Universidade de Lisboa

Faculdade de Farmácia

Applicability of cow's milk on novel drug delivery systems

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Abstract

Cow's milk has been increasingly recognized as a valuable food product to be integrated in a drug formulation. Milk's composition is very rich in proteins, presenting a high biological value; lipids, mostly organized in micelles; carbohydrates of which lactose stands out being a widely used drug excipient; vitamins and mineral salts that are present in relative minimal amounts. Naturally, each of these compounds shows biological functions in the organism that can be availed for a drug delivery purpose. The whole milk combining all the proprieties together is described to enhance the solubility of the active ingredients which is one of the biggest issues that affect their bioavailability and also to direct the active molecules to specific targets decreasing their toxicity. Gastroprotection has additionally been reported as a milk ability being beneficial in drugs reportedly aggressive to the gastrointestinal system such as non-steroid anti-inflammatory drugs. Furthermore, milk shows to be an opportunity to mask the unpleasant taste of the drugs. This has come up as a necessity over geriatric and pediatric populations that present swallowing constraints and therefore the taste is crucial for therapy compliance.

Although milk is considered a promising excipient, it has never run into a commercialized product. As a natural product, there are regulatory issue that are more difficult to be controlled such as contaminants, microbiologic growth, exact composition, among others. Its limited stability results in a short shelf-life and is mostly due to its high-water content. It has been applied technological techniques that reduce the free water with insignificant damage to the structure of milk components. Among these techniques are spray drying, freeze drying, fluidized bed processes and hybrid version techniques. This way its functionality is preserved and as well as its stability is raised. Milk allergenicity and intolerance is also an increasingly concern that must be taken in consideration.

Keywords: Gastroprotection; Milk; Targeted Drug Delivery; Taste Masking.

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1 Introductory Note

The present work consists in an adaptation of an ERASMUS project that could not be finished due to the COVID-19 pandemic disease. The initial project aimed the study of the behavior of a drug formulation when using a commercialized formulas of powder milk as part of the excipient list. On the other hand, it was expected to study the taste masking ability of milk through a spray drying coating of paracetamol pellets. The entire project was intended to be practical and has been initialized in Portugal and continued in Milan. Unfortunately, the pandemic situation caused by the virus SARS-COV2 has forbidden the access to the labs and forced the adaptation to a theoretical research project. In this, the focus is the characterization of bovine milk and its advantages as part of a drug delivery system. The biological and technological functions of the whole milk and its components are discussed as well as are presented the most used pharmaceutical technologies to manipulate formulations containing milk preserving its proprieties.

2 Introduction

Milk is a complex biological fluid produced on the mammary glands of the female mammals that has been used for centuries by animals to feed the newborns. It has a unique nutritional, hydrating, growth stimulation and strengthen the immunological barriers proprieties and it also contributes to the development of the gut microflora (1).

At room temperature, milk appears in the liquid stage with a cloudy aspect due to the fat particles, proteins and mineral salts that make part of its composition. It also presents a yellowish color due to carotenoids. It is a dynamic aqueous system with a complex composition *Figure **1***) and with high nutritional and technological value considering to be:

- A colloidal suspension of small particles made up of casein, calcium and phosphorus.
- An emulsion of fat globules and liposoluble vitamins dispersed in the whey.
- A solution of lactose, water hydro soluble proteins and vitamins, mineral salts (2).

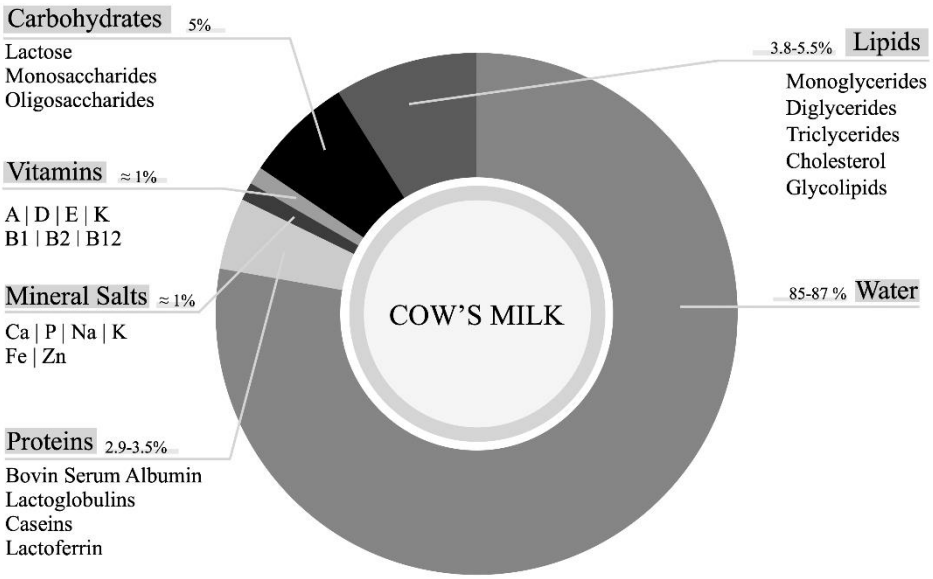

Figure 1 Composition of cow's milk, modified from (1).

Milk is highly hydrating food as it is mainly composed by water (more than 80%). Lipids represents 3.8-5.5% of its composition representing an energetic source. Among lipids, it can be found triacylglycerols, diacylglycerol, cholesterol, phospholipids and free fatty acids, the ones who are responsible for milk's distinctive flavor (3). Lipids are also a source of fat-soluble vitamins, namely A, D, E, K (4) although milk also present the water soluble ones, especially

vitamin C, B2, B3 and B5 (1). In what concerns to carbohydrates, it is constituted almost entirely by lactose which is commonly the most known milk component responsible for food intolerance (3). In contrast, it is also one of the most popular excipients found in around 60-70% of the drugs formulas. It is a versatile compound usually applied in oral dosage forms such as tablets, capsules, and dry powder inhalers in which it acts as a filler or binder, assigning flowability to the formulation as well as binding proprieties (5). Proteins are an important slice of milks composition (3-3.5%). According to their structure and physico-chemical behavior, milk proteins are classified as caseins or whey proteins. Their high biological value denotes an antimicrobial activity, absorption enabling capacity, hormonal and enzymatical function, among other biological proprieties (3). Finally, there is also a residual percentage of inorganic compounds, such as calcium and selenium, and dissolved gases (6).

Although its usage is transversal among the mammalian animals, only humans consume milk in the adult age fact that has been carried out multiple studies about its impact on the health as well as its potential as a component of a drug formulation. Either milk as a whole or each of its ingredients individually or in combination, show a wide technological applicability for the pharmaceutical and food industries. Bovine milk is considered a safe compound, with low toxicity, easy to find and low cost what make it an appropriate ingredient to be used in a large-scale production of medicines (7).

Most of the active pharmaceutical ingredients show to be very poorly water-soluble what compromises their bioavailability when administrated orally. In this sense, the formulation of this type of medicines needs to include excipients able to improve their water solubility. Over the time, bovine milk has shown this ability both by the activity of its protein and lipid phase increasing the efficacy of the medicine (8–10).

In general, the elderly people present a bigger number of diseases and therefore represents the biggest share of medicine buyers. This group, together with the pediatric population, show special requirements in what concerns to the administration route. Constraint on swallowing, attractive appearance and taste are pointed as limiting factors for the administration compliance among both of these populations (11). Most of the active ingredients show an unpleasant taste and because of that, milk has been studied to be included in drug formulations acting as a taste masker, improving the palatability of the medicines and therefore increasing the adherence to the therapy (12). There are multiple reasons pointed to explain this phenomenon such as the decreased contact of the bitter drug with the taste buds and the concealment of the bitter taste by the milk taste.

