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REVIEW ARTICLE

Human Health Effects of Lactose Consumption as a Food and Drug Ingredient

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Abstract: Lactose is a reducing sugar consisting of galactose and glucose, linked by a β (1→4) glycosidic bond, considered as an antioxidant due to its α-hydroxycarbonyl group. Lactose is widely ingested through the milk and other unfermented dairy products and is considered to be one of the primary foods. On the other hand, lactose is also considered as one of the most widely used excipients for the development of pharmaceutical formulations. In this sense, lactose has been related to numerous drug-excipient or drug-food pharmacokinetic interactions.

Intolerance, maldigestion and malabsorption of carbohydrates are common disorders in clinical practice, with lactose-intolerance being the most frequently diagnosed, afflicting 10% of the world's population. Four clinical subtypes of lactose intolerance may be distinguished, namely lactase deficiency in premature infants, congenital lactase deficiency, adult-type hypolactasia and secondary lactase intolerance. An overview of the main uses of lactose in human nutrition and in the pharmaceutical industry and the problems derived from this circumstance are described in this review.

Keywords: Lactose, antioxidant, excipient, food, interaction, intolerance.

1. INTRODUCTION: LACTOSE DEFINITION AND PROPERTIES

Lactose is a disaccharide consisting of galactose and glucose, linked by a β (1→4) glycosidic bond. It is slightly soluble in water (170 g/L at 15 °C) and is naturally occurring in the milk of mammals. In comparison with other disaccharides, lactose presents a sweetening power, which is one of the best-known properties of sugars. The sweetening power is determined in relation to sucrose as reference sugar (sucrose in a solution of 30 g/L at 20 °C is assigned a sweetening power equal to 1), of 0.15, which is six times lower than sucrose and much lower compared with other soluble sugars, such as maltose (0.4), glucose (0.7) or fructose (1.7), among others [1].

Lactose is considered as an antioxidant compound because similar to other reducing sugars (α-hydroxy carbonyl compound), it has the capability to act as a reducing agent. Reducing sugars possess a terminal aldehyde group (HC=O) which is capable of being oxidized to a carboxylate (COO-), while the oxidizing agent (cupric ions, Cu^{2+}, in alkali) is in turn reduced to a cuprous ion, Cu^{+}, in this instance, insoluble cuprous oxide [2]. This property confers to this molecule the antioxidant potential, suchother soluble sugars include fructose, glucose, galactose, and maltose, among others. The antioxidant capacity of soluble sugars and particularly of lactose was reported by Wehmeier & Mooradian [3]. These authors highlight the capacity of lactose at 5 mM to reduce free radicals to 53.5% and have some effects on the prevention of peroxyl radical formation.

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Lactose in milk can be found in two different isomers. The α-lactose and β-lactose, that differ only in the configuration of the substituents on the C4 hydroxyl group of galactose (Fig. **1**). Both the isomers are present in equilibrium in dissolution (such as in milk), although due to mutarotation, a different isoform yield can be found, being around 63% for β-lactose isoform [4, 5]. Both the isoforms differ in terms of their physicochemical properties, such as solubility, melting temperature and rotation power. As a matter of fact, α-lactose has a solubility of 70 g/L, a melting temperature of 202 °C and a rotational power of -89.4 °. While β-lactose has a solubility of 500 g/L, a melting point of 242 °C and -35 °C for rotational power. Lactose can be found in dissolution or dried because it can be obtained from milk serum by ultrafiltration, evaporation and subsequent crystallization. α-lactose isoform is obtained by oversaturated crystallization (at less than 93.5 ºC). Initially α-lactose is precipitated and a mutarotation of part of β-lactose takes place into α- isoform, thus yielding mainly α-lactose monohydrate crystals, whereas solid β-lactose is crystalized in an anhydrous way [4, 6].

Apart from the different spatial conformations, lactose can be also found in the anhydrous or hydrated form. When the rapid drying process is applied, such as spray- drying or rolled- drying, there may be insufficient time for α-lactose crystallization in the monohydrate form, obtained a mixture of anhydrous α-lactose, α-lactose hydrate and β-lactose. It is known, that anhydrous α-lactose is strongly hygroscopic and can absorb moisture from the air, increasing its volume, being responsible for caking and lumping process in many dried dairy products like milk powder, and their potential uses in the food and pharmaceutical industry [4, 6].

2. LACTOSE IN DAIRY FOODS

Dairy products play a central role in the human diet since they contain numerous nutrients necessary for the normal functioning of

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the cellular metabolism [7]. Lactose is one of the more frequently present sugars in these kinds of products.

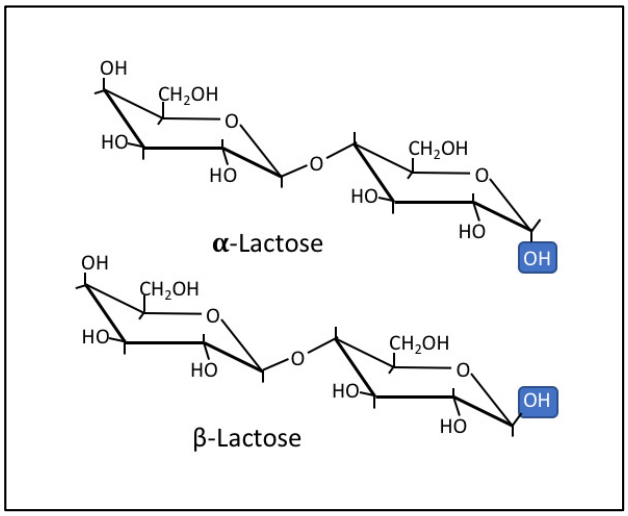

Fig. (1). Isomeric forms of lactose.

Lactose is ingested through the milk and other unfermented dairy products. The dairy products containing higher proportion of lactose include fluid, concentrated and dry milk, creams, butter and margarine, cultured milk products, frozen milk, cheeses, whey's milk. Among them, regular milk also contains fats, proteins, carbohydrates, vitamins, minerals and water. Lactose is present approximately in proportions of 4-5% depending on the cows from which it is obtained [8]. In the fermented products such as yoghurt, it was detected in concentrations of about 4.7-4.8%, with 0.8-1.0% in butter, and in different proportions in cheese, depending on the type of fermentation to which it is subjected, ranging from 0.1 to 0.5% in cheddar cheese or 1.0-3.1% in cottage cheese. Also, in ice cream, 3.6, 8.4% of lactose is present [9].

