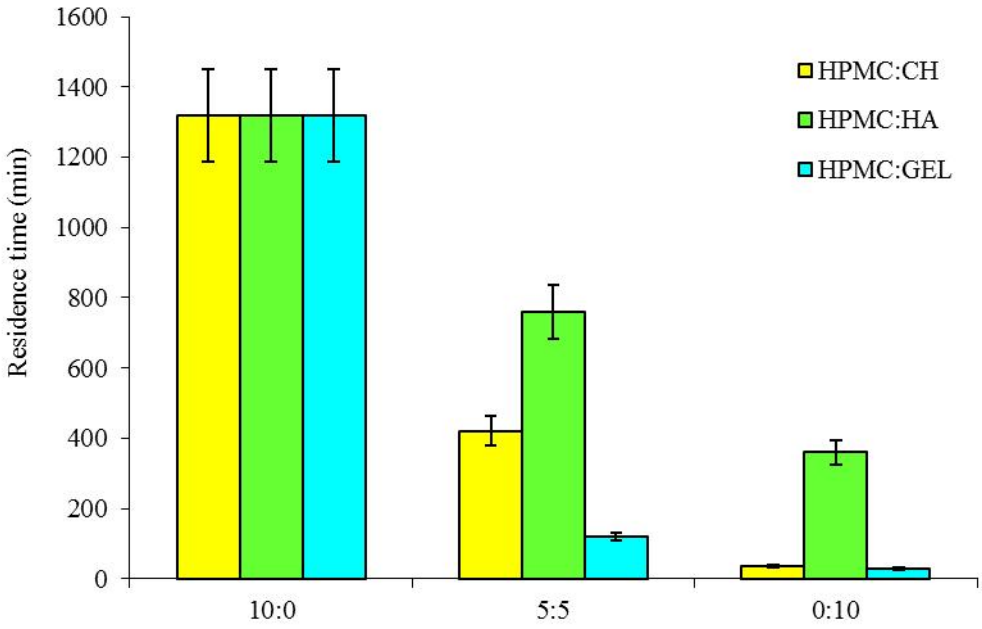


Figure 4. Residence time of unloaded buccal films on porcine buccal mucosa.

3.3.6. *IN VITRO* RELEASE STUDY

Drug release from gelled matrices is a complex phenomenon of water penetration, relaxation of the polymer chains, swelling and spreading of the matrix, interactions between drug and polymeric material, and drug dissolution and diffusion through the rehydrated matrix. The release of ODS from HPMC:CH, HPMC:GEL and HPMC:HA films were investigated; in particular, Figure 5 shows the release profiles of HPMC:CH(GEL,HA) 10:0, HPMC:CH 5:5, HPMC:GEL 5:5 and HPMC:HA 5:5, as representative formulations of the three different series. All the formulations exhibited a prolonged release of the drug. Moreover, HPMC:CH(GEL,HA) 10:0 and HPMC:CH 5:5 released the maximum amount of the drug within 45 minutes, while HPMC:HA 5:5 and HPMC:GEL 5:5 showed the maximum release of ODS after 120 minutes.

The inclusion of CH and GEL in the formulation allowed a higher cumulative amounts of ODS released from the dosage form, rather than the inclusion of HA. As described above, HPMC:HA 5:5 showed the highest molecular weight and the greatest hydration ability due to the high charge density at pH = 6.3; this permitted higher viscosity of the polymeric network in the gelled state, thus limiting the drug diffusion. HPMC:CH 5:5 and HPMC:GEL 5:5, once hydrated, created a less viscous gelled state, allowing a greater release of ODS from the dosage form.

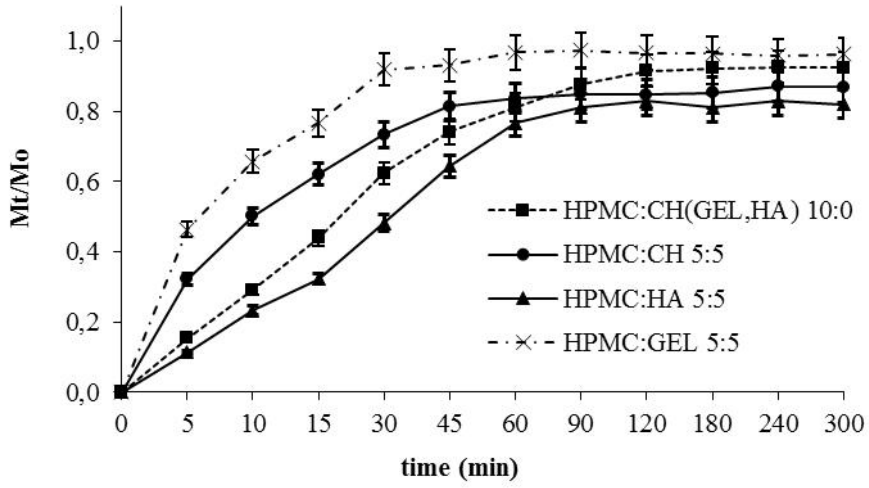


Figure 5. *In vitro* release profile of ondansetron hydrochloride from HPMC:CH(GEL,HA)10:0, HPMC:CH 5:5, HPMC:GEL 5:5 and HPMC:HA 5:5.

3.3.7. *IN VITRO* PERMEATION STUDIES

In vitro permeation studies were performed in order to establish the absorption of the drug across the buccal epithelium to the systemic circulation. Even in this case HPMC:CH(GEL,HA) 0:10, HPMC:CH 5:5, HPMC:GEL 5:5 and HPMC:HA 5:5 were chosen for the permeation studies as representative of the three different series (Figure 6). All the formulations demonstrated a sustained permeation of the drug within 6 hours. In particular the presence of HA in HPMC:HA 5:5 (J = 18.7 ± 2.5 μg/cm^2 h) did not improve the permeation ability of HPMC:CH(GEL,HA)10:0 (J = 23.9 ± 3.3 μg/cm^2 h), while both HPMC:CH 5:5 (J = 87.6 ± 14.4 μg/cm^2 h) and HPMC:GEL 5:5 (J = 99.6 ± 18.1 μg/cm^2 h) provided higher permeated drug amount at each time respect to HPMC:CH(GEL,HA) 10:0. This behaviour is in agreement with the release profiles: the more amount of drug released from the dosage form, the more absorption inside the buccal mucosa. Moreover, since chitosan is believed to interfere with lipid micelle organization in the intestine, Senel et al. (2000) explained that a possible mechanism of action of chitosan in improving the transport of drug across the buccal mucosa is the ability of interfering with the lipid organization in the buccal epithelium.

As concern the practical use of these formulations, the recommended oral maintenance dose for children of 4-11 years is 4 mg every 4-8 hours. This dosage can be achieved by use of film with a surface area of 7.7 cm^2, 9.9 cm^2, 1.9 cm^2 and 2.1 cm^2 for HPMC:CH(GEL,HA)10:0, HPMC:HA 5:5, HPMC:GEL 5:5 and HPMC:CH 5:5,

respectively. The surface area of the film was calculated according to the following equation: Css = J·A/Cl, where Css is the concentration at the steady state (39.5 ng/ml) (Simpson et al., 1992), Cl is the ondansetron clearance (0.39 L/h/Kg) (Spahr-Schopfer, 1995) and J is the permeation flux of film.

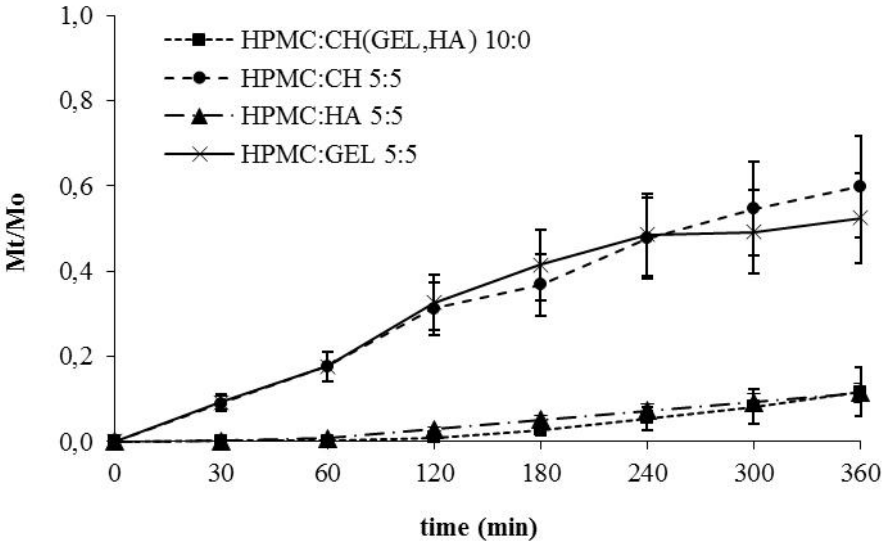


Figure 6. *In vitro* permeation profiles of HPMC:CH(GEL,HA) 10:0, HPMC:CH 5:5, HPMC:GEL 5:5 and HPMC:HA 5:5.

3.4. CONCLUSION

With polymeric buccal films, a novel solid oral dosage form was developed, fulfilling all current demands for child-appropriate dosage forms. HPMC mixtures with HA, GEL and CH can be used as materials to develop sustained release films able to allow minimal dosage and frequency, and characterized by minimal impact on lifestyle, and easy and reliable administration. The selection of suitable polymeric mixture and appropriate weight ratio allowed the modulation of the residence time of the dosage form on the application site, the release of the drug and its permeation through the buccal mucosa.

Further studies are in progress to optimize ODS release/permeation from buccal films and to improve organoleptic characteristics of the dosage form. In particular, we are applying a second film layer onto a first one to achieve unidirectional release towards the oral mucosa, avoiding drug release in the oral cavity and covering the ODS bitter taste.

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4. CASE STUDY 2

DEVELOPMENT OF FLEXIBLE AND DISPERSIBLE ORAL FORMULATIONS CONTAINING PRAZIQUANTEL FOR POTENTIAL SCHISTOSOMIASIS TREATMENT OF PRE-SCHOOL AGE CHILDREN

Trastullo, R., Dolci, L.S., Passerini, N., Albertini, B., 2015. Development of flexible and dispersible oral formulations containing praziquantel for potential schistosomiasis treatment of pre-school age children. Int. J.Pharm. 495, 536-550.

4.0. PAEDIATRIC CHALLENGE: DOSAGE FORM

4.0.1. ORAL FLEXIBLE DOSAGE FORMS

As many of the drugs prescribed for children were designed for and tested in adults, logically they are predominantly available as single dosage units suitable for adults. This implicates that in several cases tablets, capsules and suppositories are too large for the administration in newborns, infants or children, leading to the failure of the therapy. In addition, in most cases, the required paediatric dose is a fraction of the whole dose available in a single dosage unit.

The lack of commercially available, age-appropriate formulations can make them difficult to administer to children (Zajicek et al., 2013). As described in the introduction of thesis, medicines may require manipulation at the point of administration, by opening, splitting, crushing, dispersing or diluting the original dosage forms. The need for calculations and extra steps (such as dilutions and measurements), leads to an increasing of errors: the potential for error with the additional steps used during drug manipulation may exacerbate risks that have been previously recognised with other known aspects of paediatric medication administration, such as the use of unlicensed/off label drugs.

For oral medicines requiring precise dosing measurements, a new flexible platform technology was proposed to produce solid multiparticulate dosage forms (minitablets, pellets) and dosage forms dispersible in liquids or sprinkled on food (granules).

Granules were chosen in the following work thanks to their advantages:

- dose flexibility: ability to easily and accurately deliver dosing according to patient's individual needs (bodyweight);
- possibility of administration of a fine suspension in water, easy to swallow;
- manipulation of the formulation with beverages and food;
- possibility of taste masking with the use of sweeteners and flavours.

4.1. INTRODUCTION

Nowadays, there are many challenges associated with formulating paediatric medicines for developing countries and the demand of paediatric medicine still remains at large (Sosnik et al., 2012; Ivanovska et al., 2014). In particular, there is a lack of paediatric-acceptable dosage forms for most neglected tropical diseases as the majority of oral dosage forms is designed for adult patients and lacks in dosing flexibility (World Health Organization, 2007; Conway et al., 2013). Capsules or tablets are the most common dosage forms but also difficult to swallow in small children, especially between the ages of 2 and 6 years (Zajicek et al., 2013). In 2007, four children under 36 months died from choking on albendazole tablets during a deworming campaign in Ethiopia. WHO strongly recommended that manufacturers of anthelminthics for public health programs targeted at pre-school children develop formulations that are appropriate for this age group (World Health Organization, 2007). Praziquantel (PZQ) is an anthelmintic drug widely used in developing countries for the treatment of schistosome infections. Schistosomiasis is caused by the infection from parasitic worms (Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum) and results in chronic diseases such as stunting, wasting, lack of fitness, cognitive impairment, infertility and genital disease (Mutapi et al., 2011). Schistosomiasis affects more than 200 million people worldwide out of a total of 783 million at risk (in 74 developing countries) (Skopp, 2014). The affected population includes 24 million pre-school children and 65 million school-age children, at risk of a total of 72 million and 200 million, respectively. After malaria, it is the second most prevalent disease in African children (Skopp, 2014). PZQ is included in the WHO Model List of Essential Medicines for Children (World Health Organization, 2011). This drug was discovered in the early seventies and is manufactured by several companies such as Bayer (Biltricide1) and Merck (Cesol1 or Cisticid1). Nowadays, it is mostly available on the international market as a 600 mg film-coated tablet of 22 mm in length. The dosage is adjusted to the child bodyweight by administering in sets of 150 mg (a quarter of a tablet). The therapeutic regimen for the treatment of schitsosomiasis consists of 20 mg/kg three times a day at intervals of 4–6 h (Bayer HealthCare Pharmaceuticals Inc., 2011) or as a single dose of 40 mg/kg (depending on the parasite) (Sousa-Figueiredo et al., 2012a). It is a one-day treatment. Recently, height intervals instead of bodyweight for tablet division were also considered (Sousa- Figueiredo et al., 2012b). Moreover, it is reported that keeping the tablet or segments in the mouth can

reveal a bitter taste, which can promote gagging and vomiting. Therefore, the tablet should be administered with food and swallowed whole with some liquid. From a biopharmaceutical point of view, PZQ is classified a BCS class II drug (high permeability, low solubility and extensive first-pass metabolism) (Lindenberg et al., 2004). Therefore, the two main drawbacks of this drug are its bad taste and its poor water solubility, related to the high dose required. Merck has distributed more than 160 million PZQ tablets since 2007 and 38 million school children have been treated via the Merck donation since 2008 (Skopp, 2014). Moreover, since 2003, several mass drug administration campaigns have been implemented in sub-Saharan Africa treating millions of school-aged children for schistosomiasis with PZQ (Sousa-Figueiredo et al., 2012b; Coulibaly et al., 2012). However, younger children (_6 years) have been consistently excluded from access to such medication, highlighting a PZQ treatment gap for pre-schoolers and infants. WHO considers treatment with PZQ as being safe for children as young as four years of age, but as parasite eggs can be found in children within the first year of life, PZQ is widely used “off-label” (Sousa-Figueiredo et al., 2012b). Hence, a common approach in high endemicity areas is to crush the 600 mg PZQ tablets, mix with water or juice and then administer orally to pre-school-aged children at a dose of 40 mg/kg (Coulibaly et al., 2012). In July 2012, a non-profit private public partnership under Merck Serono’s leadership, named Paediatric Praziquantel Consortium, was lunched with the aim to develop a paediatric PZQ formulation for children younger than 6 years old. In particular, orodisperisble tablets of 150 mg PZQ have been developed. Therefore, this scenario clearly highlights that the current standard PZQ-based medications for pre-school children are not appropriate, making dose adjustment and swallowing a challenging process in small children (Olliaro et al., 2013). This research project focused on the development of a flexible dosage form suitable for oral administration of PZQ to children from 2 to 6 years old. According to the EMA guideline, powder, granules and pellets received “preferred acceptability” by this age group of children (EMA/CHMP/QWP/805880/2012). In this study granule formulations were investigated. Granules have the further advantage over tablets because they can be given to children as solution or dispersion in beverages or administered with food, thus improving the palatability of the formulations and the adherence to therapy. Moreover, it is reported that the bioavailability of PZQ in adults is significantly influenced by concomitant food intake and that the influence was greater with carbohydrates than with lipids (Castro et al., 2000). As reported, the effect could be related either to some

pharmaceutical factors (better tablet disintegration and drug dissolution) or to several biopharmaceutical aspects (changes in hepatic blood flow or in the metabolism of the drug during the first passage through the liver). Therefore, the development of granules dispersible in water or in other common beverages such as fruit juice and milk was investigated. The rationale for the choice of formulating dispersible granules is in line with the characteristics of the drug itself, since it allows to minimize the dissolved PZQ (acceptable palatability) in addition to maintaining the dose flexibility. The obtained granules were characterized as regards the technological properties such as moisture content, flowability, friability, particle size, drug content, solubility and dissolution behaviour. The physicochemical properties by means of Fourier transformed infrared (FT-IR) analysis, differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD) were also characterized. Finally, granules stability in the solid state (30 °C and 75 % RH, trial conditions for climatic zones IVb) (World Health Organization, 2009) and the short-term stability in milk were assessed.