Biologically, the gastrointestinal system has protective mechanisms against the internal and external hostile conditions under which it is submitted. In a long way, the non-steroid anti-inflammatory can cause gastric inflammation or even gastric ulcers, the reason why they are usually administrated in concomitance with gastric protector medicines and are recommended to be taken with food. In this sense, milk represent an opportunity to be incorporated as an excipient in these formulations by presenting gastro protective properties (13,14).

Despite the benefits, the mammary gland is also responsible for the excretion of some undesirable substances through milk that can be potentially disadvantageous for the human health (15). Moreover, the use of milk arises security questions about its microbiological growth. The high percentage of water, its chemical composition and proprieties, including its approximately neutral pH and its fluidity favors the proliferation of microorganisms increasing its deterioration rate (16). Additionally, the environment in which the milk is taken from the cow is propitious for microbiologic contamination. Considering this, the common commercialized milk is submitted to multiple processes to increase its stability and therefore increase its shelf life and health safety. Usually, raw milk is heated at 72ºC for 15 seconds. This process named pasteurization weakens the total microbiological flora and can wreck the non-spore organism. The ultra-high temperature (UHT) sterilization is other popular process in which the raw milk temperature is raised between 135-150C for 4-8 seconds and the packaging is performed under aseptic conditions. This way the inactivation of all bacteria and heat sensitive enzymes is safeguard and the shelf life at room temperature is raised to 3 months (17). However, milks potential benefits on the health can be compromised by the heating treatments described previously. The high temperatures damage the protein structure and therefore decrease their bioactivity. The same phenomenon can happen with the antioxidant agents and vitamins such as vitamin C, B6 and B1. Novel milk treatments that don't use heat, such as membrane filtration, are being considered in order to maximize its biological value (3).

The entire production chain of milk, starting from the cow's feed supplier until the sanitation practices during milk's processing, follows rigorous conditions in what concerns to the presence of aflatoxins, contaminants, pathogenic microorganisms, presence of antibiotic residues and the use of hormones on the animals (17). The regulation has been defined by FDA through the Pasteurized Milk Ordinance (18), the Canadian dairy information center through the Canadian National Dairy Code (19), the European Union through the European Regulations 853/2004 (20) , 2073/2005 (21), 1881/2006 (22), and 37/2010 (23), as the principal examples.

However, after these treatments, milk still has a huge water content that promotes its microbiological deterioration. In this view, it can be performed drying processes to remove

most of the moisture present in milk, increasing its stability. These processes include spray drying and fluidized bed drying as two examples of versatile techniques easy to preform and scale up, however require high temperatures that can damage the structure of the milk components. Freeze drying in its turn, works with very low temperatures however, it is extremely expensive, time consuming and difficult to scale-up. Considering this, the goal is to mixture the beneficial features of these process to maximize the yields.

Another emerging concern related to the milk consumption is the intolerance and allergenicity to its components. The allergenicity is generally caused by milk's proteins and usually shows up at a young age, up to 3 years, and attenuates or disappears from there. It reportedly affects approximately 2-5% of the children. The most common symptoms are wheezing, vomiting, diarrhea, fussiness and, in an extreme scenario, anaphylaxis. Actually, there have been introduced in the market special formulas without bovine milk's proteins for infants. Several times, this scenario is confused with milk's intolerance. The most common one is lactose intolerance that affects around 75% of the world's population and is caused by a deficiency in lactase, a gut enzyme responsible for the degradation of lactose in glucose and galactose (3). There are also alternatives to those who suffer from this condition, namely milk without lactose or the administration of lactase.

3 Materials and Methods

To elaborate this monography, it was done a literature review, from November 2019 until October 2020, of the literature available in the electronic databases, namely PubMed, Google Scholar and EUR-Lex.

During the research, it was preferred scientific articles published in the last 5 years and it was searched key words such as “Bovine milk”; “Milk in drug formulations”; “Milk as an excipient” and “Milk as a drug delivery agent”.

4 Milk - Composition and characteristics

Milk is considered a dynamic system because of its structural instability which is caused by the fat globule membranes; variation of compound solubility with temperature and pH; the presence of multiple enzymes responsible for lipolysis and proteolysis processes and the growth of microorganisms that can change the milk composition (6).

Milk is composed by more than 80% of water in which are dissolved or dispersed other compounds, namely: proteins, carbohydrates, hydrosoluble and liposoluble vitamins, mineral salts and lipids (24). Its freezing point is situated between -0.53 to -0.55 ºC and the pH of fresh milk is 6.6 – 6.8, approximately the same as blood (25).

The following content presents the composition and biological characteristics of each milk component that turns it a valuable excipient to be used on the pharmaceutical and food industry.

4.1 Proteins

Milk presents a high concentration in proteins (around 30-35g per liter of milk). The high biological value attributed to milk is given by the presence of every essential amino acids in its composition (26).

Milk proteins can be divided into two main groups, the ones with the most economic and technological value: caseins (80%) and whey proteins (20%) (27) (26). Besides these groups, it can also be found growth factors, enzymes, and milk fat globule membrane proteins. The last group is further explored in section "Lipids".

Caseins are the major proteins found in milk and possess the role of concentrate, stabilize and transport nutrients to the newborn (28). Being very rich in proline, they can be divided into 4 groups: αs1-casein, αs2-casein, β-casein and κ-casein (24). αs1-casein, αs2-casein and β-casein have serine–phosphate residue centers which is useful for calcium sequestration. Around 95% of caseins are found in milk as micelles, due to their natural capacity of self-assembly (29). Casein micelles have a primary role of nutrition, assuring the transport of amino acids and calcium phosphates from the mother to the newborn (9). They are described as spherical structures with approximately 100 nm in diameter that appear in milk surrounding fat globule micelles (30). It is constituted by around 94% of proteins and 6% of colloidal calcium phosphate, a group of low molecular weight compounds. The higher porosity,

hydration and hydrophobicity of micelles make them a more interesting compound rather than other serum proteins (9). There have been reported several properties of casein micelles that recognize them as a distinctive system to deliver drugs to the stomach. The abundance of these micelles in milk (around 1015 casein micelles per milliliter of milk) is one of them, representing a low-cost resource. The accessibility of casein micelles for proteolytic cleavage is other aspect pointed out, since they are open-structured rheomorphic proteins (29). As an example, β-casein micelles present an open tertiary structure susceptible to the proteolytic action of stomach enzymes maintaining their integrity when contact with saliva. This allows a drug delivery in the stomach avoiding oral/ esophagus toxicity (31). Finally, the acid-soluble calcium-phosphate bridging stabilizes the quaternary structure of the micelle improving its stability (29).

The whey proteins mostly play a carrier function for ligands, biologically. Their smaller size, comparing to caseins, gives to these proteins more stability, better digestibility and higher biovailability (25). This group includes: lactoglobulins, bovine serum albumin, lactoferrin and immunoglobulins (27).

Among the lactoglobulins there is the major whey protein of bovine milk: beta-lactoglobulin. Follows, alpha-lactalbumin, a globular metalloprotein whose functional fold depends on the presence of calcium. Its function consists in regulate lactose synthase enzyme in mammary gland. Bovine serum albumin is a large protein found in both milk and blood serum and function as a transporter of small ligand (29). Finally, lactoferrin is a nonheme iron-binding glycoprotein that has micro biostatic ability against gastric iron sensitive organisms. Lactoferrin has shown to decrease the incidence of bacterial sepsis in neonatal rats in 70% comparing to placebo. Fungal sepsis incidence has also shown a reduction (32).

After the description of the variety of milk proteins and their natural functions, it is time to consider how the pharmaceutical industry can take advantage of their proprieties, such as self-assembly, binding ability, bioaccessibility, bioavailability and biocompatibility, to improve drug formulations (25).