Depending on the process used to reduce the amount of lactose either physical or enzymatic, the residual amounts may vary. For that reason, fermented dairy products contain less lactose.

2.1. Other Lactose Uses in Food Industry

Lactose is widely used in food industry due to some of its inherent characteristics, such as texture (lactose changes crystallization characteristics of other sugars), adhesive and adsorption properties (include its ability to carry flavors and colors), hydration properties (humectant and moisturizing agent) and organoleptic properties (as mild sweetener and as browning during Maillard reaction), being used as a food ingredient (according to the European Regulation (UE) No 1129/2011 [10]) or as a food additive (according to the FDA regulation being considering as GRAS compound, FDA CFR - Code of Federal Regulations Title 21 [11]) in several food products (processed meat, breakfast cereals, margarines, ready to eat meals, among others) and in food supplements as an excipient. α-lactose monohydrate has been preferably used [12, 13]. However, The demand for anhydrous β-lactose is increasing in the food industry due to its higher solubility and greater sweetness compared with the α-lactose monohydrate.

Lactose is commonly used in confectionery and baking industry, because the reducing nature of lactose coupled with the fact that it is not fermented by bakers' yeast, makes this compound perfect for baking products. Lactose increases the browning of the crust (often highly desirable) because it plays a role in Maillard reactions.

Typical Maillard reaction is a well-known non-enzymatic browning reaction, that involves a reducing sugar and an amino acid, producing an amino acid - sugar model system that may produce colored or colorless reaction products depending on the stage of the reaction. At temperatures less than 90 °C for over a period of a few days, this reaction results in glycated proteins of darker color and more flavor due to the formation of 5-hydroxymethyl furfural (among other Maillard Reaction Products, also known as MRP). Its concentration depends on several other factors such as pH, type of reactants, temperature, water activity, *etc*. Yilmaz and Toledo reported that at specific relative humidity, temperature and time conditions, MRP from whey protein and lactose were more stable and lighter than nonglycated controls [14]. MRP constitute a group of compounds that include high molecular weight melanoidins, which are furan ring and nitrogen-containing brown compounds. These compounds were also reported by several authors to provide antioxidant properties through scavenging oxygen radicals or chelating metals. Most of these authors have understood that the complexity of MRP structures limits the determination of antioxidant activity for each compound in the whole group of MRP. Particularly, Monti and coauthors [15] reported the antioxidant capacity of the MRP from the reaction between lactose and lysine model using peroxyl radical scavenger assay.

Lactose is neither fermented by bakers' yeast, nor by other *Saccharomyces* yeasts commonly used in beers. In this way, lactose may be used to improve the organoleptic quality, because it is not fermented by beer yeasts. Different authors' review on lactose utilization outlined the use of deproteinated whey as a base for non-alcoholic beverages, animal lick blocks, lactose bricks, dried lactose, and derivatives such as lactobionic acid, lactulose, and lactitol [6].

Other relevant use of lactose focus on obtaining other widely-used sweeteners in food industry like lactitol (E 966) and tagatose (Fig. **2**):

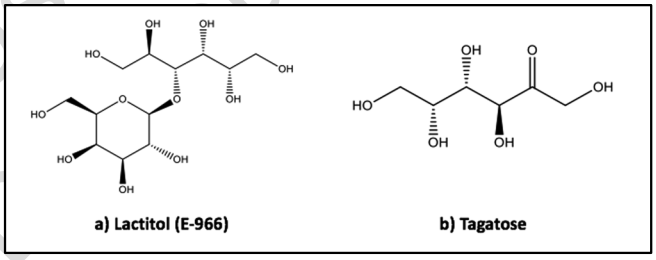

Fig. (2). Lactose-derived sweeteners. **a**) Lactitol (E-966); **b**) Tagatose.

a) Lactitol (E 966) is a food additive obtained by hydrogenation from lactose. It was discovered in 1920 and its use is widespread. It has a limited sweetening power (30-40%), so it is usually used in combination with other intensive sweeteners such as acesulfame K (E 950), aspartame (E 951) and sucralose (E 955). After ingestion, it is excreted without absorption (only 2% absorbed) or metabolization (only provide 2 kcal/g). It has a lower solubility than other polyols, such as xylitol and sorbitol. It provides a feeling of freshness and it is used as a loading agent, that is, it is added to the food to increase volume if it contributes significantly to its caloric value. It is also used for its non-cariogenic properties and as a prebiotic. Its main applications are in the manufacturing of chocolate, baked goods, chewing gums, ice creams, *etc*. [1, 16].

b) Tagatose in Europe is not considered as a food additive (sweetener), rather as a food ingredient. This compound should be considered as a semi-synthetic product since consecutive enzymatic and chemical steps are performed at an industrial scale. From a nutritional and bromatological point of view, this ketohexose is considered as a prebiotic with sweetening properties, such as sucrose with no effect on blood glucose levels, deeming it safe for diabetic individuals. Moreover, even though it is considered a sugar, it does not promote tooth decay. It is stable at pH 2-7, and shows a high solubility in water, making it ideal to

be also used as a flavor enhancer, although it lacks some stability. Furthermore, it shows humectant properties like sorbitol. It is also approved in New Zealand, Korea and in the USA, where it is considered GRAS and can be used as a low-calorie sweetener. Its applications include beverages, cereals, bubble gum, chocolate, caramels yogurts, ice creams, nutritional supplements, among others [1, 17-19].

3. LACTOSE METABOLISM

Lactose is not digested until it reaches the small intestine where it is hydrolyzed in its two main monosaccharides thanks to lactase (β-galactosidase), which is a membrane-bound enzyme located in the brush border epithelial cells of the small intestine, secreted by the villi of epithelial cells [20].