4.2. MATERIALS AND METHODS

4.2.1. MATERIALS

Praziquantel (2-(cyclohexylcarbonyl)-1,2,3,6,7,11b—hexahydro 4H-pyrazino[2,1-a]isoquinolin-4-one) was kindly donated by FATRO S.p.A., Ozzano Emilia, Bologna, Italy. Flowlac 100 (a-lactose monohydrate, Meggle batch n. L0846), Neosorb P100T (D-sorbitol, Rochette Freres batch n. E744B), Sweetpearl P90 (maltitol, Rochette Freres batch n. EMR70), GalenIQTM 721 (isomalt, Platting-De GmbH batch n. L1213921U4) and Sucrose (Platting-De GmbH batch n. 115404200) were kindly supplied by Giusto Faravelli S.p.A., Milan, Italy. PVP K30, Lutrol micro 68 (poloxamer 188) and Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil) were kindly supplied by BASF, Ludwigshafen am Rhein, Germany, while Avicel PH 102 (microcrystalline cellulose, batch n. DT353743) was purchased from FMC Biopolymer and Tween 80 (polysorbate 80) was purchased from Merk. Milli-RX20 water (Millipore, Molsheim, France) was used throughout.

4.2.2. METHODS

4.2.2.1. PREPARATION OF GRANULES

The experiments were performed using a Mini-Glatt fluidised bed (Glatt GMbH, Binzen, Germany) granulator equipped with a conical vessel volume of 0.75 L, three metallic filters, a timing filter blowing (out time fixed set 8 s) and a product temperature probe (±0.1 °C). A single granulation process (batch size 100 g) consisted of three steps: mixing-heating, agglomeration and drying. First, the granulating liquid was prepared by solubilizing in either the binder (PVP) or the surfactant (depending on the formulation) and then by suspending the drug. The final suspension was sonicated three times for 1 min each to avoid nozzle clogging during the fluid bed processing. The powders were placed at the centre of the bottom grid and were mixed by fluidising air with an inlet flow rate of 9–10 Nm^3/h at either ambient temperature or 70 °C. When the product temperature reached approximately 30 °C, the pressure of the inlet flow air was then increased to 13 Nm^3/h, reaching 18 Nm^3/h at the end of the process and the binding solution (containing or not the dispersed PZQ) was sprayed in the pressure range 1.12–1.14 bar onto the powder bed. As a consequence, the temperature of the powders first decreased but then gradually stabilized around 21–24 $^{\circ}C$. The agglomeration phase lasted about 80–120 min, according to the formulation. Once the binding solution finished, the drying phase (inlet air at 50 °C) began and proceeded for about 3–5 min. At the end of the granulation process, the granules were discharged (the temperature was around 30–35 °C), collected and stored in polyethylene closed bottles and used for the characterization. The yield of each granulation process was calculated by dividing the total weight of the granules by the weight of the initial powder (in percentage). The composition of the different batches is summarized in Table 14.

Table 14. Composition of PZQ-loaded granules.

Batches	Fluidized powder bed							Aqueous binding solution/suspension				
	PZQ	Flowlac 100	Avicel PH102	Neosorb P100	GalenIQ 721	PVP K30		PZQ	PVP K30	Lutrol F68	Cremophor RH40	Tween 80
	Amount (%, w/w)							Amount (%, w/w)				
1	10	35	45	-	-	5		-	5	-	-	-
2		40	30	-	-	-		10	-	20	-	-
3		40	-	-	-	30			-	20	-	-
4		40	20	-	-	10			-	20	-	-
5		-	20	40	-	10			-	20	-	-
6		-	30	40	-	-			-	-	20	-
7		-	-	40	30	-			-	10	10	-
8		-	-	30	45	-			-	-	15	-
9		-	-	25	40	-		20	-	-	15	-
10		-	-	25	35	-			-	-	15	5
11		-	-	35	30	-			-	5	10	-
12		20	-	35	-	10			-	-	15	-
13		35	-	20	-	10			-	15	-	-

4.2.2.2. GRANULES CHARACTERIZATION

For the detection moisture content (MC) of the granules at the end of the granulation process, the weight loss of the samples on thermal drying was measured using a Top Ray (Alessandrini, Modena, Italy) thermal balance at a fixed temperature of 105 °C. In particular, 3 g of each sample were heated in the thermal balance until a constant weight was achieved (max 15 min). The equilibrium MC of those formulations melting lower than 105 °C was ensured by keeping the batches in an oven at 40 °C overnight. Flowability tests were performed on both the raw materials (PZQ and excipients) and the finished product. The determination of bulk and tapped densities was assessed using a tap density apparatus (Erweka SVM 12). Each sample was weighted and poured into a 100 ml graduated cylinder. The apparent volume V0 and the final tapped volume Vf after 1250 taps were measured and the mean of three determinations of each granules was calculated. The Carr Index (CI %) values were calculated as follows:

$$CI\ (\%) = (V0 - Vf)/V0 \times 100$$

The mean of the three determination (± SD) was reported. The friability was determined by introducing 10 g of the granules (75–500 mm) together with 20 stainless steel beads (mean diameter 3 mm) in a friabilator (Erweka TA20, Erweka GmbH, Germany) for 10 min at a rotational speed of 25 rpm. Then, the beads were removed and the granules were sieved over a 75 mm sieve. The friability of the granules value was calculated as follows:

Friability (%) = (Initial weight - Final weight)/Initial weight x 100

The size distribution of raw materials and granules was evaluated by sieve analysis, using a vibrating shaker (Octagon Digital, Endecotts, London, UK) and 4 standard sieves (Scientific Instruments s.r.l., Milano, Italy) of 75, 150, 250 and 500 mm.

4.2.2.3. DETERMINATION OF DRUG CONTENT

The determination of the PZQ content into the granules was determined by dissolving 15 mg of each sample in 20 ml of methanol. The sample was shaken for 30 min while protected from light. Then, the solution/dispersion, depending on the batch formulation, was filtered through a 0.20 mm nylon filter syringe, diluted 1:20 with mobile phase and the drug content was assayed by HPLC. Each formulation was analyzed at least in triplicate and the mean ± SD was reported. Reverse phase HPLC method was used for the quantification of PZQ, using a method adapted from literature (Sun and Bu, 2012) and validated according to a slight modification in column length. PZQ standard solution was prepared by dissolving 10 mg of PZQ in 20 ml of methanol for few minutes and then diluting 1:200 with mobile phase in order to have a final PZQ concentration in the standard solution of 2.5 mg/L. The HPLC system consisted of two mobile phase delivery pumps (LC-10ADvp, Shimadzu, Japan) and a UV–vis detector (SPD-10Avp, Shimadzu, Japan). An autosampler (SIL-20A, Shimadzu, Japan) was used to inject samples (20 ml) onto a Kinetex 5 mm C18 column (150 mm x 4.60 mm; Phenomenex, Bologna, Italy). The mobile phase comprised of methanol and water (65:35 V/V). The flow rate was 1 ml/min and the detection wavelength was set at 220 nm. The retention time of PZQ was about 5.5 mins and the run time was set at 12 mins. Quantitation was carried out by integration of the peak areas using the external standardization method. Under these conditions, the linear calibration curve of PZQ was obtained in the range of 0.3–10 mg/L (r^2 = 0.99996).

4.2.2.4. SOLUBILITY STUDIES

Solubility measurements of pure PZQ and of all granules were performed in 50 ml of purified water, protected from light with aluminum foil. The samples were magnetically stirred at 37 °C for 72 h. The suspensions were first filtered through a paper filter and then through a 0.20 mm membrane filter and the filtrates were diluted 1:20 with the

mobile phase and finally assessed by HPLC. The measurements were performed in triplicate for each formulation and the mean ± SD was reported.

4.2.2.5. DIFFERENTIAL SCANNING CALORIMETRY (DSC) STUDIES

DSC analysis was performed using PerkinElmer DSC 6 (PerkinElmer, Beaconsfield, UK) equipped with Pyris Software. The instrument was calibrated with indium and lead for temperature, and with indium for the measurement of the enthalpy. Granules, weighting 7–8 mg, were placed in an aluminum pan and heated from 25 °C to 240 °C at a scanning rate of 10 °C/min under a nitrogen flow rate of 20 ml/min. For comparison, the same procedure was followed for the raw materials. DSC analysis studies were then repeated following storage.

4.2.2.6. FOURIER TRANSFORM-INFRARED SPECTRA (FT-IR) ANALYSIS

Studies of infrared spectra of pure drug, raw materials and granules were conducted with an IR spectrophotometer (Jasco FT- IR A-200) using the KBr disc method. The samples were diluted with KBr and then compressed into a tablet, 10 mm in diameter and 2 mm in thickness, using a manual tablet presser (PerkinElmer, Norwalk, USA) at 300 kg for 3 min. Due to the pasty form of Cremophor RH40, Nujol instead of KBR was used for the analysis of the raw material.

4.2.2.7. X-RAY POWDER DIFFRACTION (XRPD) ANALYSIS

Raw PZQ, granules and the corresponding physical mixture were studied by X-ray powder diffraction technique using a X'Pert PRO (PANanalytical, Almelo, NL) diffractometer with CuKa radiation (l = 1.5418 Å) monochromatized by a secondary flat graphite crystal. The voltage was 40 kV and the current 20 mA. The scanning angle ranged from 5 to 25° of 2u (minimum step size 0.001° of 2u).

4.2.2.8. *IN VITRO* DISSOLUTION TESTING

Dissolution tests in non-sink condition were performed to reproduce the actual condition of a child of approximately 4 years old and 16 kg weight swallows the granules. According to the therapeutic regimen of 20/mg kg three times a day, 320 mg of PZQ was required, corresponding to 1.6 g of granules (when the theoretical drug loading was 20 % w/w). As dissolution medium, a volume of 200 ml and 50 ml of water, simulating

different glasses of water, was used at room temperature. The studies ran over a period of 120 min during which 2.2 ml aliquots of the release medium were collected at specific time intervals. The samples were filtered (0.2 mm), diluted 1:20 with the mobile phase and assayed for PZQ by HPLC. For comparison, the test was performed also on raw PZQ. The mean of at least three determinations was used to determine the dissolved PZQ from each formulation. Finally, the fine suspension obtained at the end of the dissolution test in non-sink condition was added to a 500 ml of pH 1.5-buffer solution at 37 °C, simulating the fasted state of a child (Batchelor et al., 2014), using the paddle apparatus rotating at 100 rpm. The studies ran over a period of 60 min during which 2.2 ml aliquots of the release medium were collected at specific time intervals. The samples were filtered (0.2 mm), diluted 1:20 with the mobile phase and assayed for PZQ by HPLC.

4.2.2.9. LASER DIFFRACTION

The particle size measurements of the selected granules in terms of water dispersion were carried out using the laser diffractometer (Malvern Mastersizer Hydro 2000, Malvern, UK). Granules were dispersed in deionized water corresponding to 320 mg of PZQ in 200 ml and stirred for 1 min. The dispersions were then poured in the Hydro 2000 dispersion unit containing about 200 ml of dispersant medium until the obscuration reached a value between 10 % and 20 %. The results were finally expressed as the diameter (mm) at 10 %, 50% and 90 % of the distribution volume (dV10, dV50 and dV90, respectively). The Span Index, given by the expression: Span Index = (dV90–dV10)/dV50 was then calculated; a high value of this index indicated a wide size distribution.

4.2.2.10. MANIPULATION OF GRANULES WITH DIFFERENT BEVERAGES

The solubilisation test was carried out by placing about 1.6 g of granules, depending on the PZQ experimental content, in 200 ml of fruit juice (Santal mixed red fruits and Santal orange juice) and of whole milk (Granarolo, 3.6 % fat) and gently stirred at room temperature for up to 2 h. Sample aliquots were withdraw at specified times of 1.5, 3, 5, 10, 30, 60 and 120 mins. The sample taken from fruit juice (2.2 ml) was filtered with 0.2 mm syringe filter and diluted 1:20 with the mobile phase and injected in HPLC. In the case of milk, the procedure reported by our group was used (Albertini et al., 2014). Precisely 1.5 ml of an acetic acid solution (12 %, V/V) was added to the milk samples (5 ml), mixed for 1 min in a vortex (IKA-VIBRO-FIX) and then centrifuged for 12 mins at

10,000 rpm, to precipitate the milk proteins. The supernatant was then collected, filtered through a 0.2 mm filter syringe, diluted 1:20 with the mobile phase and then analysed by HPLC as previously described.

4.2.2.11. STABILITY STUDIES

To assess the granule stability, storage studies were performed according to the ICH stability zones; in particular 30 °C/75 % RH were set for accelerated trial conditions used for all the climatic zones (I–IV). Therefore, granules were stored in PE closed bottles in a forced air oven (Friocell, MMM medcenter, Germany) at 30 °C and the 75 % RH was obtained using a saturated NaCl solution (Hsieh and Taylor, 2015).

4.3. RESULTS AND DISCUSSION

4.3.1. CHOICE OF EXCIPIENTS

Focusing on PZQ characteristics, there are concerns about the palatability of the final dosage form as the racemic form (±) of the drug is characterized by a strong bitter taste together with the high dose needed. Taste masking is normally accomplished by using sweeteners and flavours. In this study different sweeteners, acting also as diluents, were assessed: a-lactose monohydrate, sorbitol, maltitol, isomalt and sucrose. Lactose mostly acting as diluent cannot be used in case of lactose intolerance and can induce diarrhoea; sorbitol has also a laxative effect at high dose and it is non-cariogenic and non-calorific. Maltitol is a polyol made by hydrogenation of the disaccharide maltose. The Joint FAO/WHO Expert Committee on Food Additives has determined the “Laxative Threshold Value” (LTV) for a number of polyols and maltitol is one of the less laxative polyols. Sucrose may promote dental caries and should be avoided in patients suffering from fructose intolerance. Isomalt, a mixture of two compounds, glucosyl-mannitol and glucosyl-sorbitol, has a great bitter suppression activity with a low cooling effect (Sentko and Willibald-Ettle, 2006). On the other hand, the use of these excipients associate with the schistosomiasis treatment limits their daily intake since it is a one day treatment. In addition, by minimizing drug in solution form, suspension improves palat- ability and allows the increase in drug load as a reduced dose volume (Stryckley et al., 2008). Moreover, in certain instances, the presence of a drug in suspension imparts more

chemical stability to the formulation (Ali et al., 2014). Stryckley et al. (2008) also reported that when the taste cannot be easily masked, a molecule can be formulated as powders/ granules that is sprinkled immediately on beverage or food before swallowing. PZQ is available as micronized form, thus with very poor flowability and wettability. To reduce interfacial tension between solid and liquid during manufacture and to promote the formation of a fine suspension before administration, several surfactants suitable for oral administration, for instance Lutrol micro 68, Cremphor RH 40 (ADI = 0–25 mg/kg/day) and Tween 80 (ADI = 0– 10 mg/kg/day), were examined. Moreover it was reported that Cremophor RH 40 can act as taste masking agent probably due to the coating of taste bud receptors in the mouth (BASF, 2011). Before proceeding with granulation experiments, the techno- logical properties of the excipients were analysed in order to identify those most suitable to the process, also in relation to their effect of taste masking, as well as safety issue in paediatric patients. The size, the powder flow and the lipophilicity of the raw materials that can be processed in the fluid bed are the main limiting factors of this technology. Very fine materials, such as micronized powders and very hydrophobic powders are certainly not ideal for this technology. In the case of PZQ, we were faced with a highly hydrophobic molecule (log P = 2.5) with limited ability to flow (CI ~ 37 %) (Table 15). The ingredients selected for this study can be divided according to their function in diluents (Avicel PH102, Flowlac 100), binders (PVP K30) and sweeteners (Neosorb P100, GalenIQ 721, Sucrose, Sweet Pearl P90). Lactose is an excellent choice of filler but can exhibit poor flow characteristics, so it is often combined with free-flowing microcrystalline cellulose in wet granulation formulations. The results of the particle size analysis and flowability are shown in Table 2. Flowlac 100 showed excellent flow properties as the 95 % w/w of the powders was lower than 250 mm and only the 10 % w/w was lower than 75 mm. GalenIQ and Neosorb showed similar flow properties (CI ~ 15 %), although the latter had lower dimensions. Sucrose and Sweetpearl P90 owing to the very fine particle size (more than 75 % are <75 mm) and to the high Carr Index values (about 21 %) were discarded by the granulation trials.

Table 15. Particle size analysis and flowability, expressed as Carr Index value, of raw materials.

	PZQ	PVP K30	Avicel PH102	Flowlac 100	GalenIQ 721	Neosorb P100	Sucrose	SweetPearl P90
Particle size (µm)	Amount (%, w/w)							
<75	100	20.38	30.13	11.52	2.51	10.49	78.00	72.41
75-150	-	52.38	39.38	34.44	23.09	53.85	18.60	20.59
150-250	-	22.38	30.25	49.50	34.34	34.87	3.40	7.00
250-500	-	4.88	0.25	3.70	39.96	0.80	-	-
>500	-	-	-	0.88	0.10	-	-	-
Carr Index (%)	36.99 ± 0.03	23.4 ± 0.31	21.70 ± 0.80	6.67 ± 0.20	14.58 ± 0.01	14.93 ± 0.32	21.05 ± 0.60	21.46 ± 2.06

4.3.2. GRANULATION EXPERIMENTS: 10 % (W/W) PZQ LOADING

In order to evaluate the performance of different excipients during the granulation process and their effect on the granule properties, preliminary experiments were performed using formulations with 10 % drug loading (batches 1–8). Among the several available granulation technologies, the fluid bed was chosen because it produces less dense and smaller granules and mostly more uniform particle size without oversized granules than high shear mixer granulators (Gao et al., 2002). These characteristics are fundamental for the PZQ granules that have to be dispersed in water and to quickly form a finely subdivide aqueous suspension. In particular, granules were produced in a top spray fluid bed using two different methods. In the traditional wet granulation the binding solution is spraying on a powder mixture containing the drug. In these studies PZQ was incorporated either in the powder mixture (batch 1), according to the traditional way, or in the liquid phase containing the wetting agents (batches 2–8). The characteristics of the obtained granules are summarized in Table 16 and their particle size distribution is shown in Figure 7.

