



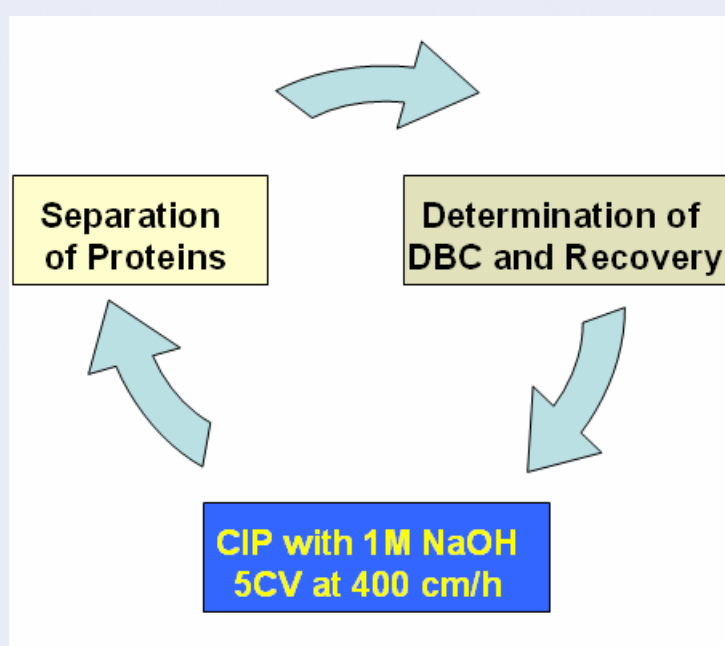
YMC BioPro IEX
Cleaning in place
CIP-stability

Stability of BioPro IEX material under CIP conditions

Author: DK
Date: 10.03.10
(iMail-Nr.10-3)

Cleaning in place (CIP) is an important part of any pharmaceutical production process. Therefore the IEX materials YMC BioPro S75 and Q75 were tested with regards to their ability to withstand the CIP procedure. For the cation exchanger material (BioPro S75), a simulated production / CIP cycle has been used, whilst for the anion exchanger material (BioPro Q75) stability was measured after prolonged contact with a basic solution

Figure 1 shows the schematic of the test cycle used. The first step in every cycle was to obtain a



chromatogram for the separation of three standard proteins. This mixture was made up of ribonuclease A, cytochrome C and lysozyme. They were separated at a flow rate of 180 cm/h using a 20 mM potassium phosphate buffer, pH 6.8 as mobile phase A with a 20 mM potassium phosphate buffer, pH 6.8 containing 0.5 M NaCl as phase B.

The next step was to determine the DBC and recovery for lysozyme. The dynamic binding was determined at 10% breakthrough. For this, the column was loaded with lysozyme in 20 mM glycine-NaOH buffer, pH 9.0 at a linear flow of 800 cm/h. For the measurement of the recovery 0.5 M NaCl was added to the same buffer system (without lysozyme). Finally the medium was cleaned in place by pumping with 1M NaOH for 5 column

Figure 1: Schematic of the test cycle

volumes (CV) at a linear flow of 400 cm/h.

Figure 2 shows the DBC and recoveries, plotted after each CIP step. For every cycle the DBC stays at a very good value, generally above 220 mg lysozyme per ml resin. The recovery remains constant at about 100%.

Finally, figure 3 shows that there is no difference in the ability to separate the protein mixture between the various runs.

