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Integrated micro-scale protein a chromatography and Low pH viral inactivation unit operations on an automated platform

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Abstract

High throughput process development (HTPD) is established for time- and resource-efficient chromatographic process development. However, integration with non-chromatographic operations within a monoclonal antibody (mAb) purification train is less developed. An area of importance is the development of low pH viral inactivation (VI) that follows protein A chromatography. However, the lack of pH measurement devices at the micro-scale represents a barrier to implementation, which prevents integration with the surrounding unit operations, limiting overall process knowledge. This study is based upon the design and testing of a HTPD platform for integration of the protein A and low pH VI operations. This was achieved by using a design and simulation software before execution on an automated liquid handler. The operations were successfully translated to the micro-scale, as assessed by analysis of recoveries and molecular weight content. The integrated platform was then used as a tool to assess the effect of pH on HMWC during low pH hold. The laboratory-scale and micro-scale elution pools showed comparable HMWC across the pH range 3.2–3.7. The investigative power of the platform is highlighted by evaluating the resources required to conduct a hypothetical experiment. This results in lower resource demands and increased labor efficiency relative to the laboratory-scale. For example, the experiment can be conducted in 7 h, compared to 105 h, translating to labor hours, 3 h and 28 h for the micro-scale and laboratory-scale, respectively. This presents the opportunity for further integration beyond chromatographic operations within the purification sequence, to establish a fit-to-platform assessment tool for mAb process development.

KEYWORDS
downstream processing, high throughput process development, micro-scale, monoclonal antibodies, scale-down

1 | INTRODUCTION

The clinical and commercial success of mAbs has created a need to develop economic, efficient, and scalable production processes. MAb downstream unit operations require significant investment in development, scale-up and validation. Therefore, an increase in efficiency, and a decrease in time and costs is a priority.[1] To address this need, high throughput process development (HTPD) has established itself as a powerful technology to accelerate development and optimization, and has shown particular promise when employed in mAb downstream processes. Current HTPD tools provide a platform approach for time- and resource- efficient chromatographic process development, through implementation of miniaturization, automation, and parallelization.[2]

High throughput chromatographic techniques are not defined by a single format nor scale. These tools include, microtiter filter plates, pre-packed micropipette chromatography tips, and pre-packed miniature packed-bed chromatography columns, all of which have successfully been employed in the development and optimization of chromatographic separations.[2–4] However, these tools are suitable for different applications; for example, microtiter filter plates were used for investigating semi-equilibrium conditions through the assessment of adsorption conditions on an objective, such as improving binding capacity or impurity removal.[5] Several other screening studies which use these 96-well filter plates have also been reported, for example operating condition screening[6] in cation exchange chromatography (CEX)[7] and hydrophobic interaction chromatography (HIC)[8] processes, as well as estimation of dynamic binding capacities.[9] The pre-packed micropipette chromatography was successfully employed in protein adsorption studies, and even in the development of a chromatography step for the purification of virus like particles.[4] Pre-packed miniature chromatography columns provide a closer representation of a packed-bed chromatography column, in terms of geometry and unidirectional flow, residence times, and protein loading capacities.[10–12]

However, the purification sequence does not only comprise the chromatographic operations in isolation, but instead is formed of several unit operations in sequence, each of which targets specific impurities and contaminants. Therapeutic mAbs are expressed in mammalian cells, which involves transfection of the genomic DNA of interest into the chosen cell, for example Chinese Hamster Ovary (CHO).[13] The transfection method occurs through viral means, which therefore poses the risk of viral contamination of the mAb product.[14] Consequently, viral inactivation must be performed in the mAb manufacturing process. While several methods of inactivation exist, such as solvent or surfactant-based methods, pasteurization, and the use of dry heat, low pH VI has established itself as a robust method for dealing with enveloped viruses encountered in mAb processing.[15] This consists of adjusting the process intermediate to the target pH, typically ≤3.6,[16] before incubating for a period of time. Low pH VI is commonly integrated with the preceding protein A chromatography operation since elution of the mAb from the column occurs at a pH value between 3 and 4.[17] Following the inactivation, the pH of the process intermediate is then increased to a value of ≥4.5, prior to further downstream purification.

mAbs are most stable in their native, monomeric state,[18] but under certain conditions, they are prone to forming non-native assemblies called as the aggregates,[19] the extent of which differs for each mAb. Aggregates are large assemblies of denatured antibody molecules that are irreversibly formed due to the biochemical and biophysical properties of the mAb itself, as well as the physiochemical environment it is exposed to during the production process.[20] Protein aggregation remains a major concern, which can negatively impact quality attributes of the mAb process such as manufacturing, titre, stability, and immunogenicity.[18,21] Therefore, (i) the process parameters must be carefully chosen in order to reduce the formation of, and (ii) the appropriate unit operations must be selected in order to remove, mAb aggregates.

Despite being a ubiquitous operation for mAb processing, the availability of micro-scale platforms for the design and investigation of low pH VI is still lacking to the best of our knowledge. This is primarily due to the absence of micro probes currently in the market, which can detect pH at the required ranges, and can also be integrated into an automated liquid handler. Consequently, inactivation variables, such as pH, conductivity, and incubation time, and their effect on protein aggregation, cannot be investigated in a high throughput platform.[14] This also prevents integration of the low pH VI operation within the purification sequence, and therefore, investigation of process intermediates.

This study demonstrates the integration of the protein A chromatography and low pH VI operations onto an automated micro-scale platform. The scale transfer was achieved by translating laboratory-scale workflows into automated workflows using Synthace Workflow Builder, before executing on the Tecan Freedom Evo 200. This platform was then used as tool to investigate the aggregation behavior of an aggregate-prone mAb during an integrated low pH VI operation, specifically, the effect of pH on aggregate formation. This paper also discusses the advantages of integrating this operation within the mAb purification sequence (Figure 1), in an effort to increase process understanding to inform on process development.

2 | MATERIALS AND METHODS

2.1 | Material source

The material source, clarified harvest of a fully human IgG1κ monoclonal antibody targeting the α-subunit of human interleukin-3 receptor or CD123, was provided by CSL Behring (Marburg, Germany). The mAb is expressed in CHOK1 SV cells using the GS expression system (Lonza Biologics, USA).