Lactase catalyzes the hydrolysis of lactose into D-glucose and D-galactose. Both monosaccharides are rapidly absorbed and enter the bloodstream. Both monosaccharides are then carried *via* the sodium-glucose transporters across the intestinal brush border. Subsequently, the monosaccharides diffuse into the blood, either passively or by means of glucose transporter 2 (protein GLUT 2). Mostly these sugars are used for energy and structural molecules used in cell-cell communications, immune functions, epithelial stabilization, and neurological development. The main use of glucose is for metabolic energy; instead, galactose is utilized by the neonate for multiple purposes, such as glycolipids and glycoproteins synthesis. For energy use, galactose must be transformed into glucose using galactokinase and galactose-1Pi-uridyltransferase [21, 22].

4. LACTOSE INTOLERANCE

4.1. Pathophysiology

Intolerance, maldigestion and malabsorption of carbohydrates are common disorders in clinical practice, lactose intolerance being the most frequently diagnosed, afflicting 10% of the world's population. Lactose intolerance was already described by Hippocrates in the 5th century B.C. and since the symptoms associated are quite unspecific, these clinical syndromes are not diagnosed properly. Therefore, a better knowledge of the symptoms and appropriate diagnostic procedures to discern lactose intolerance and conveniently treat it, is needed.

Mammals' feed is dependent on mother's milk in the early moments of life and since the domestication of the cattle, milk has been constantly used as food. Thus, the assimilation of lactose *i.e* its main carbohydrate is essential. Lactose is hydrolyzed in the small intestine by the β-disaccharidase lactase, also called lactase-phlorizin hydrolase, which is exclusively present in the glycocalyx of the enterocyte microvilli. Lactase appears in intestinal villi being maximal at the apex, whereas virtually no lactase activity is seen in Lieberkühn's crypts. The products of its hydrolysis, glucose and galactose, are normally completely absorbed in the jejunum. Lactase increases during the prenatal period to reach its peak at birth and then decreases after weaning until 2 to 5 years of age [23]; the persistence of lactase activity varies across individuals. This timed developmental step seems to serve the promotion of weaning in the infant and ovulation in the mother, thus regulating optimal birth spacing [24].

The absence or malfunction of lactase or of the enterocyte membrane proteins which transport these products make lactose accumulate in the intestinal lumen, with subsequent increase of the luminal osmolarity. This provokes a shift of plasma fluid out from the bloodstream, accelerating the passage down the intestine and causing several symptoms. Among the gut-reported symptoms in the order of frequency: abdominal pain, bloating, borborygmic, flatus, nausea, vomiting, diarrhea and constipation, and among the systemic symptoms: headache, loss of concentration, muscle pain, joint pain, stiffness, asthenia, mouth ulcers and increased frequency of micturition. Once in the colon, lactose is fermented by the microbiota resulting in short-chain fatty acids (butyrate, propionate, acetate and lactate) and gases such as hydrogen, carbon dioxide and methane, it decreases luminal pH [25]. Hydrogen gas is excreted in 85% by feces, while 15% diffuses into the blood to be excreted by the lungs through the breath. In case of inflammatory diseases, in which the absorption of fatty acids is impaired, the luminal pH decreases, further impairing water and sodium reabsorption, leading to foaming and acid feces, which may cause lesions in the anus and perineal region. An impairment of microbiota fermentation, like after antibiotic treatment, would also drive to increased luminal osmolarity leading to distension and diarrhea.

4.2. Clinical Syndromes

As stated above, the clinical condition of lactose intolerance is a consequence of lactose maldigestion and subsequent malabsorption; the intensity of clinical symptoms is correlated with the quantity of lactose ingested. Four clinical subtypes of lactose intolerance may be distinguished, namely lactase deficiency in premature infants, congenital lactase deficiency, adult-type hypolactasia and secondary lactose intolerance.

Lactase deficiency in premature infants is frequent in less than 35-week-old newborns, in which the symptoms are transient and meliorate along the first postnatal week. Congenital lactase deficiency is caused by a mutation of the LCT gene, affecting the functional capacity of lactase. It is a recessive autosomal genetic disorder leading to the inability to digest lactose, which in turn may cause watery diarrhea, growth delay, dehydration and alkalosis [26]. The histological aspect of the intestinal mucosa is preserved. Adult-type hypolactasia is present in older children and adults and is caused by reduced activity of the promotor of the lactase gene after infancy following a developmental program with race variations. Thus, lactase persistence is quite frequent in Northern European countries, while it accounts for approximately 60% of the population in Mediterranean countries and decreases to 30% in Africa and to less than 10% in Asia [27].

In secondary lactase intolerance, lactase activity is impaired subsequently to small intestine diseases such as infections, parasitosis such as giardiasis, coeliac or inflammatory bowel diseases, protein-calorie malnutrition, cow's milk protein intolerance, immunodeficiency syndrome, chronic alcoholism, functional problems such as post-surgery with a rapid stomach emptying and some pharmacological treatments such as antibiotics, colchicine and other chemotherapeutic drugs.

Due to its high prevalence, special attention must be paid to malabsorption syndrome secondary to coeliac disease. Patients afflicted by this disease show sensitivity to gluten, a protein present in cereals, against which an immunological response is triggered causing the disappearance of intestinal villi leading to flat jejunal mucosa. Hypoplasia of Lieberkühn's crypts and infiltration of the lamina propria by mononuclear cells also occur. The ileum is not normally involved. In addition to lactose intolerance, protein-calorie malnutrition or specific nutritional deficits may occur, such as anemia, bone metabolic and hemorrhagic diseases. First analytical features that appear are deficits of iron, folate and calcium and as the disease progresses, affecting longer intestine length, malabsorption of carbohydrates, fats and liposoluble vitamins (A, D, F and K) occur [28]. Therefore, besides a diet without lactose, these patients may need supplements of iron, folic acid, calcium and vitamins. The decision of lactose withdrawal from the diet in coeliac patients must be taken depending on each individual.

Although lactase is not regulated by lactose ingestion, it has been reported to meliorate its tolerance after regular intake *via* adaptation of the intestinal flora [29].

