

## ***β-lactoglobulin (Dispersome®) as a Novel Pharmaceutical Excipient for Enhancing the Aerosolization Efficiency of Dry Powder Inhalation Formulations***

**Paulo R. Lino<sup>1</sup>, Cláudia Costa<sup>1</sup>, Nichal Gentilal<sup>1</sup>, Maria I. Lopes<sup>1</sup>, Andreia Dias<sup>1,2</sup>, Mykaella Mestre<sup>1,3</sup>, Beatriz Gamelas<sup>1,3</sup>, André Ribeiro<sup>1</sup>, Joana Diogo<sup>1</sup>, Patrícia Henriques<sup>1</sup>, Filipa Ferreira<sup>1</sup>, Rui Churro<sup>1</sup>, Susana I. Saldanha<sup>1</sup>, Marco Galésio<sup>1</sup> & Alexandre J.S. Ribeiro<sup>1</sup>**

<sup>1</sup>Hovione FarmaCiência SA, Lumiar, 1649-038, Portugal

<sup>2</sup>Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Portugal.

<sup>3</sup>Institute for Bioengineering and Biosciences (iBB), Lisboa, 1049-001, Portugal

### **Summary**

This study investigates the potential of Dispersome®, a novel excipient platform using the protein β-lactoglobulin (BLG), for applications in dry powder formulations intended for respiratory drug delivery (pulmonary and nasal). BLG was used as an excipient to produce dispersible, high-density composite particles via spray drying. Key outcomes of the study include: (i) optimization of spray drying parameters to yield BLG particles with fine particle fractions reaching up to 90%; (ii) demonstrated physicochemical compatibility of BLG with active pharmaceutical ingredients (API) such as fluticasone furoate (FF) and Tobramycin (TOB); (iii) successful stabilization of amorphous dispersions containing up to 75% (w/w) drug load of FF and TOB; (iv) efficient nasal deposition of BLG-based formulations; and (v) evidence of pulmonary and systemic clearance within 24 hours post-inhalation in rats with no significant immune response. These findings support the further development of BLG as an excipient for respiratory drug delivery.

### **Key Message**

β-lactoglobulin (BLG) is a promising novel excipient for dry powder inhalation and nasal drug delivery, enabling high drug loads, excellent aerosolization, and stable amorphous formulations. Preclinical studies show rapid pulmonary clearance and minimal immune response, supporting its potential for safe and effective respiratory drug delivery.

### **Introduction**

Aerodynamic performance and long-term stability are critical quality attributes in respiratory drug formulations, as they determine the efficiency of drug delivery to targeted regions of the respiratory tract. While lactose is used in over 75% of commercial dry powder inhalers (DPIs) due to its favourable properties for carrier-based formulations [1], the limited range of safe and effective excipients restricts formulation flexibility—especially for high-dose applications, where challenges like agglomeration and poor dispersion often limit formulation development [2]. These challenges may lead to increasing the number of clinically administered dosing units to reach the intended therapeutic target. In this context, we evaluated β-lactoglobulin (BLG), a cost-effective and readily available protein, as a novel excipient for DPI formulations. Previously explored to stabilize high drug loads for formulations administered through the oral route [3], it was hypothesized that BLG could also support high drug loads in inhalable formats. To test this, we developed spray-dried BLG-based formulations with model compounds and assessed their suitability for pulmonary and nasal delivery.

### **Experimental methods**

**Materials:** Fluticasone Furoate (FF) was produced at Hovione FarmaCiência, Portugal and Tobramycin (TOB) purchased from Livzon Pharmaceutical, China. Lactose (Lactohale® 300) was purchased from DFE pharma, Germany. Leucine was purchased from Merck, Germany. The human alveolar macrophage cell models (ImmuPHAGE™ and ImmuLUNG™) were screened at ImmuONE, UK.

**Formulation:** All powder formulations were produced by spray drying in a lab-scale Buchi (B-290). FF was prepared at 1.6% w/w drug load, to enable a direct comparison against the benchmark (Breo Ellipta from GSK). The TOB was prepared at different drug loads (up to 75% w/w). The solvent system for each formulation included a mixture of water plus an organic solvent (acetone or ethanol) at different ratios. All powders were post-dried in a lab scale tray oven. Once dried, HPMC size#3 capsules were filled in a lab-scale Auger filling equipment (Quantos) or MG2 manual dosator at up to 106 mg fill weight and varying compression levels.