2.2 | Micro-scale protein A chromatography workflow design and task execution

The micro-scale protein A and low pH viral inactivation operations was designed and constructed using Synthace Workflow Builder

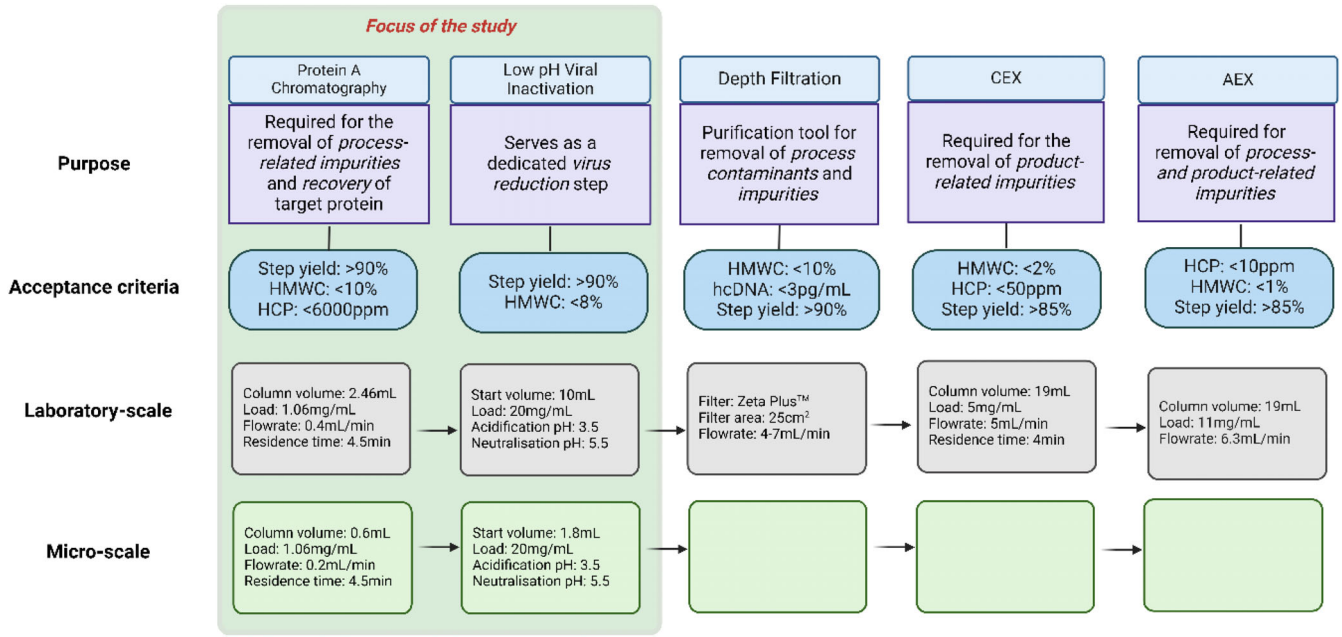

FIGURE 1 Process flowsheet of a monoclonal antibody downstream process into which the low pH viral inactivation operation will be integrated within. The purification sequence highlighted in gray and green represent the laboratory-scale and micro-scale, respectively, with the specified process parameters for each unit operation. The objective of each operation is also presented which emphasizes the importance of each within the sequence. The critical quality attributes (CQAs) for each micro-scale operation are shown. These form the criteria for which the output material must satisfy in order to demonstrate the equivalence between the micro-scale and laboratory-scale unit operations. The operations highlighted in green represent those which are investigated in this study, whereas the remaining stages will be the focus of a future study. Figure created with BioRender.com.

(v.22.10.01) (Synthace, United Kingdom); the protocol of which was based upon an existing laboratory-scale operation.[22] Once successfully simulated, the workflows were then scheduled for execution through the SynthaceHub (Synthace, United Kingdom) on the Tecan Freedom EVO® 200 liquid handler, running EVOware (v2.8.36.69–Service Pack 4) (Tecan, Switzerland). The deck layout used for the operations is presented by Figure 2a.

The layout of the deck was specified within EVOware (Tecan, Switzerland) and utilized in Synthace Workflow Builder (Synthace, United Kingdom) upon defining the labware, consumables and material. This ensures all operations were conducted robustly and reliably. The implementation of micro-scale protein A chromatography closely mimics that of laboratory-scale chromatography across the protocol steps, including (1) equilibration; (2) loading; (3) post-load wash; (4) wash; (5) elution; (6) regeneration; (7) sanitization; (8) requilibration and; (9) storage. However, the involved experimental setup differs between the two due to the fact that the transfer of solutions to the RoboColumns® (Repligen, USA) is conducted in a discrete, as opposed to, continuous manner. Therefore, these are aspirated within and between the columns by each of the eight channels of the LiHa, which ultimately plays the role of a simple inlet and outlet pump. The RoMa is responsible for moving specific plates to defined locations on the deck. Finally, the integrated plate reader fulfills the role of the detector, analyzing the plates according to a pre-defined protocol; the results of which are accessed through the Synthace Data Visualization Tool (Synthace, UK). The protein A operation will be conducted before the execution of the low pH VI step. The process flowsheet of the low pH VI step is presented in Figure 2b, and the corresponding location of each stage is highlighted in Figure 2a.

2.3 | Sample preparation and protein A chromatography

This operation utilized 8 OPUS® RoboColumns® (Repligen, USA) prepacked with MabSelect SuRe resin (Cytiva, USA). The column parameters are presented by Table 1.

The starting material for this operation was 13.9 mL of clarified harvest per column (1 g/L load), which was thawed in a water bath at 25°C, before being placed, along with the buffers, onto the deck of the Tecan Freedom EVO® 200 (Tecan, UK).

Once the task was executed on the SynthaceHub (Synthace, UK), the actions performed by the robotic station were as follows: equilibration, load, post-load wash, wash, elution, regeneration, re-equilibration, neutralization, and storage. The Liquid Handling Arm (LiHa) is responsible for aspirating and dispensing the material within and between the labware; 100 mL trough for each of the pre-prepared buffers and 96 deep-well plate for collection of the eluate, and the Robot Manipulator Arm (RoMa) is required for plate positioning prior to the collection of the eluate. The eluted fractions were collected for analysis, but only column volume 1 and column volume 2 were processed through the low pH VI operation.