4.3. Diagnosis

The first approach is the anamnesis, in which a thorough compilation of data about dietetic habits is mandatory. The next step is

the withdrawal of lactose during one week in order to observe if clinical symptoms disappear and then reintroducing it and see whether symptoms reappear. Confirmatory diagnosis is made by the breath test and other methods, namely lactose intolerance test, tissue lactase activity and genetic study. Once made the diagnosis of lactose intolerance, any of the possible intestinal diseases leading to secondary lactose intolerance must be ruled out.

The breath test detects the hydrogen and methane in exhaled air. Any hydrogen detected in the exhaled air is considered to be originated from the colonic fermentation. To perform this test, the following are required, a previous period of 7 to 10 days of a lactose-free diet, avoidance of treatments with antibiotics, purgatives, colonoscopy procedures and probiotics during at least the two previous weeks, a diet low in fermentable carbohydrates during the previous two days and overnight starving condition. Then 2 g/kg of lactose (maximum 25 g) is administered orally. The hydrogen and methane present in the exhaled air are measured basally and every 30 minutes for 3 to 5 hours. The normal increase in the basal level must be lower than 10 ppm. If it is between 10 and 20 ppm, it is considered undetermined and positive when values reach more than 20 ppm. It is the best diagnostic test for this, with a sensitivity between 76% and 100% and a specificity between 90% and 100% [30].

The lactose intolerance test is also performed by administering orally 2 g/kg of lactose in children or 50 g in adults to evaluate after 20 to 30 minutes, the appearance of symptoms such as abdominal pain, flatus and diarrhea. Caution must be taken since 10% of healthy individuals will develop gastrointestinal symptoms, dizziness, nausea and palpitation after eating 50g of any carbohydrate [31]. In addition, glycemia is measured at 30, 60, 90 and 120 minutes after lactose intake. Lactose intolerance would render an increase of glycemia lower than 20 mg/dL over the basal level in each sample. The sensitivity of this test is 76-94% and its specificity is 77-96% [25].

Tissue lactase activity measurement requires a biopsy of the enzymatic quantifications. It must be performed only if it is really necessary because of its invasiveness, high cost and the irregular distribution of lactase along the intestinal epithelium. An activity of lactase lower than 8 U/g dry weight or 0.7 U/g wet weight implies a deficiency of lactase associated with lactose intolerance.

Finally, genetic study with polymerase chain reaction for the C/T-13910 polymorphism (located upstream of the LCT encoding gene) is useful for adult-type hypolactasia since the C/C variant predicts the decline of intestinal lactase, with a sensitivity of 97% and specificity of 95% [32]. It is performed on the cleavage cells of the oral mucosa obtained by a simple swab.

4.4. Food Products for Deficiency in Lactase

As previously mentioned, deficiency in lactase production causes numerous symptoms such as diarrhea; abdominal pain and bloating that characterize lactose intolerance [33].

One of the main sources of lactose is milk and dairy products. Lactase treatment or ingestion of oral lactase supplements is one of the alternatives for the decrease or elimination of lactose in food [33]. There are three general principles for treating lactose intolerance: reducing or eliminating the intake of lactose; replacing lactose with alternative nutrients; and administering enzymatic substitutes or lactase supplements [5].

In the market, we find an increasing number of products lactose-free in an ever-wider range of dairy categories. Although the labelling rules to indicate the absence or reduced content of lactose in foods are not currently harmonized in the European Union [34], these standards should take into account the scientific opinion on the amount of lactose for people intolerant to it. It is recommended that, until there is an assurance about the absence or reduced presence of lactose in food, a maximum value of 0.01% for products "lactose-free" and 1% for those low in lactose [35].

Manufacturers are increasingly aware of the need to develop lactose-free products to prevent any long-term complications caused by untreated lactose intolerance. To design lactose-free products, it is necessary to assure the elimination of food products or food ingredients that contains lactose, for those consumers intolerant to the presence of lactose. Currently, the food industry can reduce lactose content in two main ways:

a) The first is the hydrolysis of milk lactose by means of the enzyme β-galactosidase that transforms lactose into glucose and galactose. In this sense, the enzyme is directly added to the milk and keeps incubated for hydrolysis proposed. This process is conditioned by the concentration of lactose in milk, the dose of enzyme, milk temperature and process time [36].

   The β-galactosidase enzyme can be obtained from strains of different microorganisms such as *Kluyveromyces lactis*, *Aspergillus oryzae* and *Aspergillus niger* yeasts. The first is used primarily for the lactose reduction in milk and the other two for obtaining whey of lactose-free cheese.

   The lactose hydrolysis process is carried out at a temperature between 6 and 10 ° C within 15 to 20 hours. This temperature is lower than the optimum one that would be 35 - 40 ºC, in order to avoid residual growth of psychrophilic and psychotropic bacteria. This method is best valued in the industry, although it has disadvantages such as the time required and the high cost for the production of the enzyme. The effectiveness of the process depends on the percentage of lactose hydrolysis, which, under the conditions described above, is approximately 85%. Lactose-free milks obtained by this method have higher intensities of flavor and sweetness, due to the greater sweetening flavor of glucose and galactose [37].

b) The second method consists of a variation in the previous one with previous ultrafiltration of the milk (with the loss of different minerals that are replaced after the process) followed by the action of lactase enzyme in order to reduce lactose to the desired levels [36].

However, the elimination of lactose in the diet of humans leads to a deficiency in calcium and this leads us to possible bone fractures to be more fragile. Also, they can present harmful effects such as immune dysfunction, colon health.

It is necessary to look for alternatives to achieve a good nutritional requirement and needs in men during their growing age . If we increase the amount of cheese and yogurt, the needs can be met with the right amount of fat by keeping low lactose levels (12 g) in the lactose-intolerant people with which they can fulfill the necessary calcium requirement [38]. In the market, another alternative source that we find is lactose hydrolyzed milk or oat milk, along with calcium and vitamin D supplements [39] through other food sources to get the recommended dietary allowances. A recent review of Suri and co-authors [9] highlighted lactose-free products developed from alternate sources and the effect of lactase enzymes and probiotics on the lactose intolerance.

