SARTORIUS

Application Note #13

May 01, 2010

Glycine Buffer Make-up With Flexel® for Magnetic Mixer[1] and Modeling of Low pH Viral Inactivation With Flexel® for Lev Mixer[2]

Author: Myriam Lavie
E-Mail: myriam.lavie@sartorius.com

Executive Summary

This application note presents practical implementation examples of fully single-use process solutions in downstream processing. The combination of sterile bag assemblies with single-use impeller and sensor technologies provides the capability to perform and monitor complex process steps in a fully disposable configuration. This study investigates the use of single mixing systems for the preparation of buffers, commonly used in downstream processing, and for low pH viral inactivation usually following a protein A capture step. Contained transfer of products, performances of powder hydratation and dissolution, benefits of in-line pH adjustment and low shear stress mixing are presented.

[1] This product uses Pall patented Magnetic Mixer technology.
[2] Flexel® for Lev Mixer is a trademark of Pall Corporation and this product uses Pall patented Flexel® for Lev Mixer is technology.
All information on patents can be found at www.Pall.com/patents

Introduction

Optimal buffer conditions for binding and elution steps in protein A affinity purification depends on the properties of the mAb. However, 0.1M glycine buffer is widely used as an elution buffer of the affinity column.

Following the protein A chromatography step, a low pH inactivation step is often required to inactivate enveloped viruses in the product pool.

After completion of the Protein A cycles, the eluates are pooled, the pH of the solution is adjusted to a low value, typically 3.2, and held at this value for 30 to 60 minutes at ambient temperature. The pH is then quickly re-adjusted to 5.0-5.5 to minimize exposure of the protein to low pH environment. The product is then filtered and transferred for further polishing step using for example cation exchange chromatography.

The study will investigate critical aspects of the mixing and online pH adjustment in a single-use configuration.

Purpose of the Application Study

The purpose of this application study is to develop a single-use solution for the buffer preparation at 200 L volume and for low pH viral inactivation. The following processing steps are investigated:

- Preparation of 200 L of elution buffer for a protein A column (0.1M glycine – 35mM NaCl),
- Simulation of a low pH viral inactivation in a 50 L Flexel® for Lev Mixer at the following operating volumes:
  - 12 L (minimum volume to immerse the single-use pH probe*),
  - 30 L (intermediate volume)
  - 65 L (maximum volume in 50 L Flexel® Bag for Lev Mixer)

* specific bag's handling procedure for that volume. Contact Sartorius Stedim Biotech Process Development Engineers

Materials and Methods

1. Buffer preparation

- The buffer is prepared in a standard 200 L Flexel® Bag for Magnetic Mixer ref. FMB114893,
- The mixing system is filled to 80% of the required nominal volume, i.e. 160l. The volume is monitored with the integrated load-cells of the Palletank® for Magnetic Mixer ref. FXC114153.
- The impeller speed is set to 300rpm on the Magnetic Mixer Drive Unit ref. LT-DU-006-EU for an efficient mixing,
- Powder is added in a contained way using the 15 L Powder Bag ref. FMA114008,
- After powder dissolution the final volume is adjusted to 200 L,
- The buffer is then adjusted from pH 7.2 to pH 3.8

2. Simulation of viral inactivation

- Viral inactivation is performed in a 50 L Flexel® Bag for Lev Mixer,
- The impeller speed is set to 70rpm to avoid vortex, minimize the shear forces and protein exposure to air bubbles.
- pH adjustments, is performed via introduction of acid and base from the top of the mixing bag assembly.
- In-line reading of the pH is performed with the single-use pH sensor integrated onto the mixing bag,
- pH is adjusted with HCl (1M) and NaOH (1M):
  - from 3.8 to 3.2 ± 0.1 for the incubation at low pH,
  - from 3.2 to pH 5.5 ± 0.1 for pH re-equilibration prior to the cation exchange chromatography step.
- Additional trials were performed with a bottom addition of phosphate buffers (pH 3.0 for low pH ; pH 6.5 for neutralization). pH adjustment with buffered solutions, such as Phosphate buffers, are simpler to achieve avoiding pH overshooting and local exposure to strong acid or base that may be detrimental to the mAb stability.