**Analytical Characterization:** Tapped density was calculated by dividing the weight of a material by its volume after being compacted by tapping. The material was placed in a 10 mL container and tapped

mechanically (10, 500, and 1250 times), and the volume was recorded at each stage (V10, V500, V1250). The final tapped density was based on the volume that no longer changed with more tapping. Particle size of the powder formulations was assessed by laser diffraction in a Sympatec dry dispersion unit (Sympatec HELOS/BR Rodos/M Aspiros Sympatec GmbH), 18 mm/s feed velocity and 4.5 bar pressure. Aerodynamic performance was assessed using a gravimetric Fast Screening Impactor (gFSI) with Plastiaple RS01-Mod 7 (65 L/min) and size 3 capsules containing spray-dried dispersion (SDD). Performance of dry powder formulations for nasal delivery was evaluated using the Alberta Idealized Nasal Inlet (AINI) model without flow.

**Preclinical toxicity studies:** Pharmacokinetic (PK) studies were conducted in Sprague-Dawley rats using a Battelle nose-only “flow-past” inhalation tower to deliver BLG. Plasma, bronchoalveolar lavage fluid (BAL), and lung tissues were collected at predetermined time points to assess BLG levels. Immunogenicity was assessed by quantifying antibody responses (IgG, IgE), cytokine profiles, and immune cell populations (in ImmuLUNG™) using ELISA, flow cytometry, and histology. Effects on human alveolar macrophages at 24 and 72 hours of exposure were also assessed with cytotoxicity screening (in ImmuPHAGE™) using PrestoBlue™ cell viability reagent and lactate dehydrogenase (LDH) assays, and by evaluating cytokine release Proteome Profiler Human Cytokine Array kit from R&D Systems, and phagocytic activity and macrophage polarization via flow cytometry.

## Results and Discussion

### Modulating Spray Drying Solvent Systems to Engineer BLG-Based Dry Powders with Tunable Aerodynamic Properties

Different spray-dried composite dry powder formulations incorporating BLG were developed to explore the potential of this compound as an excipient for inhalable formulations. By adjusting the solvent composition (water, acetone and ethanol) and the sequence of component addition during feedstock preparation, it was possible to produce BLG-based powders with high fine particle fractions and consistent dose delivery, both in placebo and fluticasone furoate (FF)-loaded formulations (Figure 1). A variety of biphasic solvent systems were evaluated to understand their influence on the quality attributes of SDDs, aiming to achieve particle size distributions (PSDs) suitable for inhalation. Solvent system optimization enabled the production of SDDs with fine particle fractions up to 90% and tapped densities exceeding 0.4 g/cm<sup>3</sup>. The lead formulation solvation conditions, using 70% ethanol with BLG dissolved in the water fraction, was used to prepare FF-loaded SDDs at 1.6% w/w drug load. To match the FF dose from the benchmark, capsules were filled with 12.5 mg of the BLG-based SDD. Figure 1 shows comparable fine particle fractions and density between placebo and FF formulations. Notably, the aerodynamic performance of FF formulations remained stable after six months of accelerated storage at 40°C/75% RH. Furthermore, seven independent BLG batches were tested to assess batch-to-batch variability, with consistent solid-state and aerosolization performance observed across all batches.