Taking advantage of the natural assembly and binding ability of milk proteins (33), they can be manipulated in a formulation to bind and adsorb a range of drug molecules using hydrophobic interactions, van der Waals forces or hydrogen bonds in order to achieve a specific target. It has been shown the capability of caseins to self-assembly into micelle structures able to entrap oil-soluble nutraceuticals through hydrophobic interactions, using vitamin D2 as a liposoluble model compound. Micelles has demonstrated to be able to stabilize the compound in aqueous mediums and protect them against photochemical damage (31).

In other perspective, caseins have been used to improve the bioavailability of most of the antitumor drugs enabling their oral administration instead of the intravenous one, reducing costs and increasing patients' quality of life (31). Curcumin is a food spice known to have healthy advantages namely antioxidant, antimicrobial, anti-inflammatory, antiamyloid, and particularly to inhibit carcinogenic activity in several types of cells. It is characterized as a lipophilic fluorescent molecule that provoke apoptosis in carcinogenic cells, showing no toxicity on healthy cells. Despite the promising features, curcumin shows very low aqueous solubility which compromises its bioavailability and therefore, the therapeutic target. To improve its solubility, curcumin has been successfully entrapped in casein micelles to which it connects and binds to the low polarity regions. It was concluded that curcumin is at least 2500 times more hydrosoluble after casein encapsulation and its therapeutic efficacy is not compromised (9).

Also confirming this finding, there has been conducted a study that has tested the ability of α- and β-caseins as a drug vehicle of dipyridamol. Dipyridamol was chosen due to its very low solubility, pH dependent. Along the gastrointestinal tract, the pH of the medium changes and therefore, the same happens with the solubility of this compound. It presents high solubility in the stomach (pH≈2) and, in the opposite side, a very poor solubility in the intestine (pH≈7). This affects its bioavailability and consequently limits its oral administration. The study has shown that bovine milk α- and β-caseins have high binding ability for this kind of drugs with hydrophobic character and increase its solubility and stability in aqueous solutions at neutral pH (10). Shapira *et al.*, 2010 has also reported that β-casein micelles stabilize hydrophobic chemotherapy drugs through hydrophobic and ionic interactions, increasing their solubility (31). Additionally, β-lactoglobulin has a natural binding site for fatty acids, retinol and steroids that works in a pH-dependent binding process (34).

Another strategy to deliver molecules and protect them from the external aggressors is to exploit the gelling ability of milk proteins that favors the imprisonment of different molecules. Naturally, casein micelles have the ability to encapsulate calcium and phosphate, releasing them with specific triggers such as temperature or the pH of the medium. This is possible due the formation of hydrogels which is a network system able to swell when contact with water and that do not dissolve allowing the controlled release of the drugs. Caseins and whey proteins present as advantages over other available options, their biodegradability and the avoidance of the use of chemical agents (25). F. Song *et al.*, 2010, have proved the capability to modulate the release of a molecule entrapping it in a hydrogel matrix of milk caseins when activated by a natural tissue enzyme (35).

A concern associated to the use of milk proteins is their allergenicity. It is most common in infants and young children, with a prevalence of 2-3%, and is mostly triggered by caseins and β-lactalbumin (17). The associated symptoms can early appear after milk consumption if the reaction is mediated by Ig-E, being the most severe case the anaphylactic shock. On the other hand, if the reaction is not mediated by Ig-E, the symptoms are more delayed and less severe what can compromise the diagnose (36).

There have been registered cases of allergenic reactions in children after taking medicines such as dry powder inhalers, methylprednisolone sodium succinate and diphtheria-tetanus-pertussis vaccine, polio vaccine, probiotics, and lactulose. Most of the probiotics present milk proteins in their composition. The other medicines composition present cow's lactose in common and further studies found contaminations of milk proteins such as caseins and α-lactalbumin. These findings allow the conclusion that children with cow's milk allergy should not take medicines containing milk proteins, even in small concentrations, to avoid severe reactions (37) (38).

However, although the relatively high prevalence in children, milk protein allergy tends to disappear with the age being uncommon in adults and even so, caseins are considered as GRAS by FDA.

4.2 Lipids

Milk has a very rich composition in lipids, between 3.8 to 5.5% (24), presenting monoglycerides, diglycerides, triglycerides, glycolipids and cholesterol (39). Biologically, their function consists in energetic contribution and transport of vitamins A, D, E and K, the fat soluble vitamins (4). Depending on the fat content, milk is classified as full fat milk; semi-skilled milk and skimmed milk (24). Because of their abundance and variety in fatty acids, milk fat has a stronger flavor than other fats. However, these compounds can also contribute to the modification of the flavor by participating in degradative processes namely the hydrolytic and oxidative rancidity (4). Milk lipidic phase changes over the time in its abundancy: it presents lower levels in colostrum and higher ones in mature milk (33).

Over than 98% of milk fat with polar phospholipids exist in the form of triglycerides (40). In their turn, glycolipids constitute 0.01 to 0.70% of the total milk lipids (41) and present a higher concentration of unsaturated fatty acids than the triglyceride fraction of milk (39). They are mainly found in association with the milk fat globule membrane and have an important

metabolic function in the organism (41). Comparing to other food, cholesterol has a low-level content on milk although it is the major sterol in its composition.

The majority of milk fat content is organized in globules to prevent its enzymatic degradation by lipases (39). They represent the milk's largest particles but also the lightest, with a density of 0,93 g/cm3 at 15.5°C. Original from mammary cells, the globules composition consists in a triglyceride nucleus with a multilayer lipoprotein membrane since they derive from a secretion process in which cytoplasmic lipid droplets are wrapped by the apical plasma membrane (8). It has a wide size range although more than 80% are smaller than 1μm of diameter. Different sizes of the fat globules result in different proportions between triglycerides present in the core and phospholipids present on the membrane (41). The nucleus is protected by a lipoproteic membrane of saturated and unsaturated fatty acids containing 2 to 20 carbon atoms inside their chains. Among them, there are also phospholipids, cholesterol, free fatty acids, monoglycerides and diglycerides (41). Its richness in a variety of natural lipids turns it distinctive because of its high concentration on saturated lipids that can possibly retard the degradation of water insoluble drugs due to an equal retard of the oxidation of the lipids (8). Milk fat globules comprise about 95% (42) of the total 98% of triglycerides (39).

As shown in ***Figure 2***, the globule's membrane can be divided in two layers. The internal one, evolving the lipidic core, is made up of polar lipids and proteins, and the external one consists in a polar lipidic and protein bilayer (41). Generally, this membrane is composed by 25% of proteins and 70% of polar and neutral lipids (43).

Among the neutral lipids of the globule membrane, we can find triglycerides, 95%, and monoglycerides, diglycerides and cholesterol, in smaller quantities. On the other hand, the polar lipids consist in several groups of phospholipids, such as glycerophospholipids (phosphatidylcholine (25-40%); phosphatidylethanolamine (27- 37%); phosphatidylinositol (≈5%); phosphatidylserine (≈3%); lysophosphatidylcholine, ethanolamine, diphosphatidylglycerol, in small amounts) and sphingophospholipids (sphingomyelin (20-25%)). These compounds have been reported to inhibit carcinogenic processes, lower cholesterol levels, neural signalization, blood clotting and immune and inflammatory response, among others (41). In addition, this unique composition in lipids and proteins of the fat globule membrane gives it distinct emulsification properties, lipolysis modulation as well as release of encapsulate compound ability (8).

Milk's characteristics, specifically in what concerns to the solubilizing capacity of the lipidic content, are being considered as an opportunity to face lipophilic drug formulation problems.

Alshehab *et al*., 2019 studied the ability of milk fat globules as a delivery vehicle of curcumin, a lipophilic bioactive compound (8). The study consisted in encapsulating the compound inside the globules and verify their behavior in vivo. The conclusions reported that after encapsulation, the globule maintained the shape and integrity of its membrane, as well as a uniform distribution of curcumin inside the lipidic nucleus. The in vitro studies showed that, under gastric conditions, only a small fraction, around 27%, of the compound was released. However, it has registered around 88% of compound release in simulated intestinal conditions (8).