Table 16. Granulation yield, technological characteristics and solubility of granules.

Samples	Yield (%)	MC (%)	Flowability (CI, %)		Friability (%)	Solubility (mg/L)
			Granules	Physical mixtures		
PZQ						215.00 ± 4.90
1	88.4	2.10	13.6 ± 0.57	25.20 ± 1.40	5.60	235.10 ± 11.14
2	92.0	4.80	11.2 ± 1.12	22.90 ± 2.12	4.17	232.30 ± 0.09
3	86.5	3.65	11.8 ± 0.59	18.80 ± 3.90	2.04	168.40 ± 8.30
4	89.5	4.27	8.70 ± 0.81	20.00 ± 0.00	2.04	250.30 ± 2.40
5	93.3	1.62	16.5 ± 0.24	24.70 ± 1.34	3.09	157.10 ± 9.90
6	93.4	1.64	19.10 ± 1.40	27.70 ± 2.29	-	220.80 ±12.50
8	95.4	0.42	12.2 ± 1.57	27.80 ± 2.50	-	187.90 ± 4.90
9	94.2	0.21	9.55 ± 2.62	29.11 ± 1.27	-	244.11 ± 5.07
10	92.4	1.13	16.66 ± 1.81	27.00 ± 0.00	-	198.19 ± 2.70
12	93.3	0.32	17.40 ± 0.00	27.70 ± 0.00	-	223.15 ± 0.50
13	96.0	0.31	15.56 ± 0.50	25.92 ± 1.30	3.06	218.92 ± 3.48

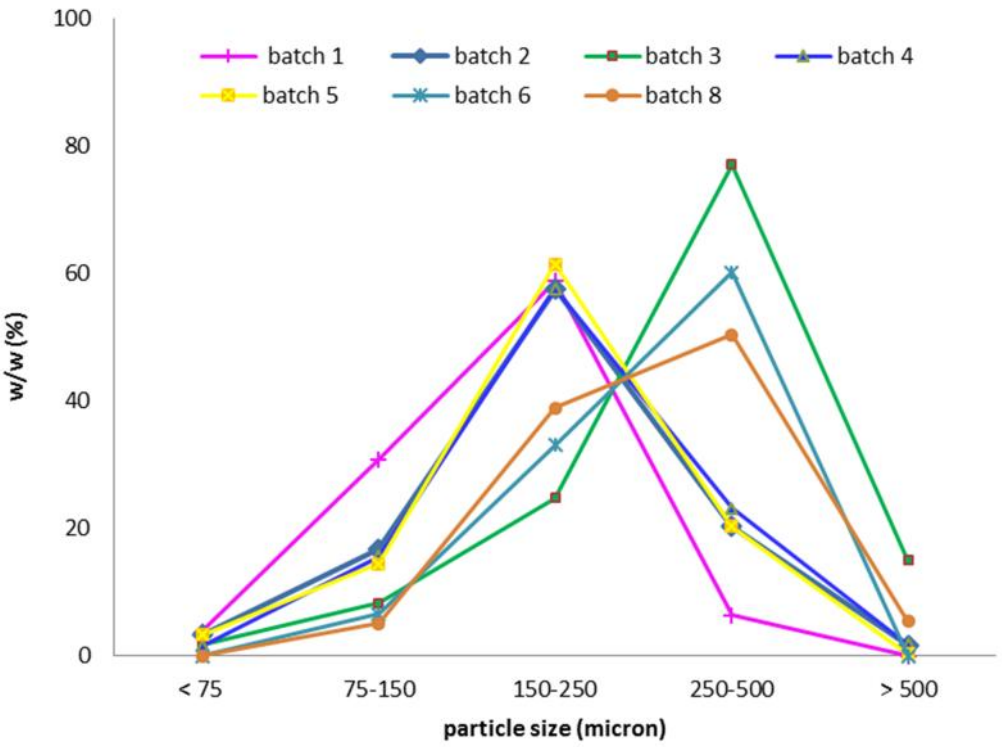


Figure 7. Particle size distribution of granules containing 10 w/w of PZQ.

As regards batch 1 formulation and its process-related parameters, a high-pressure drop and thus, an improper fluidization required a frequent filter cleaning to incorporate the fines (especially micronized PZQ) back in the process material, causing continuous interruptions in the process. Therefore, despite the experiment was conducted until the end, the yield was low (Table 16) and mostly a non-uniform distribution of PZQ within the granule size fractions was obtained, as clearly shown in Table 17.

Table17. Drug content of the granule size fractions.

Batches	< 75 µm	75-150 µm	150-250 µm	250-500 µm	> 500 µm
1	15.19 ± 0.66	8.32 ± 0.40	7.20 ± 0.62	8.05 ± 0.47	-
2	10.56 ± 0.50	10.56 ± 0.73	8.71 ± 1.09	8.83 ± 1.33	-
3	-	11.03 ± 0.11	9.62 ± 0.96	9.95 ± 0.82	9.84 ± 1.27
4	-	11.01 ± 1.54	9.75 ± 1.24	8.10 ± 1.12	-
5	13.92 ± 0.90	12.60 ± 0.69	9.80 ± 0.25	8.53 ± 0.52	-
6	-	10.45 ± 0.35	9.70 ± 0.05	8.08 ± 0.93	-
8	-	-	10.87 ± 0.63	10.26 ± 0.25	-
9	-	-	18.54 ± 1.61	19.04 ± 2.96	-
10	-	19.07 ± 0.25	16.49 ± 0.48	18.67 ± 0.28	-
12	-	-	19.53 ± 0.89	18.36 ± 0.21	-
13	-	20.23 ± 0.40	20.56 ± 0.90	19.40 ± 1.70	20.00 ± 1.40

To overcome this problem, PZQ was suspended in the aqueous liquid phase instead of using the traditional binding solution based on PVP. This second approach resulted more successful than the first one and all the other batches were prepared using this novel strategy. Lutrol F68 (20 % w/w) and Cremophor RH40 (20 % or 15 % w/w) were selected to ease the PZQ dispersion into water. Cremophor RH40 resulted the most suitable excipient to prepare the PZQ suspension, allowing a faster dispersion of the drug. All granules (batches 2-8) displayed good flow properties (Table 16), especially batch 4; the distribution of PZQ within the granule size fractions (Table 17) improved respect to batch 1 and the drug content uniformity was greater in batches containing Cremophor RH40. Conversely, these granules appeared fluffier than those containing Lutrol, resulting in negligible values of friability (Table 16). Granulation of batch 7 failed due to the severe filter clogging. Then the 15 % of Cremophor RH40 was selected as the moist suitable concentration to produce 10 % loaded-PZQ granules (batch 8). These preliminary granulation experiments showed that Flowlac 100, Neosorb and GalenIQ were all good excipients for granulating PZQ inside the fluid bed. Due to its tendency to clog the filter, a high concentration of PVP could not be used in the powder mixture. Despite the good flow properties of Avicel inside the fluid bed, it was removed from the last two experiments due to its water insolubility where in order to obtain a very fine water suspension, PZQ would be the only dispersed powder. The results reported in Table 16 evidence that the PZQ granulation with hydrophilic excipients and solubilizers did not increase the water solubility of the drug, suggesting no solid-state modification of the drug during the granulation process. To investigate the drug solid state and possible interactions between the components of the formulation, DSC, XRD and FT-IR analysis were then performed. Figure 8 shows the DSC curves of raw materials (Figure 8a) and of the batches 2–8 with the corresponding physical mixtures (Figure 8b).

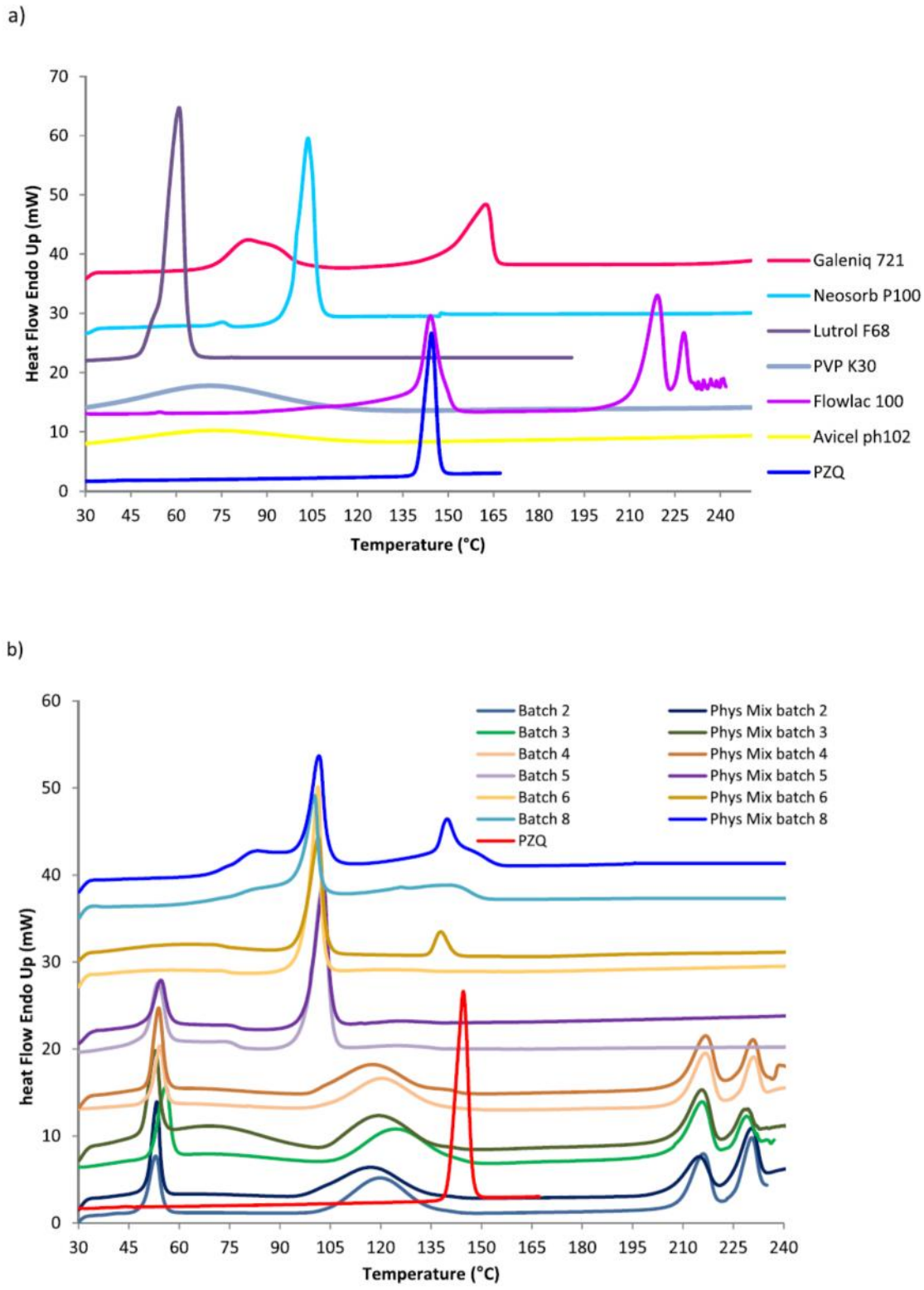


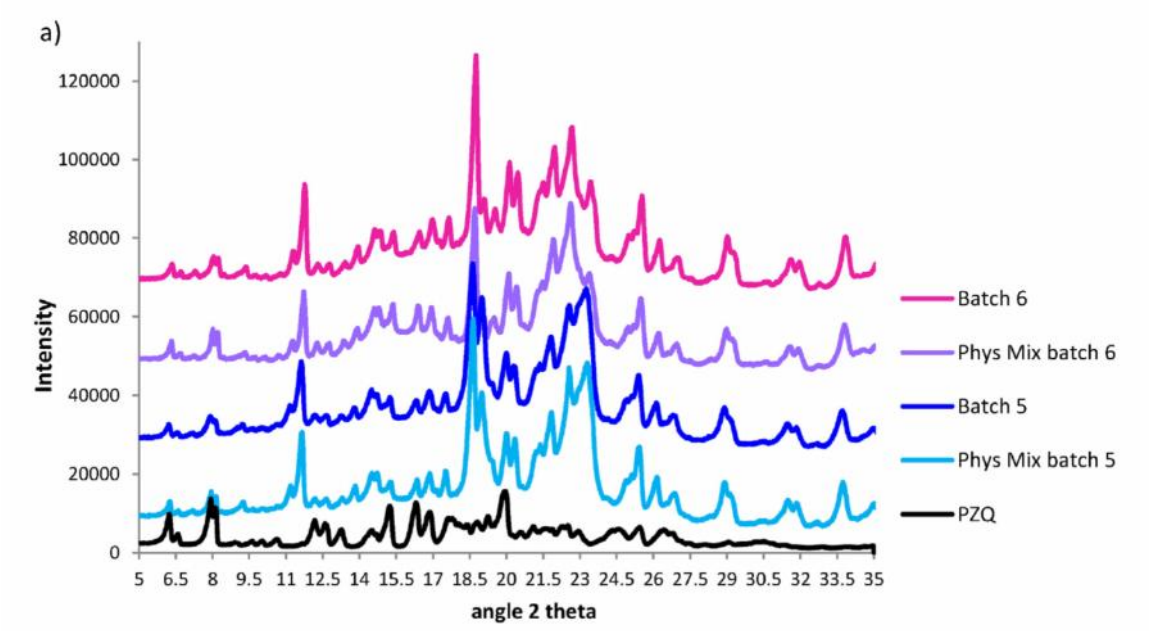
Figure 8. DSC curves of: (a) raw materials and of (b) loaded-PZQ granules in comparison with their corresponding physical mixtures and raw PZQ.

The DSC curve of PZQ showed only a single endothermic peak at 143.14 °C (ΔH = 98.3 J/g), in agreement with the melting point and enthalpy of fusion of the racemic form of the drug (El-Arini et al., 1998; Liu et al., 2004; Passerini et al., 2006). DSC thermogram of lactose monohydrate showed an endothermic peak at 143.3 °C, due to the loss of hydration water, and a melting endothermic peak at 218.4 °C with decomposition. Lutrol F 68 melted at 60.5 °C while PVP K30 and Avicel PH102 showed only a large dehydration peak between 50 ° and 100 °C. Four crystalline polymorphs and one amorphous form of sorbitol have been identified (Shur, 2009): it is reported a melting point of 110–112 °C related to the anhydrous form, a g-polymorph at 97.7 °C and the metastable form at 93 °C. As regards Neosorb P100T, it exhibited a melting peak at about 103 °C and two very small endothermic peak at 72.9 °C and at 145.8 °C, corresponding to D-iditol and maltitol, respectively. GalenIQ 721 (isomalt) revealed two thermal events: the first one at 66–99 °C and the second one at 140–165 °C, corresponding to the melting of 6-O-a-D-glucopyranosyl-D-sorbitol (1,6-GPS) and of 1-O-a-D-glucopyranosyl-D-mannitol dihydrate (1,1-GPM), respectively, as reported for a 1:3 mixture of 1,1-GPM and 1,6-GPS (Fritzsching et al., 2009). Analysing the thermal behaviour of the 10 % loaded-PZQ granules (Figure 8b), all the components evidenced a lowering of their thermal event due to the dilution effect in the formulation. Batches 2–4 clearly showed the melting peaks of Lutrol (at about 54 – 55 °C), the broader dehydration peak of lactose shifted at about 120 – 125 °C (onset at 100 °C and end at 137 °C) and the lactose melting at about 210 – 230 °C. The peak of the drug at 143 °C disappeared and may be hidden by the lactose dehydration peak and/or by the partial dissolution of the drug into the molten poloxamer. The curves of the corresponding physical mixtures were very similar to those of granules, but it was possible to see a minimum variation of the baseline around 141 °C, probably imputable to the PZQ fusion. In batch 5, the PZQ melting peak was not present in both the granulate and the physical mixture curves. Despite a higher scale magnification, a weak broad endotherm between 114 °C and 138 °C attributable to the drug was displayed in both samples and the enthalpy of fusion was very low (ΔH ~ 2 ± 0.6 J/g). In the case of batch 6, a clear peak at 138.8 °C (ΔH = 9.20 J/g) corresponding to PZQ melting appeared only in the physical mixture, while a weak broad endotherm between 111 °C and 136 °C attributable (ΔH = 1.97 J/g) to the drug can be detected at high scale magnification of the granule DSC scan. Finally, in batch 8 the PZQ thermal event was covered by the second melting peak of GalenIQ, both shifted at a lower value, while the physical mixture clearly showed a peak from 135 to 153 °C (the PZQ peak was

at about 138 °C). These results suggest either a possible modification of the drug solid state or other interactions within the formulation. To clarify the PZQ solid state within the granules, XRD analysis was then conducted on batches 5 and 6 selected as representative samples containing Lutrol and Cremophor as surfactant, respectively. Due to the complexity of the formulations, XRD analysis of granules were performed and compared to the corresponding physical mixtures. The XRD trace of raw PZQ (Figure 9a) showed the characteristic scattering peaks of the racemic drug: double scattering angles at 6.5° – 6.8° and 8° – 9.8° (2 ± θ) (El-Subbagh and Al-Badr, 1998; El-Arini et al., 1998; Liu et al., 2004). The XRD patterns of granules of batch 5 and 6 were superimposed to those of the physical mixtures, indicating the maintenance of PZQ in its original crystalline state. Therefore, interactions between the drug and the excipients could happened in those formulations. The FTIR spectrum of PZQ (Figure 9b) shows characteristic peaks at 2930 and 2852 cm^{-1}, due to the C-H and C-H_2 stretching vibration and at 1650–1600 cm^{-1}, due to the region of amide stretching vibrations. In particular, an intense band with two separate and equal spikes at 1623 cm^{-1} and 1645 cm^{-1} correspond to the C=0 and C-N stretching vibrations of the two tertiary amide groups. Moreover, the peaks in the region 3300–3700 cm^{-1} and in the fingerprint region below 1500 cm^{-1} confirmed the racemic form of the drug (Liu et al., 2004).

