A CFD model for predicting protein aggregation in low-pH virial inactivation for mAb production

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Abstract

Significant amounts of soluble product aggregates were observed in the low-pH viral inactivation (VI) opertion during an initial scale-up run for an IgG4 monoclonal antibody (mAb IgG4-N1). Being earlier in development, a scale-down model did not exist, nor was it practical to use costly Protein A eluate (PAE) for testing the VI process at scale, thus, a computational fluid dynamics (CFD)-based high molecular weight (HMW) prediction model was developed for troubleshooting and risk mitigation. It was previously reported that the IgG4-N1 molecules upon exposure to low pH tend to change into transient and partially unfolded monomers during VI acidification (i.e., VIA) and form aggregates after neutralization (i.e., VIN) (Jin et al. 2019). Therefore, the CFD model reported here focuses on the VIA step. The model mimics the continuous addition of acid to PAE and tracks acid distribution during VIA. Based on the simulated low-pH zone ([?] pH 3.3) profiles and PAE properties, the integrated low-pH zone (ILPZ) value was obtained to predict HMW level at the VI step. The simulations were performed to examine the operating parameters, such as agitation speed, acid addition rate, and protein concentration of PAE, of the pilot scale (50-200L) runs. The conditions with predictions of no product aggregation risk were recommended to the real scale-up runs, resulted in 100% success rate of the consecutive 12 pilot-scale runs. This work demonstrated that the CFD-based HMW prediction model could be used as a tool to facilitate the scale up of the low-pH VI process directly from bench to pilot/production scale.

Introduction

Viral safety and viral clearance procedures are mandatory requirements for protein therapeutics (ICH, 1998). Low-pH viral inactivation (VI), operated in either batch or continuous mode, often follows the Protein A capture step in downstream purification due to the acidic condition of Protein A eluate (PAE) (Parker et al., 2018). A typical low-pH VI operation includes an acidification (VIA) step, where PAE is adjusted to around pH 3.6 and held for up to several hours to achieve sufficient viral inactivation (Brorson et al., 2003; Mattila et al., 2016); and a neutralization (VIN) step, where the VIA pool is adjusted to a pH value close to neutral for further downstream processing (Shukla, Hubbard, Tressel, Guhan, & Low, 2007).

Even though most monoclonal antibodies (mAbs) are stable under low-pH conditions during VIA, it was reported that low-pH conditions induced denaturation and subsequent aggregation of proteins, which are pH sensitive phenomena (Brorson et al., 2003). Protein aggregates have been indicated as one of the main causes for loss of therapeutic activity and adverse immune reactions (Rosenberg, 2006). Therefore, the presence of protein aggregates in biopharmaceutical products has become a main concern for the biopharmaceutical industry and regulatory agencies (Filipe, Kükrer, Hawe, & Jiskoot, 2012). Preventing and monitoring the

formation of product aggregates is critical during process development and manufacturing of mAb products (den Engelsman et al., 2011).

A number of studies have shown that incubation at low pH induces the formation of an aggregate precursor with molten globule-like structures (Bychkova, Berni, Rossi, Kutyshenko, & Ptitsyn, 1992; Muzammil, Kumar, & Tayyab, 1999; Redfield, Smith, & Dobson, 1994). Filipe et al. (2012) described aggregation of an IgG1 under low-pH stress, where the protein molecules converted into transient, partially unfolded monomers when pH was changed from 6.0 to 1.0. The modified monomers then either refolded back to the native state or initiated an aggregation process after changing pH back to 6.0 (Filipe et al., 2012). Skamris et al. (2016) characterized the oligomerization kinetics at pH 3.3 and the reversibility upon neutralization for three immunoglobulin-G proteins (IgGs) representing typical IgG1, IgG2 and IgG4, respectively. Results revealed distinct solution behaviors among the three IgGs. At acidic pH, IgG1 retained monomeric, whereas IgG2 and IgG4 exhibited the formation of transient and partially unfolded monomers, namely oligomers. Subsequent neutralization of PAE caused IgG2 oligomers to partially reverse to the monomeric state, whereas, IgG4 oligomers tended to aggregate (Skamris et al., 2016).

