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Research Article

Detection of Excipient–Excipient Interaction in Dry Powder Inhaler Formulation Prepared by Spray Drying

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Abstract

Background: Dry powder inhalers (DPIs) are dosage forms that are used via the pulmonary route. Their formulations include two major parts; drug substance and carrier. For many years, DPIs have been made with lactose, a particularly popular carrier. Leucine has drawn more attention in recent years when it comes to DPI formulations made using the spray drying technique. Leucine was utilized in conjunction with carriers like lactose to enhance physicochemical and aerosolization properties.

Methods: In this investigation, when lactose and leucine were co-spray dried, the color of powders around the cyclone separators of the spray dryer turned brown while the produced powder in the collector was white. Both the white powder inside the collector and the brown powder around the cyclone separators were investigated by differential scanning calorimetry (DSC), Fourier transform-infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), x-ray diffraction (XRD), and liquid chromatography with tandem mass spectrometry (LC-MS-MS) to determine the identity of the degraded chemicals and to look for any potential interactions.

Results: A new peak at 177 ºC in DSC analysis is a sign of interaction. Also, FT-IR analysis shows the new peak at 1627 cm^{-1} which is related to the carbonyl group. According to SEM and XRD analysis co spray dried leucine-lactose is amorphous. Obtained data from LC-MS analysis indicates the adduct compound of leucine-lactose that resulted from the Maillard reaction was detected in both white and brown powder. Also, the reaction proceeded to form n-formyl compound.

Conclusion: There is a possibility of lactose and leucine incompatibility during DPIs manufacturing, especially in elevated temperature and humidity.

Introduction

Spray drying is a method for creating micronized particles that has lately gained popularity, especially for the manufacturing of dry powder inhalers (DPIs).[1-3] A feed solution is sprayed from a nozzle during the spray drying process while being heated to a high temperature. Chemical reactions could advance by utilizing the high temperature and dampness. Drug-excipient or excipient-excipient chemical reactions are a problem in the pharmaceutical industry that should be avoided to prevent quality problems. For many years, DPIs have used lactose as a carrier. DPI formulations have two major components: active pharmaceutical ingredient (API) and carrier. Sugars like lactose, mannitol, and glucose were used as carriers, Lactose is the most common carrier. To improve carrier properties some additives were added to lactose, leucine is an amino acid that has been used recently to improve aerosolization properties of DPIs.[4,5]

In order to formulate DPI; the drug and carrier were mixed. Also, leucine usage in DPI formulations has drawn increasing attention in recent years. To enhance the physicochemical and aerosolization performance of DPIs, API and leucine were combined, or leucine was integrated into carriers such as lactose or mannitol.[6-9] The Maillard reaction can occur when reducing carbohydrates like glucose and lactose interact with the amino groups of proteins and amino acids. This reaction frequently renders proteins inactive and gives the powder a distinctive brown hue.[10]

Few studies have been done to assess the stability of DPIs. The physicochemical instability of DPIs may result from certain particle engineering techniques and

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storage circumstances, which can also drastically alter the performance of aerosols. To choose the best formulation with sufficient stability, a rigorous analysis of physical instability processes in DPI formulations is required throughout formulation development. Additionally, it is essential to detect and comprehend physical instability when developing DPI formulations by using the proper characterization methods.[11]

In this investigation, the powders around the cyclone separators turned brown after leucine and lactose were co-spray-dried. Brown powder near the cyclone separators and white powder in the collector were both subjected to DCS, FT-IR, and LC-MS analysis to determine the type and structure of the degraded product. Regarding having a sign of the deteriorated substance connected to this incompatibility.

Methods

Materials

Leucine and lactose monohydrate were obtained from Merck, Germany (purity >99%). Formic acid, methanol, acetonitrile, and dimethylformamide (DMF) all are HPLC grade.

Spray-drying lactose and leucine

According to Molina, *et al.*,[7] spray-drying was carried out using the Mini Spray Dryer B-290 from Buchi (Flawil, Switzerland). The following were the procedure requirements: 220 °C inlet temperature, 100% aspirator, 5% pump rate, and 50% flow rate. The spray-drying solution contains 1 g of leucine and 10 g of lactose per 100 mL. This indicates that the leucine concentration in the solution was 10% w/v. Leucine and lactose were dissolved in distilled water, heated to a temperature of 75 °C, and stirred at a rate of 120 rpm. Under the aforementioned circumstances, the final solution was spray-dried.

