Access to Higher Order Structure Information of Microcrystalline Biopharmaceuticals by Solid-State NMR spectroscopy in its formulated state

A higher-order structure (HOS) is critical to a biopharmaceutical drug as the three-dimensional structure governs its function. Even the partial perturbation in the HOS of the drug can alter the biological efficiency and efficacy. Due to current limitations in analytical technologies, it is imperative to develop a protocol to characterize the HOS of biopharmaceuticals in the native formulated state. We have used an approach using solid-state (^{13}C CP-MAS) NMR methodology to demonstrate the HOS in the microcrystalline suspension drug in its formulated state. The data were further assessed by principal component analysis and Mahalanobis distance (D_M) calculation for quantitative assessment. This approach is sufficient to provide information regarding the protein HOS and the local dynamics of the molecule when combined with orthogonal techniques such as X-ray scattering. Along with, it can be an elegant tool to investigate batch-to-batch variation in the process of manufacture and storage as well as a biosimilarity comparison study for biphasic/microcrystalline suspension. However, the resolution of the 1D ^{13}C CP-MAS is not sufficient enough to investigate the structural/conformational changes in the residue level. So to overcome this challenge we have demonstrated an improvised solid-state NMR method employing a novel selective excitation scheme under fast magic-angle spinning (MAS). The applicability of the method is shown on commercial insulin suspensions at natural isotopic abundance. Selective excitation aided with proton detection and non-uniform sampling (NUS) provides the sensitivity and resolution. With the gain in resolution we could observe different conformation of insulin hexamer along with the allosteric-transition pathway.

References

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