Chromatographic approach to study protein fibrillation

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Protein fibrillation shows a significant hurdle in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and diabetes. The thioflavin T (ThT) assay is a commonly used method for monitoring protein fibrils, but it has limitations, including misinterpretation of results due to the competitive binding with contaminants.[1]
Our research focuses on an alternative approach to studying protein fibrillation using UPLC. Instead of measuring fibrillation directly, we measured the concentration of soluble protein components. The present work is devoted to studying the kinetics of insulin fibrillation at low pH and high temperature. UPLC results were comparable to those of ThT assay. Furthermore, we found the temporary generation of a deamidated insulin monomer, which cannot be detected by the ThT assay. The deamidated insulin was a product of a post-translational modification, which possibly induced insulin fibrillation. We also confirmed that some inhibitors, such as curcumin, altered the ThT results, while UPLC analysis was not affected by inhibitors.[2] In summary, our new approach provided more information to study kinetics of protein fibrillation. It is not only addressing the limitations of the ThT assay but also broadening the research scope to understand the fibrillation process. This would help in developing therapeutic strategies to combat neurodegenerative diseases.

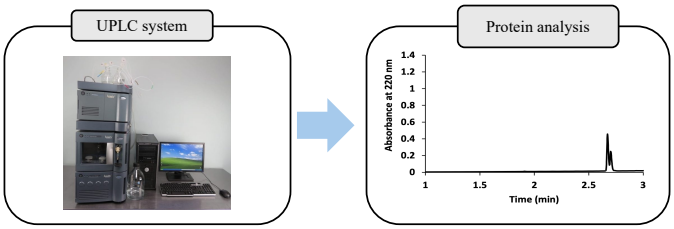

Fig. 1 Study of insulin fibrillation by UPLC .

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