5. LACTOSE AS AN EXCIPIENT FOR DIFFERENT PHARMACEUTICAL DOSAGE FORMS

Lactose is one of the most widely used excipients for the development of pharmaceutical formulations due to its inherent characteristics [40] related to its low toxicity and also due to its easy availability in the market and low price. It is also a versatile substance used mainly in solid dosage forms as a filler and diluent in hard gelatin capsules and tablets, including directly-compressible grades of lactose. It is also used in lyophilized products as lyophilization aid and as a dry powder inhaler carrier.

The top-ten most consumed drugs in Spain, alone or in combination with other drugs, are metamizole, acetylsalicylic acid, dexketoprofen, paracetamol, levothyroxine, acenocoumarol, salbutamol, ibuprofen, lorazepam and alprazolam (Tables **1** and **2**) [41]. All of them have commercially-available presentations containing lactose, although it is also true that all these medicaments have therapeutic alternatives without lactose maintaining the same pharmaceutical form, with the exception of acenocoumarol (Sintrom®), with only two presentations marketed, and alprazolam, whose alternative formulations without lactose are available in the form of oral drops. Based on these data, it is important to consider the amount of lactose consumed during the treatment (Table **3**).

Lactose can be found in two isomeric forms: alpha- and beta-lactose, depending on the orientation of the hydrogen- and hydroxyl group on carbon atom number 1 (Fig. **1**). Both isomers of lactose are also commercially available either in amorphous (anhydrous) and crystalline state (beta-lactose and alpha-lactose monohydrate). Depending on the temperature in which the crystallization process takes place, the composition of alpha or beta lactose can be modified and hence the physicochemical characteristics of the lactose crystals can be different from different batches. In a recent research work, Altamimi and co-authors [42] evidenced that variations of more than 10% can be found in different pharmaceutical-grade lactose, that can lead to serious modifications of bioavailability of the final formulations.

Lactose can also be found as pharmaceutical grade alone or in combination with other substances such as starch, cellulose, povidone or lactitol . Other possibilities include pharmaceutical lactose modified by different techniques like spray drying, micronization, granulation by different techniques, *etc*.

This wide variety of compounds leads us to the conclusion that lactose is one of the most versatile substances used as an excipient in pharmaceutical formulation. A general view of the most relevant uses of lactose-based excipients classified by dosage forms is reported.

5.1. Lactose as Filler/Binder in Solid Pharmaceutical Forms Intended for Oral Route

Lactose constitutes one of the most used excipients in tablets either by dry/wet granulation or by direct compression and other oral formulations such as granules, pellets, capsules, *etc*.

Anhydrous lactose is widely used as a capsule and tablet filler and binder. The use of anhydrous lactose as a diluent in tablets has been reported from the classic studies of Batuyios [43], even for direct compression, showing acceptable compressibility and rheological properties to be used as a diluent with high-speed tableting machines. Whiteman and Yarwood [44] demonstrated that anhydrous lactose showed the best tableting properties when it was evaluated in comparison to other lactose-based excipients for direct compression, also obtaining good results with other more complex products like Ludipress®, containing lactose, povidone and crospovidone (Ludipress® - BASF). Those results have been confirmed with subsequent studies published by Ilić *et al*. [45], who evidenced that, a commercially-available beta-lactose (DMV-Pharmatose®-DCL-21) provided better compressibility properties in comparison to alpha-lactose and superior tensile strength values. This study also evidenced that a spray-dried grade of lactose (Meggle – Flowlac®-100) resulted to be the most compressible of the lactose-based excipients analyzed. Compactability differences between crystalline and amorphous forms of lactose could possibly be explained by differences in bonding capacity [46].

Anhydrous lactose is highly recommended for those drugs exhibiting problems of stability enhanced by high moisture content. Nevertheless, although it is true that it has been traditionally described that anhydrous lactose is better than monohydrate lactose in terms of stability due to its lower water content, some recent studies have indicated otherwise. This is due to the fact that chemically bound water does not influence the stability of substances such as aspirin and niacinamide [47].

Some strategies have been implemented to improve compactibility and flow properties of lactose, such as sieving to isolate a narrow interval of particle size, granulation using different techniques, or in combination with other compounds like corn starch, microcrystalline cellulose, povidone (and crospovidone), powdered cellulose, lactitol, *etc*. In this way, granulation has been one of the most studied resources. Some studies evidenced that this process provides an improvement of the flow properties, as expected considering the modification of the particle size and shape, but associated with a decrease of the compactibility properties as a side-effect. As a matter of fact, it has also been observed that the tableting properties aredependent on the type of lactose, considering the added circumstances that the conversion of beta-lactose, present in the roller-dried powder milk form, into α-lactose was evidenced during the granulation process [48]. Partial transformation of anhydrous lactose to lactose monohydrate has also been evidenced in high-shear wet granulation processes [49].

Different results have been observed for lactose-based excipients obtained from roll compaction/dry granulation of wet granulation [50-52]. A study conducted by Grote and Kleinebudde [53] evidenced that the specific surface area was increased induced by the roll compaction/dry granulation process, whereas the granule size only had a slight influence on the strength of the subsequently produced tablets. Other authors reported that roller compaction reduces the crystallinity of α-lactose monohydrate, and the resulting material is similar to spray-dried lactose in behavior as a direct compression excipient with the advantage of a lower level of compression energy. In this way, roller compaction introduces desirable characteristics to the raw α-lactose monohydrate by inducing changes in crystallinity and particle morphology, turning it into a high-quality direct compression excipient [50, 54].

Twin-screw granulation has also been reported as one of the most promising strategies to improve the pharmacy technical properties of lactose in the last few years. It has been evidenced that this technique provides significant enhancements in different properties, mainly related to particle size uniformity, but the partial dissolution of lactose has been observed at high temperatures during the granulation [55].

The combination of lactose with other substances constitutes another alternative to improve its pharmaco-technical properties. Several authors have reported the advantages and possible limitations of the properties of co-processed excipients containing lactose [56].