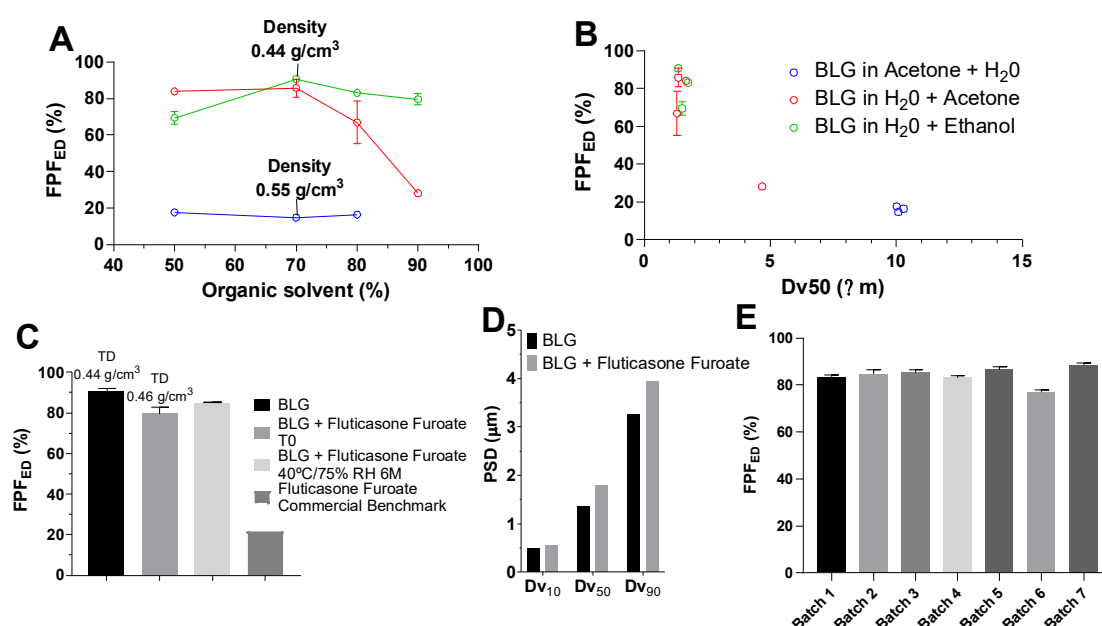


**Figure 1** (A) Fine particle fraction (FPF) of emitted dose for placebo BLG spray-dried formulations prepared under different solvent conditions (BLG dispersed in the organic phase of an acetone–water system versus dissolved in the aqueous phase). (B) Correlation between fine particle fraction and particle size distribution (PSD) at a 30 mg capsule fill weight (n=3). Color code of figure A and B are equivalent (C) FPF<sub>ED</sub> of active

BLG:fluticasone furoate (FF) spray-dried dispersions at 1.6% drug load in HPMC size 3 capsules, compared with a commercial lactose-based carrier benchmark (Breco® Ellipta®) at an equivalent FF dose per actuation. (D) Laser diffraction-based PSD analysis of placebo and FF-containing spray-dried dispersions. (E) Aerodynamic performance (FPF<sub>ED</sub>) profiles of BLG:FF spray-dried dispersions produced from seven independent BLG batches.

### Enhanced Powder Density and Dispersibility Supporting High-Dose Delivery with Superior Aerosolization Performance

Dry powder formulations containing varying concentrations (25–75% w/w) of Tobramycin (TOB) with BLG were developed using the previously optimized ethanol:water solvent system. Formulations with 75% drug load exhibited increased particle size distribution (PSD – Figure 2) and were therefore excluded from further evaluation. To assess the maximum feasible fill weight without compromising performance, a 50% MA spray-dried dispersion (SDD) was filled into HPMC size 3 capsules using both gravimetric (up to 61 mg) and dosator (up to 106 mg) methods. Despite the high fill weights and powder density, the formulations demonstrated excellent dispersibility, with emitted doses typically ≥90%, delivering up to 28 mg of MA as fine particles per actuation and achieving fine particle fractions up to 68%. Increasing the fill weight while maintaining aerodynamic efficiency enabled a reduction in the number of required actuations to reach therapeutic doses, potentially lowering patient burden. Moreover, targeting high fine particle fractions may help minimize upper airway irritation and reduce the fraction of drug swallowed.

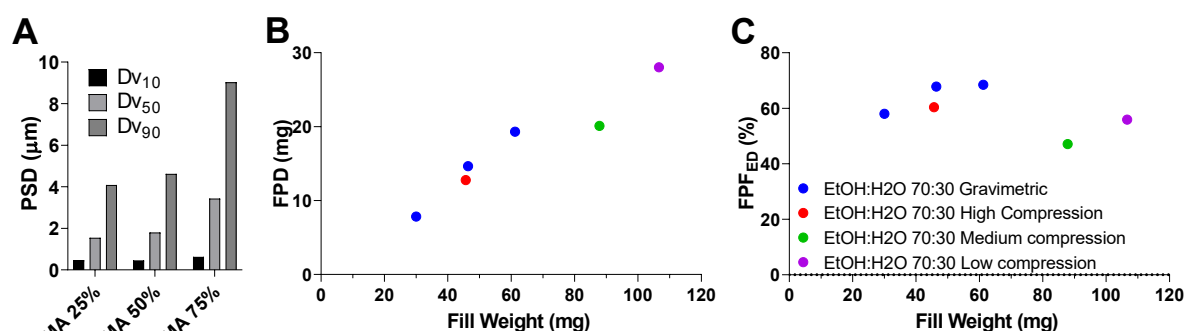


**Figure 2 (A) Laser diffraction-based PSD analysis of MA-containing spray-dried dispersions. (B and C) Aerodynamic performance evaluation of high-dose BLG–antibiotic spray-dried composite powders delivered via capsule-based systems (Plastiaple RS01), using both gravimetric and dosator filling methods across three compression levels.**

### BLG Combined with Glass Formers Enhances Amorphous Stability at Elevated Drug Loadings

Formulating inhalable amorphous drugs with high API loads can be particularly challenging, especially when dealing with compounds that have poor solubility or complex crystallization mechanism. In this study, the drug load of FF was increased to 75% to evaluate the ability of BLG to maintain the amorphous state under these demanding conditions. Crystallization of FF was detected at a concentration of 1.6% in a spray-dried formulation containing classic trehalose and leucine composite particles. However, with BLG by adding 10% trehalose—a synergistic glass-forming agent—, up to 75% FF formulation was successfully stabilized as an amorphous dispersion. This highlights BLG’s adaptability in supporting high-dose formulations of difficult compounds. Similarly, MA was also stabilized in an amorphous form at drug loads up to 75% using BLG alone.

