Universidade de Lisboa

Faculdade de Farmácia

Evaluation of fresh milk as a novel excipient

Daniela Gargana Batista

Mestrado em Ciências Farmacêuticas

2019

Universidade de Lisboa

Faculdade de Farmácia

Evaluation of fresh milk as a novel excipient

Daniela Gargana Batista

Monografia de Mestrado Integrado em Ciências Farmacêuticas apresentada à Universidade de Lisboa através da Faculdade de Farmácia

Orientador: Doutor João Fernandes de Abreu Pinto, Professor Associado
Co-Orientador: Doutor Matteo Cerea, Professor

2019

ABSTRACT

The goal of this study was to access the potential of using different types of fresh milk (half-fat, skimmed and fat milk) as a binding and taste masking excipient for manufacturing pharmaceutical dosage forms designed specifically for the paediatric population. Each batch of pellets was initially produced using paracetamol, microcrystalline cellulose (MCC) as a binder, different types of polymers between polyvinylpyrrolidone (PVP), hydroxypropyl methylcellulose (HPMC), sodium carboxymethyl cellulose (SCMC) as a super-disintegrant and deionized water as a vehicle in every single batch. An experimental batch with powder milk was also developed. Batches with fresh milk (skimmed milk, half-fat and fat milk) instead of deionized water were considered to manufacture pellets. All the products obtained were analyzed with the following parameters: size distribution, aspect ratio, LOD (Loss on Drying), friability, drug content and dissolution time.

Pellets with skimmed milk showed the highest drug content and pellets made of 30% of microcrystalline cellulose had the slowest release rate, except for the skimmed milk pellets. The presence of sodium croscarmellose in the pellets increased the release. Yet, PVP pellets made with deionized water and half-fat milk proved to be the most satisfactory ones. The study has proved that fresh milk is suitable for the production of pellets for paediatric use.

Keywords: Milk, Paediatric Formulation, Paracetamol, Pellets

RESUMO

Este estudo teve como objetivo avaliar o possível potencial inerente ao uso de diferentes tipos de leite fresco (leite magro, leite meio-gordo e leite gordo), em que estes terão a função de agente agregante e mascarante de sabor.

Cada lote de pellets foi inicialmente produzido utilizando paracetamol, celulose microcristalina (MCC) como agente aglutinante, diferentes tipos de polímeros entre os quais a polivinilpirrolidona (PVP), a hidroxipropilmetilcelulose (HPMC), a carboximetilcelulose de sódio (SCMC) e água desionizada como veículo em cada um dos lotes, sendo que o seu objetivo principal foi o desenvolvimento das melhores condições de controlo do processo. Foi ainda desenvolvido um lote experimental com leite em pó. De seguida, produziram-se lotes com os mesmos componentes, mas desta vez, substituindo a água desionizada por diferentes tipos de leite fresco (leite magro, meio-gordo e gordo). Todas as formulações foram analisadas com os mesmos parâmetros: distribuição por tamanho, aspect ratio, perda por secagem, friabilidade, teor da substância ativa e ensaios de dissolução. Pellets com leite magro apresentaram um maior teor de substância ativa e pellets com 30% de MCC obtiveram uma menor taxa de libertação, com exceção dos pellets de leite magro. As formulações com SCMC demonstraram uma maior taxa de libertação da substância ativa. Ainda assim, os pellets de PVP produzidos com água desionizada e leite meio-gordo demonstraram ser os mais satisfatórios. O estudo demonstrou que o leite fresco é um excipiente adequado na produção de pellets para uso pediátrico.

Palavras-chave: Formulação Pediátrica, Leite, Paracetamol, Pellets

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Nowadays there is a broad consensus that children deserve access to medicines that have been specifically developed and researched for their use in young patients. However, until recently, the development and testing of paediatric medicines was far from satisfactory because of the lack of specific formulations to this population. Due to the fact that medicines were often not available in a pharmaceutical form suitable to children, paediatricians had to turn to medicines authorized for adults by adapting the dosage and form. For example, by crushing adult tablets and using only a portion. This off-label use of adult medicines comes with the risk of inefficacy and/or adverse reactions in children. Side effects that may not affect adults can be important and serious in children (1). Hereupon, there was the need to implement a Paediatric Regulation in the European Union (EU) which led to an increment on the requirement of children adapted medicines.

According to the European Medicines Agency (EMA), there are some important aspects that should be considered in the selection of excipients during the development of paediatric medicines. The referred parameters are: the safety profile (both in single administration as in the long term administrations); it's palatability, which may affect therapy compliance and its potential to cause hypersensitivity reactions that can be life threatening and also the ease of administration of the final pharmaceutical forms (2).

The development of an age-appropriate formulation is a challenging task due to the wide range of pharmaceutical and clinical aspects that must be considered in order to ensure the quality, safety and efficacy of the final product. In particular, the development of pediatric formulations is complex due to the additional needs and demands of this population. The pharmacokinetic and pharmacodynamic profile of a drug varies broadly depending on the developmental stage of a child, necessitating dose flexibility to suit the dosing requirements across all age groups. Excipients commonly known as safe may represent a safety risk for children. Palatability and ease of swallowing are also considered a major issue on the therapeutic acceptability of medicines intended for children, who possess distinct preferences and swallowing abilities than the rest of the population. In many cases, the dependence on caregivers also influences the administration and acceptability (3).

Oral administration is the preferred route for the delivery of drugs, however, poor solubility of some lipophilic drugs limits their oral bioavailability, which represents one of its major problems. The majority of these drugs are also limited by their bitter taste, which may affect the therapy compliance specially in the pediatric population (2).

Studies have reported that small-sized multiparticulates can be swallowed by children over 6 months of age when dispersed in food or beverages, using appropriated medical devices (3,4).

2. STATE OF THE ART

2.1. Milk in the pharmaceutical industry

2.1.1. Milk composition

Milk is mainly composed by water, followed by fat, lactose and protein (casein and whey proteins) in smaller amounts. Milk also contains small quantities of minerals, specific blood proteins, enzymes and small intermediates of mammary synthesis. The major lipid component of cow's milk is triglyceride, which makes up a total of 98% of milk fat. The other 2% consists on mono and diglycerides, cholesterol, phospholipids, free fatty acids, cerebrosides and gangliosides. In average, milk contains about 33 g total lipid (fat)/L (5) and nearly all the fat (> 95%) exists in the form of globules (6). Bovine milk contains about 32 g protein/L (5), which makes up about 3,5% of the total. Milk proteins can be fractionated into two main groups: caseins and whey proteins (6). The casein content of milk represents about 80% of milk proteins. Caseins biological function is to carry calcium and phosphate and to form a clot in the stomach for efficient digestion. In normal milk, 95% of the casein exists as coarse colloidal particles called micelles, with diameters ranging from 80 to 300 nm (6). It has been observed that hydrophobic drug is entrapped by the casein micelles. Due to their surface-active properties, there is an improvement in the solubility of the active substance in gastric media when administered orally. These micelles have a porous structure which helps in the release of entrapped drug molecules from the inclusion complex into the dissolution media (7). On the other hand, whey proteins are globular proteins that are more water soluble than caseins, and the principle fractions are β-lactoglobin, α-lactalbumin, bovine serum albumin (BSA) and immunoglobulins (5).

	Type of milk		
	Skimmed milk	**Half-fat milk**	**Fat milk**
Lipids (g)	0,5	1,6	3,6
Proteins (g)	3,3	3,2	3,2
Carbohydrates (g)	4,9	5,0	4,8
Salt (g)	0,13	0,1	0,13
Calcium (g)	0,12	0,12	0,12

Table 1 - Nutritional values related to the different types of milk used in the investigation. The values refer to 100ml. *(Source: Esselunga, Italy)*

2.1.2. Milk as excipient

The aqueous solubility of a drug is essential for its bioavailability. Lipophilic drugs are recognized for being insoluble in an aqueous medium, resulting in a low bioavailability. In order to solve this problem, some studies refer that milk is a good carrier to this type of drugs since they have higher solubility in milk than in the aqueous medium, both in ionized as well as unionized forms (7).

In order to improve the oral bioavailability of this type of drugs, one of the common approaches has been through the use of lipid-based drug delivery systems. Milk is a natural, abundant and cheap emulsion that is commonly used daily in a child's diet and it is a patient friendly approach for the delivery of ionized and unionized hydrophobic drugs. Its ability to transport is due to the fact that casein proteins exist in a colloidal form called casein micelle thus allowing the hydrophobic drugs to be entrapped inside the micelle structure. Due to their surface-active properties such as a hydrophobic core and hydrophilic coating, there is an improvement in the solubility of the drug substance in gastric medium when administered orally. Casein micelles are also known for having a porous structure, which will help in the release of entrapped drug molecules into the dissolution medium (7). These milk characteristics lead to great solubilizing, gastroprotective and taste masking properties, which are very important features in the case of insoluble, irritating and bitter-tasting active compounds (2). Studies show that there is a direct proportion between the gastroprotective effect and the fat content of milk. The three types of milk were all significantly gastroprotective with the greatest protection seen on fat milk (8). Also, the use of lactose free milk for these formulations is still to be explored for patience with lactose intolerance (7).