Alpha-lactose combined with corn starch makes up an example of this. The product Starlac® (Meggle) is obtained by spray-drying a mixture of both components to form a monoparticulate system. This excipient combines the compaction and binding properties of alpha lactose with the disintegrant characteristics of starch. There exist some examples of the use of this excipient for fast disintegrating tablets, including orodispersible pharmaceutical forms designed to disintegrate in the mouth in less than a minute [57]. It has been reported that Starlac® also shows better functionality than microcrystalline cellulose and a well-known excipient made of cellulose and lactose (Cellactose® from Meggle) in terms of flow, compressibility, and compactibility parameters [58]. This Cellactose® has also been widely used in tablets in many formulations. It is manufactured by co-spray drying of the alpha-lactose monohydrate and powdered cellulose. It was designed especially for direct compression and shows superior properties in comparison to a simple mixture of its components in terms of dilution potential, compressibility, tensile strength, lubricant susceptibility, and subsequent tablet properties for a range of drugs [40, 59, 60]. A similar conclusion can be reached when studying Microcelac® (also manufactured by Meggle), an excipient obtained from co-spray drying of the

Table 1. Active pharmaceutical ingredients (API) most frequently prescribed in Spain, indication and maximum recommended daily dose [41].

API	Therapeutic Effect	Maximum Recommended Daily Dose
Metamizole	Analgesic and antipyretic	3450 mg
Acetylsalicylic acid (100-300 mg)	Antiplatelet	300 mg
Dexketoprofen	Analgesic, anti-inflammatory and non-steroidal antirheumatic	75 mg
Paracetamol	Analgesic and antipyretic	4000 mg
Levothyroxine	Thyroid hormones (hypothyroidism treatment)	200 mg
Acenocoumarol	Anticoagulant	Individualized doses
Salbutamol	β_2-adrenergic agonist	16 mg (oral) 800 mcg (inhaled)
Ibuprofen	Analgesic, antipyretic and non-steroidal anti-inflammatory	2400 mg
Lorazepam	Anxiolytic	10 mg
Alprazolam	Anxiolytic	6 mg

Table 2. Commercialized drugs that contain top ten most prescribed API in Spain and lactose among their excipients [41].

API	Marketed Drugs	Drugs with Lactose	Pharmaceutical Dosage Form
Metamizole	36	1	Coated tablets
Acetylsalicylic acid	32	11	Coated tablets
Dexketoprofen	63	-	-
Paracetamol	289	8	Coated and effervescent tablets
Levothyroxine	33	12	Coated tablets
Acenocoumarol	2	2	Coated tablets
Salbutamol	17	3	Coated tablets and inhalers
Ibuprofen	179	13	Coated and prolonged-release tablets, and granules for oral suspensions
Lorazepam	20	15	Coated tablets
Alprazolam	75	60	Coated and prolonged-release tablets

Table 3. Lactose included in marketed acenocoumarol and alprazolam tablets. (1) milligrams of lactose per day according to the maximum recommended daily dose [41].

Drug	Dose (mg)	Amount of Lactose Per Tablet (mg)		(1)
Alprazolam	0.25	24-98		48-965
	0.5	24-96	221.7 (prolonged-release)	
	1	24-96		
	2	48-192		
	3	221.7 (prolonged-release)		
Acenocoumarol	1	20		Individualized doses
	4	304.4		

alpha-lactose monohydrate and microcrystalline cellulose, showing better properties than a simple physical mixture of both the components [61, 62].

5.2. Lactose for Inhalation as a Dry Powder Carrier

Inhalation therapy is gaining growing interest in the potential treatment of multiple diseases, with the introduction of new drugs in diverse therapeutic areas such as chronic obstructive pulmonary disease and asthma, but also infectious diseases, diabetes or vaccination. This circumstance has led the focus towards new studies, not only on the design of new improved and easy to use devices by patients, but also on the development of novel particle technologies and excipients for respiratory drug formulations.

Dry powder inhalers (DPI) are one of the most used systems for delivering drugs in the lungs. In carrier-based DPIs, the active pharmaceutical ingredient (API) is delivered to the lungs from a static powder bed that is fluidized and entrained by the airflow generated by the patient's inspiratory effort. However, to enable the API to deposit in the lungs, the API must be micronized to achieve a particle size of less than 5 μm [63].

It is commonly accepted that a formulation intended for respiratory route should meet the following optimal characteristics for efficient pulmonary delivery: narrow aerodynamic particle size range, low surface energy and charge, non-spherical morphology, low density or high porosity as well as high physical and chemical stability [64]. If we also consider toxicity properties, we can find that the current list of excipients approved by regulatory agencies for respiratory drug delivery is very limited and the choices are confined to a few substances.

Lactose is one of the few substances that meet the above-mentioned requisites and one of the excipients approved for its use in respiratory formulations. This is the reason why lactose is often incorporated in dry powder inhalers where it can fulfill the triple function of a bulking agent, a flow aid and a carrier for the micronized drug particles [63-64]. As a matter of fact, there exists inhalation-grade lactose as one of the applications of lactose in the pharmaceutical industry [65]. Among the most widely used brand names of lactose-based excipients for inhalation, we can find Lactohale® (Borculo), Inhalac® (Meggle) or Respitose® (DFE Pharma).

The use of different isomers of lactose constitutes a point of controversy, trying to elucidate which of the forms of lactose is adequate for each formulation. Although some of the commercially available excipients are made of the crystalline form alpha lactose monohydrate (for example Inhalac®), providing good results in many industrial formulations, some studies point out that anhydrous beta-lactose can provide better characteristics to the final dry powder inhaler formulation. For example, Vanderbist and coauthors [66] reported that the roller-dried anhydrous beta-lactose possesses the most adequate surface properties, resulting in a significantly higher *in-vitro* lung deposition of nacystelyn in comparison to the conventional crystalline alpha-lactose and spray-dried lactose. Larhrib and co-workers [67], in the same year, reported that anhydrous and medium lactose resulted in a more efficient delivery of salbutamol sulfate when aerosolized from a Rotahaler® device in comparison to other grades of lactose. They hypothesized that the more efficiency obtained in terms of drug delivery from anhydrous lactose could be partly attributed to the relatively higher concentration of fine lactose in this grade of carrier, although it showed a rougher surface than other grades of lactose. Other studies [68] reported that some different available inhalation grade lactose could be used to produce carrier-based DPI budesonide formulations with acceptable powder properties and DPI performance, but with significant variations in aerosolization performance for both monohydrate and anhydrous grades of lactose.