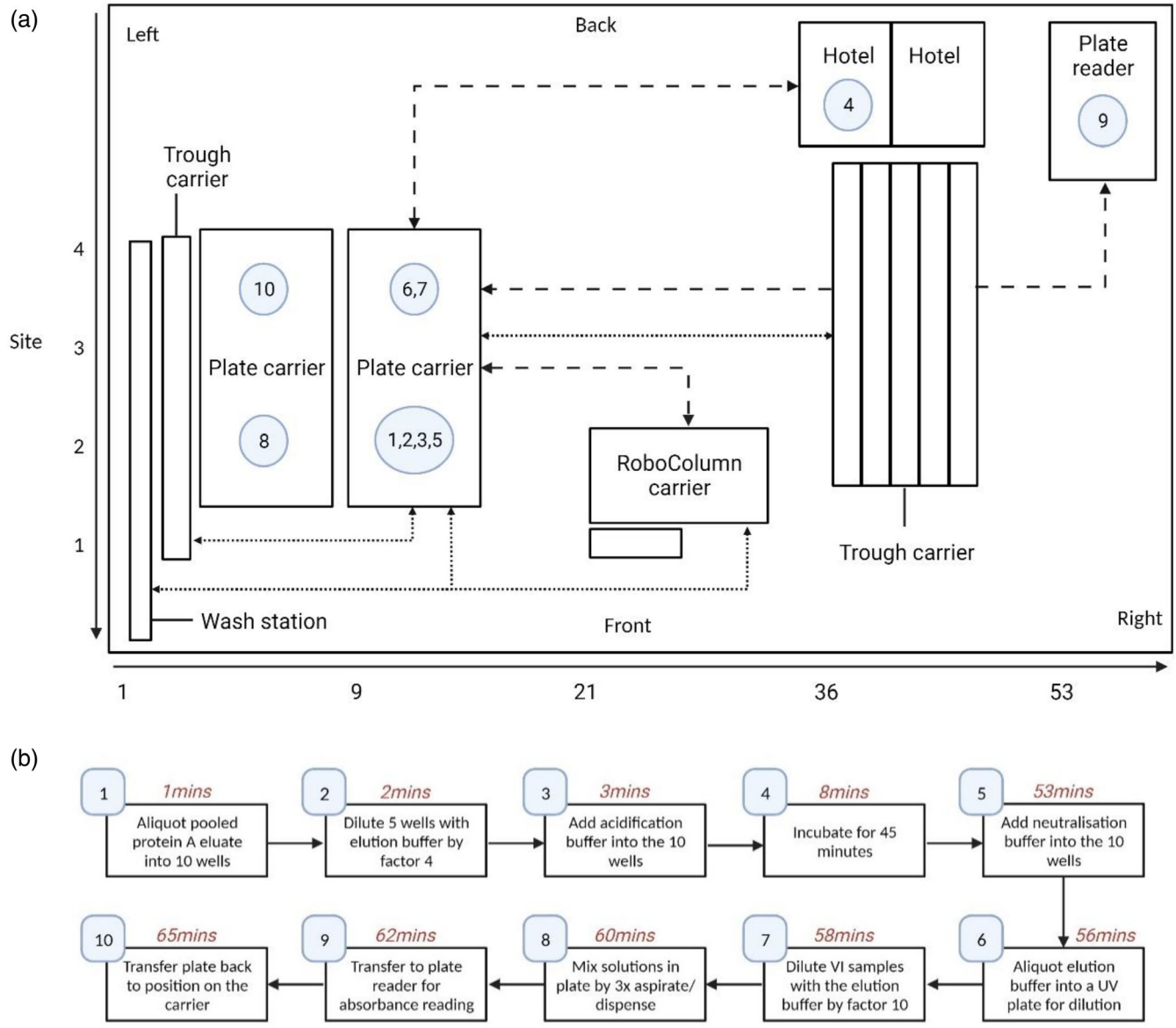

FIGURE 2 The Tecan Freedom EVO® 200 deck configuration for the implementation of the micro-scale protein A chromatography and low pH viral inactivation operations. The x-axis presents the locations of the labware and carriers on the deck, including, wash station, trough carrier for 100 mL troughs, plate carrier for NuncTM 96-Well DeepWellTM, 24-Well DeepWell, and 96-Well UV Transparent Plates, RoboColumn® carrier, hotel for NuncTM 96-Well DeepWellTM and 96-Well UV Transparent Plates, and site for Infinite M200 Pro plater reader. The y-axis corresponds to the site number, which is required for navigation to the specific labware by the Liquid Handling Arm (LiHa) and the Robot Manipulator Arm (RoMa). (a) The dotted lines represent the typical path of the LiHa during an operation, which involves movement of liquid between the wash station, trough carrier, plater carrier, and RoboColumn® carrier. The dashed lines represent the typical path of the RoMa during an operation, which involves movements of the plates between the plate carrier, hotel, and plate reader; (b) process flowsheet for the low pH VI unit operation on the Tecan Freedom EVO® 200. The timepoint and number of each stage in sequence is shown, which corresponds to position on the deck in Figure 2a. Figure created with BioRender.com.

TABLE 1 OPUS® RoboColumn® MabSelect SuRe column parameters utilized for the micro-scale protein A operation.

Column	Inner diameter (cm)	Gel bed height (cm)	CV (mL)	Load Concentration (mg/mL)	Load capacity (mg/mL)	Residence time (min)
MabSelect SuRe	0.5	3	0.59	1.06	25	3.00

TABLE 2 Buffer list for the protein A chromatography operation.

Protein A chromatography stage	Buffer composition
Equilibration	25 mM Sodium phosphate, 150 Mm NaCl pH 7.4
Post-load wash	25 mM Sodium phosphate, 150 Mm NaCl pH 7.4
Wash	50 mM Acetate, pH 5.5
Elution	50 mM Acetate, pH 3.8
Regeneration	0.1 M NaOH, 1 M NaCl
Sanitization	0.5 M NaOH
Requilibration	25 mM Sodium phosphate, 150 Mm Nacl pH 7.4
Storage	20% (w/w) Ethanol

2.4 | Low pH viral inactivation operation

The low pH viral inactivation unit operation was conducted independently, or in sequence, with the protein A chromatography operation. In the latter case, this involved processing the starting material, 13.9 mL of clarified harvest, through each of the 8 MabSelect Sure OPUS® RoboColumns® (Repligen, UK), prior to operation of the low pH viral inactivation. This resulted in 1.8 mL of protein A eluate per RoboColumn which was otherwise processed directly into the low pH VI operation, if conducted independently.

Following the design and simulation of the low pH viral inactivation workflow, the task was executed through the SynthaceHub (Synthace, UK). The actions performed by the robotic station are shown by Figure 2b. This protocol involved diluting five of the wells 1:4 with the protein A elution buffer (50 mM acetate, pH 3.8) prior to the low pH hold. The volume of acidification solution (3 M acetic acid) added to the protein A eluate was adjusted depending on the desired pH to be achieved for low pH hold. For example, ~ 10–12% (w/w) of start material amount was added to achieve a pH of 3.5 through acidification solution, and ~14–15% (w/w) of start material amount was added to achieve a pH of 5.5 through neutralization solution (2 M tris). The pH of the samples was measured off-deck using the pH electrode InLab® Micro (Mettler Toledo, USA) following acidification and neutralization, before being placed back on the deck for further processing.

Bromphenol blue was used to assess the PostMix liquid policy on the Tecan Freedom EVO® 200 (Tecan, UK), for the mixing of the acidification and neutralization solutions during the laboratory-scale low pH VI operation. The PostMix liquid policy is a liquid movement protocol, which is available in the Synthace Workflow Builder (Synthace, United Kingdom) and involves three sequences of aspirate and dispense to the defined solution. Bromophenol blue is an indicator which changes from yellow at a pH below 3 to purple at pH 4.6. The PostMix policy was considered an appropriate method for the mixing of the required solutions based on the change in color of Bromophenol blue.

2.5 | Buffer list

The following buffer compositions in Table 2 were used for the protein A chromatography and low pH VI operations:

2.6 | Laboratory-scale operation

2.6.1 | Protein A chromatography

Laboratory-scale protein A chromatography runs were conducted using the ÄKTA Avant 25 chromatography system (Cytiva, USA). An Omnifit™ EZ Chromatography Column (0.66 cm × 15 cm) (Thermo Fisher Scientific, USA) was packed with MabSelect SuRe (Cytiva, USA). The column parameters are presented by Table 3. The column performance test involved injecting 2% acetone through the sample loop. An asymmetry of 1.07, and height equivalent to theoretical plates (HETP) of 2100, was obtained, highlighting sufficient packing of the column.

The protein A chromatography method was constructed on UNICORN™ (v6.1) (Cytiva, USA) based on the laboratory-scale protocol, and therefore, consistent with the micro-scale operation. The eluted fractions were collected, and analyzed for protein concentration and high molecular weight content (HMWC). Similarly to the micro-scale protein A eluate, only column volume 1 and column volume 2 were processed through the low pH VI operation.