Results and Discussions

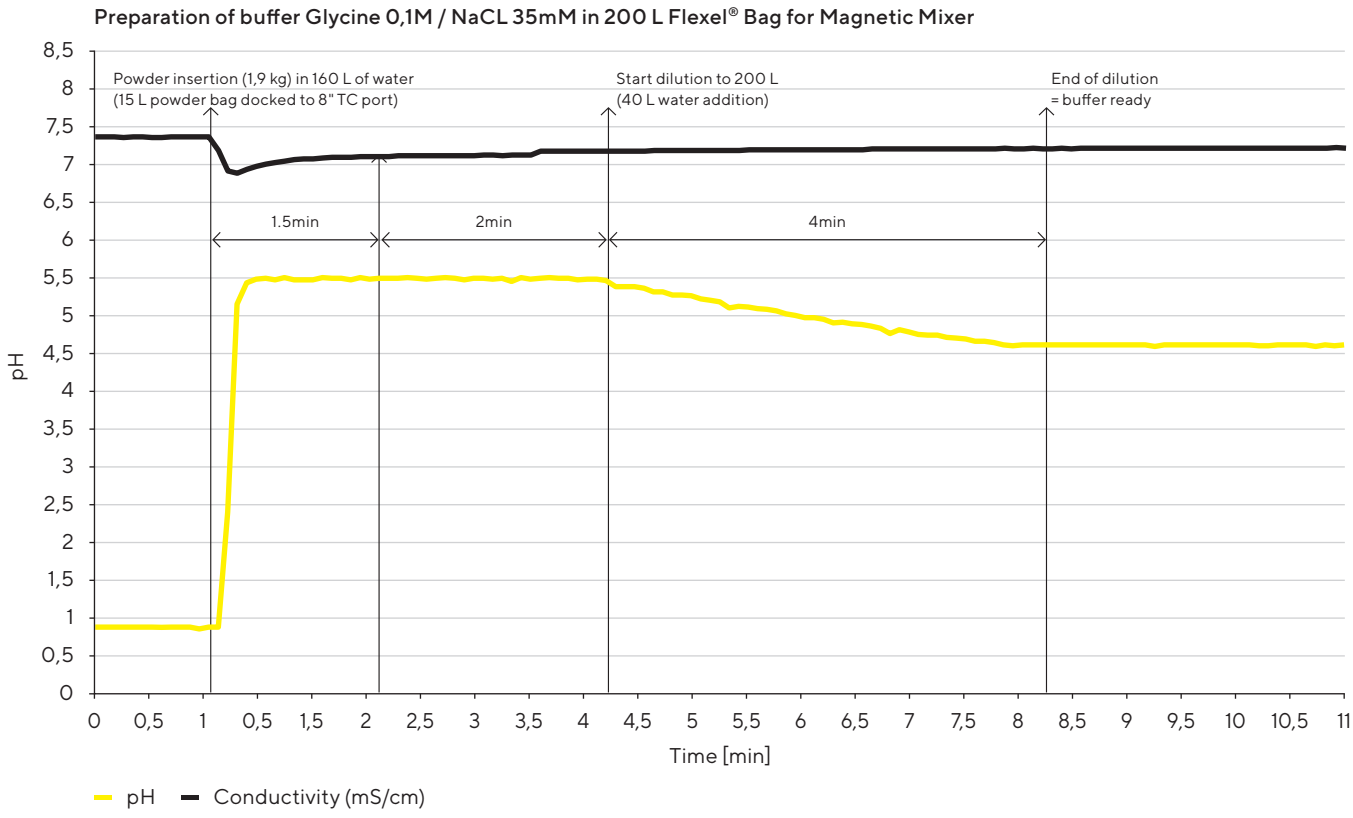
1. 200 L Glycine Buffer Preparation with Flexel® for Magnetic Mixer

Results

- Complete mixing of powders was achieved in 1.5 minutes in Flexel® Bag for Magnetic Mixer with
  - Bag filled to 80% (160 L)
  - Contained powder addition using 15 L Powder Bag
  - Impeller speed 300 rpm providing a strong vortex
  - Single-use pH sensor for in-line pH monitoring
- Total process time = 25 minutes, including:
  - Bag unpacking and installation = 2.5 min
  - Bag filling to 80% of the target volume with water using a peristaltic pump = 15 min
  - 2-point calibration of the single-use pH sensor (performed during the bag filling step)
  - Powders mixing = 1.5 min
  - Impeller rotation maintained for 2 min
  - Final dilution to 200 L = 4 min
- The pH was later adjusted from 7.2 to 3.8 to simulate the mAb eluate of a protein A column that will be submitted to low pH viral inactivation.

