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KANEKA KanCapA™ 3G Technical Note (1)

Evaluation of dynamic binding capacity, host cell protein removal and aggregation reduction properties of KANEKA KanCapA™ 3G protein A affinity resin

Monoclonal antibodies (mAbs) have emerged as clear front runners as therapeutic agents to treat variety of malignancies and form the majority share of most biopharmaceutical companies' product pipeline[1]. Typically, a purified mAb goes through several downstream processing (DSP)[2] steps; and reducing host cell proteins, DNA, viruses, endotoxins and aggregation remains a non-negotiable priority. Harvested cell culture fluid (HCCF) supernatant is subjected to several purification steps including a critical Protein A affinity step. However, advancements in upstream processing has led to higher concentration of mAbs in HCCF[2,3]. Therefore, a high dynamic binding capacity (DBC) affinity resin is a pre-requisite for efficient DSP. Ironically common affinity resins with high enough DBC require strong (low pH) elution conditions which makes the mAbs susceptible to aggregation and further degradation[4].

KANEKA KanCapA™ 3G (KanCapA 3G) is built using a cellulose-based base matrix and new Protein A ligand providing high dynamic binding capacity enabling the purification of mAbs and their Fc fusion derivatives at high titer HCCF. In addition to the enhanced DBC, KanCapA 3G is designed such that it allows the elution of mAbs and Fc fusion derivatives under mild acidic conditions and shows unique host cell proteins (HCPs) and aggregate reduction properties.

In this note we evaluate KanCapA 3G's dynamic binding capacity, elution pH, host cell protein removal and aggregation reduction properties.

Materials and methods

Evaluation of dynamic binding capacity

KanCapA 3G, KANEKA KanCapA™ (KanCapA) and high capacity agarose based (hereafter referred to as "agarose based" in the text) resins were packed into Omnifit column (0.66 cm I.D. x 7 cm height). Dynamic binding capacities of packed resins were measured using the following chromatographic conditions. Each column (1 column volume = 3mL) was equilibrated with 5 column volume (CV) of Equilibration/washing buffer (phosphate buffered saline (PBS) pH 7.4) and 28 CV of IgG solution (3 mg/mL: GAMMAGARD ® in PBS pH 7.4) was loaded at different residence times of 2, 4, 6 and 8 minutes respectively. Unbound materials were washed away with 5 CV of equilibration/washing buffer and bound IgG was eluted with elution buffer (50 mM Acetate pH 3.5). The dynamic binding capacity was calculated at 5% breakthrough.

Determination of elution pH

Eight different antibodies were used in the study: three Humanized IgG1, two Human IgG2, one Fc-fusion, and two Chimeric IgG. Elution pH of KanCapA 3G and agarose based resin were determined with the following chromatography conditions. Each resin was packed into a Tricorn column (0.5 cm I.D. x 15 cm height) that was equilibrated with 5 CV of Equilibration/washing buffer (phosphate buffered saline (PBS) pH 7.4. 0.15 mL of each monoclonal antibody were applied at 10 mg/mL and subsequently washed with 5 CV of citrate buffer, pH 6.0. Bound fractions were eluted using a linear pH gradient of 50 mM citrate buffer from pH 6.0 to 3.0 over 20 CV. Elution pH at the peak was calculated through linear interpolation between the measured pH values of the two buffers used to form the gradient.

Host cell proteins and aggregate reduction

KanCapA 3G and agarose based resin were packed into Omnifit columns (0.66 cm I.D. x 7 cm height). In order to evaluate HCPs and aggregate reduction ability of KanCapA 3G, 9.2 mL of CHO derived clarified cell culture fluid at 1.3 mg/mL IgG1, was loaded onto the column and eluted by a linear pH

gradient using 50 mM citrate buffer, pH 6.0 to 3.0 over 20 CV. Bound fractions were eluted and HCP content of each fraction was measured by ELISA using Cygnus F550 kit while the aggregation content was assessed by Size Exclusion Chromatography (SEC). A TSKgel SuperSW mAb (Tosoh, 7.8 mm × 30 cm) column was equilibrated with 0.2 M sodium phosphate, 0.1 M sodium sulfate, pH 6.7 and a total of 50 µL of affinity purified sample was injected at 0.7 mL/min. Chromatographic progress was monitored by following absorbance at 280 nm. A mAb monomer concentration was estimated by calculating and integrating the peak area under SEC chromatogram of the eluted fractions.