N-formyl leucine synthesis

One of the byproducts of the Maillard reaction is N-formyl. In Figure 1 the pathways and possible interaction were

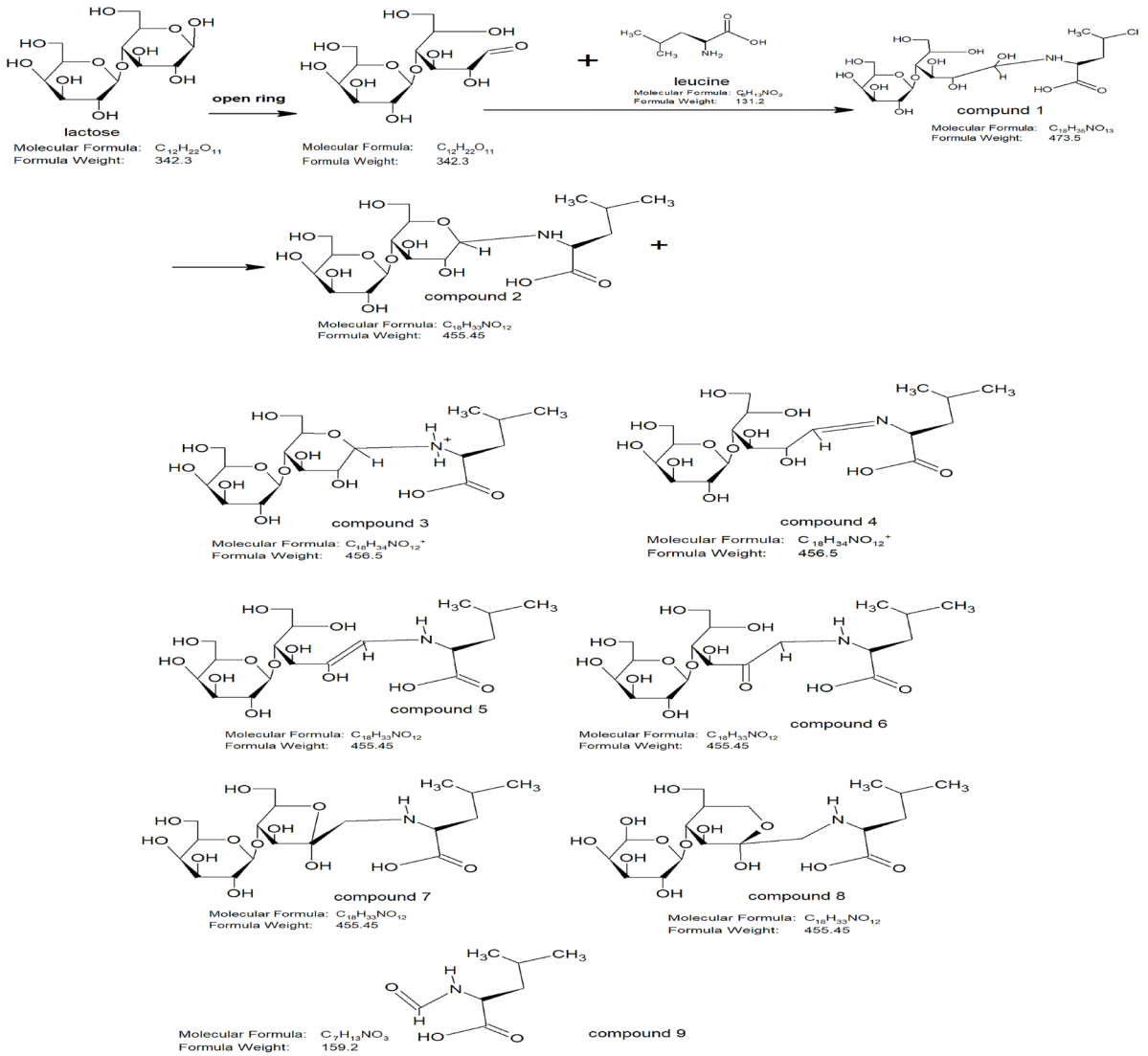

Figure 1. Possible pathway of Leucine lactose interaction.

shown.[12] The process suggested by Szalka *et al.*,[13] was used to create N-formyl leucine. DMF was used to dissolve the drug, and it was refluxed for two days at 150 °C. The end product was filtered and extracted using a liquid-liquid process. The organic layer was evaporated after the dehydration procedure, and the resulting solid powder was given the name N-formyl leucine.

