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Endotoxin testing in inhalation grade lactose—a useful quality parameter?

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Abstract

α-Lactose monohydrate is the standard carrier used for dry powder inhalation drug products. The physico-chemical characteristics of this carrier material need to be monitored and specified carefully in order to guarantee functionality of the powder formulation. But also microbiological acceptance criteria need to be considered during development and routine testing. In this study, the endotoxin content of 19 batches of α-lactose monohydrate provided by two different manufacturers was determined with a semi-quantitative LAL assay. The endotoxin content was found to be less than 18 endotoxin units (EU)/g lactose in all cases and less than 3 EU/g in most cases. Assuming that the typical lactose has an endotoxin content of 10 EU/g and that a patient inhales six times daily 25 mg of lactose and that the total amount of lactose reaches the lung, this translates to an endotoxin exposure of 1.5 EU per day. On the other hand, the proposal for endotoxins in air limits the endotoxin concentration to 50 EU/m^3 which corresponds to approximately 3333 EU inhaled endotoxins a day during normal breathing (breathing at rest conditions). The maximum endotoxin exposure by dry powder inhalations is thus two log steps lower than the recommended acceptable daily intake.

Keywords: Dry powder inhaler; Interactive powder mixtures; Lactose; Endotoxins; Inhalation exposure

1. Introduction

α-Lactose monohydrate is the standard carrier used for dry powder inhalation drug products (teWierik and Diepenmaat, 2002). The amount of lactose carrier used in a single dose varies from approximately 2 to 25 mg when the drug is formulated as interactive powder mixture or compressed tablet. In spheronized powder formulations, where lactose is used as a diluent to increase the total volume of the powder formulation, the amount of lactose per inhaled dose is in the range of 0.6–2 mg (Steckel, 2003). Lactose is a widely used excipient in the pharmaceutical industry and is of bovine origin. However, lactose derived from whey that has been pre-treated with calf rennet is so far considered as safe with regard to the TSE discussion (European Commission, 2002). Other recently discussed issues are the maximum allowable microbiological contamination and the endotoxin content of inhalation grade lactose. The FDA legitimately considers the test parameters and acceptance criteria of the USP monograph for lactose as insufficient for inhalation grade lactose (USP, 2003). Beside a detailed physico-chemical characterization of the lactose carrier as, e.g. multi-point size distribution specification and specific surface area, the FDA also asks for a lower total microbiological count, the absence of specific Gram-negative bacteria and a test and limit for

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endotoxins (FDA, 1998). The European regulatory agency does not yet require a specific test on endotoxins in dry powder inhalation drug products (EMEA, 1998). The production of pharmaceutical grade lactose is a ton-scale, multi-step process including washing, filtration and crystallization operations (German Assocoation of Dairy Industry, 1994). The manufacturing process for pharmaceutical grade lactose is summarized in Fig. 1. Briefly, the incoming raw material (in most cases cheese whey or permeate) is concentrated in a vacuum dryer until the dry matter content reaches approximately 60% (w/w). As the temperature exceeds 60 °C during this process step, the concentrate is cooled down and lactose crystallizes. The crystals are separated by means of decanters and/or screen centrifuges. Afterwards the crystals are washed several times with water of at least drinking water quality. In the refining step, the crystals are dissolved in water to a concentration of 60% under addition of activated charcoal and inorganic filter material. This slurry is heated to 97 °C over a time period of at least 30 min and afterwards filtrated at this high temperature. The filtrate is cooled down and lactose monohydrate crystals form again. These are then separated and dried in fluid bed dryers where the temperature again could reach more than 70 °C. Finally, the different lactose qualities are produced by further processing the dried lactose crystals by milling, sieving, or granulation.

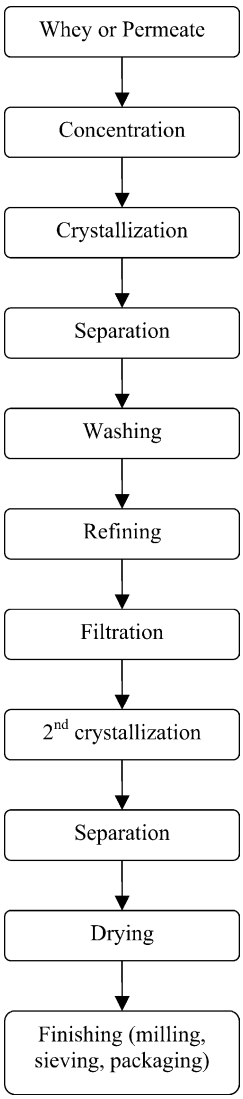

Fig. 1. Flow chart of the lactose manufacturing process.