For these reasons, milk can be very useful as an excipient: not only it masks the unpleasant flavors of some active substances, enhancing paediatric patient compliance as well it is a part of a children's diet from their birth, having high nutritional values and an important role in their healthy development (9).

3. AIM OF THE STUDY

The present work was conducted to assess the potential of using different types of fresh milk (half-fat, skimmed and fat milk) as a binding excipient for manufacturing pharmaceutical dosage forms designed specifically for pediatric population. Using fresh milk as an excipient enables to combine the technological properties of the milk such as enhanced solubility of poorly soluble drugs, good palatability and taste masking ability with its dietary-nutritional properties.

4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Paracetamol (Acetaminophen)

Paracetamol is one of the safest and most widely used analgesic/antipyretics in children. When possible, the oral paediatric dose should be calculated based on the child's weight, using 10-15 mg/kg/dose. This dose should be given every 4-6 h and should not exceed more than 5 doses, corresponding to 2.6 g in 24 h (10).

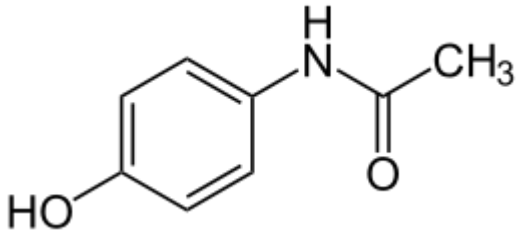

Figure 1 - Chemical Structure of Paracetamol *(11)*

4.1.2. Fresh Milk

4.1.4.1. Skimmed Milk

Skimmed milk is made when all the fat is removed from fat milk. It normally contains around 0.1% fat, which corresponds to 0,5 g of lipids in the milk that was used.

4.1.4.2. Half-fat Milk

Half-fat milk contains around 2.5% fat, which corresponds to 1.6 g of lipids in the milk that was used.

4.1.4.3. Fat Milk

Fat milk is the unprocessed milk and it normally contains around 3.5% fat, which corresponds to 3.6 g of lipids in the milk that was used.

4.1.3. Powder Milk (Nido ®)

Nido ® is a whole powder milk invented by the brand Nestlé, which has 75 years of existence. Nowadays, there are new formulas developed with minerals and vitamins, suited for each stage of childhood but, in this investigation, the used milk was the classic Nido ® milk without addition of any of these substances. Nido ® milk consists on whole milk powder, emulsifier (soybean lectin) and vitamins (A, D and E), containing 26% of milk fat, which corresponds to 3.3 g/100 g of milk fat. It also has no added preservatives or colors.

A glass of reconstituted milk (250 ml) can be prepared by mixing 32.5 g of milk powder and 225 ml of water while 130 g of milk powder and 900ml of water makes 1L of reconstituted milk with 3.7% milk fat (12).

Nutritional facts (average values)	In 100g
Fat (g)	26.2
Carbohydrates (g)	38.6
Proteins (g)	26.4
Salt (g)	0.75

Table 2 - Nido milk nutritional values referred to 100g.
(Taken from: www.saboreiaavida.nestle.pt (12))

4.1.4. Microcrystalline Cellulose (MCC)

Microcrystalline cellulose (Avicel) is a polymer prepared by acid hydrolysis of cellulose and hydrochloric acid at 105 ºC for 15 min. Aqueous suspensions of MCC have constant viscosities over a wide temperature range, are heat-stable and have good palatability. It is used to extend starches, stabilize foams and control ice crystal formation. Avicel is widely accepted in the food industry and also in the pharmaceutical industry, where it is used as a binder in tablets and cosmetics (13).

4.1.5. Polyvinylpyrrolidone (PVP)

PVP is a commonly used water-soluble polymer. Dry PVP is a light flaky hygroscopic powder and it absorbs up to 40% of water by its weight. In solution, it has great wetting properties and forms films right away. Due to these characteristics, it is recognized by being good as a binder and as a coating or an additive to coatings (14).

4.1.6. Hydroxypropyl Methylcellulose (HPMC)

HPMC belongs to the group of cellulose ethers and it is hydrophilic, a biodegradable and biocompatible polymer that has a wide range of applications such as drug delivery, dyes and paints, cosmetics, coating and agriculture. HPMC has unique solubility properties with

solubility in both hot and cold organic solvents. In the pharmaceutical industry it's used as a tablet binder and as a tablet matrix for extended release (15).

4.1.7. Sodium Croscarmellose (SCMC)

SCMC is a super disintegrant, normally used in 2% concentration in tablets made by compression and 3% by wet granulation. It is a white or greyish-white powder, practically insoluble in anhydrous ethanol, hygroscopic, insoluble in water but rapidly swells in it to 4 to 8 times the original volume (16).

Raw Material	Commercial Name	Function (17)	Company
Paracetamol	-	Active Substance	Novacyl
Skimmed UHT Milk	-	Taste-masking Binder	Esselunga, Italy
Half-Fat UHT Milk	-	Taste-masking Binder	Esselunga, Italy
Fat UHT Milk	-	Taste-masking Binder	Esselunga, Italy
Powder Milk	Nido ®	Taste-masking	Nestlé S.A.
Microcrystalline Cellulose (MCC)	Avicel PH101	Binder	FMC
Polyvinylpyrrolidone (PVP)	Kollidon ® 30	Binder Solubilizer	BASF SE
Hydroxypropyl Methylcellulose (HPMC)	HPMC AS	Binder	UniMi
Sodium Croscarmellose (SCMC)	-	Superdisintegrant Binder	UniMi
Deionized Water	-	Vehicle	UniMi

Table 3 – Raw materials considered in the experiments.

4.2. Methods

4.2.1. Preparation of Extrudates and Pellets

First, in mass preparation, the excipients were mixed with the active substance using a planetary mixer Kenwood, where the binder solution was added during the mixing process for each formulation. Pellets were prepared by extrusion and spheronization. During extrusion, the wet mass was forced through a die and shaped into cylindrical particles with a uniform diameter in a NICA Systems extruder for approximately 1 min. The resultant extrudates diameter is determined by the diameter of the die, and its length depends upon the properties of the wet mass and the extruder type.

The theoretical basis of spheronization is that the spheronizer is filled with extrudates, and due to frictional forces generated by particle-particle and particle-equipment interaction, the extrudates are initially broken into smaller cylinders and then rounded into spheres. In this investigation, a spheronizer (NICA Systems 5320, DE) with a groove plate (d=64cm) was used at 700 rpm during 5 min in order to obtain pellets (18).

Pellets were dried in an oven (PID System, type M120-VF, MPM Instrumentals, IT) at 40ºC for 24h.

4.2.2. Calibration Curve

The calibration curve was prepared using a spectrophotometer (UV/VIS Spectrometer Lambda 35, Perkin Elmer, USA) at a wavelength of 243 nm in cells with 1 mm. A stock solution was prepared dissolving 20 mg of paracetamol in 100 mL of deionized water. Withdrawals were made using graduate pipettes, and conveniently diluted. The curve was subsequently constructed to obtain the concentrations of paracetamol of each sample once the absorbance of these samples was measured (see Annex 2).

4.2.3. Percentage product yield

Product yield is based on the amount of powder that coats pellets. It is calculated with the following equation:

$$Percentage\ Yield\ (\%) = \frac{Total\ amout\ of\ pellets\ (g)}{Inicial\ powder\ weight\ (g)} \times 100 \qquad \textbf{Eq. 1}$$

4.2.4. Pellets Characterization

4.1.4.4. Size Distribution

Evaluation of particle size distribution is done by using a mechanical shaker (Endecotts Octagon 200, UK) subject to agitation (amplitude 4, time: 5 min) and sieves ASTM standard with opening mesh in the interval between 500 and 1400µm.

