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INTEGRATION OF CONTINUOUS ETHANOL PRECIPITATION AND FLOCCULATION INTO MANUFACTURING OF ANTIBODIES

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Precipitation and flocculation are optimal unit operations for continuous capture of proteins from culture supernatant. Precipitation and flocculation can be operated in a real continuous manner. The methods can be combined and after this capture step the precipitate can be stored as a concentrated solution or even as a precipitate. We have developed several precipitation/ flocculation protocols for capture of antibodies from culture supernatant. These include the combinations of $CaCl_2$ flocculation with either cold ethanol precipitation or PEG precipitation and octanoic acid precipitation with PEG precipitation. The precipitation conditions have been screened in microtiter plates or in case of cold ethanol precipitation in small scale reactors. For cold ethanol precipitation a combination with $CaCl_2$ flocculation is best suited. In the first step the high molecular mass impurities can be removed by flocculation with $CaCl_2$ while in the second step the antibody is precipitated by addition of ethanol and low molecular mass impurities are removed. The whole procedure can be repeated and then final polishing can be performed by an anion exchange step in flow through mode. Octanoic acid precipitation is also a very efficient step but an additional phase can be formed which is difficult to remove. In Figure 1 a bench top reactor for cold ethanol precipitation is depicted. The rector consists of two sections (Figure 1). First a concurrent cooling is installed to ensure a constant cooling rate while ethanol is added to the culture supernatant or pre-treated supernatant. In the second section a countercurrent cooling is installed to keep the reactors at the requested temperature. The industry standard for antibody capture is protein A affinity chromatography. Thus the properties of the antibody after cold ethanol precipitation have been compared to protein A purified material. No significant difference could be observed (Figure 2) in either composition or structure. Further purification has been tested by anion-exchange monoliths in order to remove further host cell proteins. The different protocols will be compared to other standard platforms for antibody purification. The possibilities to integrate in-process control and maintenance of steady state will be discussed. The economics of such a process will be discussed for the different scenarios of clinical phase manufacturing and how strategies can be developed when antibodies become commodities or when oral delivery becomes reality. Next steps will be shown how to scale up such a process.

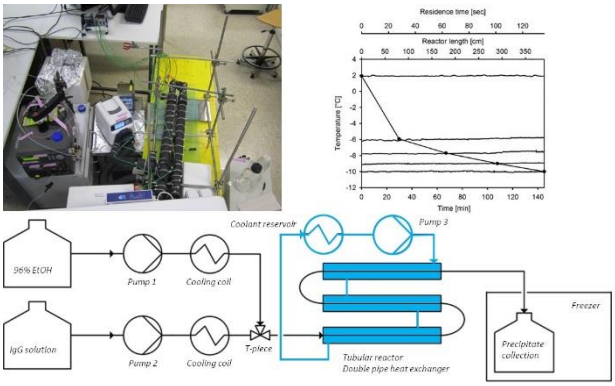

Figure 1 – Benchtop continuous reactor for cold ethanol precipitation of antibodies; top left: bench top tubular reactor, top right: temperature-profile at five different section in the reactor, bottom: schematic drawing of the reactor

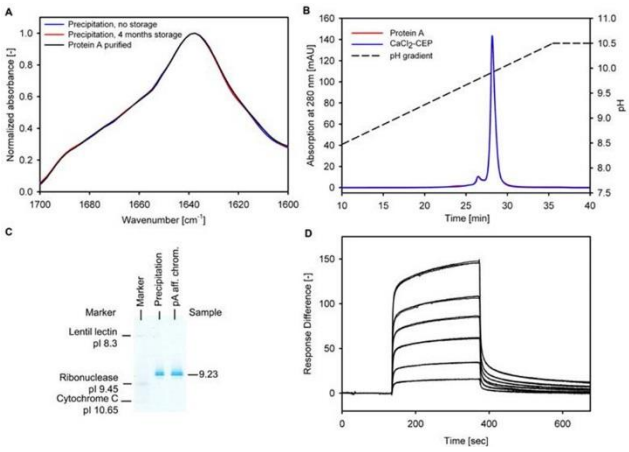

Figure 2 – Comparison of protein A purified antibody with antibody purified by CE: (A) ATR-FTIR, (B) analytical ion- exchange chromatography with linear pH- gradient, (C) isoelectric focusing (B) affinity to FcγII receptor measured by Biacore.