Differential scanning calorimeter (DSC)

By using sealed aluminum pans and a differential scanning calorimeter (DSC-60, Shimadzu, Japan), analysis was carried out. Pure leucine, pure lactose, and co-spray dried powder were employed in the approximately 5mg of samples. At temperatures between 30 and 450 degrees, the samples were heated at a rate of 15 degrees per minute. The calculations for peak temperature and enthalpy were performed using TA-60 software (Version 1.51).

Fourier transform-infrared spectroscopy (FT-IR)

To produce FTIR spectra, we used a potassium bromide disc (Bomem, MB-100 series, Quebec, Canada) to analyze the samples.

Scanning electron microscopy (SEM)

Field emission scanning electron microscope (FESEM) model MIRA3 FEG-SEM from Tescan was employed to analyze the morphology of powders. Samples were attached to aluminum stubs and sputter was coated with a thin layer of gold before being subjected to analysis.

X-ray diffractometer (XRD)

In order to measure powder crystallinity X-ray diffractometer (Tongda TD-3700, China) was used. The powder was loaded into an aluminum sample holder for analysis. A Cu Ka radiation source with a voltage of 30 kV and a current of 20 mA was utilized to scan the powder samples. The scanning was performed over an angle range of 5-35 degrees for 2 hours under ambient conditions.

Liquid chromatography with tandem mass spectrometry (LC-MS-MS)

Mass analysis was performed on the Waters Alliance 2695 (Milford, Massachusetts, USA) Micromass Quattro micro-API, at electron-spray ionization mode, positive ionization, capillary voltage 4.5 V, cone voltage 30 V, collision energy 25 eV, extractor voltage 2 V, RF lens voltage 0.1 V, source temperature (120 °C) desolation temperature (300 °C), desolation gas flow (300 L/h). Mass spectra were obtained from standard leucine, co-spray-dried leucine: lactose (both brown powder and white) and a simple physical mixture of leucine-lactose. LC conditions were as follows; C18 column (3 µ, 2.1×150 mm) was used. The mobile phase consists of [H_2O + 0.1 % Formic Acid] (40%) + [ACN + 0.1 % Formic Acid] (60%). The injection volume is 2 µL with a flow rate of 0.2 ml/min. Column temperature was kept at 35 °C. Samples were dissolved in the mobile phase and after sonication they were vortexed. Samples were injected at low and high concentrations.

Results and Discussion

Spray-drying lactose and leucine

The powder that was created after spray drying was examined visually (Figure 2). While the powder in the collector was white, the powder around the cyclone was brown. A physiochemical analysis of each powder was performed. This is similar to color change in baclofen tablets when they were mixed with lactose and dry granulated at elevated temperatures.[14]

Figure 2. Produced powder by spray drying.

DSC

Lactose monohydrate has two endothermic peaks, as shown in Figure 3. The first (145 °C) is related to water evaporation, whereas the second (215 °C) is related to lactose melting. The leucine melting point is indicated by one endothermic peak in the leucine spectrum at 268 °C. Leucine-lactose that has been co-sprayed exhibits a new, jagged peak at 177 °C. Incompatibility can be defined as the absence of a peak and the emergence of a new one. In this case, the disappearance of pure lactose and leucine endothermic peaks and the appearance of a new one could be assigned as incompatibility.[15]

FT-IR

To confirm DSC data FT-IR spectroscopy has been done. Leucine is an amino acid and thus exists as zwitterions (internal salt). It has both carboxyl and amino group functionality. Amino acid shows a very broad NH_4^+ stretch at 2958 cm^{-1}, N-H bend and COO- stretch around 3000 cm^{-1} functionalities (Figure 4).

According to Figure 4, in the structure of n-formyl leucine a new peak in 1662 related to the amide's C=O stretch or C-O bending, and one band in 3336 cm-1 related to the secondary amide's N-H stretch. Additionally, N-H bending in the secondary amide structure is associated with an absorption at 1585 cm^{-1}.[12,13]