Figure 1: Docking of the 15 L Powder Bag onto the Flexel® Bag for Magnetic Mixer

Figure 2: Vortex in 200 L Flexel® Bag for Magnetic Mixer



2. Simulation of Low pH viral inactivation step with Flexel® for Lev Mixer

2.1 low pH viral inactivation step using strong acid and base

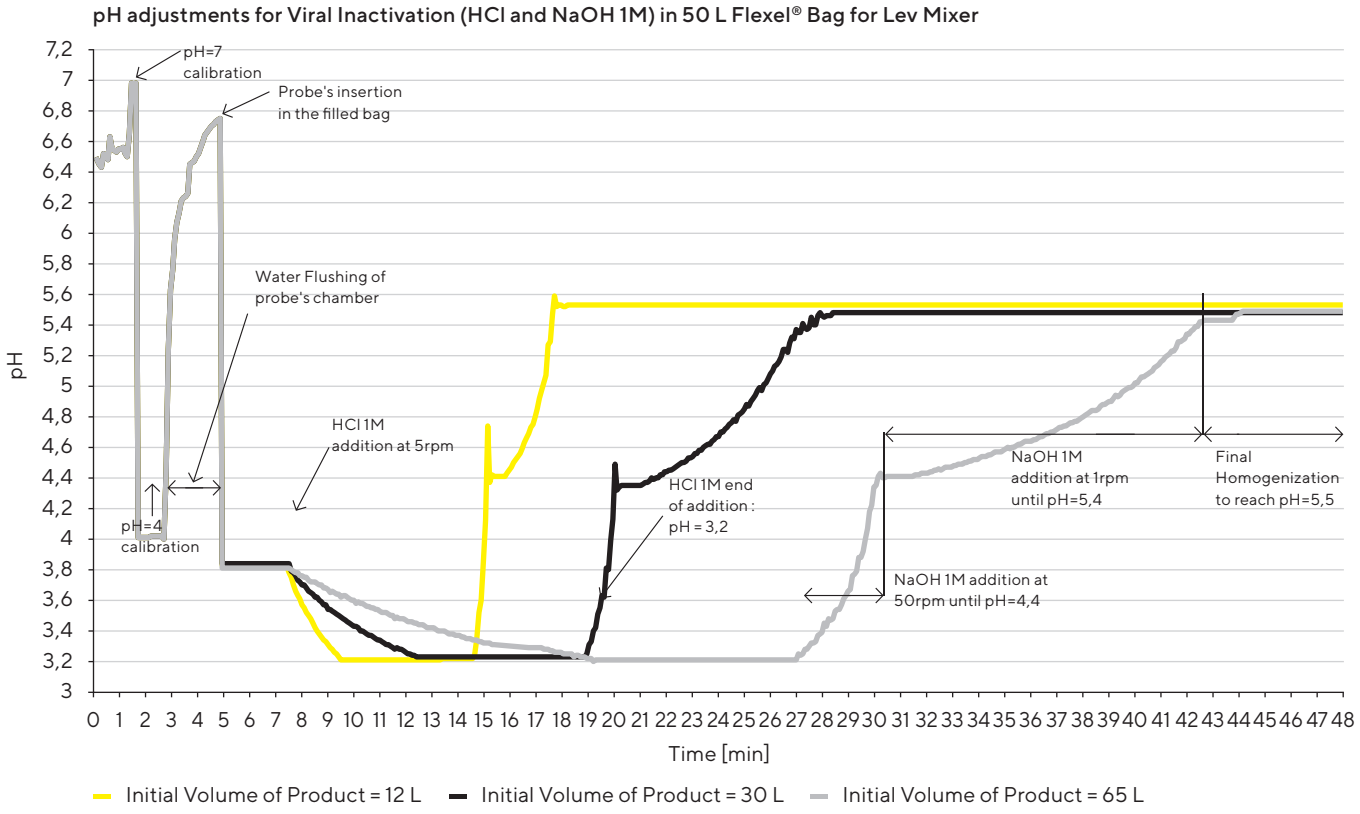
Results

- Contained top addition of HCl | NaOH (1M) through the needleless Clave connector of the Flexel® Bag for Lev Mixer,
- Impeller speed = 70 rpm (no vortex to minimize shear forces),
- Instant reading of the pH by the single-use sensor,
- All targeted pH were successfully achieved:
  - Bag filled with 12 L* at pH 3.8:
    - pH 3.21 achieved in 2.0 min
    - pH 5.53 achieved in 3.8 min
  - Bag filled with 30 L at pH 3.8:
    - pH 3.21 achieved in 4.8 min
    - pH 5.49 achieved in 10.0 min
  - Bag filled with 65 L at pH 3.8:
    - pH 3.23 achieved in 11.7 min
    - pH 5.48 achieved in 17.5 min
- No variation of pH over time during incubation.

* specific bag's handling procedure at 12 L. Contact Sartorius Stedim Biotech Process Development Engineers for details.

Figure 3: Calibration of the single-use pH probe

Figure 4: 50 L Flexel® Bag for Lev Mixer in 50 L Palletank® for Lev Mixer (weighing)



2.2 Low pH viral inactivation step using buffers

Results

