

PROTEIN AGGREGATES CONCENTRATION DETERMINATION USING ELECTROCHEMICAL METHODS

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Determining protein aggregate concentrations is important in food industry and clinical studies. Fluorescence spectroscopy, employing amyloidophilic dyes, remains a primary technique for the detection and quantification of protein aggregates [1]. However, fluorescence method faces several limitations, for example, presence of other optically active contaminants or solution viscosity can significantly interfere with method accuracy [2] – [4]. In this work, instead of fluorescence methods, electrochemical methods were employed to determine protein aggregates concentrations.

A 3 electrode system, consisting of inert platinum electrodes as working and counter electrodes and Ag/AgCl, KCl_{sat.} as reference electrode, was used. Amyloidophilic dyes, like thioflavin T (ThT), are not only suitable for fluorescence based detection, but also exhibit oxidation currents, which can be measured using electrochemical methods. Using the electrochemical activity of ThT, cyclic voltammetry curves were measured not only using different ThT concentrations, but also different protein aggregate concentrations in buffer solutions. Furthermore, electrochemical impedance spectroscopy at different protein aggregate concentrations were measured. Results demonstrate that protein aggregate addition to buffer solution, lead to measurable thioflavin T oxidation current decrease.

The obtained results suggest that using cyclic voltammetry in the potential range from 0.2 V to 0.9 V vs Ag/AgCl, KCl_{sat.}, allows for protein aggregate detection. This methodology allows for protein aggregate concentration quantification in the concentration interval ranging from 15 µg/mL to 595 µg/mL.

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