2.6.2 | Low pH Viral Inactivation

The laboratory-scale low pH VI step firstly involved removing 10 mL of the protein A eluate from storage and thawing in a water bath at 25°C. Following this, the eluate was diluted with 50 mM acetate, pH 3.8, to a target conductivity of ≤0.5 mS/cm. The pH was decreased using acidification solution (3 M acetic acid), with stirring at 115 revolutions per minute (rpm), over a time period of ≥15 min to pH 3.5 ± 0.1. The eluate was then held for 45 min, before addition of

TABLE 3 MabSelect SuRe column parameters utilized for the laboratory-scale protein A chromatography operation.

Column	Inner diameter (cm)	Gel bed height (cm)	CV (mL)	Load concentration (mg/mL)	Load capacity (mg/mL)	Residence time (min)
MabSelect SuRe	0.66	7	2.46	1.06	25	3.00

neutralization solution (2 M Tris) to a pH of 5.5 ± 0.1 with stirring of 115 rpm, over a time period of ≥15 min. The eluate was then collected, and analyzed for protein concentration and molecular weight content.

2.7 | Analytical testing

2.7.1 | Protein concentration

Monoclonal antibody concentration was determined using the Infinite M200 Pro plater reader (Tecan, Switzerland). The collected fractions from the protein A chromatography elution, as well as the low pH VI operation were diluted by a factor of 100 in elution buffer, before being transferred to a UV-Star® 96 well plate (Greiner Bio-One, Germany). These were then measured at A280, A320, A900, and A977, which accounts for pathlength correction[23]:

$$\text{Pathlength}\,(\text{cm}) = \frac{A977(well) - A900(well)}{K - Factor} * 10\,\text{mm}, \quad (1)$$

where, 0.173 was assumed for the K-Factor based upon the elution buffer used. The corrected absorbance is given by:

$$A_{\text{corrected}} = \frac{A_{\text{raw}} * K - \text{Factor}}{A_{977} - A_{900}} * 10\,\text{mm}, \quad (2)$$

Mass concentration is then determined by:

$$\text{Mass concentration}\left(\frac{mg}{mL}\right) = \frac{A_{\text{corrected}}}{\epsilon * d}, \quad (3)$$

where, A is absorbance, ϵ is extinction coefficient (molar adsorption coefficient) and d is pathlength (cm). The dilution adjusted mass concentrations were then auto calculated by the Synthace Visualization Tool which required the starting concentration, molecular weight, and molar extinction coefficient of the mAb product:

$$\text{Dilution adjusted mass concentration}\left(\frac{\text{mg}}{\text{mL}}\right) = \frac{\text{mass concentration}\left(\frac{\text{mg}}{\text{mL}}\right)}{\text{dilution ratio}}. \quad (4)$$

2.7.2 | Size exclusion high performance liquid chromatography

The presence of molecular weight content was determined by size exclusion-high performance liquid chromatography (SE-HPLC). This was done using the Agilent 1260 Infinity II LC System (Agilent Technologies, USA), which separates the molecules based upon their respective sizes while moving through the column, Acquity UPLC BEH200 (Waters, USA). The molecular components elute in the sequence of decreasing size and corresponding molecular weight. The SE-HPLC method involved diluting each sample and loading 20 µg of mAb, which was then run through the column for 30 min at a flow rate of 0.2 mL/min in 1× PBS solution. The absorbance of the eluted material was then measured at A280 to determine the relative HMWC, monomer, and low molecular weight content (LMWC) composition as area percentages.

2.8 | Statistical testing

2.8.1 | Test of significance

A paired *t*-test was used to determine if there is a significant difference between the means of two groups. The Null hypothesis (H0) assumes that there is no significant difference between the two groups, and the Alternative hypothesis (H1) suggests that there is a significant difference between the two groups. A *p*-value of 0.05 was used, which means that there is a 5% chance (or less) that the observed difference between the groups is due to random chance alone, assuming the null hypothesis is true. If the *p*-value is less than 0.05, it is often interpreted as evidence to reject the null hypothesis in favor of the alternative hypothesis.

3 | RESULTS AND DISCUSSION

3.1 | Performance development for the micro-scale protein a chromatography operation

The protein A chromatography operation has established itself as the workhorse of mAb purification, particularly as the first stage in downstream processing.[24,25] This is due to its high selectivity towards the Fc region of IgG type antibodies, high flow, and cost-effective dynamic binding capacity for removal of process-related impurities. Therefore, recovery and HMWC are the critical attributes of this operation. The values obtained must be comparable across scales, in order for the micro-scale protein A operation to perform as a suitable scale down process development platform.

Modifications were made to the micro-scale protein A chromatography operation as the recoveries obtained from the initial runs were significantly lower than the acceptable range, and from that achieved in existing micro-scale protein A chromatography experiments.[26] This was found to be as a result of the surface tension between the applied solvent and the RoboColumn's outlet, which results in dripping from each RoboColumn to occur at random. Consequently, the actual fraction volumes that are dispensed, and collected, may differ between each of the eight RoboColumns depending on the time of drop.

To account for these differences in volume, a 90-second delay was implemented following liquid dispense during the elution stage. This was in an effort to collect any residual liquid in the specified well prior to movement of the collection plate. However, the most important adjustment was made through implementing a pathlength correction of A900 versus A977 to the raw absorbance values. This involved determining the pathlength, and therefore, volume in each of the collection wells by assuming a K-Factor of 0.173.[27]

Figure 3 presents a comparison of the corrected and uncorrected recoveries across all 8 RoboColumns from the protein A chromatography operation. There is a significant difference (p-value < 0.05) across all 8 RoboColumns between the corrected and uncorrected recovery values. This highlights the importance of implementing a pathlength correction to the raw values following the protein A chromatography operation.

3.1.1 | Performance evaluation for the micro-scale protein a chromatography operation

The starting harvest material (1 g/L concentration) was thawed and placed onto the robotic liquid handler, before executing the protein A chromatography protocol (Figure 1). The eluted fractions were then collected, and measured at A280, A320, A900 and A977, before implementing a pathlength correction to the raw values. Figure 4 presents a typical chromatogram of the eluted fractions generated from a single RoboColumn, overlayed with a chromatogram generated from the laboratory-scale operation. This will allow for a comparison of the entire elution profile between the two systems.

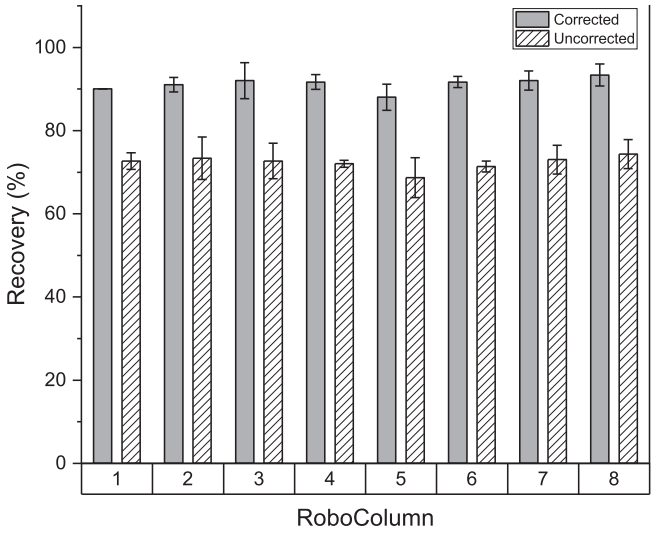

FIGURE 3 Comparison of calculated recoveries based upon corrected (pathlength correction) and uncorrected (no pathlength correction) A280 values following the protein A chromatography operation. Error bars are based upon the standard deviation from triplicate runs.