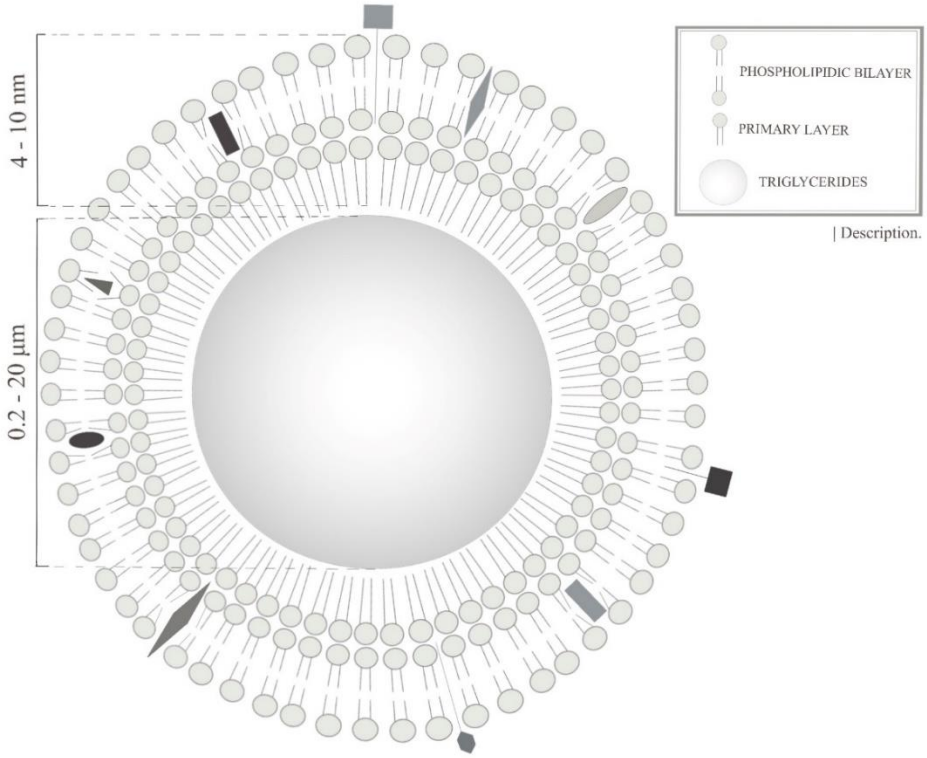

Figure 2- Milk fat globule illustration, modified from (44) .

Additionally, *in vivo* studies have reported that the solubility in the vehicle as well as the systemic exposure to the drug is enhanced when milk is used as a carrier comparing to aqueous vehicles without any fat in their composition. They also suggest that to provide a desirable solubilization environment, the performance of pharmaceutical lipid-based formulations containing triglycerides depend on their digestion to form colloidal structures of monoglycerides and fatty acids. This is explained by the fact that drugs are more likely soluble

in the colloidal self-assembled liquid crystalline structures derived from the lipolysis processes than in the original amorphous triglycerides (42).

Also, during digestion are produced fatty acids which can be one of the keys in the solubilization of weakly basic crystalline compounds since the ion pair formation together with the released fatty acids during digestion facilitates crystalline drug solubilization (42). The drug transformation passes through three stages: originally it is presented as a crystalline solid form; becomes a suspension when mixed with milk, and finally, after lipolysis, it becomes solubilized. This final step emphasizes the importance of digestion for the solubilization and availability for absorption of the drug (42).

4.3 Carbohydrates

The major carbohydrate found in milk is lactose, around 5% of the total compounds and it can only be found in this product. It is one of the most stable compounds of milk and it comprises approximately 52% of the total solids in skimmed milk and 70% of the solids found in whey. The amount of milk water and, consequently, the volume of milk produced by cows, depends on the amount of lactose secreted in the mammary gland (45). It is hydrolyzed into galactose and glucose by an enzyme present on the enterocytes – lactase. Intolerance to lactose is a relatively common condition usually caused by a deficiency in lactase, giving rise to mild to severe symptoms such as swelling, diarrhea, and flatulence (46). Through a technological vision, lactose is a versatile compound officially considered as an excipient by EMA, preforming many functions in formulations (47). It is applied as a filler to offer bulk to the formulation, as a binder to strengthen the structure of the product and as a flowability agent to ease the production processes. To produce tablets, lactose is considered one of the best diluents available since it is able to balance flowability and tableting proprieties required and can be used as a single diluent in the formulation. It is suitable to be used in direct compression, wet granulation and dry granulation processes. Furthermore, its applicability shows to be also crucial in Dry Powder Inhalers in which lactose, by providing bulk, plays a carrier role dispersing the small doses of the active ingredients. This way, the drug molecules present an appropriate flowability to leave the inhalation device, reach the inhalation airstreams and finally the lungs (5).

Additionally, lactose is considered a dry compound presenting a negligeable amount of free water what contributes to its inertia over the other compounds of the formulation. Moreover, it is water soluble which is a desirable characteristic considering oral dosage forms

that need to dissolve inside the organism. Finally, lactose can also be an important component of an encapsulating matrix due to its low viscosity even in high concentrations, flavoring capacity and its ability to convert the formulation into a glassy state when the water is removed, as it happens during spray drying, entrapping and protecting bioactive against degradation factor (34).

However, the hydrolysis of lactose can represent a problem since it promotes the adhesion of this component to the equipment, browning effect and decreasing solubility of other compounds (48).

Among the carbohydrates, we can additionally find glicose and galactose in smaller quantities.

4.4 Vitamins and mineral salts

Vitamins and mineral salts are organic substances that usually show up in low concentrations in food, although their essential value for the normal function of the organism. Milk is not an exception: even though in small percentages, it is very complete when it comes to these compounds. Among the vitamins it is possible to find vitamin A, B1, B2, B12, D, E and K. The fat-soluble ones - A, D, E and K- are associated to the fat globules of the milk (15).

Carotenoids are present in minimal amounts. Even so, they are precursors of vitamin A being responsible for around 10-50% of this vitamin in milk and for its characteristic yellowish color (4).

Although the total concentration is lower than 1%, milk is considered an excellent source of mineral salts needed for the bone's growth and general health (20). Among them are calcium and sodium – the most abundant ones- and potassium and magnesium. These appear in a phosphate, chloride, citrate and caseinate forms (49).

Usually, calcium and phosphor are associated to caseins which can explain their high digestibility. In this way, milk is considered one of the best sources of calcium that is indispensable for the skeleton growth of young people and for the maintenance of those of adults. Iron, in its turn, is present in a very low concentration, not being able to supply the organism necessities. However, it plays an important role in limiting the growth of bacteria in milk (46).

5 Whole milk properties

Previously, it was described the beneficial features of each component of milk. But the use of the whole milk can take advantage of all those proprieties at the same time allowing milk to be recognized as having valuable attributes to be applied in a drug formulation. Among them, it is identified a gastroprotection ability that is also reported to be potentially used together with drugs that are aggressive to this system. It has also been described its ability to enhance the solubility of poor soluble drugs increasing their bioavailability as well as preform a selective drug delivery preventing high toxicity to the healthy tissues. Finally, milk can mask the unpleasant taste that most of the drugs present increasing the therapeutic compliance of the patients.

5.1 Gastroprotection

Gastrointestinal mucosa receives daily injuries by strong acids, enzymes, and refluxed bile. For this reason, it owns several protection mechanisms such as the epithelial cells renewal and the gastric blood flow. Moreover, gastroduodenal epithelial cells secret a viscous mucous that confers protection to the tissues from damage. Among its composition, whose percentage varies depending on the gastrointestinal site, it is possible to find water; mucins, responsible for the viscoelastic properties; ions; salt; nucleic acids; cells; proteins and immunoglobulins that contribute to the immunologic response (50). It can also be found a layer of active phospholipids that contributes to the hydrophobicity of this protective barrier (51).