- Contained bottom addition of phosphate buffers through ½"ID silicone tubing,
- Impeller speed = 70 rpm (no vortex to minimize shear forces),
- Instant reading of the pH by the single-use sensor,
- targeted pH were successfully achieved:
  - bag filled with 30 L of product at pH 3.8:
    - 3.21 achieved in 17.0 min (15 L addition)
    - 5.52 achieved in 8.0 min (7 L addition)
- Integrated load-cells for buffer addition monitoring,
- Properties of the phosphate buffer imply less risk of precipitation compared to the hydrochloric acid.
- But the final volume in the bag of 52 L (initially 30 L) may require a concentration step before cation exchange chromatography.

2.3 Comments

- All targeted pH values were successfully reached within the tolerances of ± 0.1 pH.
- During strong base and acid additions:
  - Thanks to the pKa1 value of the glycine (2.35), the pH was smoothly adjusted during low pH and neutralization until pH = 4.4.
  - During caustic addition starting from pH 4.4: the pump speed was reduced from 50rpm to 1rpm to deal with the high sensitivity of the pH when out of the glycine buffer range, thus allowing to reach the targeted pH of 5.5 ± 0.1 within an acceptable process time.
- During phosphate buffer additions at pH 3.0 and pH 6.5, the buffer property enabled smooth pH adjustments while keeping a constant pump speed