The experimental results highlight the comparability between the two elution profiles. The elution profile of the micro-scale system is constructed using the concentration values determined every 0.5 column volume, while the laboratory-scale system is constructed from continuous data. The laboratory-scale elution profile plateaus at approximately 3500mAU due to saturation of the UV detector on the ÄKTA Avant 25. This also explains the spike prior to the plateau, which is a product of the saturation and not experimental deviation. Therefore, maximum elution occurs by column volume 1, before gradually decreasing, which further supports the alignment between the elution profiles. Benner et al. (2019) observed no significant difference between the laboratory-scale and micro-scale systems, demonstrating comparable elution profiles.[26] Furthermore, Evans et al. demonstrated comparable elution profiles between the micro-scale and laboratory-scale systems, which is consistent with the results observed in Figure 4.[10]

From Figure 5, the recovery, HMWC, and LMWC values obtained at the micro-scale and laboratory-scale are comparable for both harvest materials. While the load concentration differed between the two harvest materials, 1 g/L and 1.3 g/L, respectively, the values obtained satisfy the pre-determined process specifications. The comparability across scales is consistent with that observed in other studies.[10] This demonstrates the equivalence between the micro-scale and laboratory-scale protein A chromatography operations.

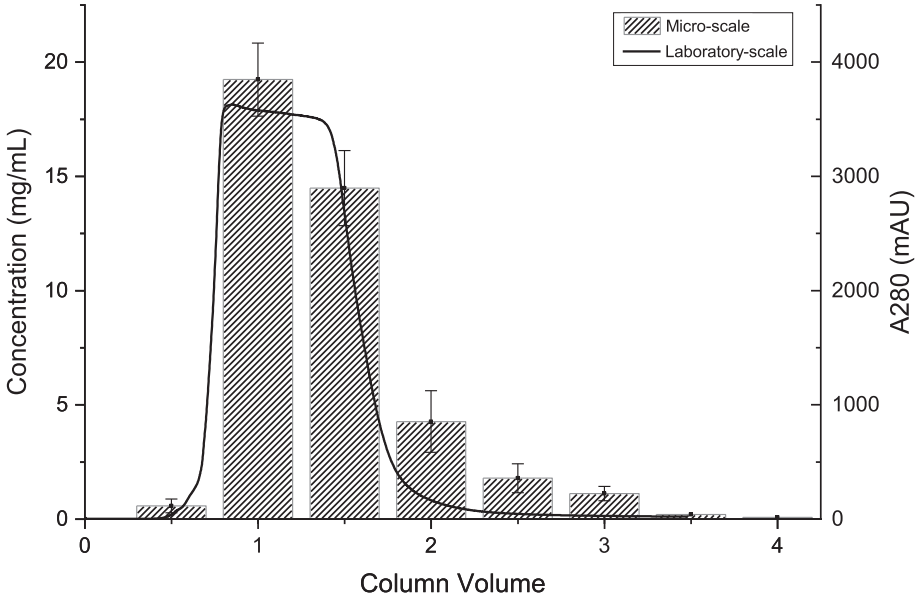

FIGURE 4 Elution profile overlays for the micro-scale and laboratory-scale protein A chromatography operations to allow for comparison between the two systems. Error bars are based upon the standard deviation from triplicate runs.

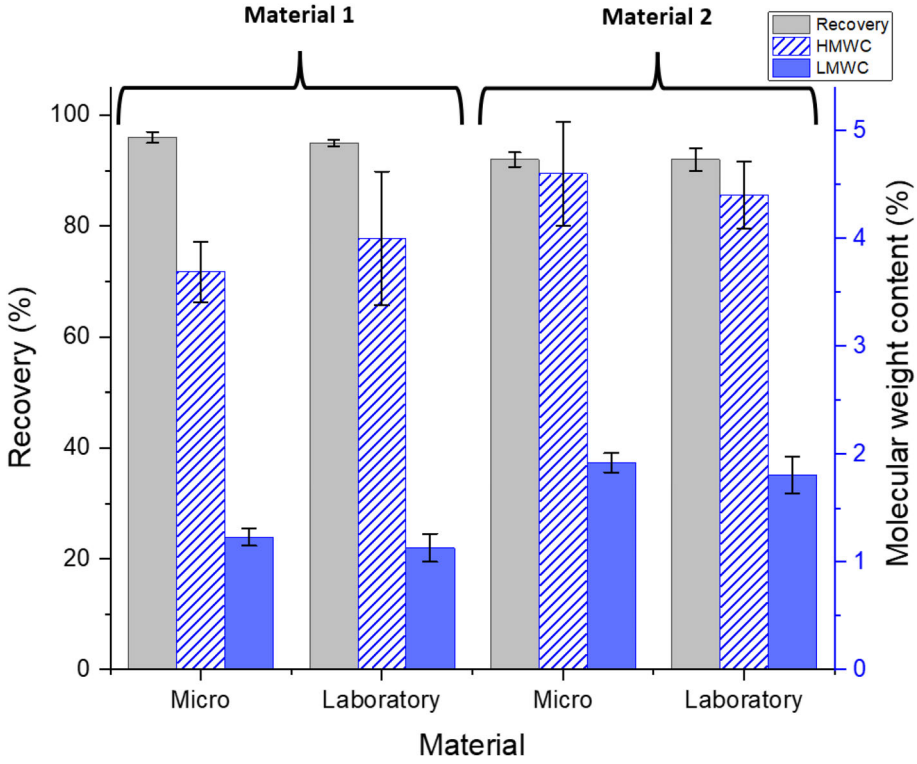

FIGURE 5 Comparison of the recovery (as defined in Section 2.8.1) and molecular weight content following protein A chromatography from the micro-scale and laboratory-scale operations. Two batches of harvest material with load, 1 g/L and 1.3 g/L, respectively, were purified to assess the equivalence between the micro-scale and laboratory-scale protein A chromatography operations. Error bars are based upon the standard deviation from triplicate runs.

3.2 | Performance evaluation for the micro-scale low pH viral inactivation

Low pH VI serves as a dedicated virus reduction step, as many enveloped viruses are irreversibly denatured at a low pH. However, the low pH hold during the VI operation has the potential to disrupt the integrity of the mAb product. Therefore, recovery and HMWC are the critical attributes of this operation. The values obtained must be comparable across scales, in order to demonstrate equivalence between the micro-scale and laboratory-scale low pH VI operations. The working volume for the micro-scale low pH VI operation was reduced by more than 5-fold compared to the laboratory-scale low pH VI step (1.8 mL vs. 10 mL) in order to integrate with the micro-scale protein A chromatography operation in the automation workflow.

