European patent EP 1627642A2 describes a method to obtain zinc-free insulin crystals for pulmonary use [18]. One of the examples in this invention describes a method to generate zinc-free insulin crystals using sodium acetate. Based on this method, purified human insulin is dissolved in TRIS buffer, pH 8.0, and ethanol mixture. The addition of sodium acetate to this solution, forms a precipitate immediately. The crystals formed after about 2 days are examined microscopically and reported to have a diameter between 0.5 and 1 μm. The crystals are washed with ice-cold ethanol in water, isolated by centrifugation, and dried by lyophilization.

Dave et al. describe a process for manufacture of a modified insulin intended for oral delivery [15]. The modified insulin is synthesized by covalent conjugation of native insulin, purified by successive reverse-phase chromatography and cation-exchange chromatography steps. The purified insulin analogue was crystallized by adding phenol and zinc chloride at a pH of about 4.8. The crystals were washed with cold, purified water to remove excess zinc and phenol and lyophilized into dry powder.

In all the above-described processes the residual moisture in the insulin crystals is removed by vacuum drying or bulk freeze-drying. Processing of Insulin crystals after crystallization typically involves a washing step which controls some important quality attributes of the freeze-dried Insulin bulk. Successful washing step ensures that the zinc content, inorganic salts and buffer residues, and residual organic solvent are controlled such that the final freeze-dried Insulin bulk has ideal quality attributes/properties (some desired properties are described in Table 1).

Crystal washing can be performed in either batch mode or continuous mode using an industrial-scale centrifuge for separation of crystals. After the crystals settle, the resulting supernatant is decanted and the crystal slurry washed several times with water for injection. The supernatant is decanted, and the crystal slurry is taken for lyophilization. The suspension and washing steps can be repeated multiple times depending on the process to achieve desired product quality.

Insulin Freeze-Dried Drug Substance a Process, Logistics, Commercial, Formulation Advantage

a. *Process advantage:* Unlike several other biopharmaceuticals which are stored as frozen liquid solutions, Human Insulin drug substance (DS) is a solid [25, 48] and has traces/residual zinc (typically less than 1% w/w). Insulin, unlike other biopharmaceuticals, is a large volume, high-value molecule with typical manufacturing yields in kilograms. The typical polishing steps in insulin manufacture are chromatography steps (reverse-phase or size-exclusion chromatography), wherein the final elution volumes can be as high as 50 L. Converting the liquid samples into a stable solid form is a definite process advantage.
b. *Logistics and commercial advantage:* A crystalline/freeze-dried solid at the end of a lengthy manufacture process is not just a process advantage but also a storage handling and logistics advantage with less volumes to store and handle at the end of the process. Solid DS is a fill–finish advantage as well, wherein cold chain transportation (and the validation of such transportation) of small volumes of freeze-dried solids becomes practically feasible. This is a practical scenario