Results & Discussion

Dynamic Binding Capacity

KanCapA 3G, KanCapA and high capacity agarose based resins were compared to evaluate the DBC at 5% breakthrough. As shown in Figure 1 that for a series of biomolecules tested, KanCapA 3G shows remarkable enhancement in DBC. At 4 minutes residence time KanCapA 3G shows approximately 25% increase (>54 mg/mL) in DBC vs KanCapA (~43 mg/mL) and ~9% increase over competitor's agarose based resin (49.8 mg/mL). At 6 minutes residence time, KanCapA 3G shows a DBC of 65.5 mg/mL while the corresponding value for KanCapA and agarose based resin is 50.1 mg/mL and 60 mg/mL respectively. This shows approximately an increase of 30% over KanCapA and ~9% for agarose based resin. Finally at 8 minutes residence time, KanCapA 3G shows a DBC of 71 mg/mL as compared to 51.8 mg/mL for KanCapA and 63.4 mg/mL for competitor's agarose based resin. In general the DBC at 5% breakthrough increases with increase in residence time.

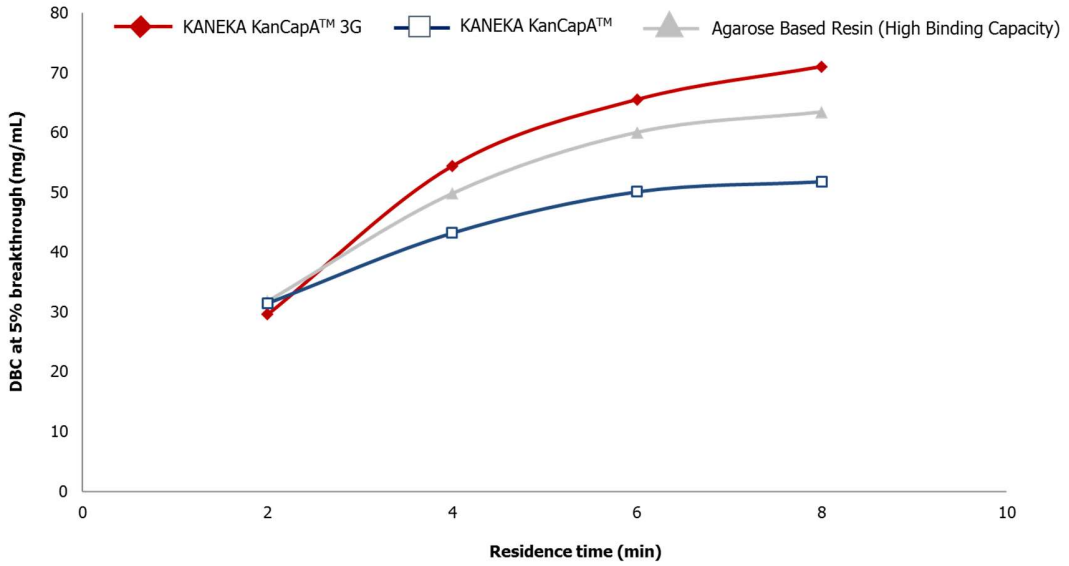

Figure 1. Dynamic binding capacity evaluation at different residence times

Mild Elution pH

Humanized IgG1, Human IgG2, Fc-fusion protein and Chimeric IgG were loaded onto KanCapA 3G, and agarose based resin. As shown in Figure 2 that all 8 monoclonal antibodies used in this study were eluted from KanCapA 3G at a slightly higher pHs (milder) as compared to the agarose based resin. Although there are subtle differences in the elution pH for some molecules (D and E) but overall trends show that elution from KanCapA 3G utilizes mild pH conditions which might be beneficial for maintaining the overall structural integrity of the target molecule.

