REAL-TIME PROCESS ANALYTICAL TECNHOLOGY: FLUORESCENT DYE-BASED MINIATURIZED SENSOR FOR AGGREGATE DETECTION

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The transition to continuous biomanufacturing is considered the next step to reduce manufacturing costs and improve process robustness in the biopharmaceutical industry, while also improving productivity and product quality. The biomanufacturing process for monoclonal antibodies (mAbs) is eligible for this continuous processing due to patent expiration and subsequent need to lower manufacturing costs (1). One of the critical quality attributes (CQAs) of interest during mAb purification is aggregate formation, a phenomenon which can lead to an adverse immune response or a decrease in product efficacy. Several processing parameters and environmental factors are known to influence antibody aggregation (i.e. pH or mechanical agitation), making their appearance unavoidable. Therefore, the development of a real-time Process Analytical Technology (PAT) tool to monitor aggregate formation is crucial to have immediate feedback and process control and achieve a continuous processing. Miniaturized biosensors (at the nL-scale) placed after each step can be a powerful solution to speed up an analytical measurement due to the inherent short operation time, while minimizing the sample volume (2).

In this work, the development of an integrated biosensing microfluidic chip for fast at-line PAT is described, using hydrophobicity sensitive fluorescent dyes (FD) to examine possible size differences of mAb species. The design of a microfluidic structure capable to effectively mix the laminar flowing mAb sample with the fluorescent dye and subsequently collect real-time information on mAb aggregation is presented. The developed micromixer presents a mixing index higher than 90% (validated with colour dyes and a fluorescent-tagged mAb molecule), detecting aggregation under 30 seconds. The proposed microstructure is then validated resorting to an UV detector, using mAb samples with diverse levels of aggregation and two fluorescent dyes, Bis-ANS and Thioflavin T (ThT). While for Bis-ANS, a hydrophobicity sensitive FD, the expected limit of detection is 3% of aggregation for a FD concentration of 0.5 µM, for ThT, a molecular rotor, the limit of detection is of 1.5% for a concentration of 1 mM. The final prototype will then be validated in a continuous chromatographic workstation on an ÄKTA™ Avant unit, operated by the control software Orbit.

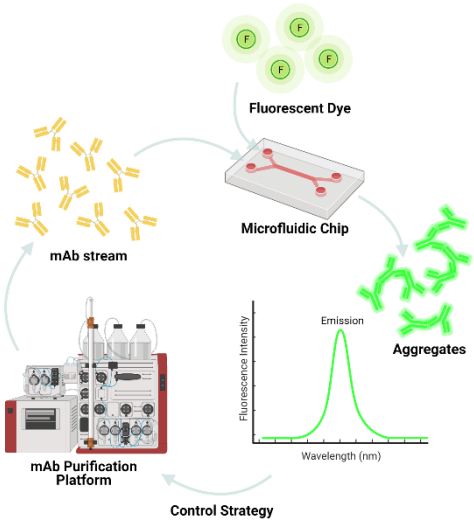

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Figure 1 – Schematic representation of the present work: a microfluidic chip is placed after each purification step in a continuous chromatographic workstation, where a sample of mAb is injected and analyzed for the presence of aggregates. A stream of a fluorescent dye is also injected into the microfluidic chip, and if an emission signal is collected by the UV sensor in the end of the channel, aggregation is occurring during the purification process. This signal can then be directed back to the continuous chromatographic workstation to create a feedback control strategy.