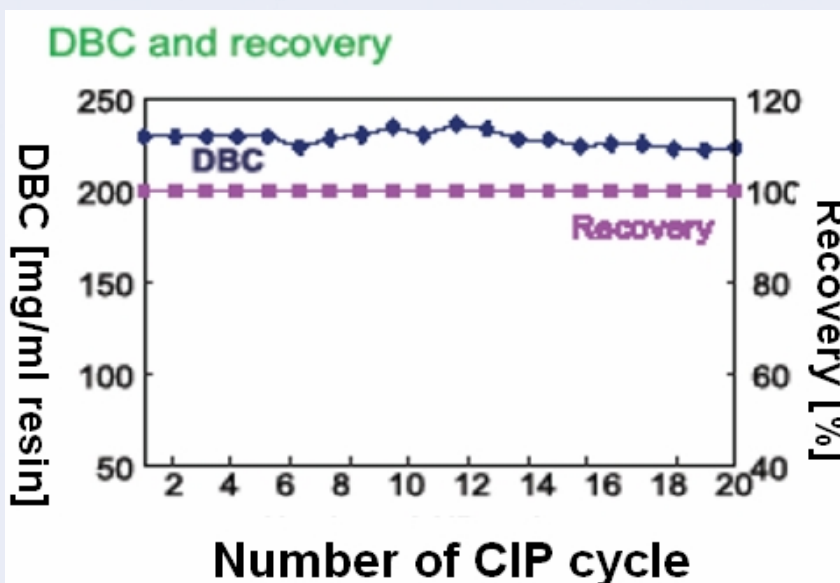


Figure 2: Values for DBC and recovery after each CIP cycle



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This demonstrates that the use of 5 CV of 1M NaOH at a flow rate of 400 cm/h is a suitable CIP procedure for BioPro S. Furthermore, the experiment shows that the YMC BioPro S75 material withstands at least 20 such CIP cycles, whilst retaining its high DBC, recovery and separation power.

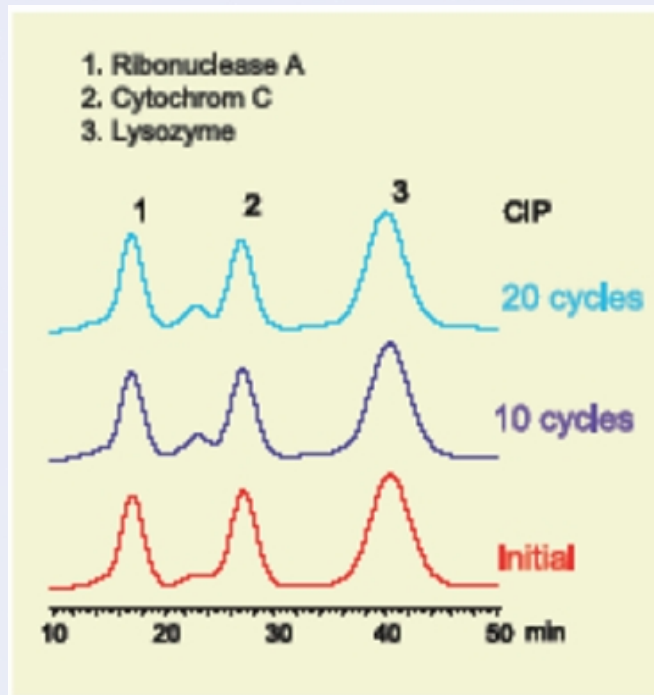


Figure 3: Chromatograms of the initial separation of the protein mixture and after 10 and 20 CIP cycles



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Stability of YMC-BioPro Q75 after contact with 1M NaOH solution for at least 6 hours

For the BioPro Q75 anion exchanger medium, stability under basic conditions was assessed by two separate experiments. In the first, the material was simply kept in 1M NaOH solution for a total of 6 hours. In the second experiment, a column was packed and flushed with 1M NaOH for 6h at a linear flow of 200 cm/h. For both experiments, samples were taken at $t = 0\text{h}$, 2h, 4h and 6h and the retention time for trypsin inhibitor was measured chromatographically. Additionally, for the first experiment, the ion exchange capacity and for the second experiment, the DBC for BSA were determined, respectively. All experiments were performed at room temperature. The time period of 6 hours was assumed to be long enough for most CIP process; however the media is stable towards 1M NaOH for more than 6 hours.

The results obtained are summarized in figures 4 and 5 and tables 1 and 2.