### Development of Spray-Dried BLG Composite Systems for Nasal Administration

Due to the high dispersibility of BLG-based formulations for pulmonary delivery, a dry powder formulation aimed at nasal delivery containing a model compound (FF in water:organic biphasic solvent systems) was developed via spray drying and tested (Figure 3). The BLG formulations demonstrated favorable deposition in the turbinate region, with minimal deposition in the nasopharynx and filter. These findings suggest a reduced risk of off-target lung deposition and oral ingestion, highlighting the versatility of BLG as an excipient for nasal drug delivery applications.

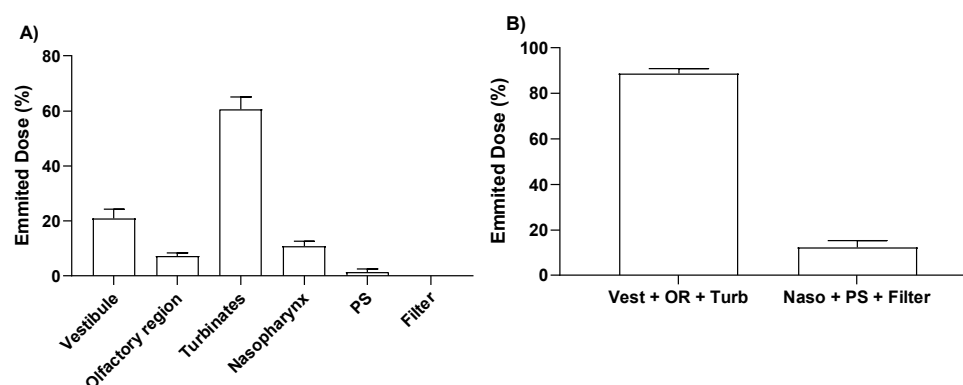


**Figure 3** Nasal deposition outcomes for BLG:FF formulations using the Alberta Idealized Nasal Inlet (AINI) model.

### **BLG was fully cleared from the lungs within 24 hours post-inhalation in a rat model, with no significant immune-related adverse effects observed**

Spray-dried BLG was administered for PK studies as a single dose at 2, 6, or 18 mg/kg to rats. The protein was largely cleared from the lungs within 24 hours, with similar trends observed in BAL. In plasma, low BLG levels were detected up to 6 hours post-exposure at 6 and 18 mg/kg, but none was found at 24 hours. To assess immunogenicity, BLG was administered daily at 18 mg/kg or at 0.67 mg/kg for three weeks. These regimens were well tolerated in rats, with no signs of irritation. Cytokine levels and immune cell profiles showed no significant differences between groups in plasma, BAL, and lung homogenates. Anti-BLG IgE and total IgE were undetectable in both plasma and BAL. While antigen-presenting cell activation remained largely unchanged, a negligible increase in MHC-II expression on alveolar macrophages was observed in the 18 mg/kg group at three weeks. Anti-BLG IgG was detected on day 14 but did not increase further by day 22 and remained only slightly elevated compared to naïve controls. In a comparative study, BLG (18 mg/kg) and lactose (20 mg/kg) were administered to Balb/c mice, with or without lipopolysaccharide (LPS) sensitization. Pre-treatment with these excipients did not alter the inflammatory response to LPS. Overall, BLG and lactose had similar effects, though BLG exposure led to a slight increase in plasma GRO (KC/CXCL1), possibly linked to endotoxin presence. Human alveolar macrophages were also exposed to non-cytotoxic concentrations of BLG to assess human-specific responses. Leucine, used as a reference excipient, was tested at lower concentrations due to higher cytotoxicity. Overall, BLG did not significantly impair macrophage function. Both BLG and leucine induced mild proinflammatory cytokine responses, more pronounced for BLG at 72 hours. Cytokine profiles differed between the two excipients. These results overall support the potential of BLG as a nasal and pulmonary excipient, though a thorough toxicological study is needed for further developing this excipient [4,5].

### **Conclusion**

In summary, our findings highlight the high potential of BLG-based spray-dried particles as pharmaceutical formulations for both inhalation and nasal delivery, particularly for achieving efficient delivery of high drug loads. Pharmacokinetic studies demonstrated rapid clearance of BLG, and the observed immune responses suggest that it is generally well tolerated, with a safety profile comparable to that of lactose and leucine. Future research will focus on evaluating additional model compounds relevant to inhalation, investigating the relationship between critical quality attributes of raw materials and formulation developability, and conducting more comprehensive toxicological assessments using human-relevant models.

### **References**

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