4.1.4.5. Loss on Drying (LOD)

Loss on drying compares the weight of a product before and after it is dried. A heating oven was used to perform this analysis (Mettler Italia, Mettler LP15, I) and it was set at a temperature of 110ºC for enough time to reach a constant weight.

$$LOD\ (\%) = \frac{Weight\ loss\ (g)}{Inicial\ \ weight\ (g)} \times 100 \qquad \textbf{Eq. 2}$$

4.1.4.6. Friability

Friability was evaluated by weighing a sample of 10 g of pellets that was introduced into a glass container of 100 ml capacity together with glass spheres. The glass container was placed into a mechanical mixer (Turbula, Willy A. Bachofen Maschinenfabrik, CH) and agitated for 10 min at a velocity of 200 rpm. Subsequently, the sample passed through a sieve and the quantity of pellets remaining on the sieve was weighed.

$$Friability\ (\%) = \frac{Weight\ loss\ (g)}{Inicial\ \ weight\ (g)} \times 100 \qquad \textbf{Eq. 3}$$

4.1.4.7. Dissolution test

Dissolution test was carried out on samples of pellets belonging to the granulometric class of 850-1000 µm in a dissolving apparatus (paddle method, Distek Dissolution System 2100B, USA) (Portuguese Pharmacopoeia, ed. 9, general chapters 2.9.3, (11). The rotation speed of the agitator was set up to 100 rpm. The dissolution medium consisted of 900 mL of deionized water, a thermostat at 37,0 ± 0,5ºC, from which samples were collected at predetermined times and then analyzed in the UV spectrophotometer (Lambda, PerkinElmer 25, USA) at a wavelength of 243 nm. Each batch was analyzed in duplicate.

4.1.4.8. Drug Content

In order to determinate the drug content, 50 mg of each batch were weighted and smashed. Subsequently, samples were diluted into 100 mL volumetric flasks. Withdrawals were made using 10 mL graduate pipettes and conveniently diluted in 50 mL volumetric flasks. Between the two dilutions, the samples were sonicated in an Ultra-Turrax for 15 min and filtered twice. The drug content was determined by measuring the absorbance in a spectrophotometer at a wavelength of 243 nm, using 1mm cells.

4.1.4.9. Aspect Ratio

For the aspect ratio analysis, 20 pellets of each batch were analyzed. The aspect ratio analysis was done with a Dino-Lite Digital Microscope Pro and a Dinocapture 2.0 software. Subsequently, the images were processed with ImageJ software. This software measured the major and minor axes of the pellet, giving the size ratio between those two axes.

5. RESULTS AND DISCUSSION

Table 4 shows several formulations with paracetamol cores made with different types of excipients and three different types of milk (skimmed milk, half-fat milk and fat milk) in order to evaluate the effect of fresh milk in each formulation.

Batches with water as a binder solution were made to test the behavior of the several excipients used and to determine the appropriate amount of water used in the wet mass, since it is the most important variable for the extrusion process due to its influence on the quality of the spheres (19). Also, pellets' structure can be markedly influenced by the composition of the granulation liquid. While many excipients are able to hydrate in the presence of water, they lack an internal water store of adequate rigidity that could be utilized to modulate the amount of water released under pressure for lubrication during extrusion and for surface plasticization during spheronization. By far, MCC is the ideal excipient that can achieve this precise control and balance of water movement in and out of the pores during extrusion/spheronization derived from its ability to absorb and retain a large quantity of water, mainly due to its large surface area and high internal porosity**,** which facilitates extrusion and spheronization (20). MCC based pellets produced via extrusion/spheronization have a good sphericity, low friability, high density and smooth surface properties. Although MCC is an ideal spheronization aid, it is associated with several limitations such as a prolonged drug release profile in the case of low solubility drugs (because of the lack of disintegration of MCC-based pellets), drug decomposition in presence of MCC and drug adsorption onto the surface of MCC fibers. Due to these limitations, various technological alternatives have been proposed and evaluated: Addition of less adhesive polymers like hydroxypropyl cellulose (HPC) and polyvinylpyrrolidone (PVP) appear to be good options to achieve a good yield with the proper rounding of pellets over an adequate range of water content. Also, the incorporation of super-disintegrants (like croscarmellose sodium or sodium starch glycolate) into MCC pellets prepared by extrusion spheronization does not lead to their disintegration in drug dissolution medium, however, they acquire a slight increase in the drug dissolution rate (21), as supported by the data (figure 3). According to Schroder *et al*. and evaluating the role of a granulation liquid in function of the disintegrants, it is known that a high amount of granulation liquid, especially water, inactivates them. If there is contact with a high amount of water during extrusion, it will lead to a full but limited swelling of the disintegrant. During the drying process, pellets tend to shrink and the disintegrant is incorporated into the matrix. After contacting with water again, pellets can swell and reach their previous size although it is not possible for the disintegrant to swell more and to build up a swelling force. The author also suggests that if there is the possibility to reduce the amount of water during extrusion, the disintegrant will swell only partially. In this case, the

full swelling after subsequent contact with the dissolution medium will result in a swelling force and will lead to disintegration (22).

PVP, HMPC and SCMC aided the process of extrusion-spheronization of MCC. However, according to Sinha *et al*, among these excipients, PVP was the most satisfactory because they had the least adhesive strength, favoring maximum yield of highest quality pellets (23). Otero-Espinar *et al.* also claims that PVP reduces the water requirements in the granulating step, improving the extrusion-spheronization process and producing more uniform and spherical pellets with a narrower particle size range when compared with the same formulations obtained without PVP. Furthermore, PVP usually has a strong effect on drug release, depending on the composition of pellets, but a generally slower release rate was observed after incorporating increasing amounts of this binder (24).

Table 4 has demonstrated that PVP is the most satisfactory excipient when used in water and half-fat milk formulations, taking every parameter into account. Skimmed milk and fat milk pellets showed a different behavior than what is suggested by the aforementioned authors hence sodium croscarmellose pellets (PARA66,7MCC23,8SCMC9,5SM and PARA70MCC25SCMC5FM) were the ones showing the best results in both types of milk. It is believed that the amount of water present in each type of milk plays a major role on pellets quality, i.e., pellets become more satisfactory if they are made with the right amount of water (19). Thus, skimmed milk presents a bigger percentage of water comparing to its amount of fat and fat milk shows exactly the contrary. There was also the need to do an experimental batch using a bigger amount of SCMC comparing to the original batch. Although it has twice the amount of SCMC, the other excipients were maintained in the same quantities.

Regarding the product yield (table 5), the results were not as expected due to many flaws during the process, inherent to the equipment. Gaps in both extruder and spheronizer resulted in large material losses. Furthermore, a blank space between the extruder and the wall of the extruder bowl retained a significant quantity of powder, contributing to the low yield of the process. Some of this lost mass was quantified and recovered but it was very difficult to reach all the holes and obtain the maximum value of loss mass. Nevertheless, the quantified values were not determinant and did not improved significantly the product percentage yield in each batch (results not shown). For the drug content test (table 5), in order to improve the results, pellets were smashed and sonicated for 15 min. Furthermore, the final solution was filtered twice. The results were not coherent with the theoretical amount of paracetamol due to the fact that between the wet mass preparation and the extrusion-spheronization process there was not an efficient homogenization of the mass resulting in discrepant values of drug content. Pellets with skimmed milk had the best results, possibly because skimmed milk has a low quantity of proteins and fat components, which might be explained by the capacity of milk proteins to retain paracetamol, which makes it unavailable to be quantified (7).

Regarding to friability test (table 5) all the values were really good, except for the formulations with microcrystalline cellulose, possibly due to the fact that the formulation should have more quantity of MCC, and also the formulation with powder milk. This may have happened due to the formulation and/or palletization process, as well as a possible variability in the raw materials (19). Friability of pellets is somehow influenced by spheronization time length and speed. The optimized formulation of paracetamol pellets cross-linked with calcium chloride solution investigated by Kulkarni et al. showed lower friability values than those that have been reported abundantly in the literature and which may also be attributed to the presence of Avicel® RC 591, having strong binding properties. (25) Nevertheless, Reynolds reported that excess extrudate friability can be overcome by incorporating more MCC, binder, or water in the granulation (26).

In terms of aspect ratio analysis (table 5 and annex 3) the values were similar with no significant variations. The ideal value for the aspect ratio is 1.0, however, in the literature, a value of 1.2 for the aspect ratio is often accepted indicating satisfactory pellets (27). The aspect ratio is a highly reproducible shape factor with a very low methodical error (coefficient of variation below 0.5% in all cases). In practice, pellets are rarely ideally spherical, so it cannot be expected that a pellet batch will meet the afore-mentioned limiting values. Here, however, the opinions of some authors appear questionable. For example, Baert et al., Vervaet and Remon, and Vervaet et al. define pellets with an aspect ratio equal or smaller than 1.2 as sufficiently round. Helle'n and Yliruusi considered pellets with an aspect ratio up to 1.55 still as round and Hileman et al. quotes a reciprocal value of the circularity of 1.2 as a limiting value for acceptable roundness (28).

For the size distribution analysis pellets were tested using sieves. Figure 2 summarizes the pellets' size distribution undersize cumulative curves of each of the formulations produced by extrusion-spheronization.