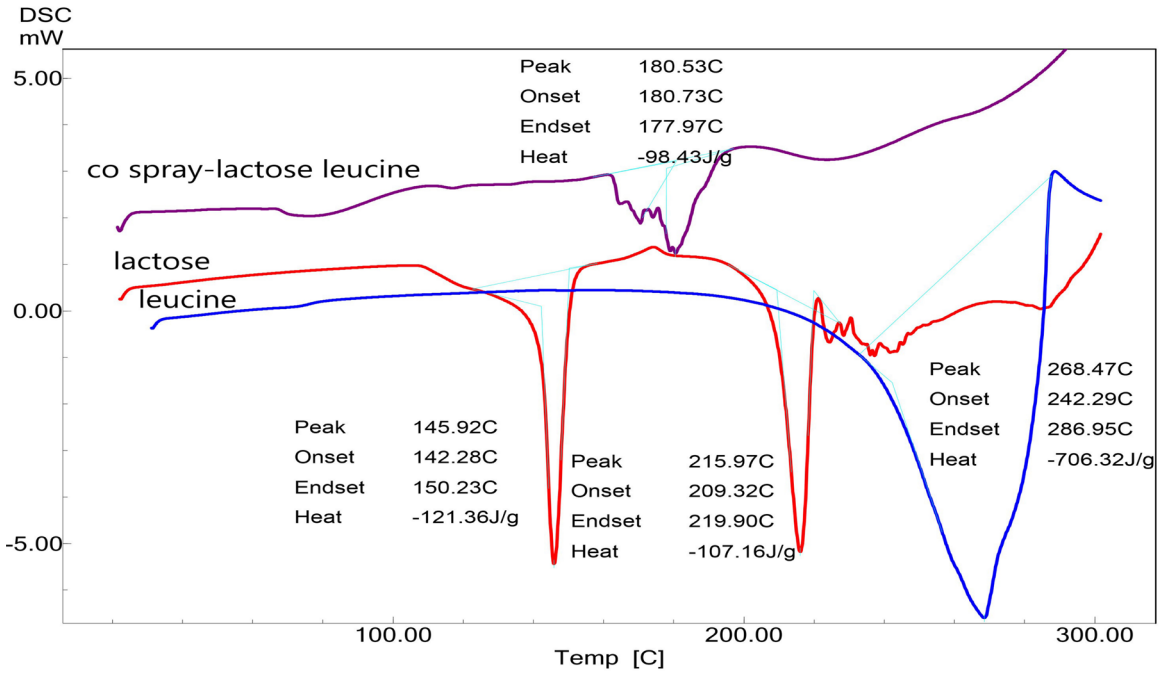

Figure 3. DSC diagram of lactose monohydrate, leucine and co-spray of lactose monohydrate and leucine.

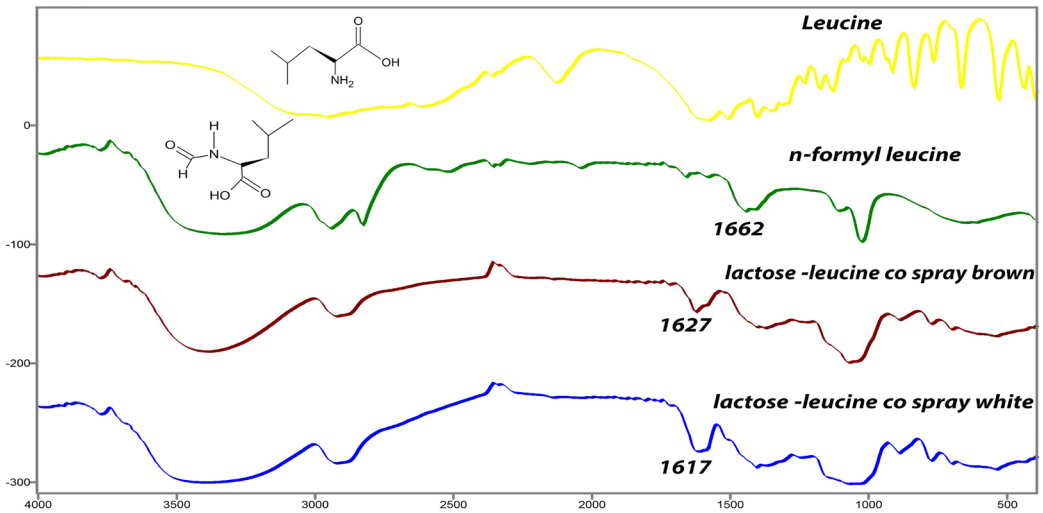

Figure 4. FTIR of leucine, n-formyl leucine, co-spray dried lactose–leucine, collected from the collector (white powder) and collected from the cyclone (brown powder).

