# Aggregation of therapeutic monoclonal antibodies by bubbling induced air/liquid interfacial agitation

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## Abstract

Degradation of therapeutic monoclonal antibodies (mAb) due to interfacial agitation through air bubbling was investigated. Samples containing mAb in phosphate buffered saline were subjected to rapid bubbling by using a peristaltic pump at an air flow rate of 11.5 mL/min. Samples were analyzed by visual observation, UV–Vis, fluorescence, circular dichroism and infrared spectroscopy, size-exclusion chromatography (SEC), dynamic light scattering, microscopy, and cell-based activity assays. The stressed samples showed increasing turbidity with bubbling time, with mAb1 showing a protein loss of 53% in the supernatant at the latest time point (240 min), indicating formation of sub-visible and visible aggregates. Aggregate rich samples exhibited altered secondary structure and higher hydrophobicity with 40% reduction in activity. The supernatants of the stressed samples showed unchanged secondary and tertiary structure without the presence of any oligomers in SEC. Furthermore, the impact of various factors that could affect aggregation was investigated and it was found that the extent of aggregation was affected by protein concentration, sample volume, presence of surfactants, temperature, air flow rate, and presence of silicone oil. In conclusion, exposure to air/liquid interfacial stress through bubbling into liquid mAb samples effectively generated sub-visible and visible aggregates, making air bubbling an attractive approach for interfacial stress degradation studies of mAbs.