Considering the described manufacturing process comprising heating cycles and a filtration step and taking into account the microbiological limits of the lactose, the endotoxin level is expected to be on a low level. It was the aim of this study to test several lactose qualities supplied by different suppliers for the presence of endotoxins in the finished product.

2. Materials and methods

2.1. Materials

Five samples of inhalation grade lactose were supplied in endotoxin-free sample tubes by Borculo Domo Ingredients (Zwolle, The Netherlands). Another 14 different lactose samples were supplied by Meggle GmbH. The latter samples consisted of inhalation grade lactose and non-finished pharmaceutical grade lactose which was sampled before the final sieving step, and were supplied in standard sample tubes which were not specifically designed for endotoxin-free product storage and transfer. The endotoxin assay was carried out with the Limulus Amoebocyte Lysate (LAL) reagent Pyrotell® (Associates of Cape Cod, Woods Hole, MA, USA) for the gel-clot method with a sensitivity $\lambda = 0.03$ EU/ml. Endotoxin-free water (Acila®), endotoxin-free test tubes were purchased from Pyroquant Diagnostik (Mörfelden-Walldorf, Germany) and sterile tips came

from Eppendorf (Hamburg, Germany). According to the Pyrotell® manual, the reagent also reliably detects fungal glucans, i.e. a negative assay also indicates the absence of cell wall components of many genera of fungi. The lyophilized reagent was dissolved in the corresponding endotoxin-free reconstitution buffer medium with phenol red, pH 7.4 (Pyrosol®, Associates of Cape Cod, Woods Hole, MA, USA), according to the user instructions.

2.2. Method

Ten grams of the corresponding lactose sample was dissolved in 90 g of endotoxin-free water. After complete dissolution of the lactose this solution was diluted stepwise by the factors of 1:10; 1:13.3; 1:20; 1:40 and 1:60. 0.1 ml of each solution was mixed with 0.1 ml of the LAL reagent and incubated at 37 °C for 60 min in a heating block. The clotting of the gel was checked manually after 60 min of incubation. The endotoxin content was calculated in EU/g based on the dilution factor of the tested solution and the sensitivity of the used reagent according to Eq. (1):

$$\frac{\mathrm{EU}}{\mathrm{g}} = \mathrm{DF}\lambda \quad (1)$$

where DF is the dilution factor extrapolated to 1 g and λ the sensitivity of the reagent. In the case of a positive result (gel formation) the endotoxin content is given as equal or higher than that of the used dilution factor multiplied with λ, and in the case of a negative result the value smaller than the product of dilution factor and λ is given as endotoxin content.

3. Results and discussion

The endotoxin contents of the 19 tested pharmaceutical grade lactose batches are summarized in Table 1. It has to be noted that those lactose samples supplied in endotoxin-free sample tubes as used in the case of the Borculo samples generally did not contain more than 3 EU/g with very good batch consistency concerning the amount of endotoxins. The samples provided in standard (non-endotoxin-free) sample tubes showed a larger variation with respect to the amount of endotoxins: eight batches contained less than 3 EU/g, three batches less than 6 EU/g, two batches less than 12 EU/g and one batch had an endotoxin content between 12 and 18 EU/g.

Table 1
Endotoxin content of the tested 19 batches of lactose monohydrate from two different suppliers

Lactose qualities	Batch number	Endotoxin content (EU/g)
Meggle GmbH		
A	0234	≥0.3, <3
B	0242	≥6, <12
C	0234	≥0.3, <3
D	0211	≥12, <18
E	0237	≥4, <6
F	0223	≥0.3, <3
G	0237	≥0.3, <3
H	0208	≥4, <6
I	0220	≥6, <12
K	0219	≥0.3, <3
L	0245	≥4, <6
M	0245	≥0.3, <3
N	0223	≥0.3, <3
O	0219	≥0.3, <3
Borculo Domo		
A	3/3008	≥0.3, <3
B	3/3009	≥0.3, <3
C	3/3010	≥0.3, <3
D	3/3011	≥0.3, <3
E	3/3012	<0.3

The amounts of endotoxins found in the lactose batches are in the same range as the limits proposed by Pharmacopoeias for sugars used for injectables. According to USP XXVI (USP, 2003) the endotoxin content of glucose for injection must not exceed 10 EU/g for glucose solutions with a glucose concentration in the range of 5–70%. Mannitol and sorbitol in injectables and a concentration higher than 10% must not contain more than 2.5 EU/g (European Pharmacopoeia, 2002).