The starting material (protein A eluate pool from harvest material 1) was thawed and placed onto the robotic liquid handler, before executing the low pH VI protocol (Figure 1). The impact of the freeze–thaw process on the aggregation behavior was observed to be non-significant, with a less than 0.4% difference in HMWC between freeze–thaw and direct processing of material (data not shown). The samples were then measured at A280, A320, A900 and A977, before implementing a pathlength correction to the raw values. Figure 6 presents the recovery and molecular weight content of the output material obtained from the micro-scale and laboratory-scale low pH VI operations.

The recovery, HMWC, and LMWC values obtained at the micro-scale are comparable to that of the laboratory-scale, and satisfy the pre-determined process specifications. Furthermore, the LMWC is also consistent across scales. This demonstrates the equivalence between the micro-scale and laboratory-scale low pH VI operations when held at a pH of 3.5.

3.3 | Use of the integrated platform to assess aggregate formation during the low pH viral inactivation operation

The performance evaluations have highlighted the success of the micro-scale unit operations through obtaining comparable results to the laboratory-scale system, and satisfying the process specifications. In order to assess process interactions, the protein A chromatography and low pH VI operations were then integrated, and executed in sequence. This automated platform was used as a tool to assess aggregate formation during the low pH VI operation, specifically, the effect of pH on HMWC formation during the low pH hold.

The starting material (Table 4) was thawed and placed, along with the pre-prepared buffers, onto the robotic liquid handler, before executing the protein A chromatography and low pH VI workflows in sequence. The protein A eluate pool was directly processed through the low pH VI operation. This is referred to as micro-scale in Figure 7. For comparison, protein A eluate obtained from the laboratory-scale operation was also pooled and processed through the micro-scale low pH VI platform. In this case, only the low pH VI workflow was executed. This is referred to as laboratory-scale. The volume of acidification solution added to the protein A eluate pool was adjusted depending on the desired pH to be achieved for low pH hold. The pH of the protein A eluate pool was then neutralized to 5.5, before being

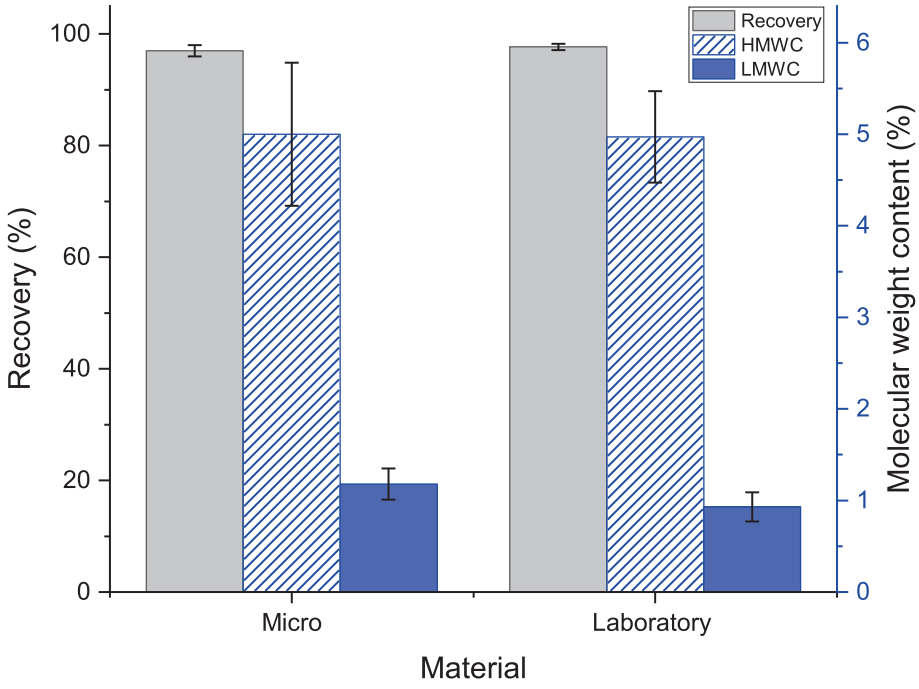

FIGURE 6 Comparison of the recovery (as defined in Section 2.8.1) and molecular weight content following the micro-scale and laboratory-scale low pH viral inactivation operations. As part of the protocol, a 1:4 dilution of protein A eluate with 50 mM acetate, pH 3.8, was conducted. Acidification solution (3 M acetic acid) was then added to achieve a pH of 3.5, before increasing to pH 5.5 with 2 M tris. Error bars are based upon the standard deviation from triplicate runs.

TABLE 4 HMWC, conductivity before dilution, and conductivity after dilution of the laboratory-scale and micro-scale protein A eluate.

Protein A eluate	HMWC (%)	Conductivity before dilution (mS/cm)	Conductivity after dilution (mS/cm)
Laboratory-scale	5.8 ± 0.8	1.8	0.5
Micro-scale	6.5 ± 0.4	1.7	0.5

Note: Errors are based upon the standard deviation from triplicate runs.

analyzed by SE-HPLC for the presence of molecular weight content. Figure 7 highlights the relationship between the hold pH and molecular weight content of the micro-scale and laboratory-scale eluate pools following the low pH VI operation.

Figure 7a highlights the overall negative correlation between the hold pH and HMWC for both the micro-scale and laboratory-scale protein A eluate pool. As demonstrated by Wälchli et al. (2020) mAbs partially denature and expose hydrophobic residues to the solvent under acidic conditions and low ionic strength.[14] The high protein surface charge, which results in electrostatic repulsion between the mAbs, prevents the formation of aggregates. However, subsequent neutralization reduces the surface charge of the mAb, while increasing the ionic strength. This results in attractive hydrophobic interactions between the surfaces of the denatured mAb, and therefore, formation of non-native aggregates.[28,29]

This suggests that at increasing acidic conditions a greater number of hydrophobic sites on the mAb molcules are exposed, resulting in higher aggregate formation following neutralization. For example, Wälchli et al. observed greater monomer fraction, and therefore, less aggregation at pH 3.5 compared to at pH 3.0 and 2.5.[14] This not only supports the findings for both the micro-scale and laboratory-scale material that at a lower pH value, increased aggregation occurs, but also presents the comparability between the two protein A eluate pools after being processed through the integrated platform. Unlike aggregation, fragmentation is not pH induced, as highlighted by the non-significant difference in LMWC across the pH range for both the micro-scale and laboratory-scale elution pools.

While there is a difference in HMWC between the laboratory-scale and micro-scale material at a low pH value (Figure 7a), which may be attributed to sample variation, negligible differences in HMWC can be expected upon a further decrease in the hold pH. This is consistent with the results obtained by Wälchli et al., where negligible differences in aggregation between pH 3.0 and 2.5 were observed.[14] This may be due to saturation of the hydrophobic sites, preventing formation of more aggregates, or that the additional hydrophobic sites at the lower pH values are not involved in the formation of the aggregates.[14] This suggests that aggregate formation will only increase to an extent, despite the acidity of the solution.