The pH of PAE pool is typically adjusted to 3.5 to 3.8 by addition of an acid solution (Brorson et al., 2003; Shukla et al., 2007) and the VIA condition expectedly should not cause mAb aggregation under the sufficient mixing condition. It held true in bench scale experiments. Using IgG4-N1 PAE, the VI process was developed at bench scale in glass beakers or in Eppendorf tubes up to 50 mL, where sufficient mixing was provided to create a homogeneous pH environment (Jin et al., 2019). Unlike setting for the bench scale VI operation, the flow velocity in a pilot/production scale mixer is usually limited to the laminar regime to minimize shear forces on the product (Parker et al., 2018). This condition is similar to the well characterized mammalian cell culture in large scale bioreactors, where insufficient mixing could lead to pH heterogeneities, characterized as low- or high-pH zone (lower or higher than pH set point) near acid or base addition points (Bylund, Collet, Enfors, & Larsson, 1998; Langheinrich & Nienow, 1999 ; Lara, Galindo, Ramírez, & Palomares, 2006; Xing, Kenty, Li, & Lee, 2009). This led to a hypothesis that a localized low-pH zone ([?] pH 3.3) might exist during VIA caused by poor mixing condition.

Computational fluid dynamics (CFD) modeling has become a useful tool to evaluate the pH heterogeneities in the VI operation to avoid at-scale tests using costly PAE. CFD modeling was employed to quantify residence time distribution in a continuous VI tubular reactor. Based on the criterion of minimum residence time for the tracer fluid elements in the reactor, the geometric and operating parameters, such as pipe length, flow direction, and the use of secondary flows, were optimized to enhance radial mixing (Parker et al., 2018). CFD modeling was also employed to simulate the steady state flow field velocity profiles of the pulse tracer experiments, which helped researchers to understand the complex flow behavior of the reactor for low-pH VI (Francis & Haynes, 2009; Kelly, 2008; Lode, Rosenfeld, Yuan, Root, & Lightfoot, 1998; Manninen, Gorshkova, Immonen, & Ni, 2013). However, those approaches were not able to predict quantitatively HMW level after the VI operation.

Most recently, Jin et al. (2019) employed CFD modeling to demonstrate the presence of low-pH ([?] pH 3.3) zone near the acid addition point of the mixer, which caused formation of a partially unfolded IgG4 monomer during VIA and led to product aggregation during VIN (Jin et al., 2019). This work focus on the details of the modeling methodology. Furthermore, based on the CFD modeling outcomes and constants obtained from protein titration experiments, a HMW prediction model was developed to quantify HMW level after the VI operation in the confined geometry of mixing vessels, where factors such as agitation speed, acid addition rate, and protein concentration were considered. The model was used for troubleshooting an initial scale-up run failed with excessive HMW formation and guiding the subsequent 12 pilot scale (50-200L) runs. This work aimed to explore the application of the HMW prediction model to facilitate the scale up of the low-pH VI process directly from bench to pilot/production scale.

Materials and Methods

Materials for virial inactivation

The IgG4-N1 used in this study was a proprietary engineered mAb from Bristol-Myers Squibb Company. The IgG4-N1 was produced from a Chinese hamster ovary (CHO) cell culture process in XDR-500 bioreactors (Xcellerex, Inc., Marlborough, MA), harvested by depth filtration, sterilized by 0.2 μm membrane filtration, and purified by Protein A affinity chromatography. The IgG4-N1 was eluted from Protein A with PAE materials in the buffer condition of 25 mM Glycine, 10 mM Succinic acid at pH 4.6. The stock PAE contained 99% of monomer and had density and viscosity of 1.0067 g ml^{-1} and 1.297 mPa s^{-1}, respectively. Different concentration of PAE solutions were obtained via either ultrafiltration/diafiltration or dilution of the stock material. All chemicals were purchased from VWR (Radnor, PA).

Mixers for viral inactivation

The 50L and 100L HyPerforma Single-Use Mixer (SUM-50 and SUM-100, Life Technology Corporation, Logan, UT) and 200L XDUO-T Quad Mixing System (XDM-200, Xcellerex, Inc., Marlborough, MA) were used in the VI operation for pilot scale IgG4-N1 production in different facilities. The dimension and geometry of the mixers are presented in Table I. The drawings of a mixer are shown in Figure 1 using SUM-100 as an example. For the SUM-50 and SUM-100 mixing system, the impeller (power number 1.21) was top mounted and inserted into solution at 15°. For the XDM-200 mixing system, the impeller (power number 1.7) was located at the bottom of bag. The VI process was operated in a SUM-100 bag (SH3B10537.01), SUM-50 bag (SH3B10534.01), or XDM-200 bag (888-0684-C) with mixing at an agitation speed. Acid or base was added at a designed addition rate through the reagent addition port. A pH probe was equipped to record online pH values.