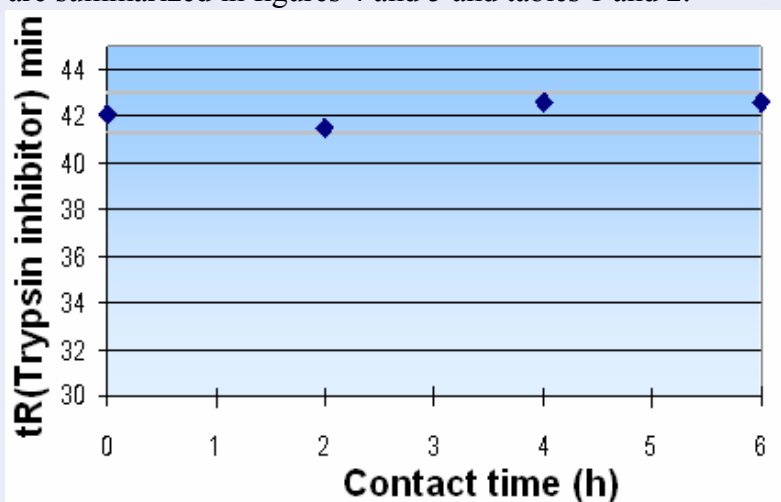


Figure 4 Retention times for trypsin inhibitor measured after 0h, 2h, 4h, and 6h contact time of the medium with 1M NaOH

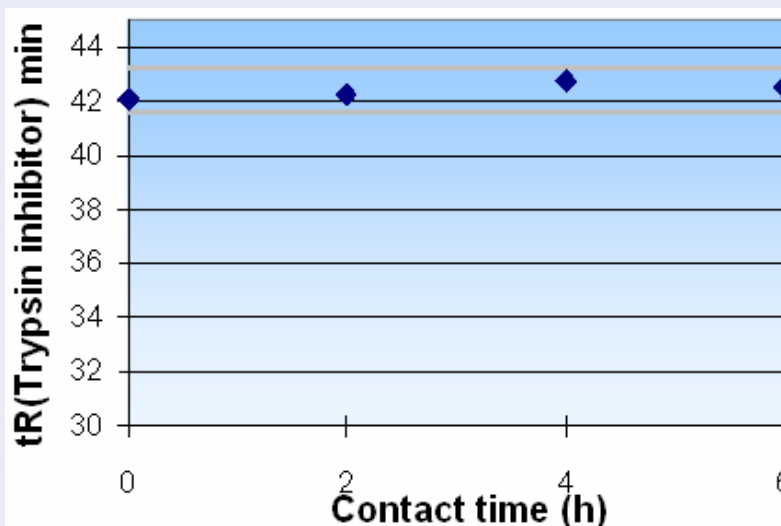


Figure 5 Retention times for trypsin inhibitor measured after 0h, 2h, 4h, and 6h of 1M NaOH solution flowing through the column



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Time (h)	Ion exchange capacity (eq/L)
0	0.16
2	0.16
4	0.16
6	0.16

Table 1 Values for ion exchange capacity following contact times of 0h, 2h, 4h and 6h with 1M NaOH

Time (h)	DBC (mg BSA/ml resin)
0	198.7
2	201.0
4	201.6
6	209.2

Table 2 Values for DBC of BSA following flow of 1M NaOH through the column for 0h, 2h, 4h and 6h

*.the apparent increase of the DBC is fully explained by the experimental error associated with the experimental methods.

As shown above the retention times following treatment with 1M NaOH stay unchanged. In figures 4 and 5 the grey lines are drawn at values 2% below and above the average of the retention times. These 2% may be assumed to be the error associated to the measurement of the retention times. All values are within this 2% level of accuracy. Table 1 shows that the ion exchange capacity is unchanged, whilst table 2 shows that the DBC does not change, remaining at the high values associated with YMC-BioPro Q75 material.

This means that all relevant values remain unchanged during the course of the experiment, proving that YMC-BioPro Q75 is stable towards 1M NaOH at room temperature for at least 6 hours.

Conclusion:

The results obtained show that both YMC-BioPro S75 and YMC-BioPro Q75 materials are stable towards 1M NaOH within the framework of a CIP procedure. Consequently, it can be said that YMC-BioPro IEX media comprehensively fulfil the requirements posed by industrial scale production processes of bio-pharmaceuticals. The resins are available as strong cation or strong anion exchangers with particle sizes of 30 or 75 µm and are produced in batch sizes of up to several hundred litres per lot.