In order to further reduce the level of aggregation, the protein A eluate pool was then diluted 1:4 with 50 mM acetate, prior to the addition of acidification buffer. Figure 7b highlights the decrease in HMWC across the pH range for the micro-scale and laboratory-scale materials compared to the undiluted protein A eluate pool (Figure 7a). Diluting the eluate pool decreases the ionic strength of the solution, which increases electrostatic repulsion. This prevents coagulation between the denatured mAb molecules following neutralization.[14] The comparability between the micro-scale and laboratory-scale material presents the investigative power of the integrated micro-scale platform which can be utilized to inform process development. For example, it can be concluded that, for this aggregate-prone mAb, which is sensitive to conductivity at a low pH, the low pH VI

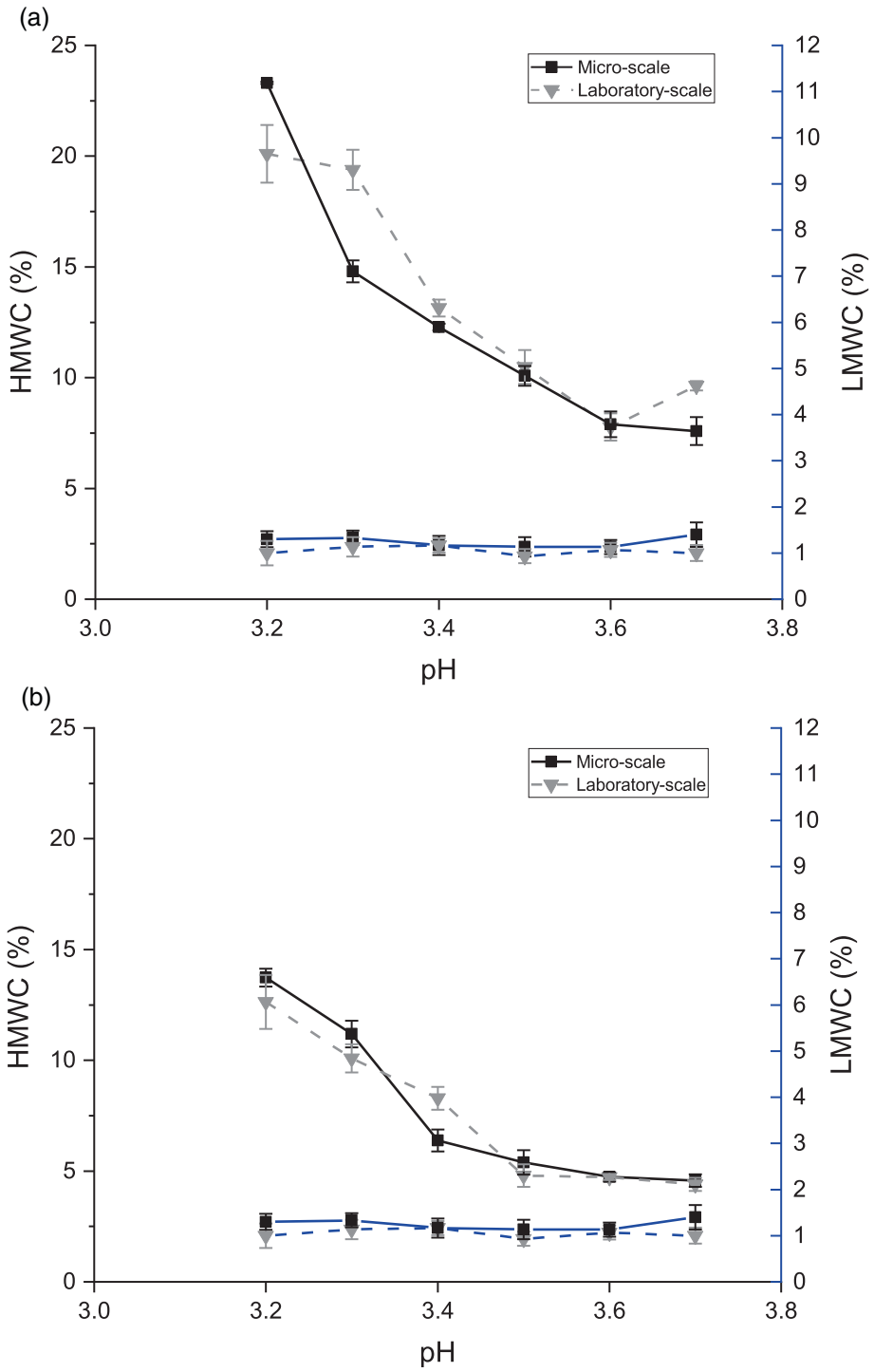

FIGURE 7 The effect of viral inactivation hold pH on HMWC and LMWC following neutralization during the low pH viral inactivation operation within the integrated micro-scale platform. This involves direct processing of the starting harvest material through the protein A chromatography and low pH viral inactivation operations. The laboratory-scale protein A eluate was also processed through the micro-scale low pH viral inactivation platform for comparison. (a) A dilution was not conducted to the protein A eluate pool. (b) A 1:4 dilution with 50 mM acetate, pH 3.8, was conducted to the protein A eluate pool prior to the addition of acidification buffer. Error bars are based upon the standard deviation from triplicate runs.

operation should be diluted 1:4 with 50 mM acetate, before incubating at a hold pH of ≤3.6. This will ensure a low enough pH to inactivate or denature enveloped viruses, while minimizing aggregate formation during the low pH VI operation.[14,16]

3.4 | Comparability assessment

In order to assess the utility of the integrated micro-scale platform, a hypothetical experiment was evaluated in terms of the resources required, including total run time and labor time. This allows for a comparison between the micro-scale platform and a typical laboratory-scale system. Such a process capability study would aim to investigate specific parameters from the protein A chromatography operation and assess the responses following the low pH VI operation, to determine the edge of failure. The two parameters for the protein A operation, for example pH and residence time, would be assessed at three settings, therefore, a total of 15 experiments to be conducted. The eluate material from each experiment would be then directly processed into the low pH VI operation, and then assessed

