Optimization of the *in-vitro* refolding of biotherapeutic Fab Ranibizumab

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Abstract

Recombinant biotherapeutics expressed as inclusion bodies require solubilization and subsequent refolding to attain a functionally active state. But refolding step often acts as a bottleneck due to a low throughput, cumbersome and expensive framework, especially in the case of complex, multi-domain proteins such as antibody fragment (Fab) molecules. Lack of comprehensive pathways and inadequate yields from conventional optimization approaches further exacerbate this. Fab Ranibizumab, with two domains and five disulphide bonds, currently presents a daunting task to be refold. Reported works exhibit sub-optimal yields and/or large time scales to refold, entailing further research. In this paper, the *in-vitro* refolding of this Fab was investigated over 24 hours, using two different methodologies. The first protocol employed the traditional two-step DoE for screening and optimization. The entire refolding process was considered as a single process step. The second approach derived inferences from the data of analytical tools like intrinsic fluorescence, zeta potential and RP-HPLC to highlight a possible time-based molecular behavior during refolding. This led to the identification of a breakpoint at the 8^{th} hour of the process, proposing initial occurrences of the native tertiary conformation. Based on this observation, segmented DoEs were conducted to optimize the two time zones of the refolding process (0-8 hours and 8-24 hours). This unconventional segment-based optimization approach led to a 55% increment over the standard conventional optimization methodology, performed for the same Fab, Ranibizumab. It prevailed with an effective yield of 32% over the conventional strategy with 20.6%.