Development of an iSPERSE™ Based Platform for the Delivery of Macromolecules via Dry Powder Formulations

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INTRODUCTION

Respiratory drug delivery of antibodies to treat local diseases such as asthma maximizes local concentration, reduces systemic exposure, and is non-invasive. The work herein presents the development of an iSPERSE (inhaled small particles easily respirable and emittable) formulation, which is defined as powders that exhibit high fine particle fraction (FPF), emitted dose (ED), and dispersibility, and particles that are geometrically small and relatively dense. This offers the advantage of an increased delivered dose of antibody per unit volume compared to alternative highly dispersible porous particles (1, 2). Macromolecules are inherently more sensitive to environmental changes than traditional small molecules. Strategies for developing dry powder-based inhalable macromolecule formulations must ensure maximal stability both during the formulation process and in the solid state. Excipient classes traditionally used in protein formulation include osmolytes, carbohydrates, surfactants and preservatives (3, 4). Particle engineering strategies such as spray drying are required to formulate proteins for inhalation in the dry powder form, but may introduce stresses such as thermal, dehydration and interfacial stresses during the process (5, 6).

To address these stability issues, previous work has used sugars to stabilize proteins, e.g., antibody formulations (7). However, the use of sugars has been reported to lead to dry powders with a low fine particle fraction (FPF) and low emitted dose (ED) (7). To address the low FPF and low ED issues, we have introduced the macromolecule into our proprietary, salt-based iSPERSE platform. These formulations have led to powders that are stable physically and chemically post-processing, and achieve iSPERSE properties of small size and relatively high density. Immunoglobulin G ((IgG), from Bovine serum) was selected as a model macromolecule for development of this platform.

METHODS

Dry powder formulations with and without IgG (Sigma-Aldrich, MO, USA) were prepared using a B-290 Mini Spray Dryer (BÜCHI Labortechnik AG; Flawil, Switzerland). The general spray drying conditions varied from an inlet temperature of 80˚C to 100˚C with compressed air as the drying medium. The concentration of the feed solution ranged from 4 g/L to 40 g/L and the liquid feed stock flow rate was 2.2 ml/min. The tap density for final formulations was assessed using a Tap Density Tester model TD1 (SOTAX; Horsham, PA). Volume median diameter (VMD) was determined using a HELOS laser diffractometer and a RODOS dry powder disperser (Sympatec; Princeton, NJ) at pressures of 1.0 bar and 4.0 bar. Powder dispersibility was determined by measuring the percentage of capsule emitted powder mass (CEPM; measured by capsule weight change) when emitted from size 3 hydroxypropylmethylcellulose (HPMC) capsules; V-Caps; Capsugel; Greenwood, SC) via a capsule-based passive DPI (RS01 Model 7HR; Plastiape S.p.A.: Osnago, Italy) and the VMD across various flow rates was measured by laser diffraction via the Spraytec (Malvern; Worcestershire, UK) at 1 kHz for the duration of the simulated inhalation. The respirable fraction (fine particle fraction (FPF) <5.6 μm) was determined using a two-stage Andersen cascade impactor method (ACI; 2 L at 60 LPM; gravimetric analysis of powder mass on glass fiber filters on inverted plate stages). The effect of processing on formulation stability was assessed using sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) in native state (no reducing agent) to monitor aggregation and integrity, SDS-PAGE in reduced conditions to assess purity of heavy and light chains, and isoelectric focusing (IEF) to investigate potential chemical degradation during the spray drying process, e.g., deamidation.

RESULTS

Placebo formulations containing a sugar and a salt were prepared to assess iSPERSE formulations for IgG development. Based on these results the lead formulation containing a non-reducing sugar, an iSPERSE salt and leucine was selected for developing the IgG platform. All subsequent formulations of IgG were composed of variations in component ratios or used modified spray drying conditions to optimize stability of the molecule through processing. The leucine was removed from the formulation after it appeared to enhance degradation of the IgG. The 100% IgG formulation showed degradation in both the heavy and light chains, was aggregated in the native state, highly charged and difficult to handle. A protein loading in excess of 25% (w/w) was developed and lot G showed good stability with iSPERSE properties, compared to 100% spray dried IgG (lot A) after spray drying (Table 1 and Figure 1). Lot G was both highly respirable and emittable (Figure 2), and was produced using a simple one-step spray drying process. The lot G formulation had a recovery yield of 61.4% from the spray drying process, enhanced stability compared to other formulations and 100% spray dried IgG, a mass emitted from the capsule greater than 80% across a flow rate of 15 std L/min and 60 std L/min (Figure 2) with a FPF <5.6 μm of 41.8% and a tapped density of 0.59 g/cc. The enhanced stability post-processing can be attributed to the formulation components and composition, and to the low spray drying temperature.