Regarding the formulations with water, most of the pellets produced ranged between 710-1000 µm, reaching about 70% as shown in figure A. Concerning the formulations with fresh milk, pellets with skimmed milk (B) and half-fat milk (C) reached 80% between 710-1180 µm, except for PARA70MCC25PVP5SM which was between 1180 and 1400 µm (B) and PARA70MCC30HFM which was between 1000-1400 µm (C); fat milk formulations (D) reached 80% between 850-1400 µm, except for PARA70MCC30FM and PARA70MCC25HPMC5FM which was between 710-1180 µm. Major percentage of most of the studied formulations range between 710 and 1204 µm, which is, for Kulkarni. *et al*., the usable size range (25). Taking this into account and comparing with data, it should be normal to have fat milk pellets in a higher size distribution class because fat milk is thicker than the other types of milk and, normally, it might increase the normal pellets' size and consequently its distribution class. There are many factors determining particle size distribution of pellets produced by extrusion-spheronization,

described by Vervaet *et al*., that may have interfered in order to cause the variability of the observed data. These factors are: content of the wet mass, the type of granulation liquid, the physical properties of the starting material, the type of extruder, its speed and its screen properties, the spheronization time, speed and load and also the drying process. (29)

Formulations made with SCMC seems to be the ones who lost more moisture content during drying. It may be explained by the fact that SCMC is a super disintegrant and it will absorb a higher quantity of water and fresh milk, comparing to the other excipients. Since this happens, it will also need a greater amount of water and milk during wet mass preparation, as shown in table 4. Loss on Drying (LOD) values for SCMC fluctuate between 0,74 and 2,62 (table 5).

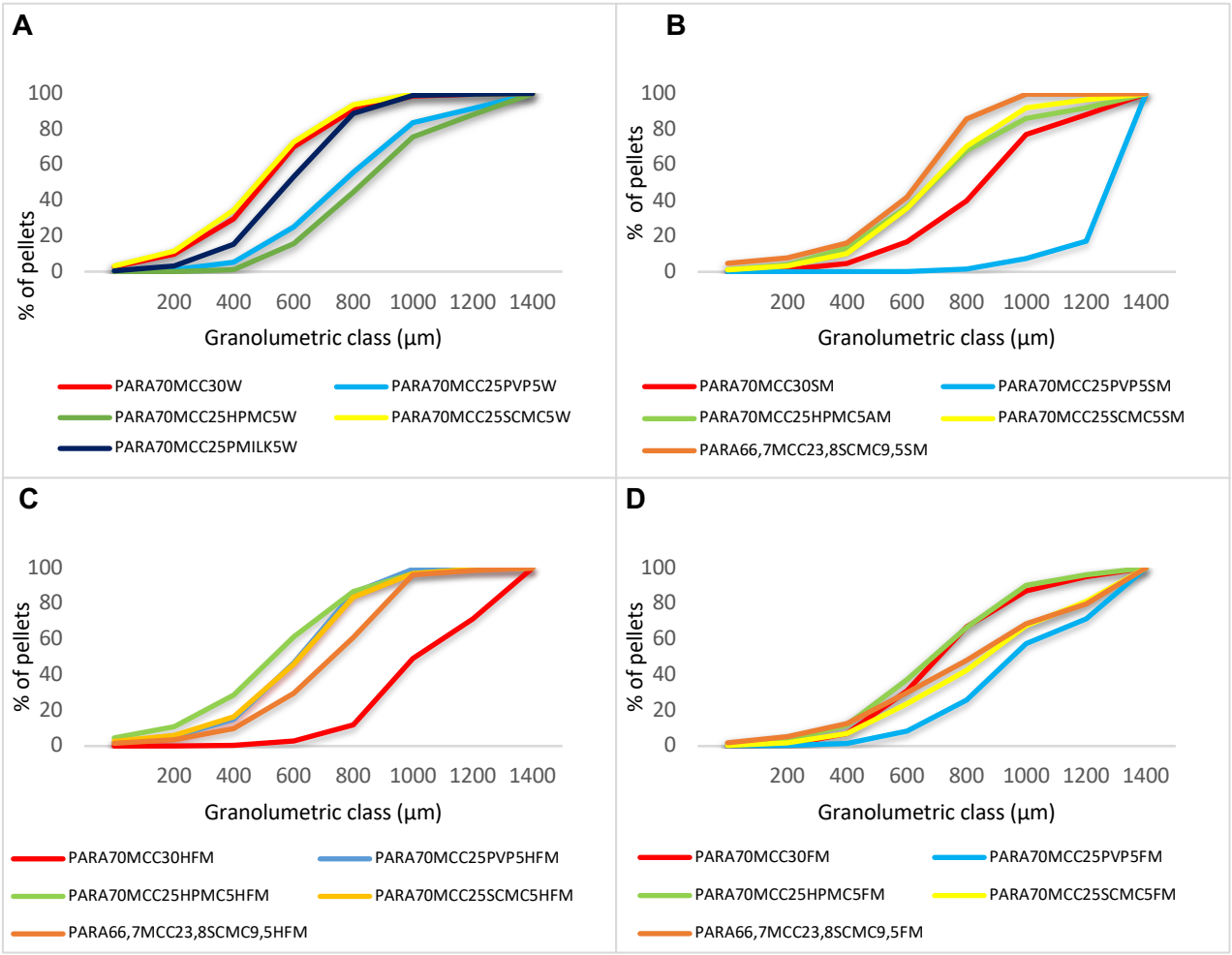

Figure 2 - Size distribution analysis of paracetamol formulations with deionized water (A), skimmed milk (B), half-fat milk (C) and fat milk (D).

For the characterization of pellets and the process several tests were made. Table 5 represents the results of pellets and process evaluation.

	Paracetamol (%)	Avicel PH101 (%)	PVP (%)	HPMC AS-LG (%)	SCMC (%)	Powder Milk (%)	Deionized Water (%)	Fresh Milk (%)
70 % paracetamol cores with deionized water								
PARA70MCC30W	70	30					55,2	
PARA70MCC25PVP5W	70	25	5				41,26	
PARA70MCC25HPMC5W	70	25		5			55,00	
PARA70MCC25SCMC5W	70	25			5		70,35	
PARA70MCC25PMILK5W	70	25				5	55,00	
70 % paracetamol cores with skimmed milk								
PARA70MCC30SM	70	30						60,80
PARA70MCC25PVP5SM	70	25	5					48,45
PARA70MCC25HPMC5SM	70	25		5				54,70
PARA70MCC25SCMC5SM	70	25			5			86,95
PARA66,7MCC23,8SCMC9,5SM	66,7	23,8			9,8			107,76
70 % paracetamol cores with half-fat milk								
PARA70MCC30HFM	70	30						61,35
PARA70MCC25PVP5HFM	70	25	5					59,70
PARA70MCC25HPMC5HFM	70	25		5				61,13
PARA70MCC25SCMC5HFM	70	25			5			95,68
PARA66,7MCC23,8SCMC9,5HFM	66,7	23,8			9,8			118,95
70 % paracetamol cores with fat milk								
PARA70MCC30FM	70	30						62,25
PARA70MCC25PVP5FM	70	25	5					46,55
PARA70MCC25HPMC5FM	70	25		5				56,15
PARA70MCC25SCMC5FM	70	25			5			111,2
PARA66,7MCC23,8SCMC9,5FM	66,7	23,8			9,8			83,95

Table 4 - Formulations made using paracetamol cores with water as a binder solution and three different types of milk: skimmed, half-fat and fat milk.

	Yield (%)	Drug content (%)	Loss on Drying (%)	Friability (%)	Aspect Ratio
70 % paracetamol cores with deionized water					
PARA70MCC30W	86,76	76,98	1,23	1,04	1,12
PARA70MCC25PVP5W	96,84	81,79	1,41	0,00	1,14
PARA70MCC25HPMC5W	79,85	94,00	0,47	0,00	1,08
PARA70MCC25SCMC5W	78,35	93,08	1,7	0,00	1,01
PARA70MCC25PMILK5W	79,35	87,76	1,25	1,96	1,11
70 % paracetamol cores with skimmed milk					
PARA70MCC30SM	87,45	104,51	1,03	1,00	1,10
PARA70MCC25PVP5SM	82,85	97,81	0,98	0,00	1,18
PARA70MCC25HPMC5SM	83,05	104,06	0,16	0,00	1,12
PARA70MCC25SCMC5SM	89,70	106,87	1,93	0,00	1,07
PARA66,7MCC23,8SCMC9,5SM	96,24	93,07	0,74	0,00	1,14
70 % paracetamol cores with half-fat milk					
PARA70MCC30HFM	78,40	92,27	0,74	1,00	1,09
PARA70MCC25PVP5HFM	92,50	88,93	1,88	0,00	1,18
PARA70MCC25HPMC5HFM	80,65	94,24	0,47	0,00	1,11
PARA70MCC25SCMC5HFM	64,95	91,31	1,61	0,00	1,09
PARA66,7MCC23,8SCMC9,5HFM	98,57	75,80	2,62	0,00	1,08
70 % paracetamol cores with fat milk					
PARA70MCC30FM	91,30	92,54	1,12	1,00	1,07
PARA70MCC25PVP5FM	77,40	91,23	0,37	0,00	1,15
PARA70MCC25HPMC5FM	63,25	91,51	2,25	0,00	1,10
PARA70MCC25SCMC5FM	89,30	89,20	1,00	0,00	1,12
PARA66,7MCC23,8SCMC9,5FM	95,81	81,29	1,75	0,00	1,23

Table 5 - Results relative to pellets and process characterization tests: product percentage yield (%), drug content (%), loss on drying (%), friability (%) and aspect ratio.

