CONTINUOUS DOWNSTREAM PROCESS OF MONOCLONAL ANTIBODY DEVELOPED BASED ON THE PROCESS ANALYSIS/UNDERSTANDING AND ITS VALIDATION

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Key Words: continuous DSP, process understanding, process analysis, mechanistic model simulations

As downstream process (DSP) of monoclonal antibody (mAb) includes several batch chromatography steps, and other operations such as virus inactivation (VI), it is important to consider how each operation is carried out in order to make an efficient continuous or pseudo-continuous DSP. Our consortium* developed a continuous DSP (CDSP) of mAb under the AMED** project "Development of platform technologies for the continuous manufacturing of biopharmaceuticals", and successfully carried out several runs at our GMP facility and laboratory. In this paper, we will present how our CDSP was developed based on the process analysis and understanding, and the purification results along with several important issues to be addressed in the future [1].
For the first "capture" step, a 2-column "periodic counter-current chromatography (PCCC)" with protein A chromatography columns was chosen. The efficiency of PCCC is strongly dependent on the operating conditions, which should be determined properly [2, 3].
After the capture step, a low pH VI was carried out. Although this can be carried out continuously by using a flow reactor, our analysis showed that the scale-down is difficult. So we decided to use a batch reactor, which can work also as a surge tank and a tank for adjusting pH for the polishing step.

The polishing step usually includes two chromatography columns of different separation modes. We decided to use a pseudo continuous operation method known as flow-through chromatography (FTC) [4]. In order to eliminate the buffer exchange between two FTC operations, the same buffer solution was employed so that the two columns (mixed mode and cation exchange) can be connected in series. Virus filter (VF) was also connected at the exit of the second column.

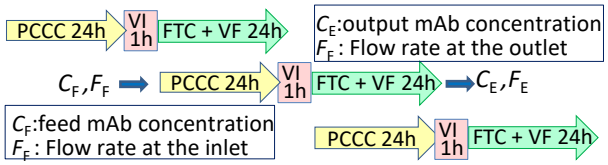

Fig.1 Continuous DSP operation schedule

Our CDSP was carried out according to the following schedule. After 24-h operation of PCCC, the recovered pool was transferred to the batch VI process, which performed one hour incubation at an assigned low pH. The solution pH and the conductivity were then adjusted for the polishing FTC step. Then, the FTC step started. PCCC and FTC were operated in parallel. After each operation for 24 hours, the lines of PCCC setup were cleaned, and the columns and/or the VF were replaced for the polishing step.
The operating conditions including the mobile phase properties for PCCC and 2-column connected FTC processes were determined based on the experimental data. The continuous operations for five days were carried out with different mAb concentrations (1–3.2 g/L), feed flow rates (0.5–10 L/d) and column volumes (1–100 mL). High yield and purity were accomplished for all runs; yield >80%, monomer purity determined by SEC > 95%, host cell protein concentration <10 ng/mg-IgG, and DNA concentration < 1 pg/mg-IgG [1]. The largest run (10 L/d and 2g/L) was carried out together with a continuous (perfusion reactor) cell culture. Our CDSP was found to be stable to feed (inlet) concentration and/or flow-rate variations (fluctuations). Further improvement of the process efficiency is possible by using mechanistic model simulations.

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**AMED: Japan Agency for Medical Research and Development https://www.amed.go.jp/en/index.html

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