The 2L and 20L bioreactors (Applikon, Foster City, CA) were used as mixers for laboratory experiments. The impellers (power numbers of 1.5 and 0.87, respectively) were top mounted and vertically inserted into solution in the biorectors. Acid or base was added at a designed addition rate through the reagent addition port. Vessels were equipped with pH probes to record online pH values.

VI process

The VI operation included acidification (VIA) and neutralization (VIN) steps. At the VIA step, 0.1 N HCl (pH 1.0, density of 0.9998 g ml^{-1}, and viscosity of 1.022 mPa s^{-1}) was added to PAE until reaching pH 3.5-3.8. The acidified PAE was held for a minimum of 60 min to inactivate virus. At the following VIN step, 2 M Tris-Base (pH 11.2, density of 1.056 g ml^{-1}, and viscosity of 1.871 mPa s^{-1}) was added to the VIA pool until reaching pH 5.3-5.7. The VIN pool was held at 4 °C overnight prior to purification. The subsequent downstream purification steps reduced the HMW level from a maximum of 3.5% in the VIN pool to $< 1\%$ in the drug substance to meet the product specification. For process robustness, the in-process criterion for the VIN pool HMW level ($HMW_{\%,\ VIN}$) was $< 2.5\%$ with 1% safety margin.

PAE titration experiments for $MF_{0.1N\ HCl}$ and $M_{pH3.3}$ measurements

Titration experiments to determine mass fraction of 0.1 N HCl *($MF_{0.1N\ HCl}$)* was performed in a 50 mL glass beaker (VWR, 10754–948) with a stir bar (VWR, 58948–138) on a stirrer station (Corning, PC-210) with initial working volume of 30 mL PAE (IgG4-N1 22.0 g L^{-1} to 35.5 g L^{-1}). Acid (0.1 N HCl) was added in 0.3 mL per bolus with the stir speed setting at 5 to 6 (equivalent to 250–300 rpm). The pH value was recorded after each bolus addition. The mass fraction was calculated based on the ratio of accumulated acid addition mass over the total mass of the acidified PAE.

Titration experiments to determine the initial HMW formation (in 10 min) at pH 3.3 ($M_{pH\ 3.3}$) were performed in 1.5 mL Eppendorf tubes (Eppendorf AG, 022363204). The amount of 0.1 N HCl to target pH 3.3 at a given IgG4-N1 concentration were pre-determined. Each pH-adjusted sample was mixed thoroughly by inverting the tube 5–7 times or pipetting up and down 5–7 times upon acid addition. The mixture was held for 10 min (including inverting time). Pre-determined amount of 2 M Tris-Base was then added into mixture to achieve pH 5.3-5.7. The neutralized mixture was held at 4 °C overnight prior to the protein soluble aggregate assay.

Protein soluble aggregate assays

Soluble product aggregates were analyzed on a Waters UPLC system (Waters, Acquity H-Class PLUS) with UV detection at 280 nm, using an Acquity UPLC protein BEH SEC column (Waters, 125 Å, 1.7 μm, 4.6 mm×150 mm) and an Acquity UPLC protein BEH guard column (Waters, 4.6 mm × 30 mm). All samples were 0.2 μm filtered to remove potential large particles prior to applying a total mass of 50 μg protein to the SEC-UPLC system. The mobile phase (200 mM sodium phosphate, 150 mM sodium chloride, pH 6.8) was run at a flow rate of 0.4 mL/min. Results were analyzed using Waters Empower 3 chromatography data system software (Waters, version 3).