Apart from different considerations concerning the particle size distribution and its relevancy to the efficacy of lactose as a carrier, Vanderbist *et al*. [66] reached to a conclusion that the presence of fine particles could play an important role in obtaining reproducible *in-vitro* deposition results. It is also well known that the extrinsic addition of lactose fines to the coarse lactose carrier significantly enhances the aerosolization performance and delivery of the API [63]. Recent studies [69] suggest that the presence of intrinsic lactose fines in the formulation influences the performance and their role and interactions between the lactose carrier and the micronized drug, which are still not fully understood.

Another important parameter to determine the efficacy of a carrier for inhalation is the permeability of a powder bed; a property related to particle shape and hence, packing patterns, mean particle size, size distribution, cohesivity and flowability, and tensile strength. In this way, recent studies reported the comparative permeability of eight lactose materials as DPI carriers, covering a broad range of particle sizes, shape, crystalline form, and porosity [70]. Aerolizer® was used for the eight specimens of lactose as a model turbulent-shear inhaler dispositive with fluticasone propionate as an active drug. Although the influence of permeability is still controversial, it has been demonstrated that inhalation-grade lactose showing different permeability, showed differences in their performance as a carrier.

5.3. Other Uses: Lactose as Lyophilization Aid

Lactose is also used in the pharmaceutical industry as an excipient included in some formulations designed to be lyophilized. The use of lactose as a bulking agent for solutions, in general, has been widely described. For example, some patents published in 1978 reported the use of lactose when the solutions require a bulking agent for ease in the lyophilization process. These inventors claimed that it was generally accepted that large molecules like lipoproteins due to their large molecular size did not require such additional bulking agents. The importance of lactose as lyophilization aid is demonstrated by the fact that forty years later, there are still many examples of new patents describing the use of lactose for this purpose [71, 72].

Anhydrous lactose is used as a bulking agent in many lyophilized formulations like amphotericin-B, alprostadil or doxorubicin HCl. However, there are other additional interesting actions that justify the presence of anhydrous lactose. An important parameter to be considered when developing a formulation intended for its lyophilization is collapse temperature. It is well known that the lyophilization of amorphous material requires the primary drying temperature to be kept below this collapse temperature of the formulation. Anhydrous lactose, showing a collapse temperature of -31 ºC and a glass-transition temperature around -28 ºC, can also act as a collapse temperature modifier [73], facilitating the first steps of the freeze-drying process.

6. LACTOSE AND POLYMORPHISM

Polymorphism should be defined as a molecular or atomic arrangement within the unit cell in the solid-state as a consequence of the thermodynamic and/or kinetic behavior of materials.

From a pharmaceutical point of view, it includes amorphs states and pseudo polymorphs such as solvates, hydrates and anhydrous polymorphs [74]. This phenomenon can affect the inherent pharmacotechnical physicochemical and pharmacological properties such as solubility or stability. This fact can give rise to important differences in the bioavailability or unexpected side effects of the pharmaceutical product [75].

From the perspective of chemical synthesis, it can be very expensive and time-consuming to produce only a polymorphic state of a specific drug, since thermodynamic and kinetic crystallization factors involved in transitions between different polymorphs of the same substance can vary little in many examples, but it must be checked previously. For that reason, a polymorph can be converted

into another one with the lowest free energy and the best stability in certain circumstances:

a. Physiological or pathological changes,
b. Inherent production variations in feeding rate, pressure, run time, torque or temperatures variations during manufacturing,
c. Drying phases in more complex process manufacturing like tablets obtained for wet granulation, extrusion, freeze-drying, spray drying, *etc*.,
d. Changes in brands of "inert" excipients from different suppliers, different degrees or differences between batches of the same manufacturer,

In this sense, these aspects should be considered in the frame of scale-up studies.

Many analytical techniques have been reported for characterizing polymorphs like spectroscopic methods (FT infrared, NMR or Raman spectroscopy), atomic force microscopy, X-ray diffraction techniques or thermal methods (differential scanning calorimetry or thermogravimetric analysis is able to detect transitions between phases due to changes in enthalpy) [76].

Numerous substances of pharmaceutical interest show polymorphism. Cimetidine, paracetamol, and ibuprofen or theophylline are only three well-known examples of active pharmaceutical ingredient (API) polymorphs [77].

Polymorphism is a phenomenon that affects, not only actives drugs, but also other substances. Several researchers have also demonstrated that some excipients, such as lactose, have polymorphs. In fact, lactose has four principal structures in the solid-state that should be considered as real polymorphs, α-lactose monohydrate, the anomeric equivalent β-lactose, the anhydrous stable form of α-lactose and the unstable hygroscopic form of α-lactose.

New manufacturing strategies like continuous manufacturing can also affect in a different way than the classical batch-processing manufacturing on the polymorphic transitions of lactose. Continuous processing, quite usual in other productive sectors like food or chemical industries, is not yet relevant for the traditional pharmaceutical dosage form production, although this strategy has been adopted for water or air production in pharmaceutical laboratories. Continuous manufacturing has advantages over batch production in terms of reduction of time-to-the-market, up-scaling, process optimization, validation or minimum operators. Nevertheless, pharmaceutical companies are reluctant to include it, since it requires investments in new specific equipment, training of the operators and above all because of the uncertainty of approval by the regulatory authorities. Authors understand the lab´s nervousness about taking the first steps, but from our point of view, regulatory agencies such as FDA or EMA are doing their best to support them. In 2016, the Executive Office or the President and National Science and Technology Council, published a document urging pharmaceutical companies to make an effort in this regard [78]. Following this strategy, the Center for Drug Evaluation and Research (CDER) has recently published draft guidance with their "*current thinking on the quality considerations for continuous manufacturing of small molecule, solid oral drug products*" [79]. Also ICH guidelines Q8-Q10, strongly encourage the companies to include this approach in their classical manufacturing lines [80-82]. In this context, several research groups have published numerous scientific studies in the last years; most of them focused on the influence of process parameters on granule quality [83-85].

