

## **Characterization of Insulin NPH Microcrystals in Pharmaceutical Suspensions**

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Insulin preparations with an extended action profile consist of microcrystalline suspensions that slowly dissolve and release the insulin into the blood stream. The action profiles are partly dependent on the crystal form and on the composition of crystals and soluble insulin. The intermediate-acting preparation, NPH (Neutral Protamine Hagedorn) is an insulin-zinc solution co-crystallised with the basic peptide protamine, consisting mainly of arginine residues. The crystals form hexameric insulins with two zinc atoms and one protamine peptide per hexamer. Structural characterisation of the crystals in the pharmaceutical preparations have until now been hampered, mainly due to their microcrystalline nature. Single crystal x-ray analysis has been used after modifications of the crystal growing media to promote larger crystals. However, such modifications may influence the crystal contacts, packing and structural arrangement and may therefore not reflect the true structure in the microcrystals.

In this study, we have used x-ray powder diffraction to analyse several insulin products, and in house preparations of microcrystals. We are able to distinguish between different crystal systems and to check for homogeneousness between different batches/preparations. Furthermore, the micro diffractometer at SLS (Swiss Light Source, Switzerland) has been used to collect single crystal data of the 25 x 5 x 5  $\mu\text{m}^3$  large NPH microcrystals to a resolution of  $\sim 3\text{\AA}$ .

**Keywords:** microcrystals, crystal characterization, macromolecular crystallography