Computational fluid dynamics

The CFD simulations were performed using ANSYS software (ANSYS Inc., Cannonsburg, PA). The geometry and mesh of a mixer was generated with Workbench ANSYS 18.1 or newer versions (ANSYS Inc., Cannonsburg, PA). For demonstration, the drawings of a SUM-100 mixer are shown in Figure 1 as an example. The computational domain of a mixer was divided into an inner rotating cylinder zone centered with impeller and an outer stationary zone. The diameter and height of the cylinder rotation zone were [?] 1.2-fold diameter of impeller (D) and [?] 3-fold impeller blade height, respectively. The interface between the rotating and stationary zones was defined as Frozen-Rotor interfaces. Since the impeller has an angle from the vertical center line of the mixer, tetrahedral elements were used in both zones of the mixer. The complete mesh consisted of > 30,000 elements per liter, except for XDM-200 vessel that had 2,647 elements per liter due to its simple cubic shape. In all cases, the minimum orthogonal quality was > 0.15 and the maximum skewness was < 0.90 to ensure acceptable mesh quality.

It should be noted that two outlets shown in Figure 1 do not really exist in a mixer and they were added as the outflow boundaries to drain out excessive fluid due to acid addition and keep a constant working volume for simulation. The port diameter of an outlet equals to that of the acid inlet. For these two outlets, one (outlet-1) was located at the liquid surface as the mirror image of the inlet, while the other (outlet-2) was located on the sidewall at the height of the impeller clear distance. The volume fraction of two outlets were determined by simulation tests.

CFD simulations were performed with ANSYS FLUENT 18.1 or newer version using the k -ϵ turbulence model coupled with the species transfer model. The first step simulation started with k -ϵturbulence model using the PAE as the only fluid under the steady condition. All solid walls were no-slip boundaries. The top liquid surface was the boundary of a flat surface with free-slip wall. The agitation speed was assigned to rotation zone and impeller shaft. All other set-up parameters of the first step simulation used FLUENT default values. The solution was converged when all residuals reached < 0.0001 or simulation reached 10,000 iterations. The relax factor of turbulent kinetic energy (k) and turbulent dissipation rate (ϵ) were *the first order of windup* in the first 2,000 iterations, and then shifted to *the secondary order of windup* if the solution was not converged.

After converge of the initial k -ϵ turbulence model, the species transfer model was added for the second step simulation, the VIA simulation. The FLUENT set-up parameters are presented in Table II. The boundary of inlet was changed from *wall* to*mass-flow-rate-inlet* using the PAE as the inlet fluid at the target flow rate, while the boundaries of outlet-1 and outlet-2 were changed from *wall* to *outflows* with volume fractions of designed values. The simulation was performed under steady condition for 2,000 iterations. This transient sub-step was required to introduce and stabilize inlet flow. The simulation was then shifted into the transition condition and the inlet fluid was changed from PAE to 0.1 N HCl. The simulation time step was 0.05 s. At each time step, the solution was converged when the values of all residuals reached $< 5 \times 10^{-5}$ or iterations reached 20.$MF_{0.1N\ HCl}$ was defined as the volume-averaged mass fraction of 0.1 N HCl and its value was recorded at each time step during simulation. Iso-surface of a $MF_{0.1N\ HCl}$value could not be defined yet at 0 s, which would be done at 1 s flow time. The simulation was paused at 1 s flow time. Iso-surface of pH 3.3 was defined by the corresponding $MF_{0.1N\ HCl}$ value as the boundary of low-pH zone. The simulation was then resumed and the vertex-averaged area of pH 3.3 iso-surface ($A_{pH3.3}$) value was recorded at each time step during simulation. The simulation was complete when the flow time reached the VIA duration.

Numerical analysis

The alignment of mixing of different type/scale of mixers was based on equivalent power input per unit volume (P/V). This value were either predicted from CFD simulation or directly calculated from the impeller power number (N_p) provided by the vendor. The moment (M) of impeller surfaces (N m) obtained from the converged first-step model was used to calculate the simulatedP/V value by Eq. 1.

$P/V = 2\pi \bullet N \bullet M/V$ (1)

Where, N is the agitation rate (s^{-1}); $V$is the liquid volume (m^3).

The N_p derived P/V value was calculated by Eq. 2.

$P/V = N_P \rho N^3 D^5/V$ (2)

Where, D is the impeller diameter (m); and ρ is the liquid density (kg m^{-3}).