Both FTIR spectra of co-spray-dried lactose-leucine (white and brown) show peaks at 1627 cm^{-1} and 1617 cm^{-1}, which may be related to the amide's C=O stretch in the n-formyl-leucine structure, and one band at 3383 cm^{-1}, which may be related to the secondary amide's N-H stretch in the n-formyl-leucine structure (Figure 4). Overall, the spectrum of N-formyl leucine and co–spray dried samples are similar. It could be concluded that the reaction proceeds to N-formyl formation (Figure 1).

FT-IR data demonstrate that the spectrum in both co-spray-dried powders is quite comparable, even though the color of the powder in the collecting part did not change visibly (Figures 2 and 4). However, the reduction of band intensity shows that the mixture of lactose leucine after spray drying became amorphous.[16] In order to confirm FT-IR data SEM and XRD were performed. Also, FT-IR of the lactose-leucine physical mixture and heated lactose–leucine over a stirrer (75 °C) show that the interaction occurred after spray drying (Figure S1 in Supplementary Data).

SEM and XRD

As shown in Figure 5, the co-spray-dried leucine–lactose powder has amorphous nature. The morphology of the powder was spherical with a smooth surface in comparison with untreated powders. The amorphous powder is vulnerable to degradation.[17] As the surface of powder was increased the possibility of reaction has been increased. XRD result confirmed the SEM findings (Figure 5). There is no sharp peak in the XRD spectrum which indicates the co-spray dried leucine–lactose powder is amorph.

LC-MS

These m/z are the abundant m/z of standard leucine: 132, 86, 43 (Figure 6). When samples were injected into LC-MS-MS at lower concentrations the main peaks were just related to 141 m/z and 183 m/z (Figures S2 and S3 in Supplementary Data) which could be related to N-formyl leucine (MW: 159.2). The peak at 141 m/z may relate to N-formyl which loses a water molecule (Figure 7). The nucleophilic attack of carboxylic acid oxygen to the

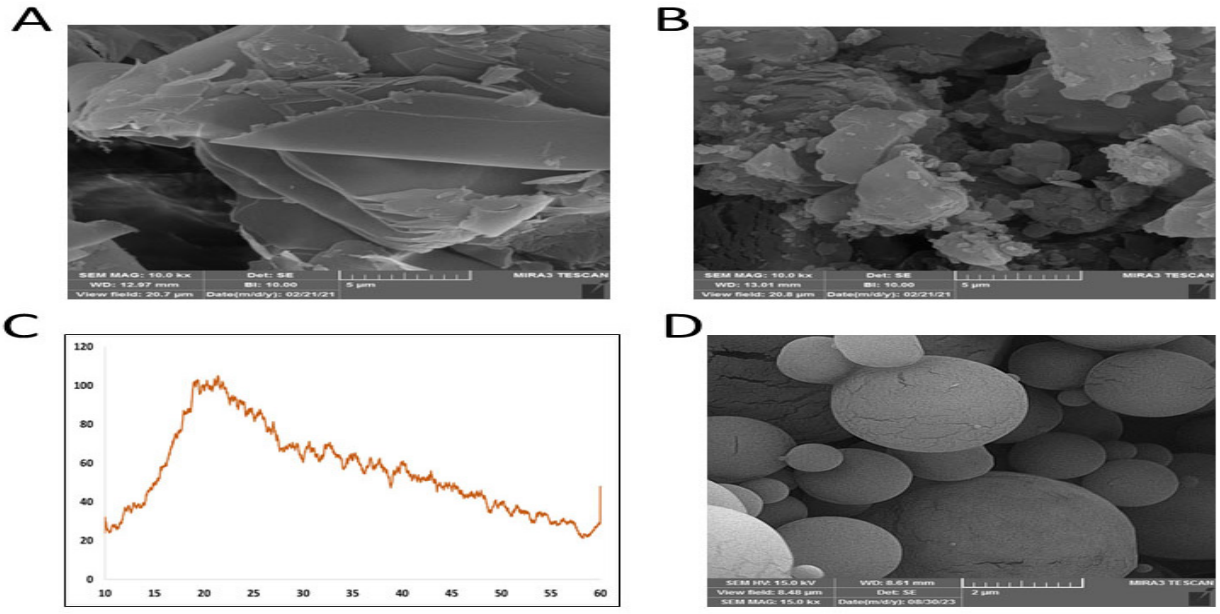

Figure 5. SEM and XRD results of powder. A: raw leucine, B: raw lactose monohydrate. C: XRD of co-spray dried leucine – lactose, D: SEM of co-spray dried leucine – lactose.