The IR spectra of physical mixture and granules (batch 6) displayed the major bands of each component alone, a broad band between 3000 and 3600 cm^{-1} due to -OH stretching vibrations and to the formation of hydrogen bonding within the formulation and no new bands or peak shifts were seen. The only functional groups in the PZQ molecule are two tertiary amide moieties. These would only be expected to form weak intramolecular interactions. In fact, looking at the amide region of batch 6 and of its physical mixture, a clear splitting of the band appeared (1625 cm^{-1} and 1648 cm^{-1}) and the stretching vibration at 1625 cm^{-1} resulted in a higher intensity, especially for granules. Such difference suggests that the carbonyl group could serve as proton acceptor in hydrogen bond formation with the several -OH groups of the other excipients. This interpretation can be supported by the smaller values for ΔH of granules than that of the physical mixture, as shown in Figure 8b (Prankerd and Ahmed, 1992). This behaviour was also observed for the other granule formulations (not shown). To confirm this hypothesis different binary mixtures between PZQ and each excipient were prepared at the weight ratio of the batches 1–8 (PZQ:Flowlac 1:4, PZQ:Neosorb 1:4, PZQ:GalenIQ 1:4, PZQ:Lutrol 1:2 and PZQ:PVP 1:1 and 1:3) and analysed by DSC. The results (graph not

shown) evidenced that Lutrol was the excipient that most influenced the PZQ enthalpy of fusion and its melting peak, followed by Neosorb and GalenIQ.



b)

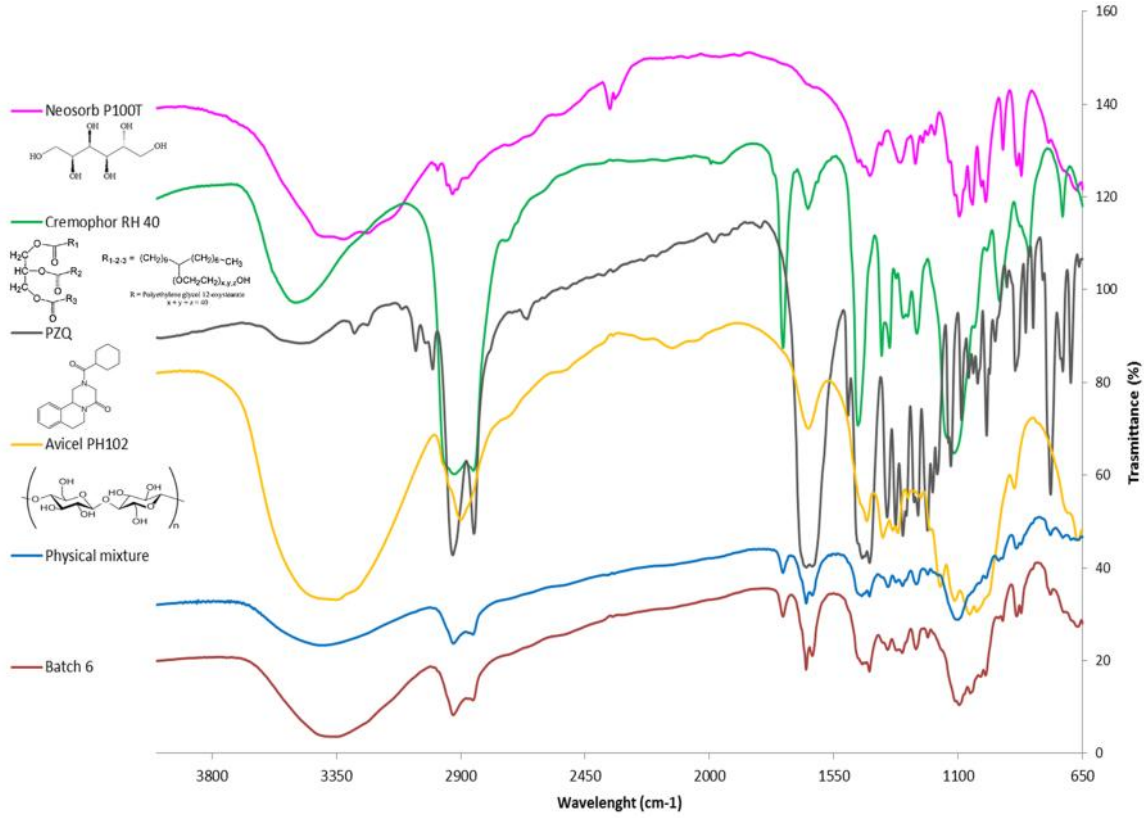


Figure 9. (a) XRD pattern of 10 % loaded-PZQ Granules (batch 5 containing Lutrol F68 and batch 6 containing Cremophor RH40) in comparison with physical mixtures and raw PZQ; (b) FTIR spectra of batch 6, physical mixture and raw materials.

4.3.3. GRANULATION EXPERIMENTS: 20 % (W/W) PZQ LOADING

To reduce the dose burden, subsequent studies were carried out to increase the drug loading up to 20 % w/w. Five different batches (9 – 13) were thus prepared and Flowlac 100, Galeniq 721, Neosorb P100 and PVP K30 were incorporated in the powder mixture (Table 14). Due to better processability, Cremophor RH 40, calculated at 15 % w/w on the solid material was first selected. In particular, batch 9 was produced with similar formulation to batch 8, to evaluate the feasibility of the process with double PZQ amount. The granulation process was well controlled despite that the system was very sensitive to the particle movement in the bed, to the spraying of the liquid and to the drying capacity. The main disadvantage was the adhesion of the particles on the walls and upon

filters. Nevertheless, the yield remained high at 94 % (Table 16). Then, the addition of a different surfactant, Tween 80 (batch 10) and the combination of Cremophor RH40 and Lutrol (batch 11) was also examined to evaluate their influence both on the formation of the PZQ suspension and on particle aggregation and granule densification. In fact, it is reported that surface active agents improve the fluidized bed granulation (Parikh and Mogavero, 2005). Comparing the different surfactant-based suspensions, the stirring rate must be low in presence of Tween 80 to reduce foam; while Lutrol and Cremophor do not produce foam but required enough time to obtain a fine PZQ dispersion, especially with Lutrol at 20 % w/w. In fact, in batch 13 the amount of Lutrol was decreased to 15 % to ease the formation of the suspension. Experiment 11 failed due to the whole adhesion of the powder bed on the walls of the equipment. PVP was then reintroduced within the formula (batch 12 and 13) to increase the granule strength and reduce granule friability. All granulation experiments had a high process yield and granules displayed a very low relative humidity and good flow properties (Table 16). The main particle size of granules (50 – 70 % w/w depending on the formulation) ranged from 150 to 250 mm (Figure 10) and all granules had uniform distribution of the drug within the size fractions (Table 17).

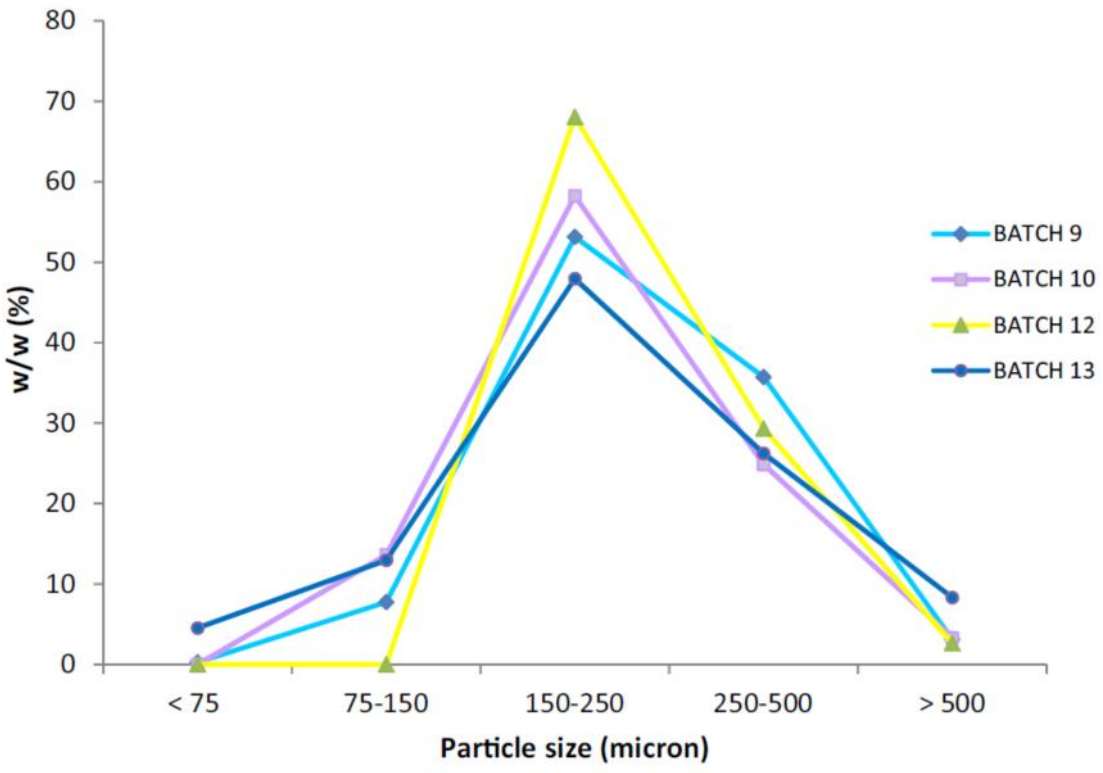


Figure 10. Particle size distribution of granules containing 20 % w/w of PZQ.

This fact indicates size uniformity of the atomizing suspension and confirms the optimum selection of the liquid spray rate and the nozzle atomizing pressure. Results from detailed analysis of batches 9, 10 and 12 containing Cremophor RH40 show evidence that the addition of a second surfactant (batch 10) did not improve the granule characteristics as both the drug content uniformity and PZQ solubility decreased respect to batches 9 and 12. Finally, in the last experiment, Cremophor RH40 was replaced with Lutrol and the lactose amount was increased to enhance the powder flow in the fluid bed. The obtained granules were denser than batch 12 with a good friability (Table 16) and a uniform PZQ distribution within the size fractions (Table 17). Moreover, doubling the drug concentration, the solubility of granules was similar to that of raw PZQ and of 10 % PZQ-loaded granules. The thermal analysis results from these batches is reported in Figure 11.

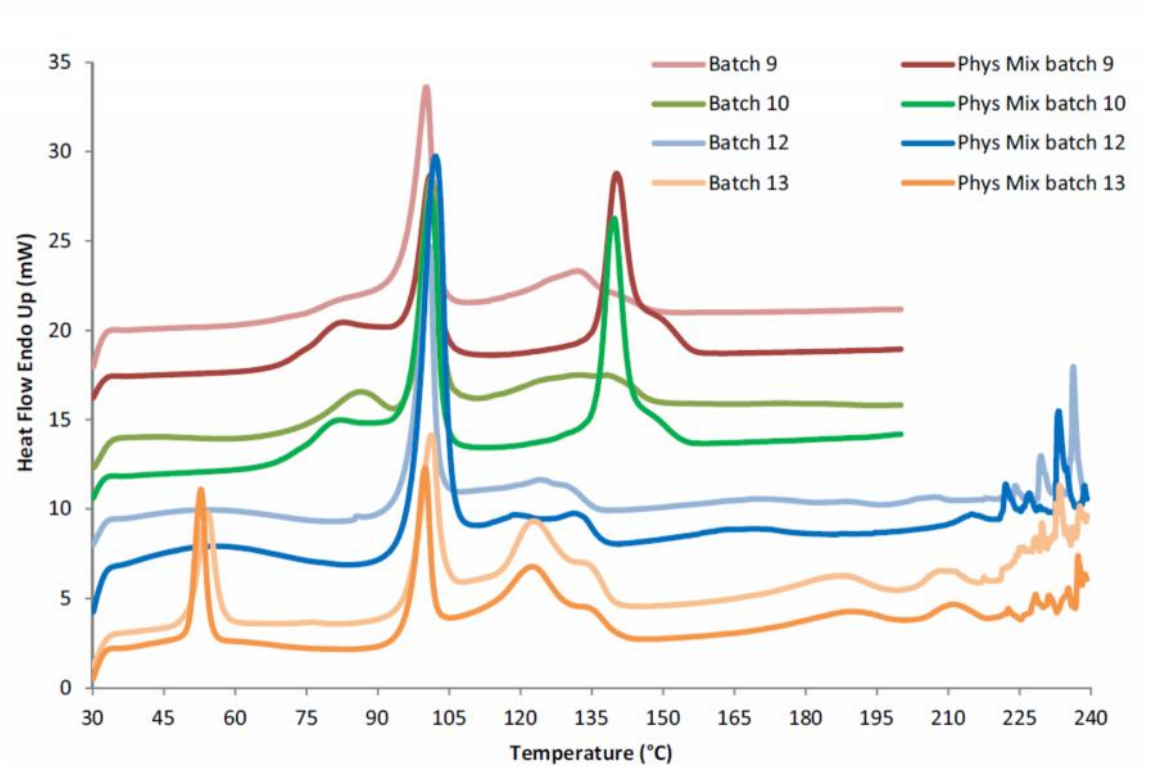
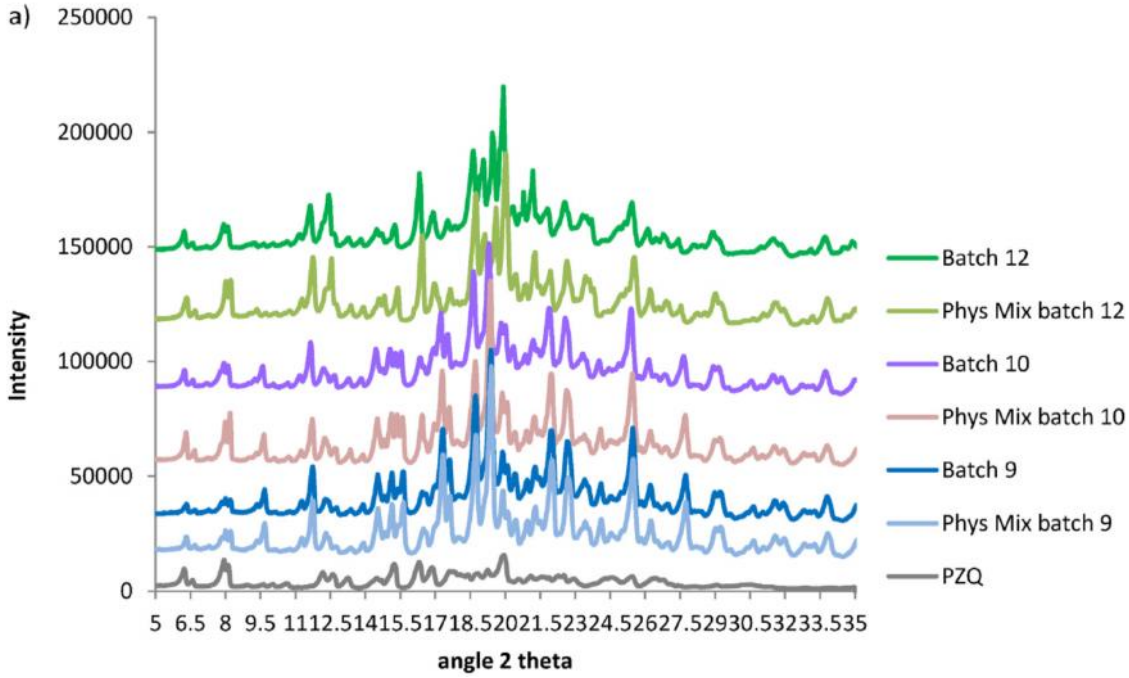


Figure 11. DSC curves of 20 % loaded PZQ granules in comparison with their corresponding physical mixtures.