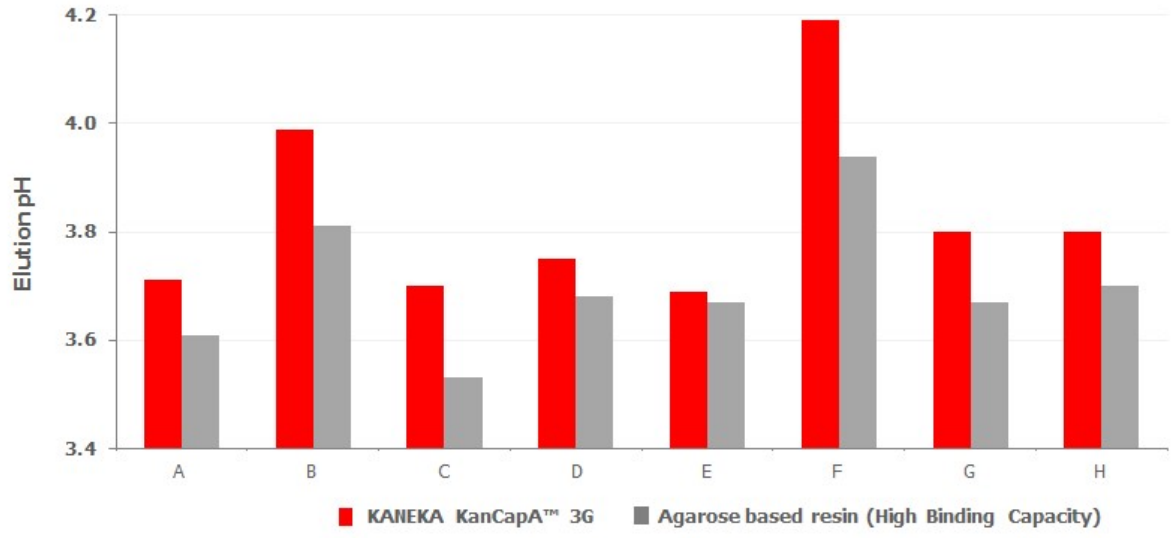

Figure 2. Elution pHs of eight antibodies from KANEKA KanCapA™ 3G compared with high binding capacity agarose based resin. Molecules A, G, H = Humanized IgG1; B, F = Human IgG2; C= Fc-fusion; D, E = Chimeric IgG. Elution pH gradient is using citrate buffer from pH 6 to 3.

Host cell protein removal and aggregate reduction

Due to higher fermentation titer in recent mAb process, the HCCF as loading materials on Protein A chromatography contains high concentration of not only mAb but also HCPs, which increase HCP contents in Protein A elution pool. In addition, low elution pH induces aggregation of target mAb because of its high concentration. Therefore, a major challenge in Protein A chromatography step is to reduce HCPs and aggregation effectively.

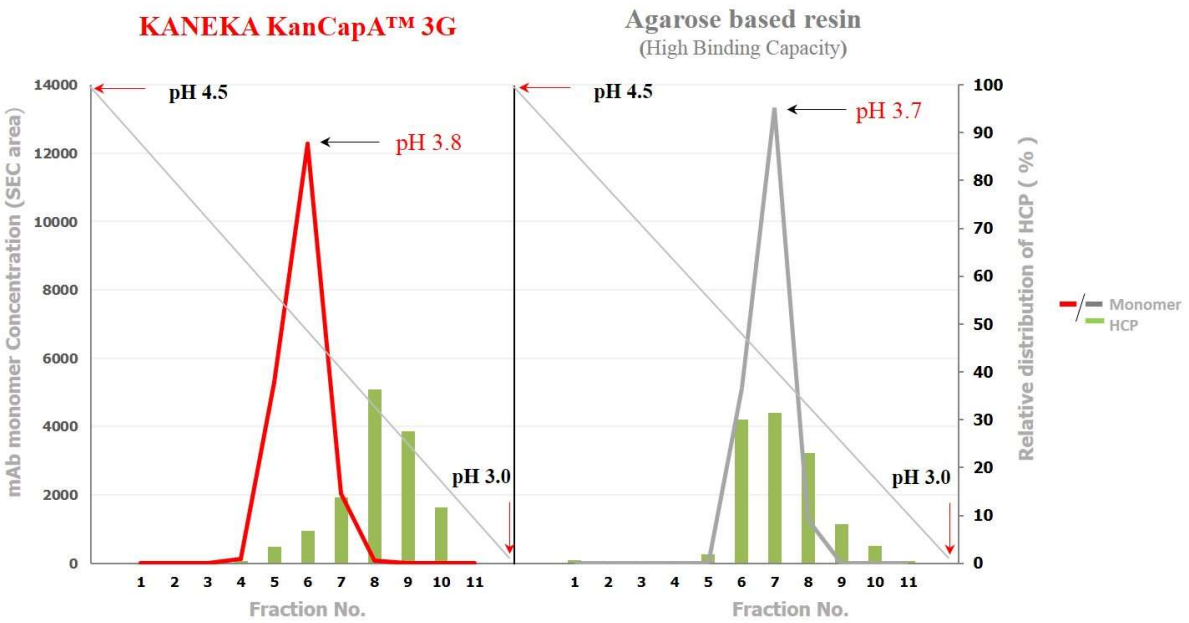

Figure 3. Host cell proteins separation from target mAb using a linear pH gradient