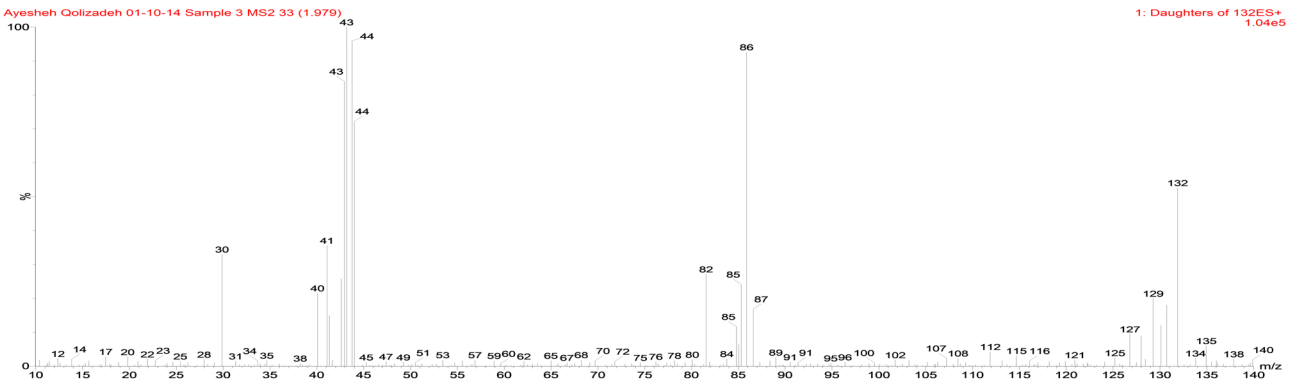

Figure 6. LC-MS-MS standard leucine.

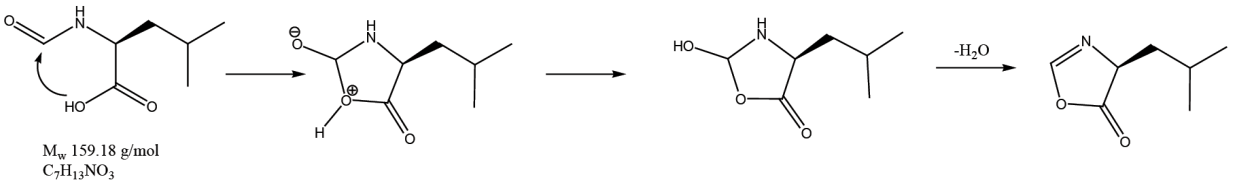

Figure 7. Water removal mechanism from n-formyl leucine.

carbonyl group of the amide functional group can lead to the formation of an oxazole ring and the removal of water. Also, 183 m/z (159 + 24) could relate to N-formyl + Na^+ molecule.

In Figure 8, A and B samples were injected at high concentrations and the peak at 457.5 m/z was obvious which can be related to lactose-leucine adduct (MW 456.5, Figure 1, compounds 3 and 4) that a water molecule loss. It could be concluded that the adduct amount in the sample is so low but N-formyl concentration is high. So, the interaction has proceeded to form an N-formyl compound which is in agreement with achieved data by FTIR. Browning could be a result of the Maillard reaction between lactose and leucine. As shown in in a simple physical mixture of lactose – leucine there is not any sign of incompatibility. It could be concluded that the presence of heat and moisture proceeds the Maillard reaction.

Prasad Vinjamuri *et al.*,[18] reported that screening design showed at outlet temperatures >130°C Maillard reaction between leucine and lactose is possible which is confirmed by achieved data in this study.

Conclusion

Lactose is a common carrier of DPI formulations. To improve aerosolization results, lactose is combined with other excipients such as leucine. In this study when lactose and leucine were co-spray-dried the powder around the cyclone separator turned brown while the powder in the collector was white. Following analysis of the powders by DSC, FTIR, SEM, XRD and LC-MS results show obtained powder is spherical and amorphous, so the possibility of interaction is increased. Degradation compounds were discovered in both the brown and white powder, proving that white powder is capable of containing degradation compounds. Although the lactose-leucine physical mixture does not show any degrade compound. In this investigation by using LC-MS analysis the interaction between excipient – and excipient has been confirmed. This type of interaction not only influences the DPI performance but also may affect the safety of the final product and may influence active pharmaceutical agent stability as well. This type of study in DPIs is new and exclusive. The interaction may alter the physicochemical characteristics of the product which decreases DPIs efficiency, stability and appearance change, so the product is not usable or efficient. This study provides information about excipient–excipient interaction which is important for the pharmaceutical industry.