Batches 9 and 10, having similar composition, showed analogous DSC traces: granules displayed a broad endothermic peak comprised of the PZQ melting peak and of the 1,1-GPM moiety of GalenIQ; while physical mixtures clearly evidenced a sharper peak referable to both PZQ and GalenIQ in the range 130– 155 °C. The DSC curve of granules of batch 12 was similar to that of corresponding physical mixture. In both cases, a weak

broad peak included the dehydration peak of lactose at about 120 °C and the PZQ peak from 126 to 136 °C, both hardly distinguishable. Considering the higher drug content of this batch than the 10 %-loaded ones, the lowering of the drug melting event and of its enthalpy suggest that some modification/interactions might be occurred, even in the physical mixture. To verify the solid state of the drug in batches 9, 10 and 12, X-Ray Powder Diffraction (Figure 12a) was assessed and the results confirmed that PZQ remained in its original crystalline state both within granules and physical mixtures. The FTIR analysis (Figure 12b) show similar spectra of batch 12 granules and the corresponding physical mixtures indicating the formation of hydrogen bonding between the components of the formulation, as previously observed for batch 6. These results show the absence of strong interactions between PZQ and excipients after the granulation process. On the contrary, in batch 13 (Figure 11) the endothermic peaks of each component were lowered due to the presence of Lutrol but clear and quite distinct: Lutrol at 54 °C, Neosorb at 100 °C, dehydration peak of lactose at 120 °C, PZQ at 135 °C (ΔH = 3.3 J/g) and the melting peaks of lactose at 188 and 210 °C. The DSC curve of the physical mixture was superimposed, suggesting the absence of drug modifications and of further chemical interactions, apart from hydrogen bonds.



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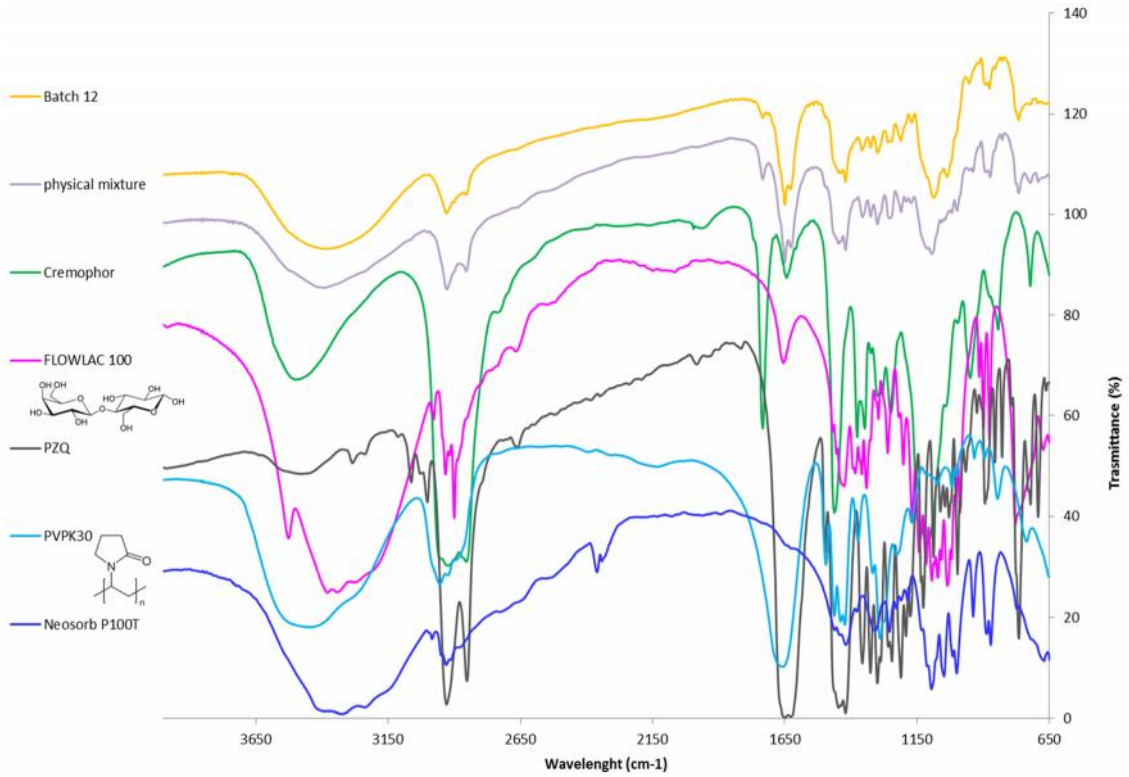
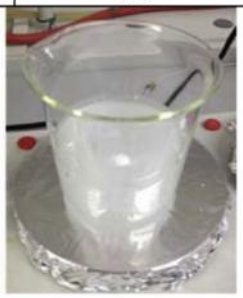
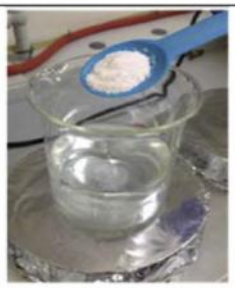
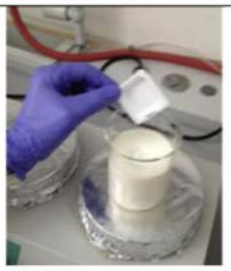
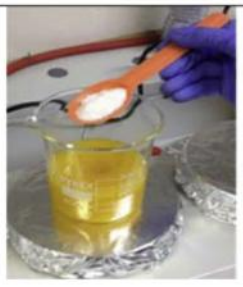
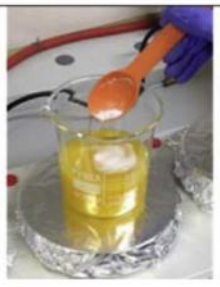


Figure 12. (a) XRD pattern of 20 % loaded-PZQ granules in comparison with physical mixtures and raw PZQ; (b) FTIR spectra of batch 12, physical mixture and of the raw materials.

The next step of the study tried to reproduce the actual condition in which a child of 4 years and of 16 Kg weight might assume the granule suspension. According to the therapeutic regimen, 320 mg of PZQ are required three times a day, corresponding to 1.6 g of granules. Therefore, in these experiments non-sink condition was used as the PZQ dose was higher than its saturated solubility (Table 16). Batch 13 (main fraction) was selected for these experiments, having a drug loading equal to the theoretical one. The solubilisation test was first performed in 200 ml of water simulating a glass of water. The system was stirred at room temperature and granules formed a very fine suspension easy to disperse in water unlike the pure drug, as shown in Table 18.

Table 18. Images of PZQ and of the granule dispersion in different liquid substrates taken at different time.

Suspension	to	t1	t30
PZQ in water			
Batch 13 in water			
Batch 13 in milk			
Batch 13 in red fruit juice			
Batch 13 in orange juice			

In particular, PZQ formed a coarse dispersion in which particles were still floating after 30 min, while granules formed a very fine opalescent suspension. The analysis of the particle size distribution revealed that dispersed particles had d_{V10} = 6.40 mm, d_{V50} = 16.80 mm and d_{V90} = 39.92 mm with a Span Index = 1.994. The amount of PZQ solubilized in the aqueous suspension is shown in Figure 13.

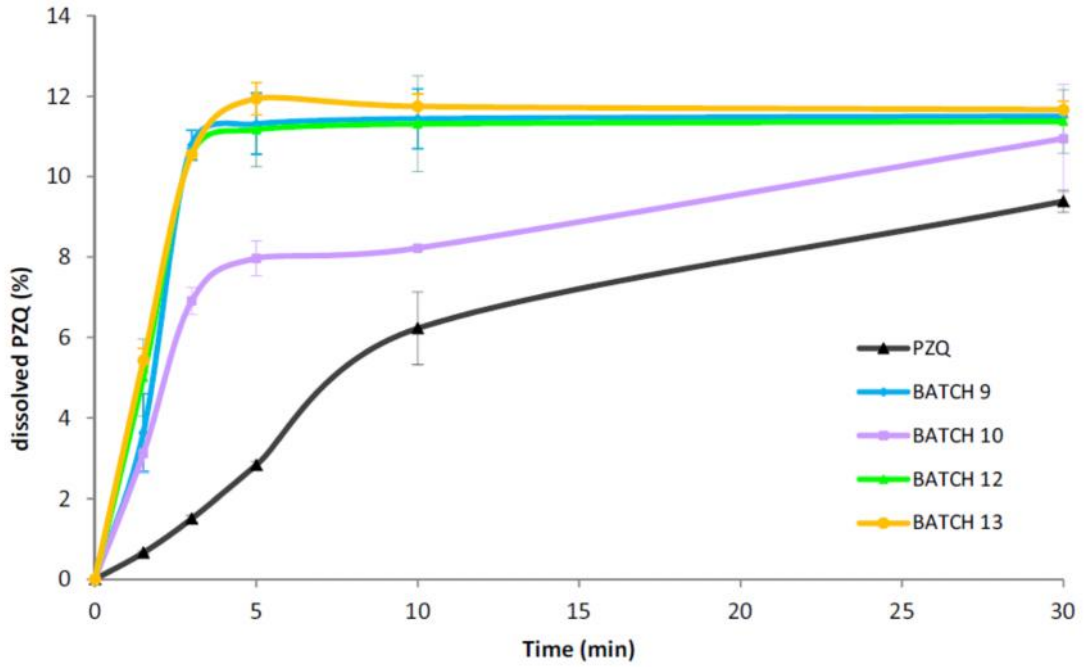


Figure 13. Dissolution test of granules and raw PZQ in water in non-sink condition.

After 5 min the PZQ dissolved from batches 9, 12 and 13 was about 12 % while only the 3 % of drug was dissolved in the same time. In the case of batches 9 and 12, the dispersion in water was very prompt: the granules did not float and were immediately wetted due to the presence of PVP and Cremophor without the formation of foam. Already after few minutes, the suspension was free of coarse particles and aggregates, and the walls of the beaker did not show residues of particles. Batch 10 dispersed well, but less quickly than batches 9 and 12, probably due to the not synergistic effect of the two surfactants. As regards batch 13, granules showed a similar behaviour to batch 12 and were quickly wetted. At the end of the test, it was possible to observe the formation of a very fine foam, mainly adherent to the walls of the beaker without any residual particles. Considering the granule dispersion in a lower volume (for instance 50 ml), the PZQ saturation solubility was promptly reached displaying a plateau after 5 min and the amount of PZQ dissolved from granules of batch 13 was about 3 % (data not shown).

The appearance of the final suspension was similar to that obtained in 200 ml of water, with a milky appearance. Therefore, batch 12 and 13 displayed very similar characteristics and a similar solubilisation ability towards poorly soluble PZQ. In both cases, Cremophor RH 40 and poloxamer 188 did not demonstrate a high solubilisation capacity, but they were very useful to improve the PZQ wetting and dispersion during the granulation set up and to enable the formation of the final water suspension prior to oral administration. After granule dispersion the amount of PZQ available for taste perception was very low (3 %) or using a higher volume at least reached the 12 %, which could make the medicine unpleasant to take. Adding the finely subdivide 200 ml aqueous suspension (batch 13) to 500 ml of buffer solution pH 1.5 (Figure 14) simulating the fasted state of a child (Batchelor et al., 2014), the dissolution profiles revealed that after 30 min about the 50 % of the drug was dissolved, reaching the equilibrium solubility of the drug (batch 13) in this medium. An increase of dissolved drug available for adsorption might create sink condition and might saturate the PZQ biotransformation pathways leading to an increase of the PZQ bioavailabilty. Therefore, the potential effect of enhanced dissolution of PZQ might require a dose adjustment and a specific paediatric clinical study design.

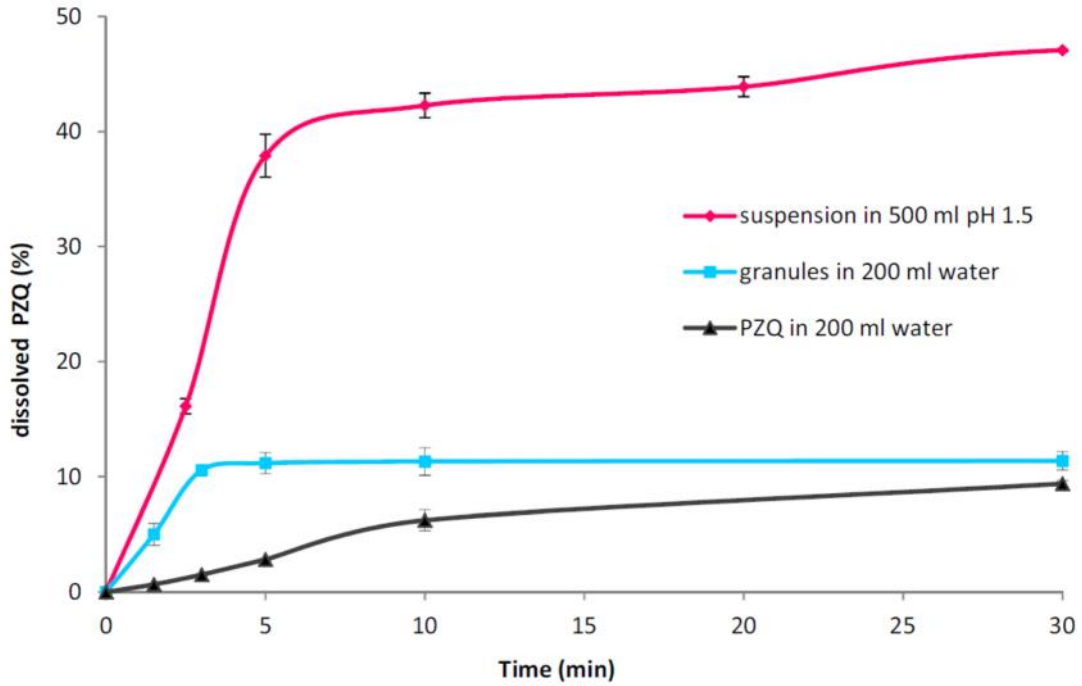
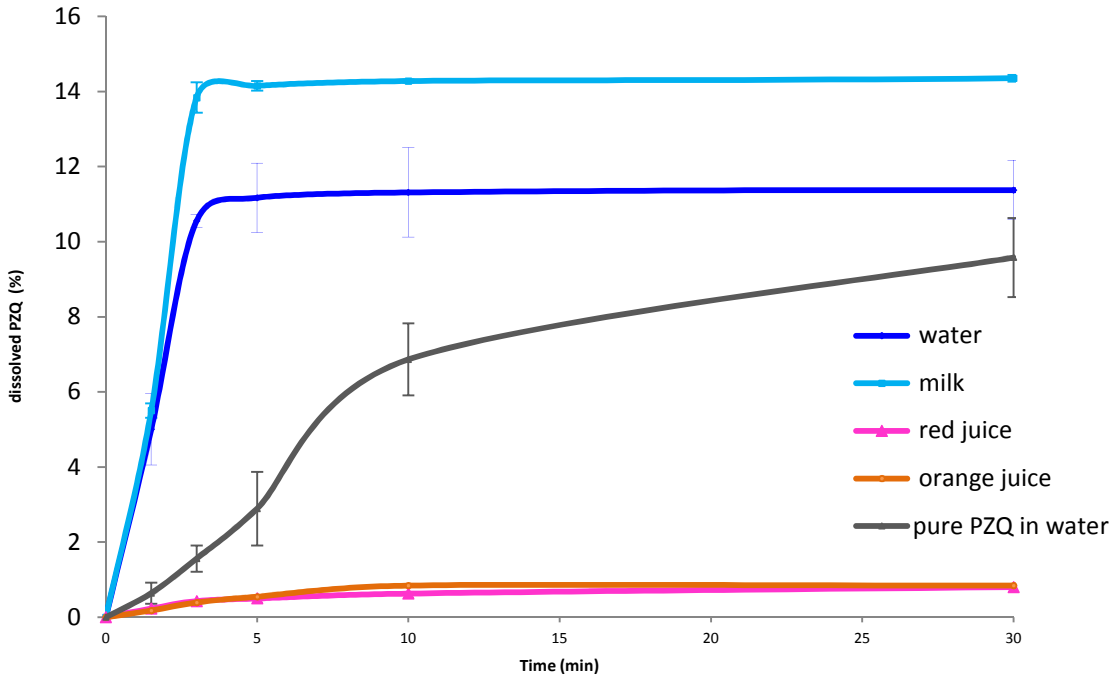


Figure 14. Comparison between the dissolution rate of the reconstituted suspension in pH 1.5 buffer and of granules (batch 13) and raw PZQ in water.

4.3.4. MANIPULATION OF GRANULES IN DIFFERENT FOOD SUBSTRATES

To ameliorate children's compatibility, the dosage form was manipulated with different beverages. A current practice of mixing drug products with food/beverage involves the use of milk or other common foods as ice cream, yoghurt, fruit puree and jam, used as a prequel or chaser for the medicine either as a reward or to aid taste masking. Even EMA recognizes that the practice of dosing drug products with food does occur (EMA/CHMP/QWP/805880/2012). On the other hand, manipulation can also involve several risks of wrong dosage, instability and incompatibility, if not previously studied and indicated on labels and a simple process of reconstitution of oral powder can become a risk even for safety (World Health Organization, 2007). Therefore, the study focused the attention on the granules manipulation with milk and on the short-term stability of the samples in this substrate. Moreover, in addition to milk, different fruit juices, which are commonly used to dose medicines to children, were tested. The pH of these beverages is likely to vary across types of juice and brands of juice and may have a severe impact on the stability of the active ingredient. In Figure 15a are reported the dissolution profiles of the granules (batch 13) in the various substrates compared to PZQ in pure water.