For the dissolution test, 300 mg of pellets were placed on vessels with 900 mL of deionized water. According to data (figure 3), formulations with sodium carboxymethylcellulose (SCMC) showed a higher drug release due to the fact that SCMC is considered to be a super disintegrant whose swelling properties cause expansion of the disintegrant particles and also pushes the adjacent particles apart (30). Therefore, SCMC is normally introduced to shorten pellets' disintegration time. Iosio *et al*. claims that this strategy resulted in a dramatic reduction of disintegration time from 37 to 14 min (31). Nevertheless, formulations with a higher quantity of microcrystalline cellulose showed a slower release, except for the formulations with skimmed milk, in this case PARA70MCC25PVP5SM had the slowest release rate. These results, regarding to the formulations made with 30% of MCC can be explained by the fact that MCC is working only as a binder. It is also not surprising that the formulations with high microcrystalline cellulose content had the slowest release profiles, as the MCC causes the formation of a hydrophilic matrix sustained release delivery system that forms a very hard cover around the pellets, which improves their resistance (31,32). Several authors also reported that the higher the water content the formulation was, the longer the disintegration time.

Hence, it must be complemented with a disintegrant to increase the release rate, as proven in the other formulations (33). Nevertheless, the formulations released completely the drug within 90 min.

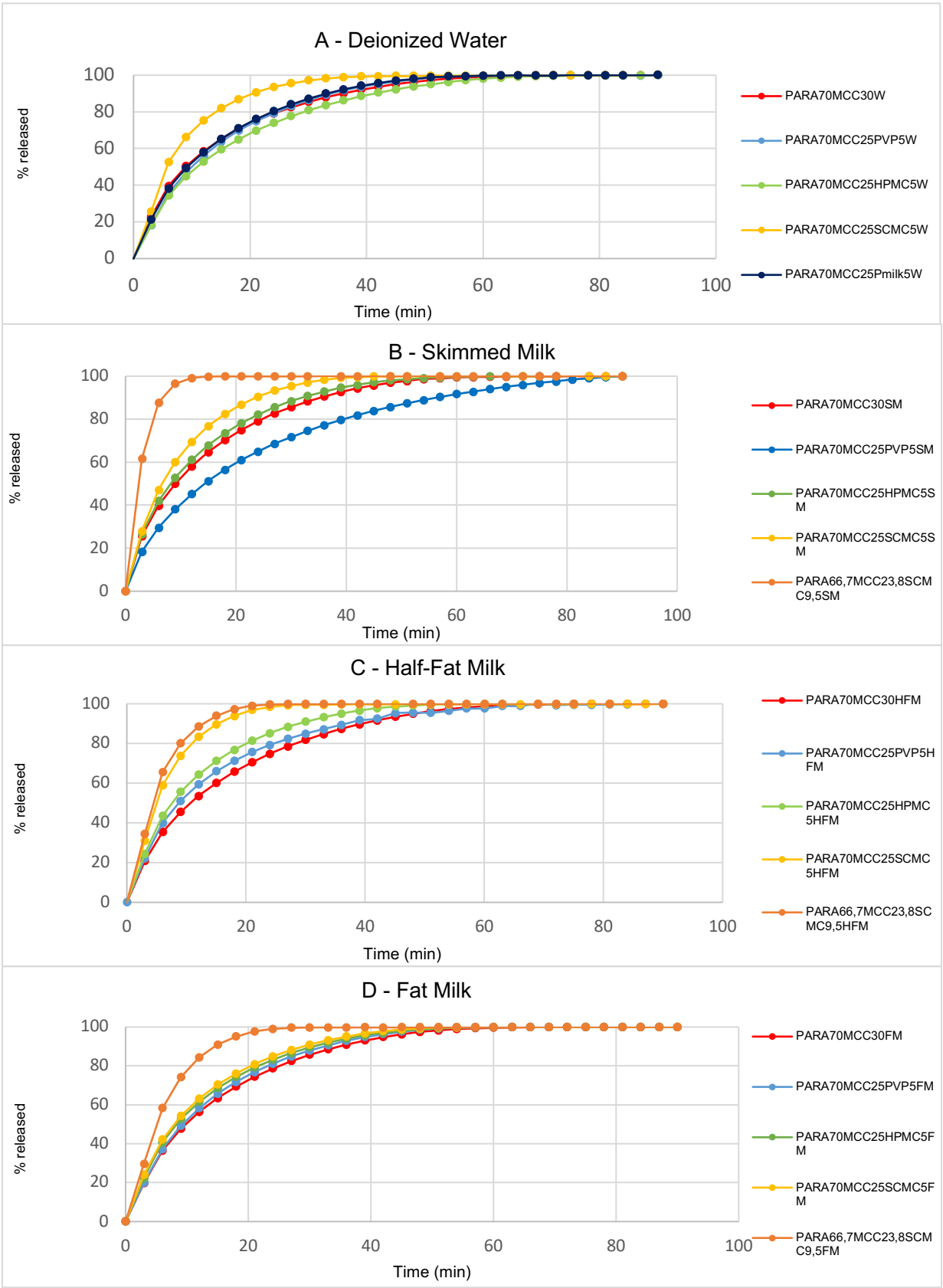

Figure 3 - Dissolution profiles relative to paracetamol formulations with deionized water (A), skimmed milk (B), half-fat milk (C) and fat milk (D).

6. CONCLUSION

During this project, fresh milk has been proved as a novel excipient for the preparation of pellets that can be used by the paediatric population.

MCC showed to be an ideal spheronization aid but it is associated with several limitations, leading to the need of introducing less adhesive polymers like PVP and HPMC or even a super-disintegrant like SCMC. PVP demonstrated to be the most satisfactory excipient when used in water and half-fat milk formulations, taking every parameter into account. Skimmed milk and fat milk pellets showed a different behavior, where sodium croscarmellose pellets showed the best results in both types of milk. Turns out that the amount of water present in each type of milk plays a major role on pellets quality, i.e., pellets become more satisfactory if they are made with the right amount of water. Thus, skimmed milk presents a bigger percentage of water comparing to its amount of fat and fat milk shows exactly the contrary.

The assessment of the drug content in general was not as expected. Formulations with skimmed milk had the best drug content results possibly due to the fact that skimmed milk has a minor quantity of fat and protein components when compared with the other types of milk that were used. Nevertheless, pellets obtained were characterized by regular shape and 70-80% of similar size range between the different types of milk (between 710-1180 μm), except for the fat milk formulations, which was between 850-1400 μm. Pellets were also evaluated on the dissolution time. Formulations with 30% of microcrystalline cellulose showed a slower release, except for the formulations with skimmed milk, which had the slowest release rate. On the other hand, formulations with sodium croscarmellose had the highest release rate.

7. FUTURE WORK

In next steps of the project, it would make sense if the batches were repeated in order to improve even more the formulations as well as the process parameters. Once the right quantity of excipients is adjusted, some of the parameters might be improved. Also, it would be interesting if palatability tests were conducted in order to realize that fresh milk pellets really are well accepted by the paediatric population.

It should also be considered the lactose intolerant population, finding a way around to overcome this situation and make the therapy available to this group as well.

