ABSTRACT

Antibodies and recombinant active substances of the second generation, which also include the Fc-fusion protein, represent a high percentage of the global market of biologically active substances as there is a wide range of their use in numerous applications. Because biological medicines are produced by living organisms, the healing substance in the final biological medicine can express a certain lower stage of variability (microheterogeneity), which has to be within a certain acceptable range. One of the more important post-translation modifications, that has a vital influence on their structure, solubility, stability, function, locality, folding, and interactions with other proteins, is glycosylation. The production of therapeutic glycoproteins thus takes place under controlled conditions, which must be constantly monitored to ensure a consistent, reproducible and predictable pattern of glycosylation. With this aim, we wanted to develop chromatographic techniques for determining different isoforms o antibodies, which we could include in a bioprocess analysis. In the first part of the doctorate dissertation, we characterized lectin ligands for affinity chromatography. For this purpose, we modified the lectins for a more successful immobilization to carriers and better exposure of the active region for binding. Biological activity of lectins was checked by biolayer interferometry (BLI). The modified lectin rPA-ILNME6, which we named rPE6, bonded to a carrier showed better bonding kinetics in comparison to the first. In the second part of the doctorate dissertation, we further determined the conditions for partial antibody unfolding, which is needed, for the carbohydrates to expose themselves and with that become accessible to bond with lectins. We exposed the antibodies to temperature stress with the presence of a reductant and detected changes with the help of tryptophane intrinsic fluorescent measurements, DLS measurements, size-exclusion chromatography, and ionic exchange chromatography. According to the results of intrinsic fluorescence and BLI measurements, the unfolding of antibodies was achieved, but the proportion of unfolded molecules, as well as their stability, was low, as they could not be detected by chromatographic methods. Because of the unsuccessful unfolding of the anty bodies we used Fc-fusion proteins, as representatives of glycoproteins, with known glycan structures exposed for bonding, for the development of chromatographic carriers with lectins. This way we were able to characterize a poliHIPE carrier with the bonded lectin rPE6 in the third part of the doctorate dissertation. We estimated the carrier capacity to 0.57 mg/ml and the limit of detection between 0.19 mg/ml and 8 mg/ml. We showed that the binding kinetics of the immobilized lectin rPE6 is fast enough so that it doesn't affect the Fc-fusion protein binding. This allows us to use the developed carrier for fast and efficient separation of glycoprotein isoforms. We optimized the separation time to 10 min, at a mobile phase rate of 8 ml/min, and the carrier maintained stability for a further four months after immobilization. In the fourth part of the doctorate dissertation, we showed an example of the use of this carrier for monitoring a perfusion bioprocess production of an Fc-fusion protein. The concentration pattern obtained with poliHIPE carrier matched with the results obtained with the protein A column isolation. The components of the medium, HCPs and DNA molecules did not interfere with the detection with our carrier, which makes it suitable for separating isoforms of glycoproteins that have oligosaccharides bound by lectin ligand in the glycan structure. The carrier was also used to determine layer thickens by measuring pressure drop, which allows us to track the aggregate formation of the Fc-fusion protein, but their concentration was below the limit of detection. However, we used this method to show lectin aggregation during immobilization.

Keywords: lectins, monoclonal antibody, Fc-fusion protein, chromatography, biolayer interferometry.