(a)



(b)

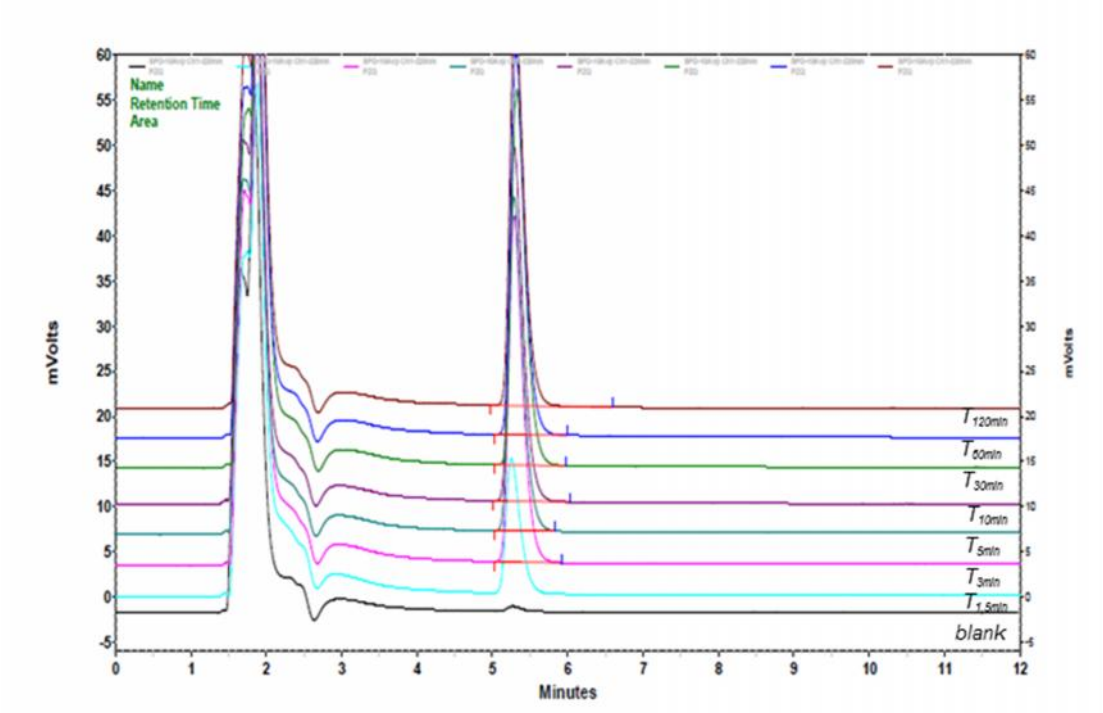


Figure 15. (a) Solubilisation rate of PZQ from batch 13 in different drinking media; (b) Chemical stability of PZQ and of granules (batch 13) in milk.

The recovery in milk was about 14 % after 5 min, indicating that the solubility of the PZQ was higher to that in water and fruit juice. This fact can be attributable to the ability

of the milk to emulsify the drug by forming micelles. In both the fruit juices, the solubilisation of PZQ decreased (≤ 1 %) due to the lower amount of free water because of the high concentration of dissolved sugars within the juice. Further, the pH of milk (5.75) and of juices (red juice = 3.24, orange juice = 3.28) did not change after the addition of granules. The chemical stability of the dosage form in milk was then investigated in 2 h by HPLC (Figure 15b). Due to minimum amount of PZQ dissolved after granule dispersion into the juices, their short-term stability was not assessed. No modification of the drug peak was observed during time (if not an increase of the peak area related to the solubilisation of PZQ in the first minutes), indicating that PZQ was stable in this medium, at least for the examined time.

4.3.5. STABILITY UPON STORAGE

Granules of batch 13, which showed the best properties, were subjected to stability in different conditions and possible solid-state modification and/or interaction within the components of the formulation were investigated by means of DSC. The results reported in Figure 16 show that the DSC trace of the physical mixture and granules at t_0 were very similar. In particular the following peaks, Lutrol F68 melting peak at 55 °C, slight deviation of the baseline at 74 °C and endothermic peak at 100 °C due to Neosorb melting, two narrow spikes at about 120 °C and 133 °C due to Flowlac and PZQ and the final melting peaks of Flowlac at 185 °C and 207 °C followed by decomposition, remained unchanged. The DSC traces of granules stored at ICH zone IVb conditions (30 °C/75 % RH) reveal no significant modifications of the DSC curves apart from the disappearance of the small peak at 74 °C. Furthermore, a slight shift of the first melting peak of lactose at higher temperature was evidenced. Therefore, these results indicate that PZQ remained in its original crystalline form and the storage conditions at high temperature and humidity did not alter the stability of the granules. In fact granules displayed the same flowability (CI % = 15.80 ± 0.75), despite the permeability of the polyethylene container to water vapour.

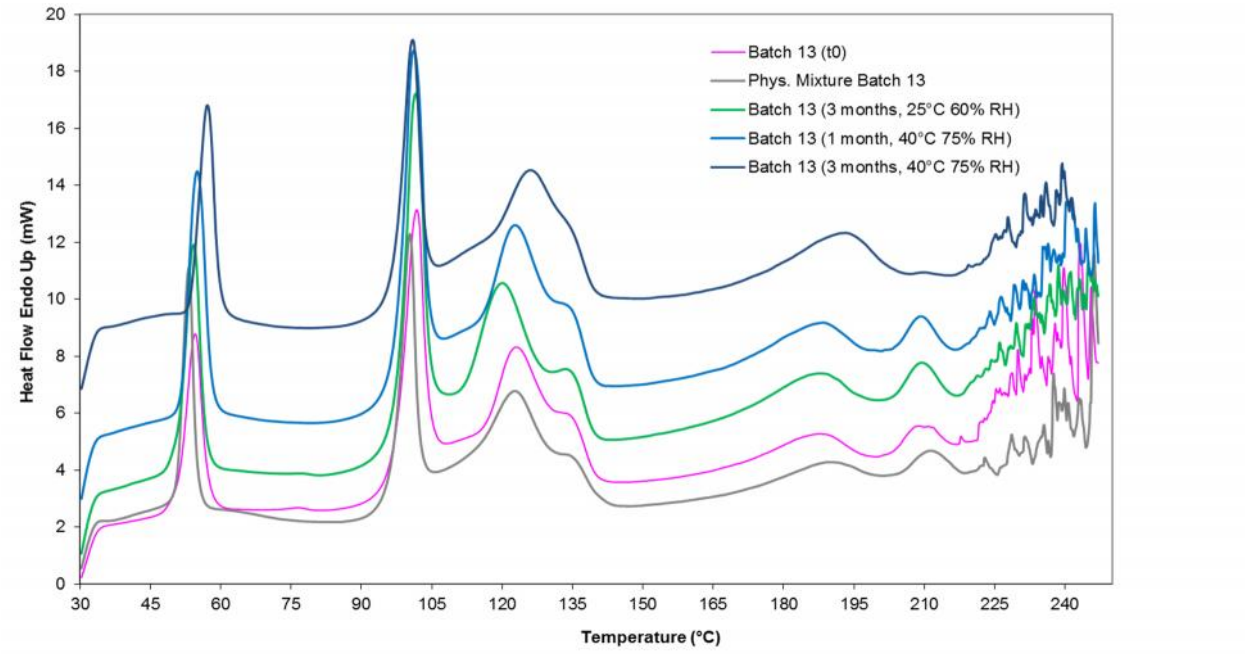


Figure 16. Stability of batch 13 at different storage conditions.

4.4. CONCLUSION

An age-appropriate flexible dosage form intended for the oral administration of PZQ to paediatric patients was developed. A number of formulation challenges related to PZQ characteristics (e.g. low solubility, high dose, bad taste and very poor flowability) were faced. The formulations were considered in relation to dosage and pharmaceutical form and targeted to younger children, which have difficulties in swallowing tablets currently available on market. In particular, safe excipients (lactose, sorbitol and poloxamer 188) were used to obtain granules loaded with 20 % w/w of PZQ. The production process based on fluid bed wet granulation was rapid and easy to control. Granules displayed good flow properties and a uniform distribution of the drug within the size fractions. Granules administered with either water, milk or fruit juice formed a fine suspension that can be easy to swallow and their ease of dose manipulation may contribute to increased compliance and drug bioavailability. Finally, the results of the short-term stability in milk

and upon storage can support the use of this formulation for the potential treatment of schistosomiasis in children of 2–6 years old. Further studies are in progress in order to optimize praziquantel treatment for preschool-aged children focusing on different technological strategies able to enhance the drug solubility and hence to reduce the final dose to be administered.

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5. CASE STUDY 3

DEVELOPMENT OF POLYELECTROLYTE COMPLEXES AS POTENTIAL TASTE MASKING SYSTEMS IN PAEDIATRIC-FRIENDLY FORMULATIONS

5.0. PAEDIATRIC CHALLENGE: TASTE MASKING

5.0.1. TASTE MASKING BY POLYELECTROLYTE COMPLEXES

Following the implantation of European Regulation with respect to medicinal products for paediatric use, scientist community has to speed up for making medicine available for children by encountering multiple problems of paediatric formulation. Indeed, the taste of oral medicine is one of the most crucial factors influencing adherence to therapeutic regimens and therapeutic outcomes. Bad taste of medicine may represent the failure of the treatment.

As reported in the introduction of the thesis, numerous methods are employed for the assessment of the taste and for effective taste masking, for example, use of flavours and sweeteners, microencapsulation, complexing with ion exchange resin, use of insoluble prodrugs, formation of inclusion complexes, gelation, liposome, multiple emulsions, granulation, and so forth.

The following work focused on polyelectrolyte complexes based on natural molecules, protein and polysaccharide, as potential encapsulating agent of active pharmaceutical ingredients characterized by bitter taste. The drug molecule fits inside the complex, through chemical bonds or by physical entrapment. If the complex is stable in the environment of the oral cavity and at the salivary pH, it covers the molecules of the drug, decreasing the amount of particles exposed to taste buds, thereby reducing the perception of bad taste.

5.1. INTRODUCTION

Nowadays, there is a strong interest in designing supramolecular structures to provide multifunctional particles with potential stabilizing, encapsulating and texturing properties for both pharmaceutical and food applications (Jones et al., 2010). These innovative systems can be useful for the encapsulation of pharmaceutical compounds, nutraceuticals, vitamins, flavors, bioactive peptides, colors and preservatives. In the specific case of active pharmaceutical ingredients, the main purposes of their encapsulation are both the delivery to specific sites of action and the taste masking of their bitter taste (McClements, 2015). In fact, once the drug has been encapsulated, through electrostatic interactions, hydrophobic or covalent binds, depending on the nature of the molecules involved, it may be possible to design the particle properties as to deliver the drug to a specific site of action, such as mouth, stomach, small intestine or colon (Jones et al., 2011). Moreover, this system is also of particular interest for the administration of drugs in specific groups of patients, like the paediatric population, in which the unpleasant taste of the drug administered by oral route represents a big challenge. In fact, the drug entrapped in the complex is not able to interact with the taste buds, and thus the bitter taste is masked.

Another property of the multifunctional particles is the stabilization of the so called 'Pickering emulsions', system in which they can surround the oil droplets, reducing the interfacial tension (Guzey et al., 2007, Binks, 2002, Frasch-Melnik et al., 2010).

In fact, over the past decades, surfactant-free emulsions have attracted increasing attention to avoid the well-known adverse effects associated with some small molecules surfactants on human health (Soltani et al., 2015). Solid particles can replace surfactants, since they are able to stabilize the emulsions by means of their adsorption at the interface. Partial wetting of the surface of the particles by water and oil is the origin of the strong anchoring of them at the oil-water interface; thus, particles do not need being amphiphilic to ensure the stabilization of the emulsion (Chevalier et al., 2013, Guzey et al., 2004, Moreau et al., 2003).

Multifunctional particles include polyelectrolyte complexes, based on proteins and polysaccharides, which are able to encapsulate active pharmaceutical ingredients and surround the oil droplets of emulsions. Architectural manipulation of the oil-water interface through conjugation of proteins with polysaccharides is a widely attended approach for increasing emulsion stability. In this way, a protein-stabilized interface is

deposited electrostatically with an oppositely charged polysaccharide, leading to an increase of steric hindrance (Hu et al., 2015, Hu et al., 2015).

Pickering emulsions have been studied also for their ability to encapsulate lipophilic drugs, to control their delivery in specific site of action and to ensure their protection against the external environment. Even in this case, drugs entrapped inside the droplets of oil are not able to interact with the taste buds in the mouth, ensuring the masking of bitter taste and improving the compliance of children (Frelichowska et al., 2009, Simovic et al., 2007).

The main idea of the work is based on the development of an innovative structuring approach that enables the simultaneous encapsulation and targeted delivery/release of two actives within/from simple oil-in-water (o/w) emulsions. The system is based on an external aqueous phase and an internal oil phase, stabilized by a polyelectrolyte complex able to place at the interface between the two phases and loaded with the first active. The second active is solubilized into the oil phase of the emulsion.

In particular, the present work is mainly focused on the development of proteins/polysaccharides complexes loaded with the first active. Whey protein and pectin were chosen as natural and nontoxic compounds, especially suitable for the administration in children. Whey proteins are a group of milk proteins classified as globular proteins consisting of mostly β-lactoglobulin and α-lactoglobulin. They can adsorb to the oil droplet surface, reduce the interfacial tension and form a protective membrane around the droplets to prevent the aggregation (Teo et al., 2015, Gunasekaran et al, 2007). Pectin is a nontoxic, biodegradable, biocompatible and anionic polysaccharide extracted from the cell walls of most plants, such as apples, oranges and pears. It is characterised by gelling property and branched heteropolysaccharides, which consists predominantly of linear chains of partially methyl-esterified (1,4) -d-galacturonic acid residues. Depending on the degree of substitution of d-galacturonic carboxyl groups by methoxyl groups (OCH3), defined as the degree of esterification (DE), pectins are classified as high-esterified pectins (DE > 50 %) or low-esterified pectins (DE < 50 %) (Marciel et al., 2015). Caffeine, a methyl xanthine alkaloid, was chosen as a model drug for its bitter taste. Complexes were loaded with caffeine and analysed in terms of the particle size, the zeta potential, the encapsulation efficiency, the stability and the *in vitro* release profile in different fluids simulating the gastrointestinal tract.

The second part of the work will consider the preparation and characterization of emulsions as a system for the simultaneous encapsulation and release of two different active compounds.

5.2. MATERIALS AND METHODS

5.2.1 MATERIALS

Pectin Betapec RU 301 (PEC, DE > 50 %, pKa = 3.5) was donated by Herbstreith & Fox KG (Neuenbürg, Germany). It is a beige powder of neutral smell, extracted from sugar beet chips and soluble in water to a viscous and colloidal solution.

Whey Protein (WPI, MW 14000-18000 Da, IEP = 5.2) was purchased from Volac (Orwell, United Kingdom). The composition of the whey protein was: 91.0 % w/w protein, moisture 4.0 % w/w, fat 1.0 %, ash 3.5 % w/w and lactose 0.5 % w/w. Mineral content of the WPI was: 0.50 % w/w Ca, 0.65 % w/w P, 0.10 % w/w Na, 0.15 % w/w K, 0.02 % w/w Mg, and 0.02 % w/w Cl. Caffeine (anhydrous) (CAF, MW 194.19 g/mol) was purchased from Sigma-Aldrich (UK).

Sodium hydroxide pellets (NaOH) and hydrochloric acid (HCl) concentrated were purchased from Fisher Scientific Chemical (Loughborough, UK) and used to prepare diluted solutions in distilled water.

All the other chemicals and solvents used for the preparation of buffers were of analytical grade and purchased from Sigma-Aldrich (UK).

5.2.2. METHODS

5.2.2.1. PREPARATION OF BIOPOLYMER SOLUTIONS

In order to obtain polyelectrolyte complexes, preliminary solutions of the two biopolymers were prepared. PEC and WPI were both solubilized in Millipore water, at double the concentration with respect to the final concentration needed in each case. PEC

was stirred with a magnetic agitation for at least 2 h and slightly heated to facilitate the dissolution and ensure the hydration of the powder in order to obtain 0.4 % (w/w) and 0.8 % (w/w) solutions. The pH of the final solutions was around 4, and adjusted to 7 with diluted NaOH. WPI was dissolved at room temperature until complete dissolution of the powder for at least 2 h, using a magnetic stirrer to obtain 1.0 % (w/w) and 2.0 % (w/w) solutions. The final pH of the solutions was adjusted to 7 with few drops of diluted NaOH.