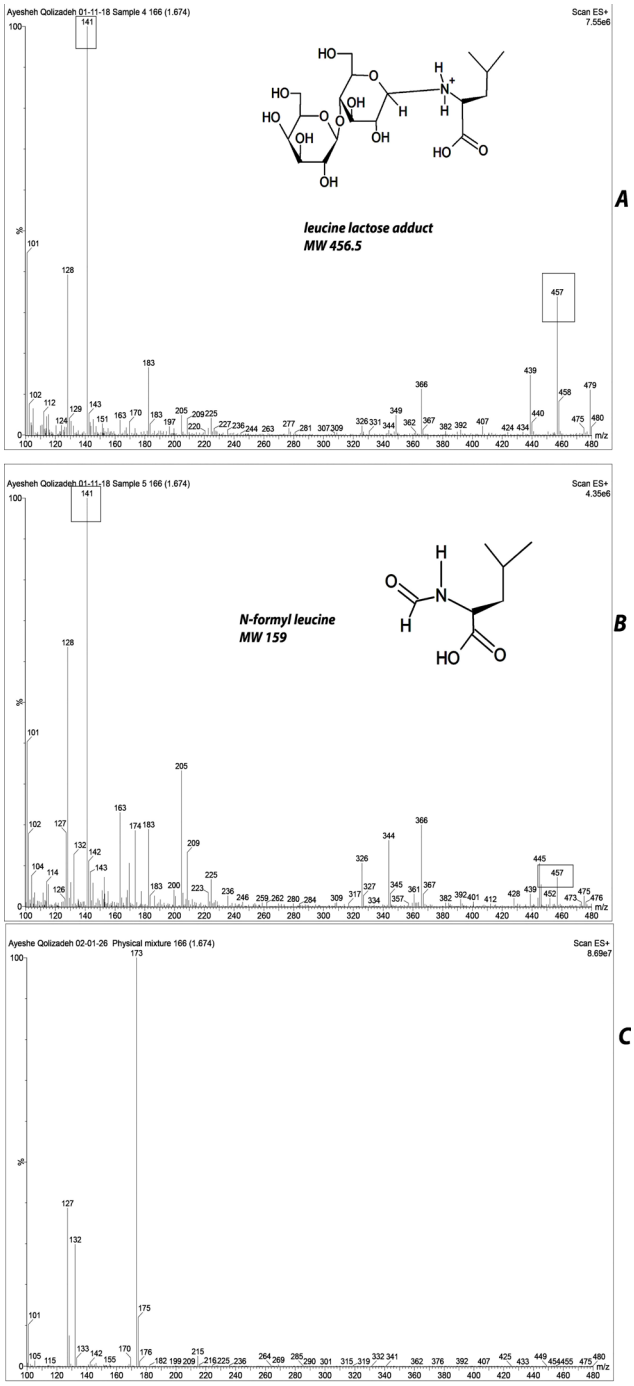

Figure 8. LC-MS analysis of co-spray dried lactose – leucine A: brown powder, B: white powder, C: physical mixture lactose – leucine.

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Author Contributions

Aiesheh Gholizadeh-Hashjin: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - Original Draft. Hamed Hamishehkar: Funding acquisition, Methodology, Resources, Project administration, Supervision; Validation, Writing - Review & Editing. Farnaz Monajjemzadeh: Conceptualization, Funding acquisition, Methodology, Resources, Project administration; Supervision, Validation, Writing - Review & Editing.

Conflict of Interest

The authors have no conflicts of interest.

Supplementary Data

Supplementary data, Figures S1-S3, are available at https://doi. org/10.34172/PS.2023.20.