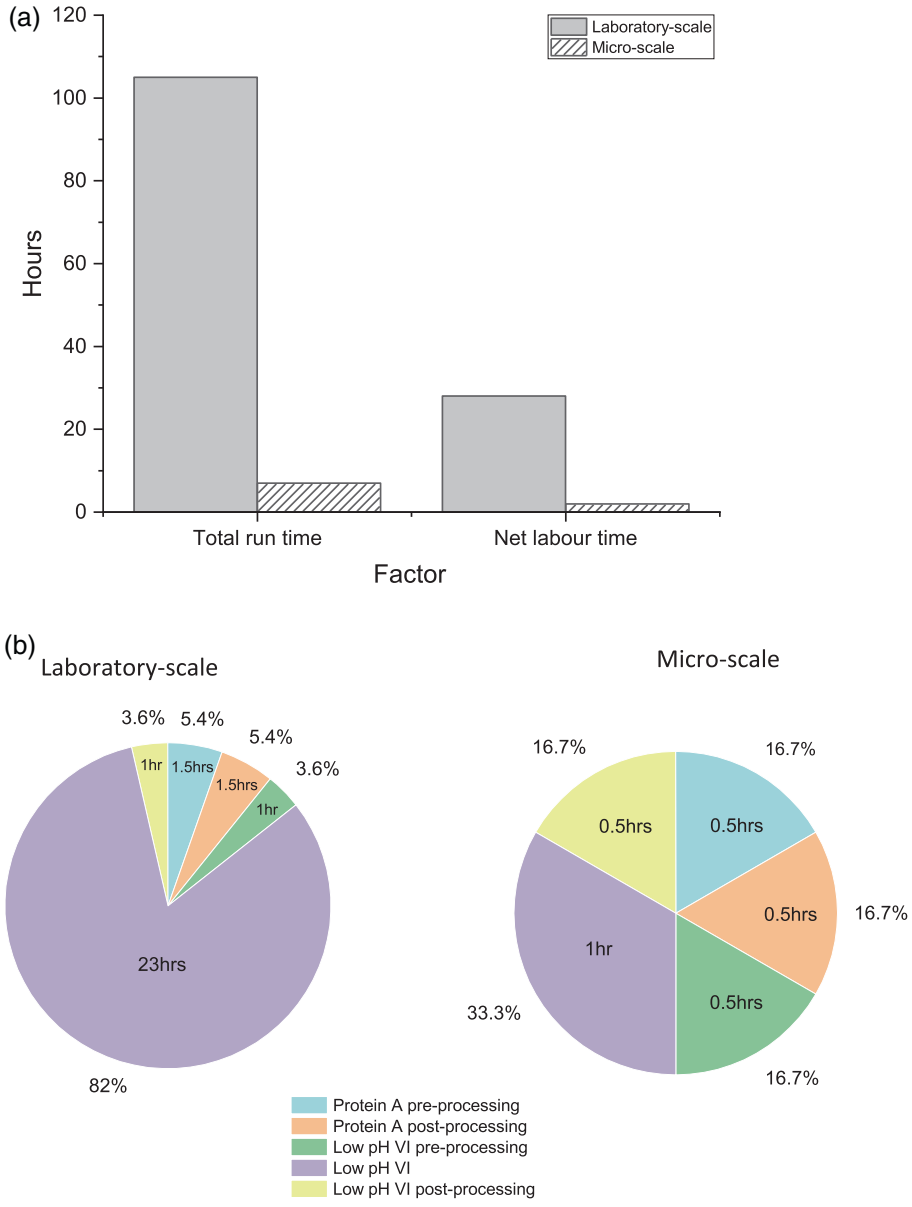

FIGURE 8 Comparison between a typical laboratory-scale system and the integrated micro-scale platform based upon the hypothetical experiment, which comprises 15 protein A chromatography runs before processing through the low pH viral inactivation operation. (a) the processing hours and labor hours are the criteria for comparison; (b) breakdown of the labor hours between the pre-processing, post-processing, and unit operations. The hours within the pie charts refer to the run time of each part of the process, and the percentages outside refer to the proportion of the total run time each constitutes. Figure created with BioRender.com.

for two responses, maximization of yield, and minimization of HMWC. The micro-scale platform would be considered as a single system to truly reflect the integration.

The advantages of the micro-scale system arise from the miniaturization and parallelization of experimentation. To conduct the aforementioned study, less load material would be required to for all 15 protein A chromatography runs, 855 mL compared to 209 mL, for the laboratory-scale and micro-scale systems, respectively. Furthermore, the parallel throughput capability of the micro-scale platform allows for the entire experiment to be conducted within a significantly lower total run time, which translates to less labor hours, unlike the laboratory-scale system, which requires the runs to be performed sequentially (Figure 8a). While this highlights the lower resource demands, decreased processing time, and greater labor savings achieved by the micro-scale system, the productivity gains accentuate the advantages of the integrated platform. For example, increasing the productivity (chromatograms generated per hours) of the protein A chromatography operation by greater than 13-fold.

From Figure 8b, each stage of the integrated platform requires less labor hours compared to the stages of the laboratory-scale system. The largest difference arises from the low pH VI operation, which accounts for 82% of the labor hours, as opposed to 33.3%, for the laboratory-scale and micro-scale systems, respectively. This highlights the significant time and labor savings that can be achieved when leveraging the integrated platform beyond chromatographic operations, which will ultimately result in further productivity gains. For example, increasing the generation of response datasets (HMWC and recovery) per hour by almost 15-fold.

Integrating the micro-scale operations within an automated platform utilizes the parallel throughput capability of the micro-scale system, allowing for assessment of process interactions between the unit operations to increase process knowledge and understanding. This

highlights the effectiveness and utility of the integrated micro-scale platform, where the productivity gains, coupled with capability to inform process development, presents the potential of the integrated micro-scale platform as a resource- and time- efficient fit-to-platform assessment tool for mAb production.

4 | CONCLUSIONS AND FUTURE OUTLOOK

This research presents the integration of the protein A chromatography and low pH VI unit operations within an automated micro-scale platform. Micro-scale workflows were designed, constructed, and simulated using Synthace, before executing on the Tecan Freedom Evo 200. The success of the micro-scale workflows were shown by demonstrating the equivalence between the two scales for each individual operation. The material output quality obtained from the protein A chromatography and low pH VI operations were comparable across scales, achieving recovery and HMWC values that satisfy the predetermined process specifications.

The integrated micro-scale platform was then utilized as a tool to assess aggregate formation during the low pH VI operation, specifically, the effect of pH on HMWC formation during the low pH hold. The laboratory-scale and micro-scale protein A eluate showed comparable HMWC across the assessed pH range of 3.2–3.7. It was then observed that aggregate formation was minimized by diluting the protein A eluate by 1:4 with 50 mM acetate, which was again consistent across both scales. The comparability between the micro-scale and laboratory-scale material presents the success of the integrated micro-scale platform which can be utilized to inform on process development and optimization.

The utility of the integrated micro-scale platform was assessed and compared with a typical laboratory-scale system, by evaluating the resources required to conduct a hypothetical experiment comprising 15 runs. The advantages of the integrated platform were realized through the miniaturization and parallelization of experimentation. This results in lower resource demands, increased labor efficiency, and greater productivity compared to the laboratory-scale system. For example, the entire experiment can be conducted in 7 h, as opposed to 105 h, which translates to labor hours, 3 h and 28 h for the micro-scale and laboratory-scale systems, respectively. While each stage of the integrated platform requires less labor hours compared to the stages of the laboratory-scale system, the largest difference arises from the low pH VI operation, which accounts for 82% of the labor hours, as opposed to 33.3%, for the laboratory-scale and micro-scale systems, respectively. This highlights the significant time and labor savings that can be achieved when leveraging the integrated platform beyond chromatographic operations.

This presents the utility of the integrated micro-scale platform as a resource- and time- efficient assessment tool for mAb process development. Further integration of non-chromatographic operations, such as depth filtration, as well as on-deck pH measurement, will enhance the utility of the platform, which can then also be coupled with Design of Experiments (DoE) to create a HTPD strategy based upon process analytical technology (PAT).

AUTHOR CONTRIBUTIONS

Paras Sharma: Investigation; conceptualization; writing – original draft; methodology; data curation; writing – review and editing; software; formal analysis; visualization; validation. **Lars Robbel:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision. **Michael Schmitt:** Conceptualization; funding acquisition; methodology; project administration; supervision; resources. **Duygu Dikicioglu:** Conceptualization; writing – review and editing; project administration; supervision; resources. **Daniel G. Bracewell:** Conceptualization; funding acquisition; supervision; resources; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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