5.2.2.2. COMPLEX FORMATION

The complex formation involves the mixing of 50/50 (v/v) WPI and PEC solutions at pH 7, a value at which they are both negatively charged and the electrostatic attraction does not occur. In particular, 0.4 % (w/w) of PEC solution was added to 1.0 % (w/w) of WPI solution and 0.8 % (w/w) of PEC solution was added to 2.0 % (w/w) of WPI solution, by mixing with magnetic stirrer at room temperature for 30 min. The final contents of the solutions containing the two biopolymers were 0.2 % (w/w) PEC and 0.5 % (w/w) WPI, 0.4 % (w/w) PEC and 1.0 % (w/w) WPI respectively, with total biopolymer concentrations of 0.7 % w/w and 1.4 % w/w respectively. Complex formation was achieved by acidifying the mixtures at pH 4 with few drops of HCl 1M and leaving them at room temperature for 1 h under gentle magnetic agitation. In these conditions, electrostatic interactions can occur between PEC, negatively charged, and the positive charges of WPI (Cooper et al., 2005, Hong et al., 2007, Santipanichwong et al., 2008, Peinado et al., 2010). Caffeine (CAF) was added in the protein solutions before complexing, at a final concentration of 5 mg/ml, 10 mg/ml and 20 mg/ml. A thermal treatment of the complex was also investigated. It has been applied in order to overcome some obstacles that limit the widespread application of complex coacervation, as the low stability over a large range of pH values, the possible increase in the mean particle size and eventually the macroscopic phase separation. (Kruif et al., 2014, Hong et al., 2007, Jones et al., 2009). For this reason a controlled heating of electrostatic complexes conducted after complexation, has been also evaluated. This has been proposed to increase the stability of complexes to environmental conditions such as changes in pH and ionic strength. One part of each complex at pH 4 was immediately stored at 4 °C, one part was placed in water bath at 95 °C and heated at 87 °C for 1 min. The solutions were kept stirred using a submersible magnetic stirrer, cooled down with ice and stored at 4 °C.

5.2.2.3. COMPLEX CHARACTERIZATION

Particle size measurements.

PEC-WPI complex size analysis was performed by dynamic light scattering (DLS) using the Zetasizer Nano Series (Nano ZS, Malvern Instruments, Worcestershire, United Kingdom). The instrument infers the size of the particles from measurements of their translational diffusion coefficients. Two drops of concentrated PEC-WPI complex suspension were diluted into 50 ml of water at pH 4; this was immediately transferred to a polystyrene cuvette and analyzed. The analyses were conducted on at least three freshly diluted samples.

Zeta-potential analysis.

Zeta-potential analyses were performed on the Zetasizer Nano Series (Nano ZS, Malvern Instruments, Worcestershire, United Kingdom), equipped with MPT-2 multipurpose titrator. The zeta-potential was determined by measuring the direction and velocity that the molecules or complexes moved in the applied electric field. A mathematical model was used by the software to convert the electrophoretic mobility measurement into zeta-potential values. For the analyses of complexes, the suspension was diluted in the same way as for the size analyses and added to a zeta cell. Zeta-potentials were reported for triplicate readings of three freshly diluted samples.

5.2.2.4. ENCAPSULATION EFFICIENCY

In order to evaluate the amount of CAF entrapped inside the complexes, encapsulation efficiency (EE %) was determined for each sample. 1.5 mL of complex suspension were transferred to separate Eppendorf tubes and centrifuged for 60 min at 15,000 rpm and 20 °C; this centrifugation time was determined in preliminary experiments as sufficient to separate the complex as an insoluble pellet. The supernatant was isolated, filtered with 0.2 µm filters and analysed by HPLC, using an Agilent 1100 series chromatography system. 20 µl of each sample was injected onto a reverse phase Onyx monolithic C18 column, (100 x 4.6 mm). The mobile phase was prepared by mixing 500 ml of deionized water and 500 ml of methanol and the flow rate was 0.8 ml/min. UV detection was

performed at 275 nm. A calibration curve showed that the peak area was linearly related ($r^2 = 0.999$) over the CAF concentration range 0.125-10 mg/ml.
EE % was then calculated with the following equation:

$$EE\ \% = (1- C_{sup}/C_{max}) \times 100$$

Where C_{sup} represents the concentration of the CAF isolated in the supernatant after centrifugation (it is assumed that this concentration is that which was not complexed), and C_{max} is the theoretical concentration of CAF previously added into the system. Some preparative conditions were modified in order to increase the CAF encapsulation efficiency, as the total biopolymer concentration (TBC), the concentration of CAF and the temperature of complexation. All the analyses were conducted in triplicate.

5.2.2.5. STABILITY STUDIES

Once biopolymer particles have been formed, it is important to establish their stability in particular conditions. This type of information is useful to determine the range of commercial products where the biopolymer particles can be successfully utilized. In order to assess the stability of the complexes, each sample was stored at 4 °C for 30 days in closed glass bottles and the zeta-potential, the size and the encapsulation efficiency have been evaluated in predetermined time points, by using the same methods described above.

5.2.2.6. *IN VITRO* DISSOLUTION ANALYSES

In vitro dissolution studies of CAF from the complexes were carried out using a USP II paddle apparatus (Tablet Dissolution Tester DIS 6000, Copley Scientific), rotating at 100 rpm and at constant temperature of 37 °C. Glass vessels of convenient capacity and size, and covered with lids to minimize and avoid the evaporation of the fluids were used. Different biorelevant media were chosen to mimic the gastrointestinal tract and to evaluate the potential of these complexes to enable drug release at specific locations (Kostewicz, et al, 2014). The oral cavity has been mimicked using 25 ml of buffer at pH = 7.4 (potassium dihydrogen phosphate 12 mM, sodium chloride 40 mM, calcium chloride 1.5 mM, sodium chloride to pH = 7.4, demineralized water to 1 L). Dissolution was monitored over a period of 60 sec in this medium. The stomach has been simulated with 50 ml of Simulated Gastric Fluid (without pepsin) USP-30NF45 at pH < 2 (sodium

chloride 2.00 g, HCI 1.0 M 70.0 mL, deionized water to 1 L), during a period of 30 min. Finally, 1000 mL of Blank Fasted State Simulated Intestinal Fluid (FaSSIF) was used at pH = 6.8 to mimic the intestinal environment (sodium hydroxide pellets 0.348 g, sodium dihydrogen phosphate 3.954 g, sodium chloride 6.186 g, deionized water to 1 L) over a period of 2 h. At predetermined time points 2 ml samples were withdrawn, filtered (0.2 µl), analyzed for drug content using HPLC and replaced with fresh 2 ml (preheated at 37°C) dissolution media after each sampling. Each experiment was performed in triplicate.

5.3. RESULTS AND DISCUSSION

5.3.1. COMPLEX FORMATION

Polyelectrolyte complexes based on whey protein and pectin were prepared by electrostatic attractions between oppositely charged groups, anionic polysaccharide and cationic protein surface groups, under pH controlled conditions. Complexes were formed at pH 4, which is a value less than the isoelectric point of WPI (IEP = 5.2), where protein possesses a net positive charge and less than the pKa of PEC, (pKa = 6.3), where pectin is negatively charged. This provides a system in which electrostatic interactions can occur, eventually resulting in charge neutralization of the protein-polysaccharide complex and subsequent coacervation or precipitation.

The thermal treatment has been applied in order to overcome some obstacles that limit the widespread application of complex coacervation, as the low stability over a large range of pH values, the possible increase in the mean particle size and eventually the macroscopic phase separation. (Kruif et al., 2014, Hong et al., 2007 , Jones et al., 2009). For this reason a controlled heating of electrostatic complexes conducted after complexation, has been also evaluated. This has been proposed to increase the stability of complexes to environmental conditions such as changes in pH and ionic strength. The compositions of the different formulations are reported in Table 19.

Table 29. Composition of CAF loaded complexes.

Formulation	WPI (%)	PEC (%)	CAF (mg/ml)	heated	unheated
F_{1a}	0.5	0.2	1.0		X
F_{1b}	0.5	0.2	1.0	X	
F_{2a}	0.5	0.2	5.0		X
F_{2b}	0.5	0.2	5.0	X	
F_{3a}	0.5	0.2	10.0		X
F_{3b}	0.5	0.2	10.0	X	
F_{4a}	1.0	0.4	10.0		X
F_{4b}	1.0	0.4	10.0	X	
F_{5a}	1.0	0.4	20.0		X
F_{5b}	1.0	0.4	20.0	X	

5.3.2. COMPLEX CHARACTERIZATION

Particle size measurements.

Particle size distribution is one of the most important factor influencing complex physicochemical properties and functional performance, such as stability and release characteristics. The mean particle diameter of the nanoparticles was measured at pH 4 and the results are shown in Figure 1. There are no significant differences among the formulations; unheated complexes demonstrated a slight increase in the particle diameter from F_{1a} to F_{5a}, with a mean value of about 363 nm. In general, there are no differences between unheated and heated complexes, for each formulation, except for $F_{3,}$ where heated complex has a diameter significant less than unheated complex. Heated complexes apparently remained more stable respect to the others with a mean value of about 304 nm. This means that the biopolymer weight ratio, the total biopolymer concentration and the concentration of CAF in the system did not affect the size of the particles. The thermal treatment instead seemed to permit more stability and a reduction of the main particle size (Jones et al., 2011).

Zeta potential analysis.

The electrical characteristics of biopolymer particles, depending on the type, concentration and localization of any ionized groups at their surface, and measured at pH 4, was not strongly affected by the modifications of the preparative conditions (data

showed in Figure 17), showing no significant differences among the different formulations. Zeta potential values resulted around -22 mV and -22.5 mV for unheated and heated complexes, respectively. The negative charge of the complexes confirms the core-shell structure and is the result of the placement of the anionic PEC molecules onto the surface of cationic WPI core. The fact that the net charge on the nanoparticles was negative is due to the formation of an outer coating and not all the anionic groups of the pectin molecules were bound to the cationic groups of the protein molecules (Hu et al., 2015). The CAF was included inside the complexes and it did not affect their electrical surface properties.

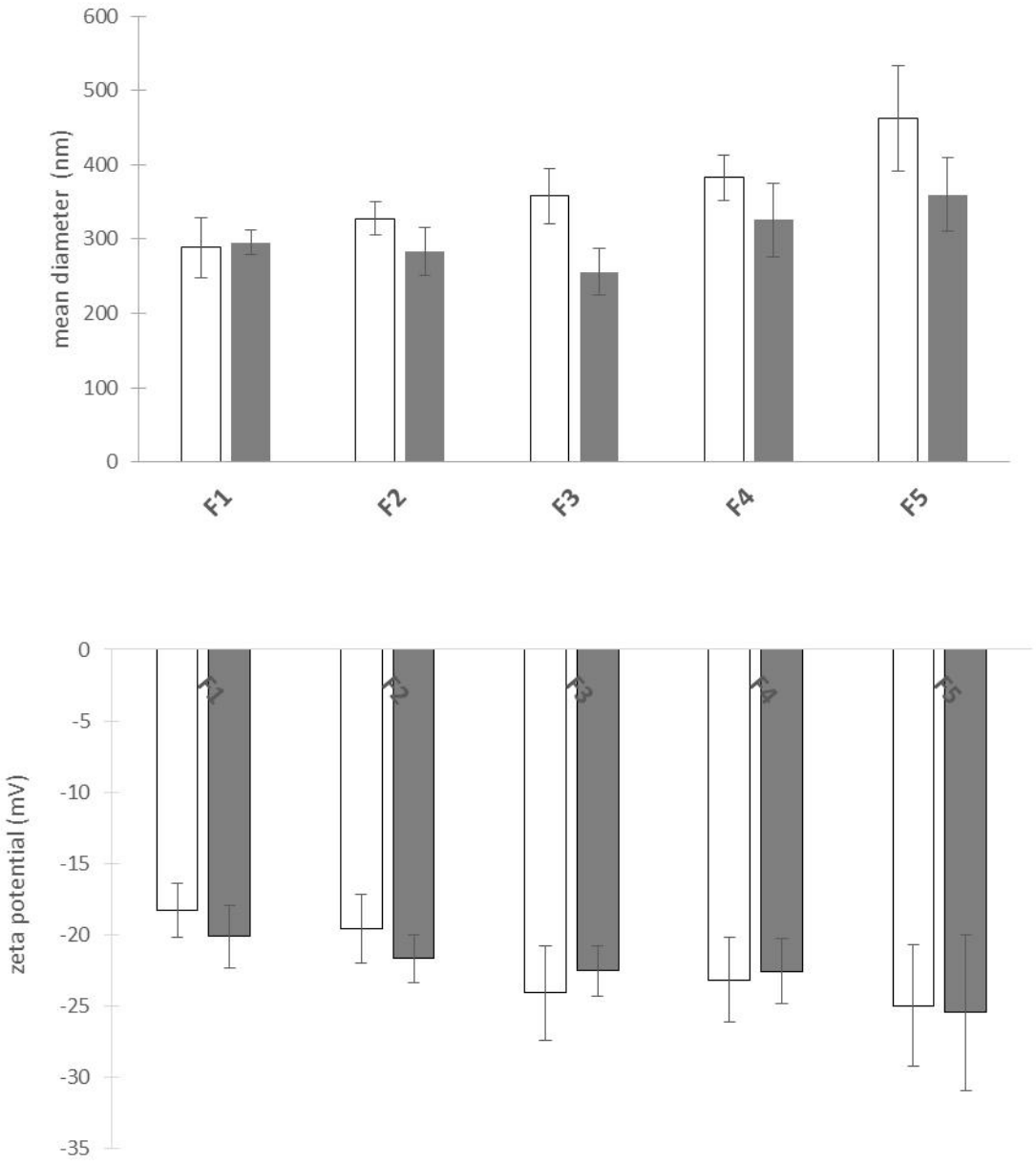


Figure 17. Complex characterization: particle size and zeta potential of unheated (white) and heated (grey) complexes.

5.3.3. ENCAPSULATION EFFICIENCY

Biopolymer particles could be developed to encapsulate, protect and release active pharmaceutical ingredients, such as CAF. The drug should be mixed with protein-polysaccharide solution at ambient temperature, before the formation of the complex and the heating of the system. Preliminary experiments were carried out in order to verify the stability of the drug in our experimental conditions, and they showed that CAF does not undergo any modifications at 87 °C (T_m= 234-236.5 °C).

The measurement of encapsulation efficiency is an efficient method to verify the ability of the system to incorporate the active and to determine its concentration within the nanoparticle. PEC-WPI complexes were prepared with a final concentration of 5 mg/ml, 10 mg/ml and 20 mg/ml, as showed in Table 19, and the results suggest that the increase of CAF concentration, in both unheated and heated complexes, led to an increase of the encapsulation efficiency of the system (Figure 18). In particular, complexes prepared with 20 mg/ml of CAF, achieved an encapsulation efficiency around 50 % (52.66 ± 1.07 and 49.11 ± 3.16 for unheated and heated complexes respectively). This behaviour could be mostly attributed to the fact that the more concentration of CAF, the more chances to create physical and chemical interactions between the active and the biopolymers. All the other modifications of the preparative conditions, did not significantly affect the encapsulation efficiency of CAF inside the complexes.

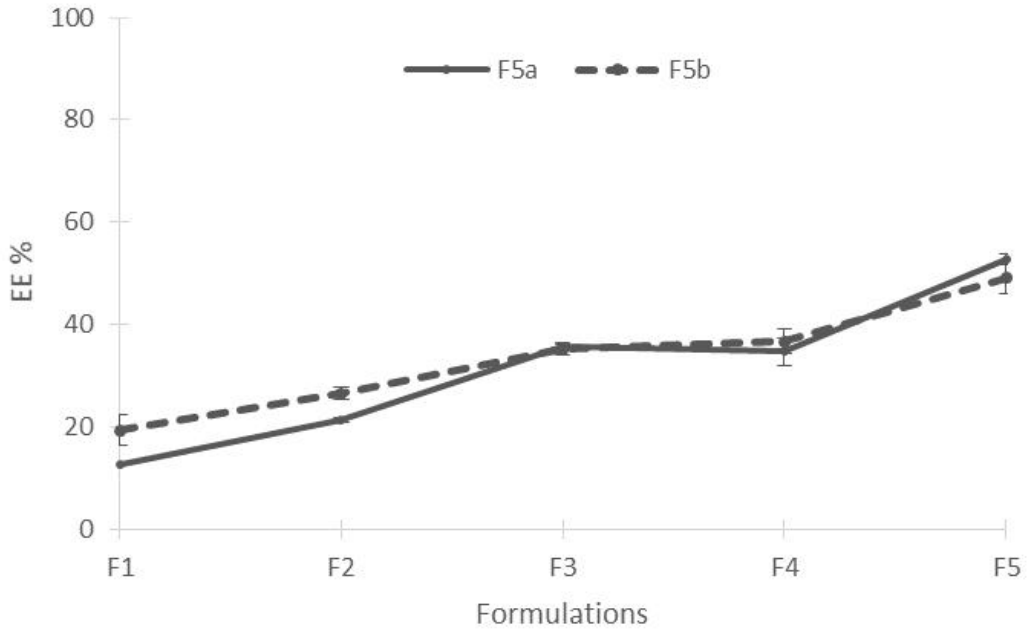
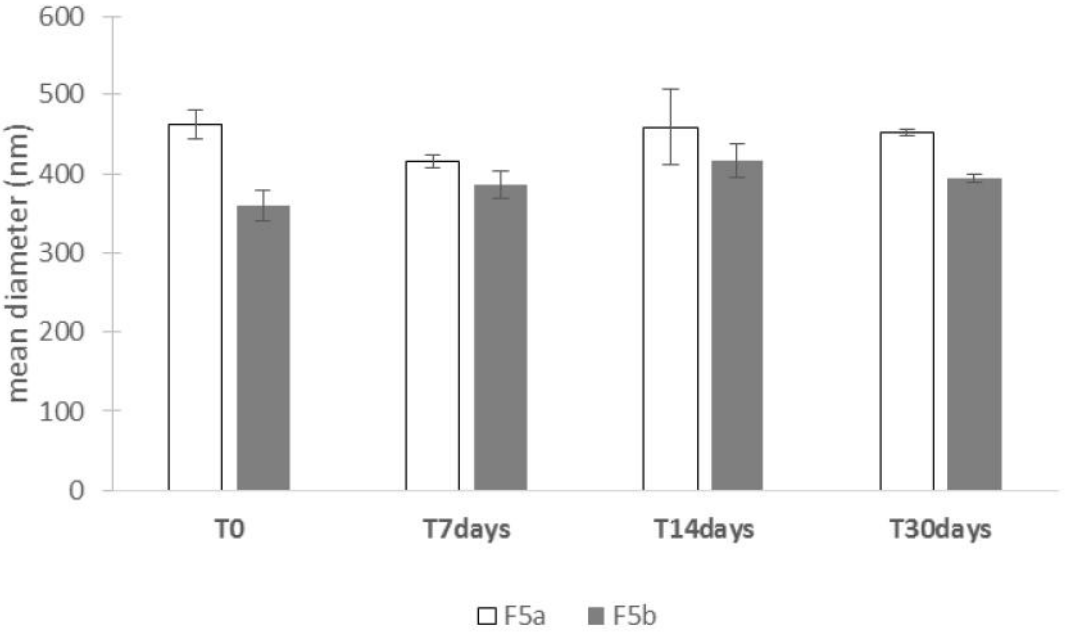


Figure 18. Encapsulation efficiency % of CAF inside complexes for each formulation.