8. BIBLIOGRAPHY

1. Parliament E. State of Paediatric Medicines in the EU: 10 years of the EU Paediatric Regulation. 2017;
2. Soulele K, Macheras P. Milk as a medium for pediatric formulations : Experimental findings and regulatory aspects. Int J Pharm [Internet]. 2015;492(1–2):344–5. Available from: http://dx.doi.org/10.1016/j.ijpharm.2015.05.015
3. Lopez FL, Ernest TB, Tuleu C, Gul MO. Formulation approaches to pediatric oral drug delivery : benefits and limitations of current platforms. 2015;1727–40.
4. Krause J. Improving Drug Delivery in Paediatric Medicine. 2008;22(1):41–50.
5. Haug A, Høstmark AT, Harstad OM. Bovine milk in human nutrition – a review. 2007;16:1–16.
6. Bylund G et al. Dairy Processing Handbook. Tetra Pak Group; 1995. 241–262 p.
7. Kamal SS, Kaur D, Singh S, Sharma A, Katual MK, Garg AK, et al. An Investigative and Explanatory Review on Use of Milk as a Broad-Spectrum Drug Carrier for Improvement of Bioavailability and Patient Compliance. 2016;8(2):72–5.
8. Dial EJ, Romero JJ, Lichtenberger LM. Gastroprotection by Dairy Foods Against Stress-Induced Ulcerogenesis in Rats. 1995;40(I):2295–9.
9. Bennett SM, Zhou L, Hayes JE. Using Milk Fat to Reduce the Irritation and Bitter Taste of Ibuprofen. 2012;231–6.
10. Farinde A. Pediatric Acetaminophen Dosing [Internet]. Medscape. [cited 2019 Aug 17]. Available from: https://emedicine.medscape.com/article/2172407-overview
11. INFARMED. Farmacopeia Portuguesa 9.0. Lisboa; 2008.
12. Nestlé. Saboreia a Vida [Internet]. [cited 2019 Aug 24]. Available from: https://saboreiaavida.nestle.pt/produtos/nido/leite-em-po-nido/#gs.xydqbk
13. Holtzapple MT. CELLULOSE. In: Encyclopedia of Food Sciences and Nutrition. 2003. p. 998–1007.
14. Kariduraganavar MY, Kittur AA, Kamble RR. Chapter 1 - Polymer Synthesis and Processing [Internet]. 1st ed. Natural and Synthetic Biomedical Polymers. Elsevier Inc.; 2014. 1–31 p. Available from: http://dx.doi.org/10.1016/B978-0-12-396983-5.00001-6
15. Deshmukh K, Ahamed MB, Deshmukh RR, Pasha SKK, Bhagat PR, Chidambaram K. 3 - Biopolymer Composites With High Dielectric Performance: Interface Engineering [Internet]. Biopolymer Composites in Electronics. Elsevier Inc.; 2017. 27–128 p. Available from: http://dx.doi.org/10.1016/B978-0-12-809261-3/00003-6
16. Eyjolfsson R. Chapter One - Introduction. In: Design and Manufacture of Pharmaceutical Tablets. Academic Press; 2015. p. 1–28.
17. Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients. 6th editio.

London and New York: Pharmaceutical Press; 2009. 119, 130–131, 326–327, 581,582 p.
18. Nguyen TTL. Extrusion-spheronization of pharmaceutical products: system for the delivery of active ingredients which are poorly soluble by oral route. Université de Strasbourg; 2017.
19. Muley S, Nandgude T, Poddar S. Extrusion – spheronization a promising pelletization technique : In-depth review. Asian J Pharm Sci [Internet]. 2016;11(6):684–99. Available from: http://dx.doi.org/10.1016/j.ajps.2016.08.001
20. Liew C V, Gu L, Soh JLP, Heng PWS. Functionality of Cross-Linked Polyvinylpyrrolidone as a Spheronization Aid : A Promising Alternative to Microcrystalline Cellulose. 2005;22(8).
21. Dhandapani NV, Shrestha A. Pelletization by Extrusion-Spheronization: A detailed review. 2012;10–23.
22. Schroder M, Kleinebudde P. Development of Disintegrating Pellets Obtained from Extrusion/Spheronization. 1995;5–8.
23. Sinha V, Agrawal M, Agarwal A, Singh G, Ghai D. Extrusion-Spheronization: Process Variables and Characterization. Critical reviews in therapeutic drug carrier systems; 2009. p. 275–331.
24. Otero-Espinar FJ, Luzardo-Alvarez A, Blanco-Méndez J. Non-MCC materials as extrusion-spheronization aids in pellets production. 2010;20(4):303–18.
25. Kulkarni RB, Amin PD. Masking of Unpleasant Gustatory Sensation by Cross- Linking of Dehydrated Paracetamol Alginate Pellets Produced by Extrusion-Spheronization. 2008;(2004):199–205.
26. Augsburger LL, Dressman JB, Hughes JA, Junginger HE, York P, Topp EM, et al. Extrusion/Spheronization as a Granulation Technique. In: Parikh DM, editor. Handbook of Pharmaceutical Granulation Technology. 2nd ed. Taylor & Francis Group; 2005.
27. Chopra R, Newton JM, Podczeck F. Preparation of Pellets of Different Shape and Their Characterization. 2001;6(4):495–503.
28. Podczeck F, Rahman SR, Newton JM. Evaluation of a standardised procedure to assess the shape of pellets using image analysis. 1999;192:123–38.
29. Vervaet C, Baert L, Remon JP. Extrusion-spheronisation: a literature review. 1995;116:131–46.
30. Hoag SW. Capsules Dosage Form: Formulation and Manufacturing Considerations. In: Developing Solid Oral Dosage Forms. S Edi. Pharmaceutical Theory and Practice; 2017. p. 723–47.
31. Iosio T, Voinovich D, Grassi M, Pinto F, Perissutti B, Zacchigna M, et al. Bi-layered self-emulsifying pellets prepared by co-extrusion and spheronization : Influence of formulation variables and preliminary study on the in vivo absorption. 2008;69:686–97.
32. Pinto JF, Buckton G, Newton JM. The influence of four selected processing and formulation factors on the production of spheres by extrusion and spheronisation. 1992;83:187–96.

33. Chaerunisaa AY, Sriwidodo S, Abdassah M. Microcrystalline Cellulose as Pharmaceutical Excipient. In: Pharmaceutical Formulation Design - Recent Practices. 2019.
34. World Health Organization (WHO). 4th WHO Model List of Essential Medicines for Children. 2013.
35. Martino M De, Chiarugi A. Recent Advances in Pediatric Use of Oral Paracetamol in Fever and Pain Management. Pain Ther. 2015;4(2):149–68.
36. Information NC for B. PubChem Database [Internet]. Acetaminophen, CID=1983. [cited 2019 Jul 15]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Acetaminophen
37. Prescott LF. Kinetics and Metabolism of Paracetamol and Phenacetin. 1980;291–8.

Annex 1: Drug under study – Paracetamol

Paracetamol is an analgesic and antipyretic drug that is commonly used, being the drug of choice of many international guidelines and recommendations when it comes to the treatment of fever and pain. It is on the list of essential Medicines for Children of the World Health Organization and it is listed as a safe drug for this population (34). Actually, it is the only analgesic that can be used in children below 3 months of age, according to WHO guidelines.

Due to the heterogeneity of this group, it is important to administer the correct dose according to body weight and age, therefore using the milligram per kilogram (mg/kg) dosage. The optimal dose for treating paediatric fever above 3 months old is 15 mg/kg every 4-6 h and it should not be administered more than 4 times per day. For children below 3 months, the optimal dose should be 10 mg/kg every 4-6 h (35).

Chemical Formula (11)	$C_8H_9NO_2$
Molecular weight (g/mol)	151.16
Melting Point (ºC)	169-170
Boiling Point (ºC)	> 500
Water solubility (mg/ml) at 25 ºC	14
Density (g/cm^3)	1.3
LogP	0.49
pka	9.38
UV Wavelength of maximum absorption (nm)	250
Taste	Slightly bitter taste
Odour	Odourless

Table A1 - Paracetamol chemical properties. (36)

Taking into account it's chemical properties, paracetamol has a very fast rate of absorption from the small intestine and therefore, the rate of absorption will depend on the gastric emptying. Paracetamol is rapidly distributed and extensively metabolized, with the biotransformation occurring in the liver. Also, large doses of paracetamol can cause acute hepatic necrosis (37).