Figure 3 and 4 showed unique separation behaviors of target mAb from HCPs and aggregates of KanCapA 3G as compared to high capacity agarose based Protein A resin. Each fraction from the linear pH gradient elution was evaluated with HCP-ELISA and SEC by which mAb monomer contents of resulting fractions were quantified by calculating the area under peak. Results from Figure 3 and 4 showed that KanCapA 3G was more efficient in HCP and aggregates separation as compared to industry leading Agarose based resin. Although the precise molecular basis of this action is not clear but it is tempting to speculate that the synergistic effects of unique KanCapA 3G ligand design and mild pH elution could be one reason for the separation.

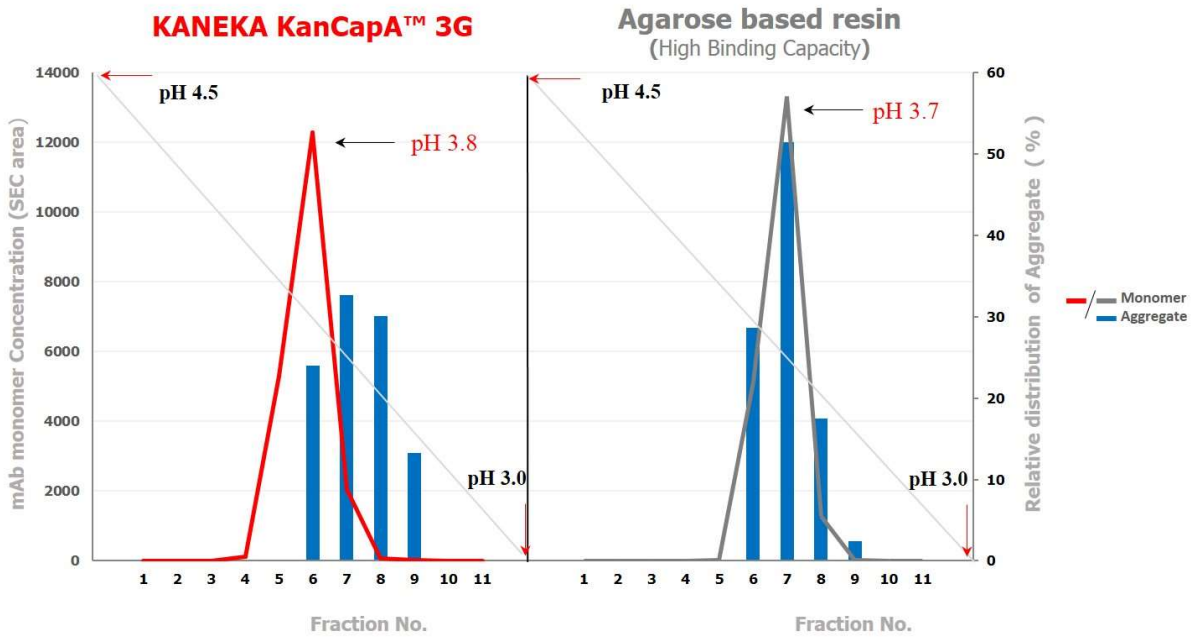

Figure 4. Aggregates separation from target mAb using a linear pH gradient elution.

Conclusions

The data presented in this note reveals that KANEKA KanCapA™ 3G offers a high dynamic binding capacity of more than 60 mg/mL (at 6 min residence time). We show that KANEKA KanCapA™ 3G offers unique binding properties that allow biomolecule elution at mild acidic pH and is efficient in host cell protein and aggregate removal. Taken together, the unique properties of KANEKA KanCapA™ 3G given by its proprietary ligand makes it an attractive resin for monoclonal antibodies manufacturing and is thus poised to help solve challenging downstream bioprocess issues.

References

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