Results

Development of the CFD model to simulate VIA

A CFD model was initially developed for troubleshooting the excessive product aggregation in the VI operation of the pilot lot, S100_1. The operating condition is presented in Table III. As previously reported (Jin et al., 2019), the lot was performed in a SUM-100 mixer containing 66.5 L of PAE (28 g L^{-1}IgG4-N1, pH 4.6) with a 50 rpm agitation speed and 0.1 N HCl addition at 2.62 L h^{-1} L^{-1} to achieve pH 3.6 (i.e., VIA). The acidified VIA pool was held at room temperature for 60 min for viral inactivation and followed by neutralization to pH 5.5 with 2 M Tris-base (i.e., VIN). The HMW levels of the VIN pool was 7.1%, which exceeded the level specified for in-process pool and led to lot rejection of the final drug substance produced. In the preliminary troubleshooting investigation, experiments and the CFD model simulation suggested that the localized low-pH ([?] pH 3.3) zone during VIA was the root cause for the product aggregation.

The CFD model for VIA simulation was modified from the well-established$k$ -ε turbulence model coupled with the species transfer model in literature (Spann et al., 2019). Unlike the original model that used a single bolus tracer addition to simulate mixing time, the modified model mimicked continuous addition of 0.1 N HCl to simulate VIA. To develop the new model, simulations were performed to screen different volume fractions and the screening criterion was based on the agreement between the model predictions and the experimental pH values at the end of the VIA step (EOVIA).

The pH of the acidified PAE resulted from the amount of 0.1 N HCl added, i.e. 0.1 N HCl mass fraction ($MF_{0.1N\ HCl}$) of the acidified PAE pool as shown in Figure 2A. The data showed a linear correlation between $MF_{0.1N\ HCl}$ and the pH of the acidified PAE pool. Furthermore, this correlation was protein concentration dependent. As the protein concentration increases, so does the requirement of 0.1N HCl for a given pH adjustment. For example, it required $MF_{0.1N\ HCl}$ of 0.078 to bring pH down from 4.45 to 3.65 for the protein centration of 22 g L^{-1}, while it required $MF_{0.1N\ HCl}$ of 0.115 to achieve the same pH adjustment for the protein concentration of 35 g L^{-1}.

The simulation results for screening volume fraction of outflows are shown in Figures 2B-2D. Since the overall volume fractions was the unit for the two outlets, for a given outflow-1 volume fraction (VF_{out1}), the outflow-2 volume fraction (VF_{out2}) equals to 1 - VF_{out1} . Therefore, onlyVF_{out1} needed to be tracked for data analysis. The screening was first performed with simulations for lot S100_1, of which EOVIA $MF_{0.1N\ HCl}$ was 0.1215. TheVF_{out1} value was initiated from 0.1, and stepwise (0.1 per step) increased to 0.9 in the simulations. As shown in Figure 2B, there was a clear linear correlation between the simulated EOVIA$MF_{0.1N\ HCl}$ and VF_{out1}value. The best agreement between the simulation and the experimental EOVIA $MF_{0.1N\ HCl}$ was observed at$VF_{out1} = 0.7$ (0.1214 of simulation vs. 0.1215 of measurement). Using $VF_{out1} = 0.7$ for lot S100_1, the simulated $MF_{0.1N\ HCl}$ profile appeared to overlap with the experimental profile as shown in Figure 2C.

To consolidate the CFD model with $VF_{out1} = 0.7$ for VIA simulation, the model was applied to 5 additional VI runs in 2L or 20L Applikon reactors. The conditions for VI experiments are presented in Table III. The experimental and simulation results are shown in Figure 2D. The variations of the simulations and the experimental EOVIA $MF_{0.1N\ HCl}$ values were within 10%, demonstrating that the CFD model was generally applicable to mixers of different types and scales for VIA simulation.

In summary, the modified CFD model for VIA simulation was the$k$ -ϵ turbulence model coupled with the species transfer model, which facilitated continuous tracer addition and the constant working volume during simulation using two outflows. The preferred VF_{out1} and VF_{out2} are 0.7 and 0.3, respectively.

Defining ILPZ as the CFD modeling output to predict HMW level

The integrated low-pH zone (ILPZ) is the critical value to predict HMW level in the VI operation. The variables and constants of the equation for ILPZ calculation were studied. It was reported that although product aggregates were detected in VIN pools, their precursors in deed were generated during VIA due to the exposure of the PAE pool to the localized low-pH zones (pH [?] 3.3) (Jin et al., 2019). Therefore, $A_{pH3.3}$ was defined as the boundary of low-pH zone as the critical variable of the equation for ILPZ calculation.