Annex 2: Calibration Curve

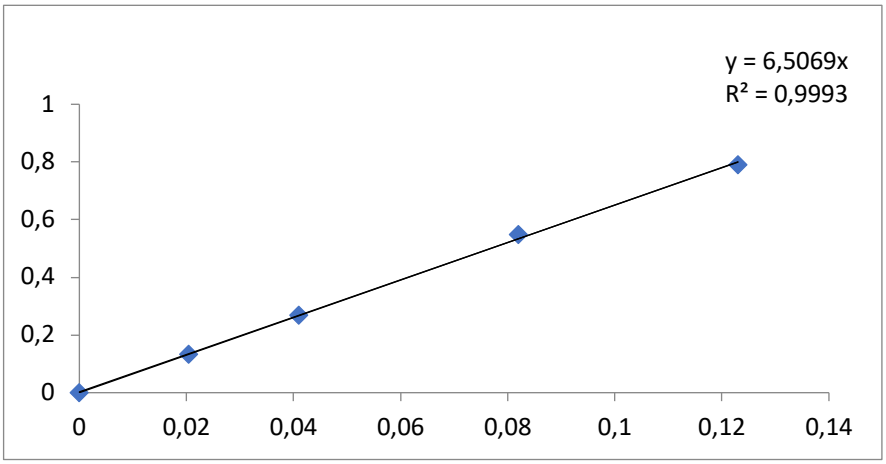

Figure 4 - Calibration Curve

Annex 3: Aspect Ratio

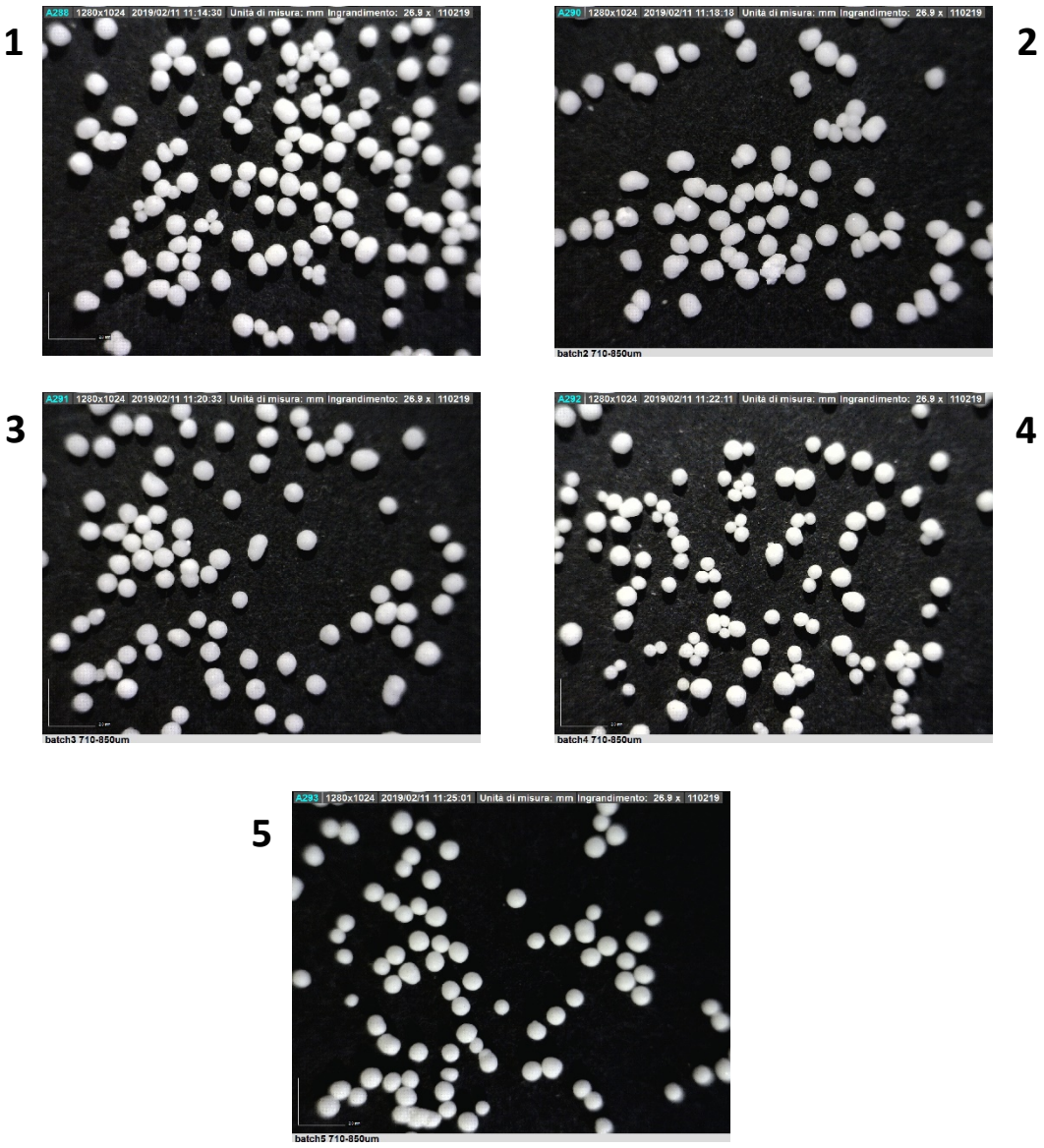

Figure 5 - Paracetamol cores with deionized water: **(1)** - cores composed by paracetamol and MCC only (PARA70MCC30W); **(2)** - cores composed by PVP (PARA70MCC25PVP5W); **(3)** – cores composed by HPMC (PARA70MCC25HPMC5W); **(4)** – cores composed by SCMC (PARA70MCC25SCMC5W); (**5)** – cores composed by powder milk (PARA70MCC25PMILK5W).

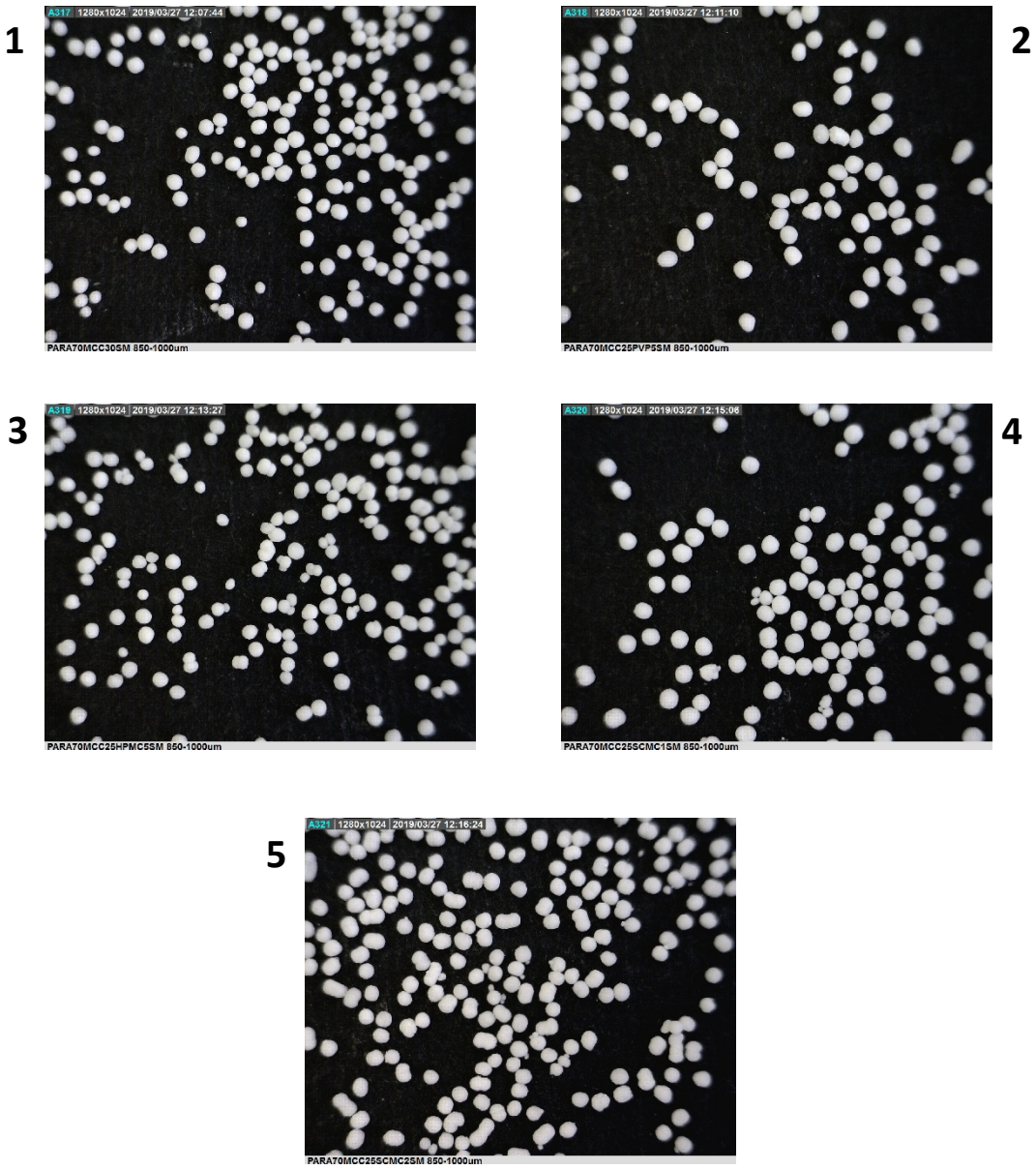

Figure 6 - Paracetamol cores with fresh skimmed milk: **(1)** - cores composed by paracetamol and MCC only (PARA70MCC30SM); **(2)** - cores composed by PVP (PARA70MCC25PVP5SM); **(3)** – cores composed by HPMC (PARA70MCC25HPMC5SM); **(4)** – cores composed by SCMC (PARA70MCC25SCMC5SM); **(5)** – cores composed by a bigger amount of SCMC (PARA66,7MCC23,8SCMC9,5SM).