References

1. Shepard KB, Pluntze AM, Vodak D. Simultaneous spray drying for combination dry powder inhaler formulations. Pharmaceutics. 2022;14(6):1130. doi:10.3390/pharmaceutics14061130
2. Mancini L, Paolantoni M, Schoubben A, Ricci M. Development of spray-dried n-acetylcysteine dry powder for inhalation. Int J Pharm. 2023;631:122550. doi:10.1016/j.ijpharm.2022.122550
3. Xu Y, Harinck L, Lokras AG, Gerde P, Selg E, Sjöberg C-O, et al. Leucine improves the aerosol performance of dry powder inhaler formulations of sirna-loaded nanoparticles. Int J Pharm. 2022;621:121758. doi:10.1016/j.ijpharm.2022.121758
4. Rahimpour Y, Kouhsoltani M, Hamishehkar H. Alternative carriers in dry powder inhaler formulations. Drug Discov Today. 2014;19(5):618-26. doi:10.1016/j.drudis.2013.11.013
5. Alhajj N, O'Reilly NJ, Cathcart H. Leucine as an excipient in spray dried powder for inhalation. Drug Discov Today. 2021;26(10):2384-96. doi:10.1016/j.drudis.2021.04.009
6. Li L, Leung SSY, Gengenbach T, Yu J, Gao GF, Tang P, et al. Investigation of l-leucine in reducing the moisture-induced deterioration of spray-dried salbutamol sulfate power for inhalation. Int J Pharm. 2017;530(1-2):30-9. doi:10.1016/j.ijpharm.2017.07.033
7. Molina C, Kaialy W, Chen Q, Commandeur D, Nokhodchi A. Agglomerated novel spray-dried lactose-leucine tailored as a carrier to enhance the aerosolization performance of salbutamol sulfate from dpi formulations. Drug Deliv and Transl Res. 2018;8(6):1769-80. doi:10.1007/s13346-017-0462-8
8. Thiyagarajan D, Huck B, Nothdurft B, Koch M, Rudolph D, Rutschmann M, et al. Spray-dried lactose-leucine microparticles for pulmonary delivery of antimycobacterial nanopharmaceuticals. Drug Deliv and Transl Res. 2021;11(4):1766-78. doi:10.1007/s13346-021-01011-7
9. Molina C, Kaialy W, Nokhodchi A. Technology. The crucial role of leucine concentration on spray dried mannitol-leucine as a single carrier to enhance the aerosolization performance of albuterol sulfate. J Drug Deliv Sci Technol. 2019;49:97-106. doi:10.1016/j.jddst.2018.11.007
10. Weers JG, Tarara TE, Clark A. Design of fine particles for pulmonary drug delivery. Expert Opin Drug Deliv. 2007;4(3):297-313. doi:10.1517/17425247.4.3.297
11. Shetty N, Cipolla D, Park H, Zhou Q. Physical stability of dry powder inhaler formulations. Expert Opin Drug Deliv. 2020;17(1):77-96. doi:10.1080/17425247.2020.1702643
12. Siahi MR, Rahimi S, Monajjemzadeh F. Analytical investigation of the possible chemical interaction of methyldopa with some reducing carbohydrates used as pharmaceutical excipients. Adv Pharm Bull. 2018;8(4):657. doi:10.15171/apb.2018.074
13. Szalka M, Lubczak J, Naróg D, Laskowski M, Kaczmarski K. The maillard reaction of bisoprolol fumarate with various reducing carbohydrates. Eur J Pharm Sci. 2014;59:1-11. doi:10.1016/j.ejps.2014.04.005
14. Monajjemzadeh F, Hassanzadeh D, Valizadeh H, Siahi-Shadbad MR, Mojarrad JS, Robertson T, et al. Assessment of feasibility of maillard reaction between baclofen and lactose by liquid chromatography and tandem mass spectrometry, application to pre formulation studies. AAPS PharmSciTech. 2009;10:649-59. doi:10.1208/s12249-009-9248-8
15. Ghaderi F, Monajjemzadeh F. Technology. Review of the physicochemical methods applied in the investigation of the maillard reaction in pharmaceutical preparations. J Drug Deliv Sci Technol. 2020;55:101362. doi:10.1016/j.jddst.2019.101362
16. Ciolacu D, Ciolacu F, Popa VI. Amorphous cellulose—structure and characterization. Cellul Chem Technol. 2011;45(1):13.
17. Chang RYK, Chen L, Chen D, Chan H-KJEOoDD. Overcoming challenges for development of amorphous powders for inhalation. Expert Opin Drug Deliv. 2020;17(11):1583-95. doi:10.1080/17425247.2020.1813105
18. Vinjamuri BP, Haware RV, Stagner WCJAP. Inhalable ipratropium bromide particle engineering with multicriteria optimization. AAPS PharmSciTech. 2017;18:1925-35. doi:10.1208/s12249-016-0668-y