The growth of low-pH zones ([?] pH 3.3) in the acidified PAE pool during VIA are shown in Figures 3A-3B using lots S100_1 and A2_1 as examples. The conditions of these two lots are presented in Table III. Both of two lots had high HMW levels ([?] 7%) in VIN pools. The$A_{pH3.3}$ profiles of all training runs are shown in Figure 3C, indicating that the two examples are representatives of two types of the training runs. In the case of S100_1,$A_{pH3.3}$ increased 17-fold from 0.00233 m^2 at 1 s to 0.0390 m^2 at 192 s. Exposure to a large low-pH zone even with only a short duration caused excessive product aggregation. Conversely, in the case of A2_1,$A_{pH3.3}$ increased 5-fold from 4.34×10^{-5} m^2 at 1 s to 2.20×10^{-4} m^2 at 2,580 s. The long exposure duration to the low-pH zone also caused excessive product aggregation. Taking into consideration both the size of and the exposure duration to the low-pH zone, a time integral of low-pH zone over the VIA duration was chosen as the backbone of the equation for ILPZ calculation.

The time span between the instant $MF_{0.1N\ HCl}$($MF_{0.1N\ HCl,t}$) and $MF_{pH3.3}$also affected the growth of low-pH zone. As shown in Figure 3D, linear$MF_{0.1N\ HCl}$ growth profiles were observed during VIA in all training runs. Diffusion of 0.1 N HCl into PAE is driven by background HCl concentration, presented as $MF_{0.1N\ HCl,t}$. The closer $MF_{0.1N\ HCl,t}$ approaches to$MF_{pH3.3}$, the slower 0.1 N HCl diffuses to PAE, which was the main factor that caused the expansion of the low-pH zone with the progress of VIA processing. Taking into consideration the exponential growth of $A_{pH3.3}$ shown in Figure 3C, the variable $MF_{0.1N\ HCl,t}$ and constant$MF_{pH3.3}$ were combined into the term$MF_{pH3.3}$ /($MF_{pH3.3}$ −$MF_{0.1N\ HCl,t}$) in the equation for ILPZ calculation.

As previously reported, HMW formation also impacted by the PAE protein concentration (Jin et al. 2019). Titration experiments were performed to determine HMW level (%) after a 10 min hold at pH 3.3 ($M_{pH3.3}$). This value was used as the measure of initial HMW formation rate. It appears that there was the secondary order correlation between $M_{pH3.3}$ and protein concentration as shown in Figure 4A. For a given VIA run, the$M_{pH3.3}$ value would be determined from the correlation curve and used as a constant in the equation for ILPZ calculation to reflect the impact of PAE concentration.

Based on the above data analysis, Eq. 3 was defined to calculate ILPZ, which captures the CFD modeling output and PAE property. The ILPZ profiles are shown in Figure 3E. Exponential growth of ILPZ profiles were observed during VIA. The total HMW amount of the VIN pool ($HMW_{total,VIN}$, g) of the training runs are shown in Figure 3F. It appears that the higher ILPZ value corresponds to the higher $HMW_{total,VIN}$.

$$\text{ILPZ} = \sum_{t=0}^{n} M_{\text{pH3.3}} \bullet A_{pH3.3}^{1.5} \bullet t \bullet F_{pH3.3}/(F_{pH3.3} - \text{MF}_{0.1N\ HCl,\ t}) \quad (3)$$

Establishing equations to predict HMW formation

The HMW prediction equations were then developed using the training data set of 6 VI runs as presented in Table III. Considering that the HMW assay variation could cause a relative standard deviation (RSD) of 25% for samples containing the VIN pool HMW level ($HMW_{\%,VIN}$) of 1%, the lots selected as the training

data set had a criterion of $> 2.8\% HMW_{\%,VIN}$ in order to ensure a RSD of $< 10\%$. The training data set included VI runs using different agitation speeds and acid addition rates in mixers of various scales and types.