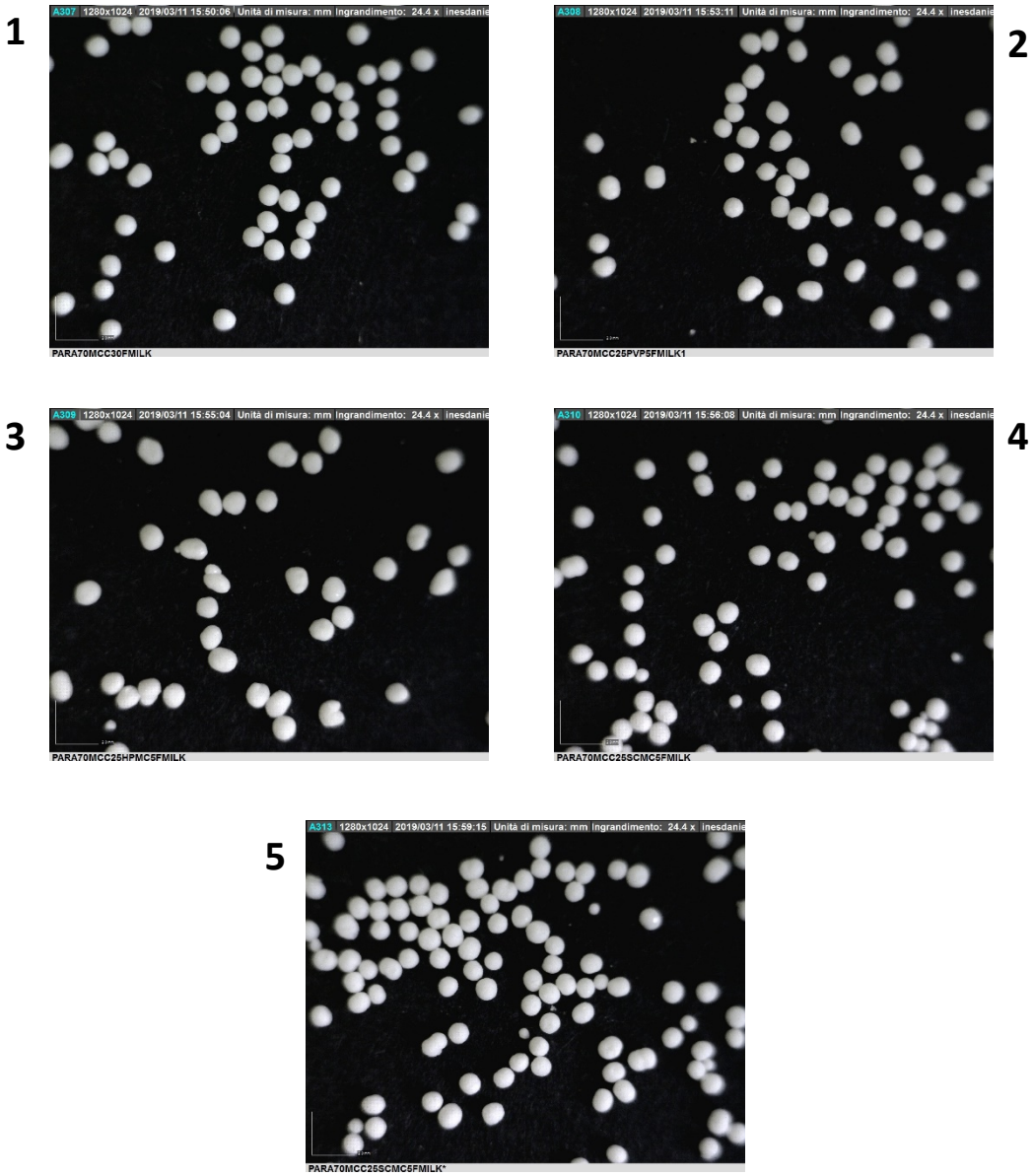

Figure 7 - Paracetamol cores with fresh half-fat milk: **(1)** - cores composed by paracetamol and MCC only (PARA70MCC30HFM); **(2)** - cores composed by PVP (PARA70MCC25PVP5HFM); **(3)** – cores composed by HPMC (PARA70MCC25HPMC5HFM); **(4)** – cores composed by SCMC (PARA70MCC25SCMC5HFM); **(5)** – cores composed by a bigger amount of SCMC (PARA66,7MCC23,8SCMC9,5HFM).

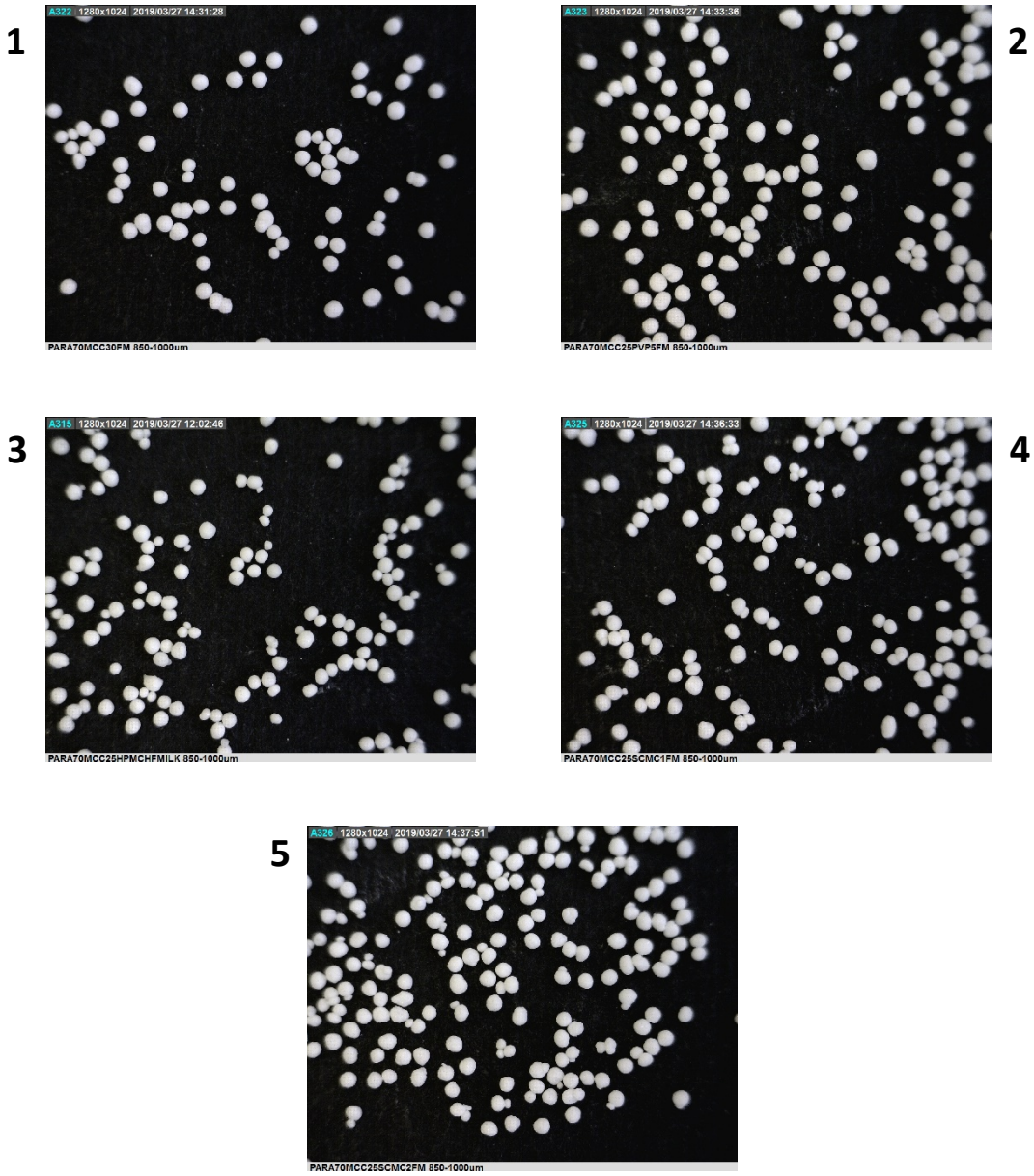

Figure 8 - Paracetamol cores with fresh fat milk: **(1)** - cores composed by paracetamol and MCC only (PARA70MCC30FM); **(2)** - cores composed by PVP (PARA70MCC25PVP5FM); **(3)** – cores composed by HPMC (PARA70MCC25HPMC5FM); **(4)** – cores composed by SCMC (PARA70MCC25SCMC5FM); **(5)** – cores composed by a bigger amount of SCMC (PARA66,7MCC23,8SCMC9,5FM).