Besides the biological aggressors, there are many medicines that can potentially cause harm to this system. There is a consensus about the gastric damage that non-steroidal anti-inflammatory drugs (NSAIDs) cause when taken orally in all pharmaceutical forms. To minimize the injuries, on the summary of the characteristics of these medicines, it is recommended to take them after meals, with water or milk (52).

Several studies have been conducted in order to show the ability of dairy food to prevent gastric lesions, through the characteristics of its lipidic and protein content. The main human phospholipids of the gastric mucosa are phosphatidylcholine and phosphatidylethanolamine, the same ones as in milk (51). Considering this, it was suggested that milk enhances the protective properties of the epithelium by preserving the hydrophobic gastric layer (53) and it is even reported as a protector factor against gastric cancer (54). Additionally, α-lactoalbumin

has been described to diminish the probability of gastric diseases by increasing prostaglandin and mucin synthesis (14).

Further data describes a treatment with acidified ethanol to induce gastric ulcers that was preceded by the ingestion of liquid bovine milk by mice. It was registered a decrease of gastric myeloperoxidase and malondialdehyde levels, substances potentially aggressive to the mucosa, and the decrease of the expression of proinflammatory genes. On the other hand, it was shown the increase of gastric mucus content and antioxidant enzymes- catalase and superoxide dismutase- in the stomach of mice (14).

Sanka *et al.*, 2014 preformed an experiment aiming the study of an inclusion complex of piroxicam with skimmed milk, in order to enhance its solubility, dissolution release profile and reduction in ulcerogenicity (13). Regarding the gastric aggression, it compares the performance of piroxicam alone, the mixture between piroxicam and milk and an inclusion complex of piroxicam in milk. The inclusion complexes of piroxicam in milk has left the gastric mucosa almost intact proving a better result than the simple mixture of the drug in skimmed milk which caused partial loss of the mucosa. Finally, the administration of piroxicam alone caused total loss of the mucosa, confirming the benefit of the inclusion of milk for this purpose.

Beyond these physicochemical mechanisms, it seems that casein hydrolysates have the ability of gene regulation. Although it was shown a downregulation effect of the gene that expresses NFκB, a pro-inflammatory factor, it was also registered a downregulation of the gene responsible for the production of TGF-β1, a factor that contributes to the barrier function. The contradictory conclusions taken by this study suggest a loss of protein activity after a necessary treatment with corolase, during the process (55).

5.2 Enhancement of drug solubility and targeting delivery system

When a medicine to be taken orally is formulated, there is a special concern about its bioavailability that affects the minimum concentration of the drug to achieve the therapeutic goals and its toxicity to the tissues that demands a targeted deliver of the molecules to limit the side effects. Are pointed as explanations for the low bioavailability the poor water solubility of the majority of the drugs since they are required to be in a solution state to be absorbed; the drug partition coefficient that can affect the cells lipidic membrane permeation and the first-pass metabolism (56).

To overpass drug solubility issues, there have been described multiple systems including liposomes, polymeric nanoparticles, solid lipid nanoparticles and lipid nanoemulsions.

However, those alternatives have proved not to be viable due to the high costs, toxicity limitations and lack of scalability (57). Considering these limitations, milk has started to be considered as an asset ever since it has demonstrated to enhance the solubility of drugs in aqueous media and therefore to increase the systemic exposure of those drugs (42). Beyond the casein micelles and the milk lipidic strategies described previously, this can also be supported by the existence of exosomes- small vesicles able to enhance solubility and deliver drugs into a specific target. Milk's exosomes show characteristics of an ideal drug delivery system such as low cost, easiness to scale up, having similar inter-tissue biodistribution when given orally, biocompatibility, long circulating half-life time, and minor immune responses have been shown as well as no significant toxicologic and genotoxic effects have been reported (57–59).

Exosomes and micro vesicles are part of a group called extracellular vesicles (60). Exosomes distinguish themselves in the group through the size – diameter between 40 and 100 nm; floating density in sucrose gradient - 1.1-1.19 g/mL; cup-shaped form and a biochemical composition. The minimum size of an exosome depends on the structure of the lipid bilayer, which is around 5 nm thick, requiring a rigid structure to form vesicles. (43). Despite the nano size, exosomes can carry different types of molecules such as nucleic acids, lipids and proteins and exchange them between cells, representing an opportunity to build a drug delivery system (59).

Furthermore, the biogenesis process of exosomes consists in the formation of vesicles through double invagination of the plasma membrane giving birth to multivesicular bodies, inside the cell, that in their turn, contain intraluminal vesicles. Finally, the last ones are expelled by the cell, by exocytosis, originating the exosomes. Biologically, those vesicles are released by multiple types of cells and they act as a mediator of communication between them by allowing the exchange of their load (59) and, it contributes to angiogenesis and inflammation processes as well as to the immune response. From a pharmaceutical technology perspective, this capacity can be availed to choose a drug as a cargo of the vesicle to achieve a specific target. Regarding to the sources of exosomes, milk has a huge advantage since the animals, such as cows, horses, sheep, goats or even humans, have the capacity to produce several liters per day instead of what is verified when it is used fluid or blood plasma cell cultures (43). Also, they have shown to be very versatile since they are distributed in blood, urine, breast milk, tears and cerebrospinal fluid and can even cross the biological barriers as plasma membranes and blood brain barrier (59)(58). Due to their biological function, the composition of exosomes protect the cargo against degradation and facilitate its cellular uptake (60).

Since naturally, the exosomes act as a carrier of miRNA and mRNA between cells influencing their gene expression, the same mechanism was expected and then confirmed to happen with siRNA by its encapsulation in cow's milk exosomes (58). It has been demonstrated a protective effect from digestive juices and an increased delivery to Caco-2 cells compared to the control group – siRNA alone (43). After the submission to aggressive conditions such as low pH, different temperatures and digestive enzymes mimeking human digesting conditions, the exosomes' biomarkers showed insignificant differences confirming its stability in extreme conditions (60,61). The intercellular delivery of siRNAs has multiple limitations due to their instability in circulation, their size, and their negative charge that difficult the passage through the cell membrane and therefore, this approach has proved to be an opportunity to work around this problem. Still regarding targeting delivery, to solve the high toxicity that many drugs present to the tissues, such as chemotherapeutics, the goal would be to direct the drug to targeted cells, minimizing the side effects. In this sense, a successful methodology consists in modify the membrane of milk exosomes, attaching factors that are overexpressed in cancer cells to improve specificity, suppressing their toxicity to the other tissues. As an example, this type of modification has been successfully reproduced by the addition of a tumor-targeting ligand, the folic acid. Another method showed that exosomes, by increasing the bioavailability of either hydrophilic and lipophilic drugs, allow the use of smaller drug doses what directly impact, in a positive way, the cytotoxicity and therefore, it minimizes the side effects (7).

Trying to prove the potential of milk's exosomes as part of a hydrophobic drug molecule delivery system, it was tested the encapsulation of curcumin, a water-insoluble molecule, in buffalo's milk exosomes. The experiment showed that it binds the exosomes, being associated to their membrane. It was also reported the increasing of curcumin stability in salivary, gastric pancreatic and bile juice, being successfully up taken and trans-epithelial transported in Caco-2 cells. The same behavior was verified with other hydrophobic drugs such as paclitaxel, doxorubicin, among others (43).