The values of ILPZ and $HMW_{total,VIN}$ of the training runs were plotted in Figure 4B, which suggests an exponential correlation between ILPZ and $HMW_{total,VIN}$ as defined by Eq. 4. The $HMW_{total,VIN}$ was then converted into $HMW_{\%,VIN}$ using Eq. 5, where the C_{PAE} and V_{PAE} represent protein concentration (g/L) and volume (L) of the PAE pool, respectively.

$$\mathrm{HMW_{total.VIN}} = 2.364e^{1.179IPTZ} \quad (4)$$

$$\mathrm{HMW_{\%,VIN}} = 100 \bullet \mathrm{HMW}_{total,VIN} / \left(C_{\mathrm{PAE}} \bullet V_{\mathrm{PAE}}\right) \quad (5)$$

The model predictions and the experimental $HMW_{\%,VIN}$ values of the training runs are presented in Table III. The model predicted $HMW_{\%,VIN}$ values ranged from 2.7% of Lot A20_1 to 7.9% of Lot S100_1, which were in good agreement with the experimental $HMW_{\%,VIN}$ values (2.9% of Lot A20_1 and 7.1% of Lot S100_1).

Furthermore, the simulation of lot S100_1 not only predicted well the $HMW_{\%,VIN}$ value but also demonstrated the time cause of low-pH zone growth as previously shown in Figure 3A, suggesting that product exposure to the localized low-pH zone resulted from poor mixing were the root cause of the HMW formation observed during the VI operation.

HMW prediction model verification and application to VI process scale up

To verify the HMW prediction model, different pilot VI runs (50-200L) were used as the validation data set. The data set covered 7 conditions and 12 runs. For some conditions, multiple runs were performed using the same operating parameters in the same mixer. The PAE protein concentration, working volume, agitation speed, and the acid addition rate were taken into consideration as the operating parameters, which were examined by the model simulations to mitigate the risk of excessive product aggregation ($HMW_{\%,VIN}$ of $<$ 2.5%).

The geometry and operating conditions of the validation runs are presented in Table IV. The EOVIA $MF_{0.1N\ HCl}$ values were first examined to validate the CFD model for VIA simulation. As presented in Table IV, variation between the model predictions and the experimental EOVIA $MF_{0.1N\ HCl}$ values were $< 9\%$. Like trends of the training runs, a linear growth profile of $MF_{0.1N\ HCl}$ was observed in a validation run as shown in Figure 5D.

The low-pH zone profiles were then examined to validate Eq. 3. The growth of the low-pH zones was visualized by CFD simulations shown in Figures 5A and 5B using the conditions of S100_2 and X200_1 as examples. Quantitatively, the $A_{pH3.3}$ and ILPZ profiles are shown in Figures 5C, 5E. Across the validation runs, the EOVIA $A_{pH3.3}$ values were of comparable magnitude in the order of 10^{-4} m^2 and much less than the value of failed pilot lot S100_1 (0.0399 m^2). Nevertheless, like the trends of training runs, exponential growth profiles of both $A_{pH3.3}$ and ILPZ were observed in all validation runs.

The $HMW_{\%,\ VIN}$ values were eventually examined to validate the HMW prediction model. The $HMW_{total,VIN}$ values of the validation runs are shown in Figure 5F. The difference between the model predictions and the experimental $HMW_{\%,\ VIN}$ values ranged from 0.03% in X200_3 to 0.98 % in X200_1 as presented in Table IV. Furthermore, according to the model simulations, all 12 validation runs would have $HMW_{\%,\ VIN}$ of [?] 1.41%, which met the in-process criterion of $< 2.5\%$ $HMW_{\%,\ VIN}$. Agreed with the model predictions, the 12 validation runs did achieve [?] 2.0 % $HMW_{\%,\ VIN}$ with a 100% success rate.

These results suggested that the model developed from training runs were applicable to the validation runs for mitigating the risk of excessive HMW formation. This case also demonstrated the application of the HMW prediction model to facilitate the VI process scale up directly from bench to pilot/production scale.

Discussion

We reported here on the CFD-based HMW prediction model. The model includes two components. The first component is the CFD model for VI simulation, which provides profiles of $A_{pH3.3}$ and$MF_{0.1N\ HCl}$ to calculate ILPZ. The second component is the HMW predicting equations from ILPZ. The model was established using a training data set of 6 VI runs and verified with a validation data set of 12 VI runs.