Table 1.

iSPERSE analysis of IgG formulations. The iSPERSE formulations were spray dried with different component ratios and molar concentrations, with spray drying parameters altered based on the stability assays in this table.

Lot	Formulation	Spray Drying Yield	RODOS D50 (µm)		Water Content	Aggregation / Poor Integrity of IgG	Degregation of Heavy and/or Light Chains
			1 bar	4 bar			
A	IgG alone	48.8%	2.99	2.62	N/A	Yes	Yes
B	NaCl: Trehalose: Leucine: IgG	64.7%	2.00	1.72	3.5%	Yes	Yes
C	NaCl: Trehalose: IgG	80.2%	2.31	2.04	2.9%	Yes	Yes
D	NaCl: Trehalose: IgG	62.4%	2.35	2.17	3.3%	Yes	No
E	NaCl: Trehalose: IgG	63.7%	2.43	2.03	3.5%	Yes	Yes
F	Sodium Citrate: Sorbitol: IgG	64.0%	2.44	2.23	N/A	Yes	Yes
G	NaCl: Trehalose: IgG	61.4%	3.10	2.91	N/A	No	No

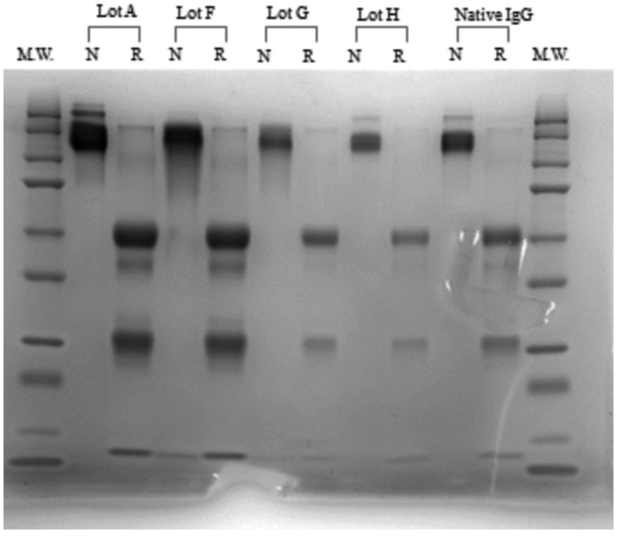

Figure 1. Stability analysis of spray dried IgG formulations under native and reduced conditions in a 12% Poly-acrylamide gel. The native state analyzes the integrity of the IgG and possible covalent aggregation whereas the reduced samples evaluate purity and integrity of the heavy and light chains.

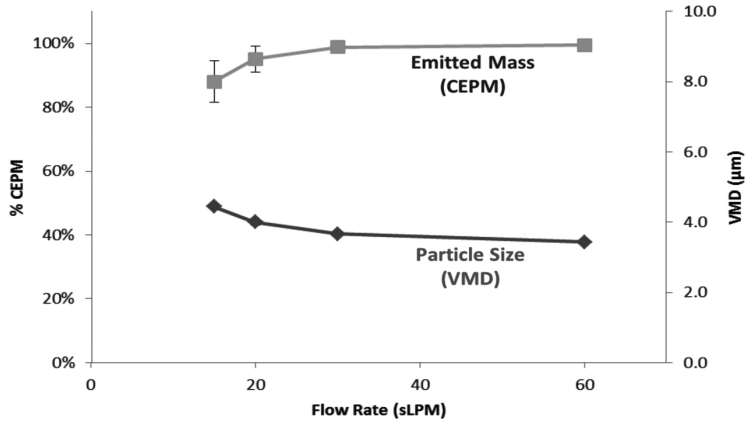

Figure 2. **Dispersibility properties of lead IgG iSPERSE formulation across flow rates in terms of emitted mass from capsule and particle size, n=5 (lot G). The formulation had an IgG loading of greater than 25% and showed good stability by SDS-PAGE and IEF.**

CONCLUSION

The iSPERSE platform described herein is capable of both stabilizing macromolecules during the spray drying process and in the solid state and imparts a highly dispersible and respirable characteristic on them. The dense nature of these particles also facilitates the delivery of a high payload to the lungs with a simple inhaler, which is highly desirable for macromolecules. These demonstrated properties establish iSPERSE as an alternative delivery route for macromolecules for respiratory disease.

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