In a different approach to test the impact of milk on drug solubility, Ahin *et al.*, 2007 characterized the solid dispersion of prednisolone in skimmed milk. Prednisolone was used as a hydro insoluble API, with a limited bioavailability, when administered orally. It was observed the formation of an inclusion complex between the surface of prednisolone particles and the amino acids of the milk which showed the ability to increase the solubility of the drug. It was not possible to confirm all milk components responsible for this phenomenon but it is believed that the main players are the casein micelles, due to the significant surface activity that increase the solubilization of prednisolone in their interior and whey proteins, due to their hydrophilic

sites that interact with the drug molecule. Furthermore, the inclusion complex revealed an inferior particle size than the pure drug which is an important factor to increase the solubility of the drug and therefore its bioavailability (62). The same event was verified by Sanka *et al.*, 2014, in the experiment with piroxicam described previously (13). Comparing the behavior of the drug alone, a mixture of the drug and milk and the inclusion complexes of the drug in milk, it was observed an increase of solubility of the mixture which showed to be even higher in the inclusion complex. The fact that the inclusion complex reduces the size of the particles and turn the drug into an amorphous state was given as a possible explanation for the enhanced solubility. The introduction of milk in the formulation has also demonstrated to improve the dissolution rate showing higher results when using inclusion complexes. This is explained by the fact that the carrier prevents particle aggregation and inhibits the crystal growth which increase the dissolution (13).

An invention has been patented reporting a device that applies milk as a medium with high solubilization skill used with the purpose of increasing the bioavailability of drugs with poor solubility in water. The device includes two, three or more compartments in which, one of them contains a solution of the active ingredient in a buffer or alcoholic solution, and the other one contains milk. The patent suggests a device design that separate the two compartments by a membrane. Before the administration, the membrane can be easily broken to mix the ingredients and be taken by the patient. Whole milk can reportedly be used in a sterilized or homogenized form or in a reconstituted form from powder or even in a skimmed and lactose free form. The patent uses mefenamic acid, acid salicylic and cyclosporin as examples of hydrophobic actives that, because of this characteristic, are known to be difficult to granulate, tablet and dissolve. The micronized or granulated lipophilic active ingredients showed to dissolve successfully in milk due to their high surface area and solubility in the milk fat globules (63).

5.3 Taste masking

A considered part of the active ingredients presents a bitter taste that highly influences the taste of the final medicine. This fact represents a concerning by the time a new drug is formulated, especially when the target is the pediatric and geriatric populations (12). These populations have especial administration requirements to achieve compliance to the therapeutic considering that they are the most sensitive to flavors and who suffer the most from dysphagia and swallow coordination (11,64). Regarding the pediatric population, the EMA recommends

the formulation of a medicine with a neutral flavor and that preferably has a pleasant taste by itself, without the need to be administered with food or drinks (18).

Milk is a very complete nutritious food that is widely used since the baby is born, being generally well accepted. It also seems to have taste masking properties that has awaken an special attention for its inclusion in these types of formulations. This option can replace the use of sweeteners representing advantage to the ones who have sugar diet restrictions and also reduce toxic effects caused by its overconsumption due to the pleasant taste (65).

Bennett *et al*., 2012 has proposed on a study that ibuprofen- an API known to have bitter taste and cause gastric irritation- would be partitioned on the lipidic phase of the milk, reducing taste perception by decreasing the interaction between the drug and the sensory receptors, although the bitterness is not modified. Milk was chosen due to its abundance, the high stability of the commercialized emulsion formulas and its availability in different fat levels, what makes it a very versatile option. During the study, it was verified a decrease of the sensory response in a dose dependent way of the milk fat content. However, it was concluded that this phenomenon was not fully explained by the lipid partition of the drug since a constant amount of ibuprofen was distributed on the aqueous phase. This study also suggested that an increased viscosity also influence the palatability of sweetness as well as bitterness, showing to be another important factor.

The measurement of the taste masking ability of a compound is essential to ensure the success of the formulation but represents a challenge. The most common resource used is the human panel, however, it can also be chosen animal models and analytical processes (64). Considering the analytical procedures, it has emerged the need of an equipment with higher selectivity, easier to perform and with lower costs. In this sense was developed the electronic tongue which consists in "low- selective sensors and uses advanced mathematical procedures for signal processing based on the pattern recognition (PARC) and/or multivariate analysis" according to IUPAC (67). The signal of the sensors is influenced by several characteristics of the sample such as pH, ion strength, viscosity and type of counter ion (67). It is assumed that electronic tongues are able to determine the taste of sweetness, bitterness, salty, sourness or tasty (68). The biological structure of the mammalian olfactory and taste systems is mirrored into the several sensors that constitute the electronic tongue which seems to be a viable and ethical alternative to the experiment in humans and animals, especially when testing drugs whose toxicity is not well known (69). The biggest disadvantages pointed are the massive number of measurements required to calibrate the equipment (68), the susceptibility to environmental factors and the limited quantitative information acquired (70).

6 Milk-based formulations stabilizing techniques

Although all biological and technological benefits of milk there are a few disadvantages that need to be considered by the time this compound is chosen as an excipient. Milk is a perishable compound mostly because of the high content in water. This requires special conditions of preservation and limits the shelf life of the final drug. Considering that milk water content does not contribute directly to its beneficial attributes, the removal of moisture is a solution that highly increases the stability of the compound.

In this view, spray drying, freeze drying, and fluidized bed drying processes are described below as valuable techniques to stabilize most dairy ingredients.

6.1 Spray-drying

Spray Drying is a widely used process in the pharmaceutical and food industry mostly with the intuit of preservation, stabilization, and microencapsulation (48).

Generically, this process can be described as the transformation of a fluid state to a solid dried state through a process in which the feed is sprayed into a heated drying solvent. The feeding fluid can assume the form of solution, suspension, emulsion or even a semi-solid form (71).

The spray drying process ***Figure 3***, begins with the atomization of the feed, in which is produced a spray inside an atomizing chamber. The goal is to optimize the evaporation conditions turning the process more economic. Considering a co-current flow design, the drying air is cooled, and the evaporation process is very quickly whereby the product does not suffer thermic degradation. This design represents a big step facing one of the disadvantages of spray drying – the thermo degradation- that is critical in experiments that involve thermolabile compounds (72). The viscosity of the feed concentrate increases with the percentage of proteins, total solid content and crystallized lactose (73).

After this first stage, the droplets forming the spray contact with the heated gas, starting the evaporation of the solvent (74). After evaporation, the dried particles formed are collected by a cyclone, a filter bag or an electrostatic precipitator.

The spray drying can be performed through a multiple stage process.

The one-stage drying process takes place in a drying chamber where the product is dried to the final moisture. During this process occurs a big heat and mass transfer in a short period

of time, what can promote product degradation. When the liquid particles are decelerated by the friction against the air is when the drying takes place and consequently, the droplets lose weight and volume. It is also registered an increasing on the solid content and therefore, an increasing of the viscosity and surface tension of the particles. The contraction of the particles is influenced by the amount of air in the droplets and the air temperature (73). However, it was observed that the dryness of smaller particles is more uniform than the bigger ones. In these cases, the particle can be overheated due to slower evaporation rates what, in extreme cases, can lead to a hardening phenomenon – a hard crust on the surface of the particle is formed. Usually, hardening occurs when the particle has a residual moisture content what makes the proteins very vulnerable to heat damage, denaturation and, what consequently, decreases powder solubility. The amorphous lactose is another component that contributes to the hardening effect by becoming almost impenetrable to water (73).