Each individual is exposed to endotoxins during normal breathing as bacteria and their constituents are associated to dust particles and ultra fine water vapour droplets. Thorn (2001) has summarized the results of several studies analysing the effects of inhalation of endotoxins and organic dust particles. He concluded that the symptoms occurring after acute inhalation of endotoxins are comparable to those observed after a chronic inhalation of organic dust particles. Especially workers of certain occupational groups, like workers in waste treatment or waste disposal, waste water treatment, agriculture and forestry or natural

substance processing are exposed to contaminated air. The clinical symptoms caused by the inhalation of endotoxin containing dusts vary from mucosa irritations, headache, tiredness, vertigo, diarrhoea to fever. Lundholm and Rylander (1980) were the first who described this disease as 'sewage worker syndrome'; more recently, organic dusts are being made responsible for a series of diseases, such as the toxic pneumonitis or the organic dust toxic syndrome (ODTS) (Müller-Barthelmeh, 1999). Whereas the endotoxin exposure in the environmental air is relatively low (0–50 EU/m^3) and difficult to measure, workers in the corresponding occupational groups are exposed up to an endotoxin level of 30,000 EU/m^3 (Kraus, 2003). So far, there are no generally accepted working levels or exposure limits for endotoxins in air. Experimental and epidemiological studies describe dose–response curves based on acute and chronic effects with no-effect levels of 90–1800 EU/m^3 (Linsel, 2003); a limit of 50 EU/m^3 has recently been proposed and discussed as maximal admissible concentration (MAC) in the environmental air at the working place (Heederik, 2003; Smola, 2003).

For the individually inhaled endotoxin quantity, the exposure time is also relevant. For a rough calculation of the maximum acceptable endotoxin quantity during the use of a dry powder inhaler, a continuous exposure of 24 h/day has been assumed. The alveolar ventilation is in the range of 7 l/min (at rest) but increases during physical exercises due to the increased oxygen demand up to 120 l/min due to the increased inhalation volume and breathing frequency (Thews et al., 1999). Assuming the proposed maximal tolerable endotoxin concentration of 50 EU/m^3, a human would be exposed to approximately 500 EU per day based on the alveolar ventilation at rest of 7 l/min. Assuming further, that a patient inhales six times a day from a dry powder inhaler which delivers 25 mg of lactose monohydrate per shot results in a maximum of 150 mg of inhaled lactose a day. Provided that during each inhalation the total amount of lactose is delivered to the lung (which does not happen in practice), the endotoxin content of the inhaled 150 mg of lactose should not exceed the proposed limit of 500 EU corresponding to an endotoxin content of 3333 EU/g lactose. As has been shown in Table 1, the actual values for lactose monohydrate are more than two log steps below this endotoxin threshold.

4. Conclusions

The batches tested in this study were manufactured to match at least the pharmacopoeial requirements of lactose monohydrate, including the need to conform to the microbiological specifications for use in inhaled preparations: not more than 10^2 total microbial count per gram, not more than 10^1 Enterobacteria and absence of specific organisms like *Pseudomonas aeruginosa* and *Staphylococcus aureus* (European Pharmacopoeia, 2002). The endotoxin level of 19 different batches from two lactose suppliers was on a negligibly low level. Accordingly, inhalation of lactose is not expected to result in adverse effects due to the simultaneously inhaled endotoxins. In addition, the fact that only about 10% of the lactose (1/10 of the assumed high mass of 25 mg lactose per inhalation) will be inhaled, whereas the remaining portion will be swallowed after impaction in the oropharynx, was not considered in this theoretical calculation.

The authors conclude that a test and a limit for endotoxins in inhalation grade lactose would not result in an improved product safety.

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