The CFD model for VIA simulation was described in our previous report (Jin et al., 2019). We report here the details of the modeling methodology. Furthermore, in the previous work, the volume fractions of the outflows in the CFD model were fixed, where VF_{out1} of 0.1 was used. The model provided acceptable accuracy to simulate VIA ([?] 5% variation between the predictions and the experimental EOVIA $MF_{0.1N\ HCl}$) in SUM-100 and SUM-50 mixers (n = 3) (Jin et al., 2019). Being screened whole spectrum of VF_{out1} values (0.1 - 0.9), theVF_{out1} of 0.7, appeared to applicable to broader types/scales of VI mixing vessels, including SUM-100, SUM-50, XDM-200, Applikon 20L, and Appilkon 2L mixers (n = 18).

The CFD model for VIA simulation was modified from the original species transfer model (Spann et al., 2019), of which the tracer was patched to the fluid and distribution of the tracer was then tracked in a duration for mixing time calculation. This bolus addition model is not the case of VI condition at pilot/production scale, where acid is typically continuously added during VIA. To simulate volume expansion due to acid addition during VIA, one thought was to use the gas-liquid two-phase model and patch air in headspace that would enable liquid expansion during simulation. However, the ANSYS FLUENT software is incapable to run the species model coupled with the multiple phase model. To have continuous acid addition in the liquid phase model while avoid volume expansion, two outlets boundary was introduced into the modified model. With this modification, the CFD model is capable to simulate the VIA processing with the continuous acid addition.

This work aims to mitigate the risk of product aggregation in the VI operation. This would ensure the designed protein loading at subsequent chromatographic polishing steps while achieving satisfactory step yield, final product quality, and overall robust downstream performance. A purification platform for mAbs typically includes two chromatographic polishing steps after VI operation (Fahrner et al., 2001; Shukla et al., 2007), e.g., AEX/HIC, AEX/CEX, and etc. (Shukla, Leslie, Wolfe, Mostafa, & Norman, 2017). In the case here, a polishing step yield was positively correlated the protein loading but inversely correlated with $HMW_{\%,\ VIN}$ (data not shown). The reliable control of $HMW_{\%,\ VIN}$ to $< 2.5\%$ significantly improved downstream throughput, resulting in substantial reduced cost of goods of the manufacturing.

We demonstrated here on the application of the CFD-based HMW prediction model to assist troubleshooting and guide scale-up of the VI process. Like other common situations during commercial process development, representative scale down model (SDM) was not established yet when the initial scale-up run was operated. The at-scale test using PAE was impractical due to prohibitive study cost and large material requirement (Kateja, Kumar, Sethi, & Rathore, 2018). In this work, the CFD-based HMW prediction model was used to quantify and minimize the localized extreme low-pH zones, especially for large-scale VI operation, to reduce the mAb aggregation risk. Agreed with the model predictions, the validation data set of 12 pilot runs (50-200 L scale) achieved 100% success rate. The results suggested that the CFD-based HMW prediction model may be general applicable to optimize the scale-up parameters of VI process from bench to pilot/production scale for mAbs and potential biologics of other modalities.

Symbols and Nomenclature:

$A_{pH3.3}$ area of pH 3.3 iso-surface (m^2)

AEX anion-exchange chromatography

CEX cation-exchange chromatography

CFD computational fluid dynamics

D diameter of impeller (m)

EOVIA the end of VI acidification

$MF_{pH3.3}$ $MF_{0.1N\ HCl}$ at pH 3.3

HMW high molecular weight

$HMW_{\%,VIN}$ the VIN pool HMW level

$HMW_{total,VIN}$ the total HMW of the VIN pool (g)

HIC hydrophobic interaction chromatography

ICH International Council for Harmonization

IgG immunoglobulin G

ILPZ integrated low-pH zone

k turbulent kinetic energy (m^2s^{-2})

mAb monoclonal antibody

M moment (N m)

$MF_{0.1N\ HCl}$ 0.1 N HCl mass fraction

$M_{pH3.3}$ HMW level (%) after a 10 min hold at pH 3.3

Np impeller power number

N agitation speed ($m\ s^{-1}$)

PAE Protein A eluate

$P\ /V$ power input per unit volume ($W\ m^{-3}$)

T diameter of vessel (m)

SDM scale down model

SEC size exclusion chromatography

VF_{out1} volume fraction of outflow 1

VF_{out2} volume fraction of outflow 2

VI viral inactivation

VIA acidification of the viral inactivation operation

VIN neutralization of the viral inactivation operation

ρ liquid density ($kg\ m^{-3}$)

ϵ turbulent dissipation rate (m^2s^{-3})

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