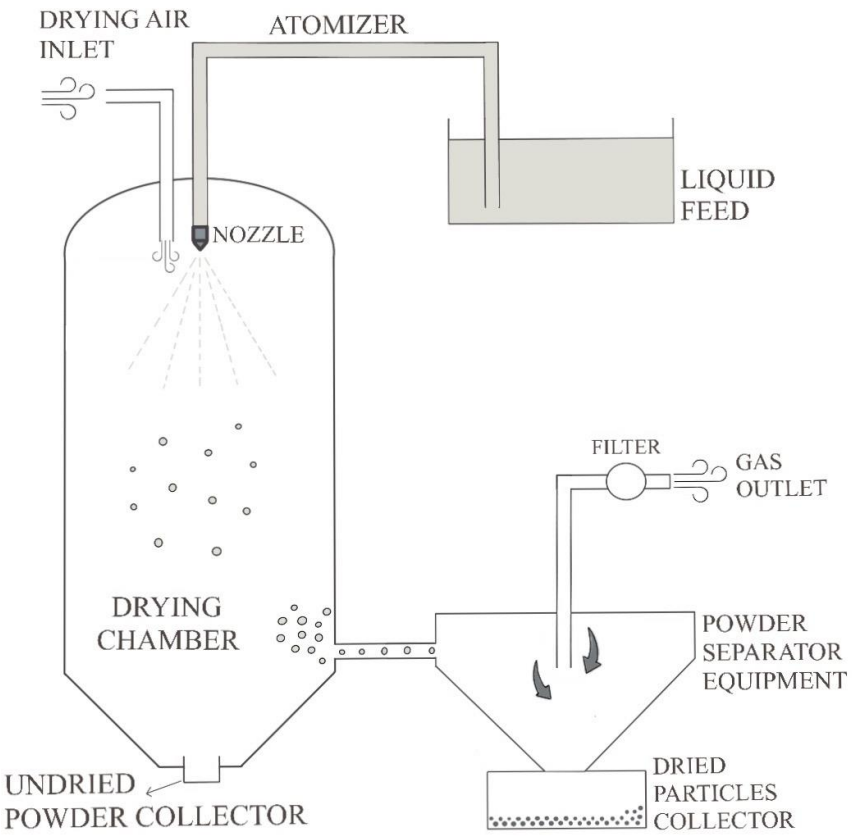

Figure 3 Spray drying equipment design, modified from (75)**.**

The drying process explained above, leaves a residual water content (2-10%) on the final particles. The two-stage drying process has been designed to eliminate this remaining

moisture. For this purpose, a longer residence time of the powder on the system is required. To achieve this goal, it can be used a hot air in a pneumatic conveying system which increases the driving force. The final powder produced by this method presents higher solubility, higher bulk density, lower free fat, lower content of occluded air, less dust waste and higher process yield. When the powder is dried, it is separated in a cyclone and cooled in other pneumatic conveying system with cold and dry air (73).

There are multiple factors influencing the characteristics of the final dried product, standing out powder moisture content, size distribution and particle morphology (74). Among those factors are the physicochemical properties of the feed and of the drying solvent, the control parameters of the process (e.g. air temperature, solution or suspension feed rate, aspirator flow and spray air flow (74,76)), as well as the design of the dryer (71).

The spray drying technique is recognized to increase storage stability and ease the handling of milk based products (77). However, it presents other advantages including the reproducibility (72), drying speed (74), simplicity to perform, continuity, and automaticity of the process possibility of determining the desired dried particle size and humidity; the applicability to heat sensitive materials, such as milk components; maintenance of the products chemical integrity and sensorial properties of fresh milk after the reconstitution of the powder in water (74).

However, one of the detected problems of this technique is the deposition of the particles on the chamber walls that leads to their degradation and consequent contamination of the final product. An appropriate selection of the setting controls of the operation can minimize this effect. It has been concluded that if the air inlet temperature remains constant, there is an increase of feed flow rate and, consequently increases the deposition of particles on the walls. The same way, the greater the amount of product fed, the shorter the residence time of the particles inside the chamber which reduces the time available for drying. Consequently, the deposition rate increases due to the increase of wettability and cohesiveness of the powder particles (71).

Additionally, the energy consumption required for spray drying is considerably higher than the one required for a simple evaporation. It is considered that the increasing of the concentration of the feed can minimize the impact of this problem on the overall yield. In practice, this solution has not shown to be feasible since the viscosity of the feed is a limiting factor for the performance of the machines available.

Nese *et al.*, 2020 has conducted a study of spray dried milk based formulations for theophylline delivery, in which, when milk was used with higher percentages of fat, it has been

observed a higher adherence of the dried powder to the wall of the cyclone, negatively impacting the yield of the process. Additionally, the process of drying the powders with high fat milk content at low temperatures was found to be harder comparing to higher temperatures that allows a greater mobility of fat particles facilitating powder drying. Microbial growth was also analyzed in this study being registered to be faster in low fat milk samples (74).

As presented before, the high temperature usually required to perform spray drying processes is also a concerning factor considering the milk's stability. Milk's proteins can suffer structural damage and consequently lose their functionality due to heat exposure. Since proteins represent a considerable percentage of milk compounds (20%) and are present in many important structures for drugs vehicle, this is a particular point to take in consideration.

In this sense, there have been reported an innovative device able to process viscous products at low temperatures, based on a spinning cone evaporator model. Centritherm® CT is a device primary designed for food industry, to protect the color, flavor and active ingredients proprieties since it requires only one second of heat exposure, using temperatures below 35ºC. The spinning cone design applies centrifugal forces to the product which allows high concentrations in a single step. It also presents the possibility to introduce a pre-evaporator stage that allows a reduction in energy consumption. The results of a pilot-scale study using concentrated skim milk dried with this device, did not show protein denaturation. Also, solubility index of 100% and dispersibility index over 80% were verified (77).

If drying parameters are not optimized, the bigger molecules can slowly migrate from the nucleus of the particle to its surface, causing segregation of the components which can affect the final product stability during conditioning and storage. In this sense, this phenomenon will change the properties of the powder surface, the particle segment that interacts the most with the environment and therefore is fundamental for its functionality (78).

Studying how the parameters of the process affect the quality of the final product, Nikolova *et al.*, 2015, concluded that the increasing of the spraying pressure, decreases the size of the droplets and therefore, promotes a better drying. A better dried particle shows lower water activity what results in higher stability. The same effect was registered when the outlet temperature was analyzed, being this parameter the most influent on powder moisture. It was also concluded that the increasing of the outlet air temperature, increases the tapped density of the dried powder since higher drying rates origin smaller and more spherical particles which increase compressibility during tapping. The drying temperature influences the particle porosity and therefore, the bulk density of the powder (78).

6.2 Freeze-drying process

Maintaining the goal of improving stability of milk-based medicines by removing moisture, freeze drying is a popular process for drying sensitive materials since it does not need high temperatures to be performed as in spray drying processes. The process works with very low temperatures and minimal exposure to oxygen assuring the preservation of the biological and physico-chemical conditions specially of thermolabile products such as milk based formulations (79). Moreover, it allows the processing of milk directly from the liquid form and transform it into the solid one, which is less expensive to store and easier to transport (80).
This drying process is divided in three steps: freezing, first drying stage and second drying stage (81).

During freezing, the product is submitted to very low temperatures during a short period of time to origin small ice crystals. Although bigger crystals shorten the drying time, since the sublimated vapor is removed by diffusion and it is improved by the pore size - smaller crystals avoid a potential physical damage of the product and therefore they are preferred. Then, the first drying stage is launched by decreasing the pressure below the vapor pressure of ice and the temperature of the chamber is raised to promote sublimation (82,83). The moisture of the product exists in a free state and in a protein bound state. Free water content is easily frozen being ready for sublimation during this first drying. However, the remaining bounding unfrozen water molecules still promotes the degradation of the product (81) what raises the need of a secondary drying stage. In this, the moisture is removed by desorption. Desorption is faster in smaller crystals as they present bigger surface area reducing time consumption of this last step (82). Summarizing, the first drying step is usually the most cost and time-consuming step what constitutes one of the disadvantages of the process. Additionally, it is pointed out high costs and scale-up issues what limit its use in large scale productions (81).

Considering the advantages and limitations of the spray drying and freeze-drying processes, the spray freeze-drying concept has arisen. It is a mix between those two methods and includes atomization, freezing, primary drying, and secondary drying. It starts with the atomization of the product in a liquid stage as it happens in spray drying. However, the atomization is made into a cryogenic medium instead of a heat gas. This step allows an instant freezing of the sprayed particles. Then, the iced droplets are collected and transferred to another chamber to be dried in two stages as it occurs in freeze drying. This novel drying approach is able to dry products avoiding high temperatures and, as it provides a bigger surface area of the

frozen starting material, the two drying processes turn more rapid and consequently less expensive (84).