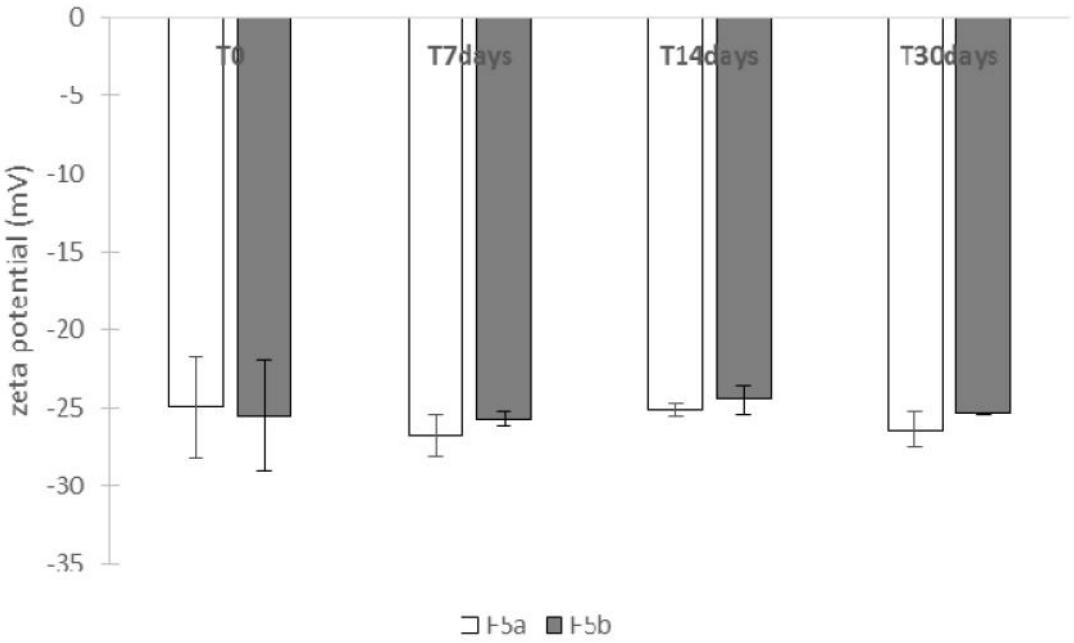
5.3.4. STABILITY STUDIES

Formulations F_{5a} and F_{5b}, which showed the best properties in terms of encapsulation efficiency, were subjected to stability studies, under storage in closed glass-bottles at 4 °C for 30 days. The stability, evaluated in terms of zeta potential, particle size and encapsulation efficiency at predetermined time points, is reported in Figure 19. The results showed that both the complexes, unheated and heated, remained stable after 7, 14 and 30 days, without any significant modifications as regards the main properties and the encapsulation efficiency of the system. This means that the structures of the two biopolymers, their interactions, the core-shell structure of the complex and the encapsulation efficiency of the active inside the system are maintained during time, allowing the possible use of the formulations one month after their preparation.

(a)


600
500
400
300
200
100
0
mean diameter (nm)
T0
T7days
T14days
T30days
F5a
F5b

(b)


0
-5
-10
-15
-20
-25
-30
-35
zeta potential (mV)
T0
T7days
T14days
T30days
F5a
F5b

(c)

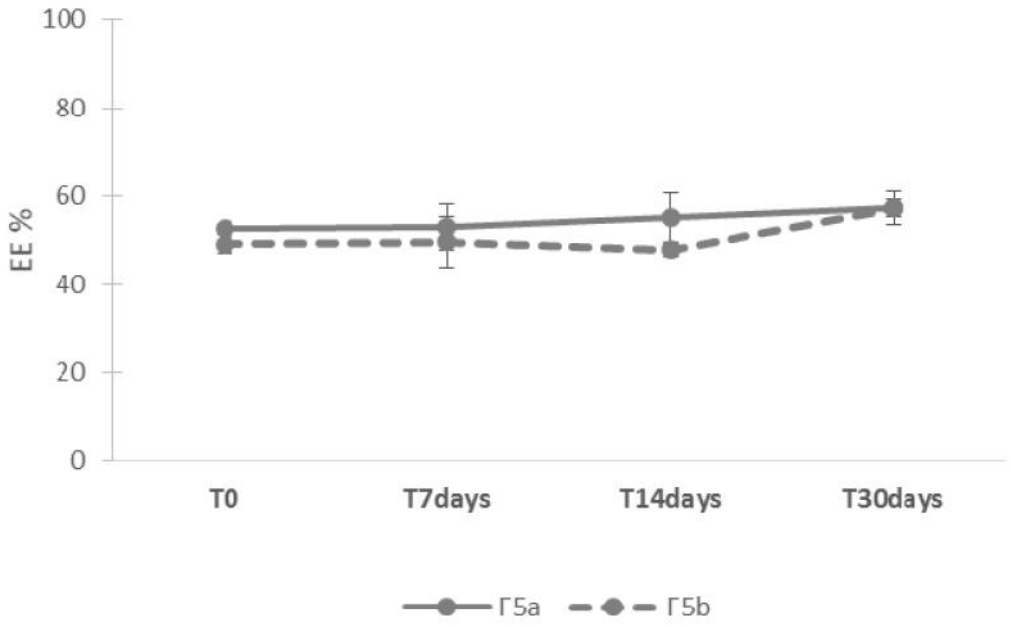


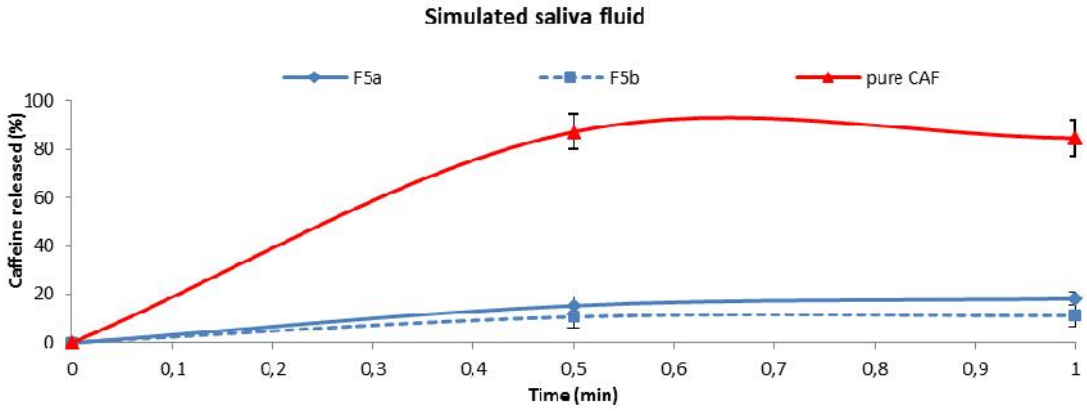
Figure 19. Stability studies: (a) particle size, (b) zeta potential, (c) encapsulation efficiency.

5.3.5. *IN VITRO* DISSOLUTION ANALYSIS

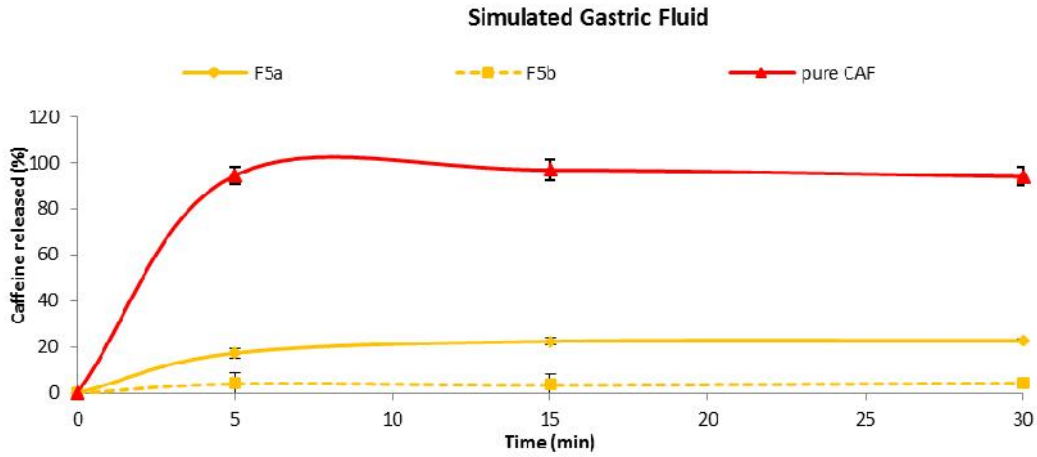
Liquid formulations are usually the preferred model of delivery bitter drug, especially in pre-school aged children (Nunn et al., 2005), and in particular, the delivery system may have to release the functional component at a particular site of action (Mc Clements et al., 2009). In fact, the structure protecting the bitter ingredient must breakdown not in the mouth, but during later digestion to release the active compound so that it becomes bioavailable (Coupland et al., 2014). In order to verify these conditions, F_{5a} and F_{5b} were selected for *in vitro* dissolution studies, as the best formulations in terms of encapsulation efficiency and for the good stability upon a storage of 30 days at 4 °C. For comparison, an equivalent amount of pure CAF was analysed by using the same buffers and the same conditions. Sink conditions were used to study the dissolution behaviour in fluids simulating the whole gastrointestinal tract. The solubilisation test was first performed in 25 ml of simulated saliva fluid (SSF). More than 85 % of pure CAF was solubilized in SSF in 30 seconds (Figure 20a), while the amounts of CAF released from the complexes were of 18.2 % and 11.2 % after 60 seconds, for F_{5a} and F_{5b}, respectively. Then the analysis was conducted in 50 ml of simulated gastric fluid (SGF) (Figure 20b); in these conditions 100 % of the pure active was dissolved after 5 minutes, and only 17.4 % and

4.2 % were released from the complexes in 5 minutes for unheated and heated complexes respectively. Finally the dissolution studies carried out in 1000 mL of fasted state simulated intestinal fluid (FaSSIF), revealed a complete solubilisation of the pure active within 5 minutes, while 81.4 % and 45.1 % of CAF were released from the complexes after 5 minutes for F_{5a} and F_{5b} respectively (Figure 20c). The dissolution analyses highlighted a decrease in solubilisation and dissolution rate of CAF for F_{5a} and F_{5b} formulations, when compared with the pure active. CAF was completely dissolved in each medium in a short time while both the formulations permitted a controlled release of the drug, especially in the media simulating the oral cavity and the stomach. This behaviour could be related to the charge density of the system, in fact at high pH values, the system is characterized by an excess of negative charges that promote the water uptake, the hydration of the biopolymer, and the subsequent release of the active substance. Another important consideration concerns the difference in the dissolution profiles of unheated and heated complexes; in all the simulated fluids, heated complex demonstrated a lower release of CAF respect to the unheated complex. This behaviour is in agreement with literature data for which the heating of protein-polysaccharide complexes leads to the aggregation of the biopolymer through hydrophobic interactions and/or disulfide bonds and the formation of more stable particles in a large range of pH (Jones O. et al., 2010).

(a)



(b)



(c)

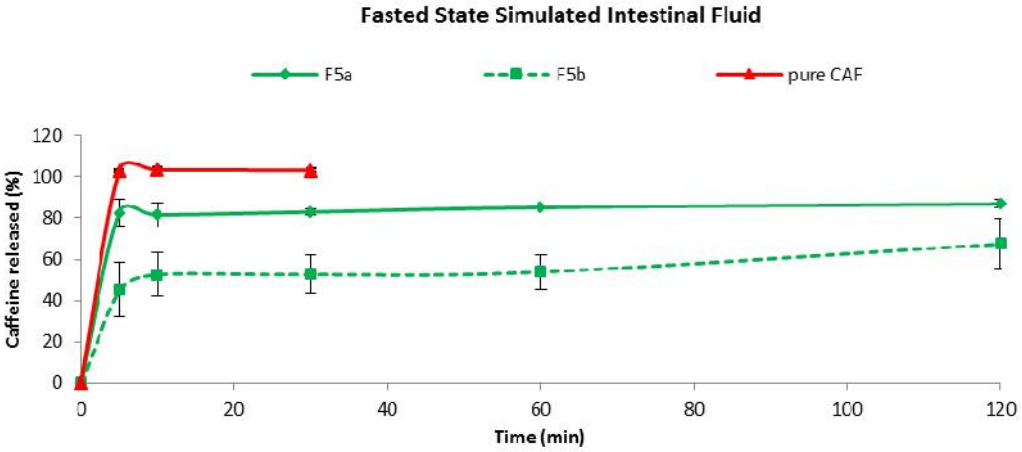


Figure 20. Dissolution test of complexes and pure caffeine in different media: (a) simulated saliva fluid, (b) simulated gastric fluid and (c) fasted state simulated intestinal fluid.

5.4. CONCLUSION

Biopolymer particles intended for the encapsulation and oral administration of caffeine to paediatric patients were developed. Polyelectrolyte complexes based on whey protein and pectin, loaded with different concentrations of caffeine and stable during one month were prepared with a rapid and easy production process, by mixing the aqueous solutions of the two biopolymers and adjusting the pH at 4, value at which the electrostatic interactions can occur. Complexes displayed uniformity in terms of the mean particles size and the zeta potential, whereas the encapsulation efficiency increased with the increase of the concentration of the drug in the system. Heating the complexes did not affect the particles size, the zeta potential and the encapsulation efficiency respect to the unheated complexes, but the heated system were more stable, allowing a lower release of caffeine in the three different biorelevant media. Dissolution profiles of caffeine from these systems in simulated saliva fluid demonstrated that a low amount of drug was released from heated and unheated complexes, with respect to the other media, providing the confirmation about the fact that these biopolymer particles is a suitable system for the taste masking of molecules characterized by unpleasant taste. Nevertheless, further work is needed for the assessment of the compliance of paediatric patients.

The second part of the study will be focused on the ability of biopolymer particles based on polyelectrolyte complexes in stabilizing emulsions, intended for the simultaneous encapsulation and delivery of two different drugs.

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FINAL CONCLUSIONS

The considerable attention that paediatric medicines has gained in the last few years is mainly due to the lack of appropriate formulations suitable for the administration in children.

Taking into account the peculiar needs, together with anatomical and physiological characteristics of the paediatric population, it was possible to discover novel technological platforms and improve formulations and dosage forms already available on the market, with the ultimate goal to ensure greater adherence to the therapy and success of the treatment, avoiding the risk of errors.

The results discussed in the first 'case study' have showed that transmucosal route was successfully employed for systemic delivery of ondansetron hydrochloride, through the buccal mucosa, by polymeric mucoadhesive films. The retention time of the dosage form on the application site and its ability to provide a sustained release, improved the absorption of the drug into the systemic circulation, allowing a decrease of dosage and frequency of administration and minimum impact on children lifestyle.

The second 'case study' have demonstrated that a flexible dosage form intended for the administration of praziquantel to paediatric patients is a valid alternative to the conventional tablet already available on the market. Granules can be administered with water, milk or fruit juice, forming a very fine suspension easy to swallow. Their ease of dose manipulation may contribute to increased compliance and drug bioavailability.

The third 'case study' have highlighted the possibility to develop polyelectrolyte complexes as potential systems for the taste masking of active substances characterized by bitter taste, leading to paediatric-friendly formulations, better tailored to children.

Finally, the results presented in this thesis demonstrated that some of the predominant issues related to paediatric administration of medicines can be overcome by using specific formulation strategies, in order to guarantee the complete adherence of children to the therapy and thus the success of the treatment.

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