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Characterization of excipients for their application during purification of monoclonal antibodies and other therapeutic proteins

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Antibodies are an integral part of the human immune system. They are mostly Y-shaped monomeric molecules with different roles in the destruction of specifically targeted cells or molecules. Therefore, their specific targeting and binding ability has been explored by researchers and pharmaceutical industry for their application as drug substance. Ever since 1975, when the first hybridoma technology was invented by Köhler and Milstein, to produce pure mouse antibody, technologies involving antibody production has increased significantly.
Today, mostly IgG monoclonal antibodies with their subtypes are used as drug substance and their production is done through genetically engineered CHO cells in a cell culture, followed by a multi-step purification train. As part of this purification platform, cation exchange is often used to mildly separate aggregates and other product- and process-related impurities, that could potentially elicit an immune response from the patient. However, in the recent years, more and more publications showed a phenomenon during cation exchange chromatography, the two-peak elution behavior. In mostly a salt gradient elution, and sometimes after a certain hold time on the column, the protein elutes in two distinct peaks, instead of just one, as expected. These publications also show that this phenomenon is mostly protein-dependent and has several different explanations.
In my project, I used a standard approach to elicit this two-peak elution behavior with a monoclonal antibody held on a cation exchange resin for 1000 min before elution in a salt gradient to 1 M NaCl. To elucidate what exactly the second peak is, I used size exclusion chromatography. It was found that the second peak is mostly comprised of monomer, which are probably conformationally altered, but also ~30% aggregates, which are reversible, depending on the buffer pH value.
I further investigated several influential factors on this phenomenon. Bead size, tentacle technology, buffer composition, weak vs. strong cation exchange ligands, and load density were found to have an effect on the two-peak elution behavior. Interestingly, especially temperature and hold time had a strong influence on the amount of second peak created. This is a clear indication that the unfolding and aggregation on the resin surface and in the resin pores is a kinetic process.
Since SE-HPLC analysis showed a correlation between pH value of the buffer system and stabilization of the resulting aggregates, I wanted to test different ligand densities on the cation exchange resin. I found that, when the Donnan effect is calculated for the specific buffer pH value, counterion concentration, and the ligand density of the resin, the monoclonal antibody is subjected to a significantly lower pH value during the 1000 min hold time than originally thought of. The lower pH value inside the resin pore has a significant impact on the conformational stability of the antibody, which was shown with nano-DSF (intrinsic fluorescence) measurements.
Another big impact on the second peak had the addition or substitution of excipients into the buffer systems. Excipients are mostly used at the end of the purification train to stabilize the protein, increase shelf-life, and facilitate easier application to the patient. In this case, I wanted to study the effects of excipients on the surface-induced unfolding and aggregation. In a first smaller study I used 5 common excipients added or substituted during the three phases of the chromatography (only during load and hold phase, only during elution or present throughout the chromatography). It showed that the biggest influence of excipients can be achieved during either only the load and hold phase or when present during the entire chromatography step. I then added several more excipients to further study the effects on this phenomenon and again, I used nano-DSF measurements to compare these effects with a more "traditional" in-solution screening approach. Some excipients showed similar stabilizing/destabilizing effects in both systems, while others had completely opposite effects. Nevertheless, this approach,

following a guideline to avoid other influential parameters, can deliver more diverse insight into protein-excipient interactions, while the protein is surface-bound.