Fontayne *et al*. [86] focus their study on process analytical technology tools accomplished by a continuous twin-screw high-shear wet granulation process of the ConsiGma^{TM}-25 system. Anhydrous theophylline was included as an active pharmaceutical ingredient, while lactose monohydrate 200 mesh as a filler and polyvinylpyrrolidone as a binder were added as excipients. NIR and Raman spectroscopy probes showed real-time conversion between anhydrous, monohydrate and metastable forms of theophylline as a consequence of changes in equipment, product or process variables during production.

It has been demonstrated that polymorphic transition from excipients (lactose, mannitol, *etc*.), which have been reported during classical size reduction, granulation, coating, tableting, encapsulation or drying production techniques, also occurred during continuous granulation [87].

7. DRUG-LACTOSE INTERACTIONS

The absorption of orally-administered drugs may be conditioned by different factors that can be classified into two main groups [88]:

a. Endogenous factors (these factors cannot be modified).

- Physiological factors [89, 90].

Gastric emptying: it can be the limiting factor that alters the incorporation of the drug into the organism since it directly influences the lag time prior to absorption. Gastric emptying depends on emptying modulating mechanisms linked to the volume and type of diet and gastric peristalsis that should be divided into four phases [91]:

- Phase I: relative stillness for 30 - 60 minutes. There are small contractions widely spaced over time.
- Phase II: it is the acceleration period that lasts 20 - 40 minutes. The contractions increase in frequency and intensity, increasing intragastric pressure.
- Phase III: the period of sudden and regular contractions that lasts approximately 10 minutes. Undigested solid residues are removed from the stomach.
- Phase IV: decreasing motility (<5 minutes).

This fact has a direct and negative impact on the absorption of BCS class I drugs (high solubility and high permeability). Biowaivers simulation studies show differences of 25% in C_{MAX} values in some rapidly dissolved and absorbed drugs of this class, due to variations in excipients and gastric emptying rates [92].

Intestinal transit: the absorption process occurs mainly in the intestine, so the mean residence time is related to mean absorption time [93]. In general, without degradation, subjects with lower gastrointestinal transit show a high bioavailability, which was related to high absorption [94].

- Pathological factors [95]:

Functional changes such as gastric or duodenal ulcer, which affect gastric emptying and intestinal transit, usually slow it down.

Total or partial resections: They are especially important if they affect the drug absorption area. The decrease in absorption should also be evaluated in pharmaceutical forms of modified release.

b. External factors (these factors can be modified) [96]:

- Factors that depend on the physicochemical characteristics of the active substance or formulation.

Particle size: Atkinson, Bedford, Child and Tomich [97, 98] in two classical manuscripts published in 1962, confirmed the better absorption of griseofulvin, a BCS class II drug (low solubility and high permeability), of smaller particle size in volunteers.

Polymorphism: Ciprofloxacin is one of the most prescribed urinary antiseptics and it is available as the free base or as the hydrochloride salt. Singh and Chadha [99] focused on developing a new polymorph of ciprofloxacin saccharinate with enhanced biopharmaceutical properties with respect to the available drug.

- Drug-drug, drug-excipient, drug-herb or drug-diet interactions occur when a drug and an excipient, or an associated prescribed or over the counter (OTC) drug, and a herb or a component of

the diet alters the dissolution, absorption, distribution, metabolism or elimination of a coadministered agent. The interactions are not always detrimental to therapy, although in other situations, they are one of the commonest causes of adverse drug reactions and represent a common dangerous clinical problem. In this frame, the interaction is classical due to the co-administration of P-gp inhibitors (*e.g.* sildenafil) and BCS class II or IV drugs [100].

In this frame, lactose being considered as a nutrient or as one of the most commonly used excipients in pharmaceutical oral dosage forms, should be considered in the preformulation stage of the development of numerous drug substances [101]. Several authors have reported incompatibility between free primary amino drugs and lactose. Isoniazid is a first-line bactericidal agent active against *Mycobacterium tuberculosis* and other related *Mycobacterium* and is used alone or as part of combination therapy during prophylaxis or treatment of these illnesses. Chemically, isoniazid or an isonicotinic acid hydrazide has a free primary amine group that is able to form less permeable hydrazones with lactose and other reducing sugars (Fig. **3**), which can modify its pharmacokinetic properties and condition its therapeutic efficacy. According to the Biopharmaceutics Classification System, isoniazid should be on the border between BCS class I and III due to inconsistent data of permeability [102].

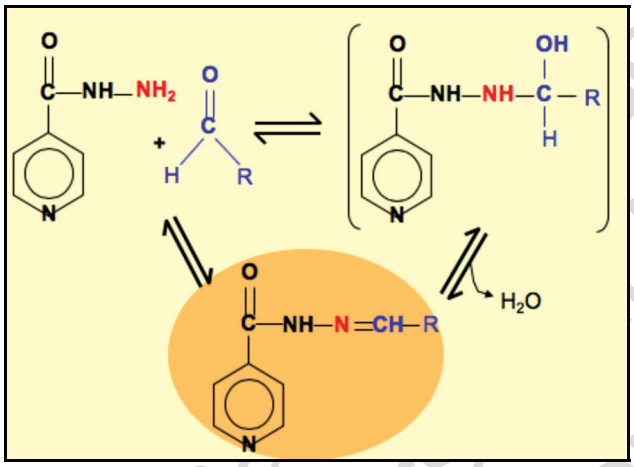

Fig. (3). Hydrazone obtained by making isonicotinic acid hydrazide (isoniazid) react with lactose.

CONCLUSION

In conclusion, it is evidenced that lactose is a widely used excipient in the food and drug industry and it is also present in many foods products. This is the reason why the potential activity of lactose should always be taken into consideration in terms of metabolic reactions, side-effects, as well as interactions of lactose present in the diet or even in the same formulation, provoking reactions concomitantly with other excipients or drugs, which can modify their therapeutic efficacy and their toxicity [103].

CONSENT FOR PUBLICATION

The authors declare that our manuscript does not provide individuals´ data, so, no consent for publication is needed.

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The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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