6.3 Fluidized-bed process

Fluidized bed is a technique with a wide applicability in pharmaceutical production and drying of granules. The final product produced by this method presents as advantages a favorable flowability, compressibility, an homogeneous coating thickness (85), high heat and mass-transfer rates, high yields, lower residence times and a more stable final product (86).

To carry out the drying process, a high temperature air is injected inside the chamber under vacuum conditions. Milk powder is then fluidized from the bottom of the equipment over a current of air. The hot dry air promotes the evaporation of the moisture present in the powder particles, increasing their stability (80).

In fluidized bed granulation after the fluidization of the powder inside the chamber due to an air flow, a binder component is sprayed causing the agglomeration of the particles in granules. When compared with other granulation techniques, the fluidized bed shows to be simpler and demonstrate higher yields, uniform thickness, and distribution of the binder component on the surface of the particles, higher granule compatibility and higher drying rates. The coating of pellets or small tablets is also possible. In this process, the solid particles are fluidized and subjected to a coating spray (85).

In general, this technique presents disadvantages such as the use of high temperatures that can damage the properties of milk components, specially proteins. The excessive agglomeration of the particles is also a relatively common phenomenon and therefore can represent another disadvantage. When the binding solution is sprayed, the surface of the particles is wetted and consequently gets more sticky, either because the film of the binding solution is not dried or due to a modification of the viscosity of the surface of the particle. Collisions between sticky particles promotes undesired agglomeration, in particular cases (87). In dairy products, the use of fluidized bed after spray drying is common.

Generally, it is difficult to determine optimal parameters when the intent is to fluidize amorphous powders, such as milk. Amorphous components present some advantages such as porosity, good solubility, and bioavailability but they also seem to be highly unstable due to their higher tendency to adsorb water and forming bridges with other particles originating agglomerated powders (86). Amorphous lactose is one of the most unstable components of the milk, being its crystalline alpha monohydrate form the most stable one and, consequently more

fluid, with less probability to agglomerate (88). This lactose form can be obtained by crystallization below 93.5°C (47).

During the processes of fluidized bed or spray drying, the hot humid gas used for the fluidization contributes to the stickiness of milk powder containing amorphous lactose due to its tendency to absorb moisture and to form bridges with the other component's particles. This phenomenon can lead to cake formation. To solve this issue, it is necessary to balance the temperature and humidity to achieve a better powder crystallinity in the least time and therefore, a better powder fluidization (88). Yazdanpanah *et al.,* 2011 has studied the performance of a process able to increase the crystallinity of milk powder through two stages. This process includes a Humid Loop spray drying stage in which the powder become partial crystalized, differently from the classical process that produces a more amorphous powder (86). For this, it was added to the traditional equipment a closed humid loop for the inlet/outlet air that allows a higher control of the humidity levels during the process. Lastly, a fluidized bed stage that, due to the previous step, it can be performed in less stages and in shorter cycles since the powder has a higher fluidizing ability. In a process that includes sorption, crystallization, and desorption, the phenomenon of crystallization and drying is finished in multiple steps. The conclusions of this study were positive, showing that the combination of the two processes has industrial advantages to manipulate milk powders (86).

7 Conclusion

Milk is not only a nutritional product. Each of its components plays different biological roles in the newborn organism that can be availed to apply on pharmaceutical formulations (6).

Milk presents multiple advantages over other excipients. As a biological fluid broadly used at least in the first years of age, it is not expected to cause significant adverse reactions. Cow's milk protein allergy and lactose intolerance are the only conditions that have been reported but only in a non-significant incidence that only justify a limitative use of milk in drug formulations for children with cow's milk allergy (26,37) . Another advantage pointed out is the fact that cows are able to produce multiple liters of milk per day which turns this biological fluid in a low cost and widely accessible excipient (43).

Milk based formulations are a novel promising approach to achieve a target delivery of lipophilic drugs either in an ionized or unionized form. This is possible thanks to lipids that play an important role by solubilizing the lipophilic molecules through the encapsulation into the milk fat globules. Moreover, the lipidic digestion creates a greater environment for solubilization and therefore promotes the absorption process (89). Additionally, proteins give their contribute by establishing hydrophobic and ionic interactions with the drug molecules and through their self-assembly proprieties and gel formation capacity that enable the entrap of bioactive molecules increasing their solubility, controlling their release and directing them to a specific target (8,29). Still with this purpose, researchers have shown a special interest in milk exosomes. These exosomes present the characteristics of an ideal drug delivery system not showing cell toxicity or immune responses, having similar inter-tissue biodistribution, high bioavailability and long circulating half-life time. It shows to be an opportunity for targeted gene delivery in anti-tumor therapies being a strong candidate for further investigation in the future (43).

The gastroprotective proprieties of bovine milk are conferred by strengthen the gastric mucosa and increase of prostaglandin, mucin and antioxidant enzymes, which are protective substances (14), and also by modulating the expression of inflammation-related genes (55). Most of the studies focus on the gastric benefits when milk is drunk in concomitance with medicines, however, more studies should be done to confirm the greater gastroprotective performance of milk directly in the formulation.

The taste masking mechanism performed by milk is still not fully understood. The partitioning of the drug molecules into the milk fat phase and an increased viscosity of the

formulation seem to explain in part this propriety, but not in its entirety (66) . The lack of studies regarding the taste masking ability of milk can potentially be explained by the difficulty in measure the impact on the flavor of the formulation. While human and animal methods present ethical issues, the analytical equipment remains an expensive and relatively inaccessible, although the progress in the viability of results and in the overall costs (68,69). These factors can be one of the causes of the reduced information about this theme.

Nonetheless, milk shows practical limitations specially in what concerns to its microbiological stability due to its high-water content. There are multiple drying processes available to remove the moisture of the milk. However, milk is a thermolabile compound mostly because of its content in proteins and lipids and, many of these processes requires high temperatures. Freeze drying overcome these concerning since it uses low temperatures, although its applicability is limited due to high costs and time-consuming issues. Spray freeze drying seems to be a good option to manipulate milk-based medicines, since it combines the low temperatures of freeze drying and the speed and easy scalability of the spray drying. This way, it is possible to reduce the costs and maximize the yields and the biological value of milk in the formulation (81,82,86).

In general, milk is a strong candidate to be used as part of a targeted delivery system of drugs being necessary additional in vitro and in vivo studies to clarify the behavior and the physicochemical characteristics of the milk-based final formulation.

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Annex

A1. ERASMUS programme

This project has started in Portugal with the intuit of being finished, in a practical way, in Italy, following the ERASMUS program. In Portugal, the objective established was to study the performance of granules and pellets using fresh milk and powder milk. After these steps, it was also intended to coat the material produced with a solution including fresh milk, through a spray drying process. After several tries, it was accomplished a suitable formulation for paracetamol pellets, using commercialized powder milk. The powder bulk resulting from the formulation described on ***Table 1***- Formulation 1 was, after a hydration period of 24 hours, extruded and espheronized successfully (maximal strength = 35kN and velocity = 110 mm/s). Although the strength has been stabilized at 4kN which was a low value for an optimal performance according to the literature, the pellets were spherical and powderless.

In Italy, the differences in the equipment has required quantitative modifications in the formulation that are described in ***Table 1***- Formulation 2. In a visual analysis, the produced pellets were spherical and powderless. During this part of the project were done a few formulations tries to improve the knowledge about the equipment. During this process, were done granulometric, friability and dissolution tests.

Table 1 Milk-based formulations

	Content (%)	
Ingredient	**Formulation 1**	**Formulation 2**
Paracetamol	30	30
Croscarmellose	5	5
Lactose	20	20
Microcrystalline Cellulose	30	30
Powder Milk	10	10
Polyvinylpyrrolidone	5	5
Water	50	55
	(based on solid content)	(based on solid content)