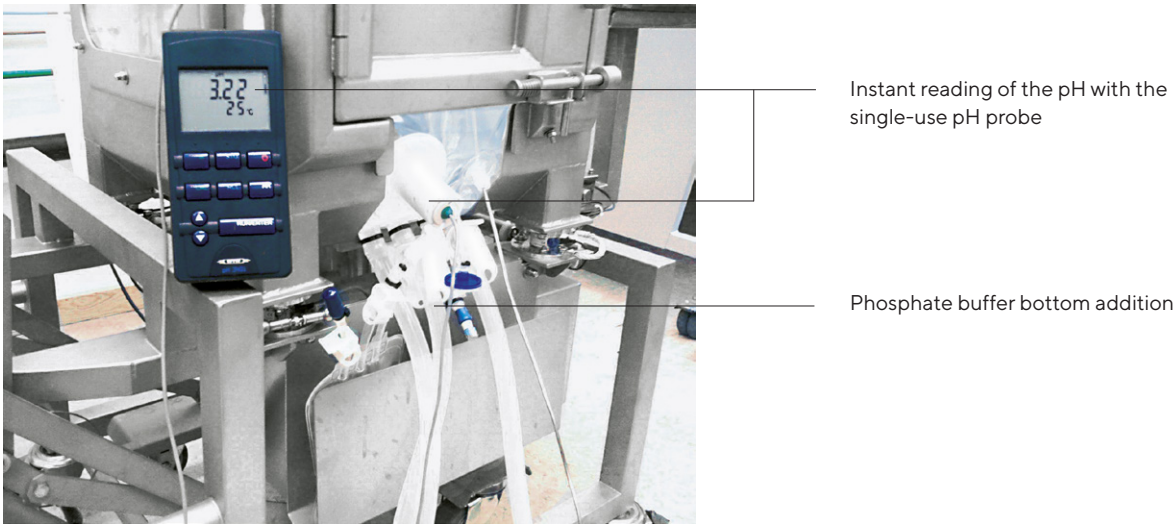
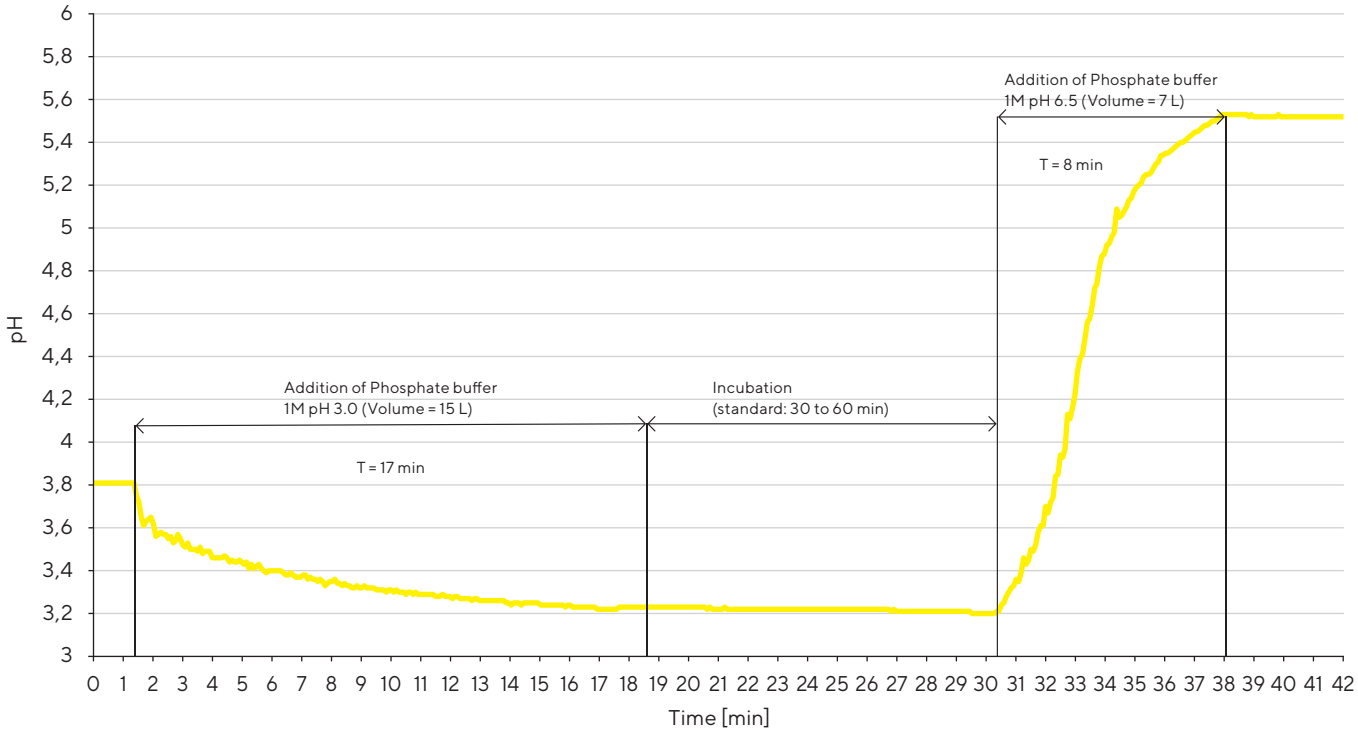


Figure 5: 50 L Flexel® Bag for LevMixer® in 50 L Palletank® for Lev Mixer (weighing)

pH adjustments for low pH Viral Inactivation
with phosphate buffers 1M (pH 3.0 and 6.5) in 50 L Flexel® Bag for Lev Mixer filled to 30 L



Conclusion

The 200 L of glycine buffer is efficiently prepared with the strong mixing provided by the Flexel® for Magnetic Mixer technology. A low bioburden is guaranteed by the contained processing conditions. Furthermore, containment of the buffer salts in the powder transfer bag protects the operator from exposure to chemicals. The combination of sterile bag assemblies with a single use impeller and pH sensor simplifies the buffer preparation operations and provides the ability to monitor the pH of the solution.

Low pH viral inactivation was performed with the Flexel® for Lev Mixer technology. The levitated impeller technology provides low shear stress and particulate free aseptic mixing conditions required for processing high value products, such as mAb. The range of Flexel® Bags both for Magnetic Mixer and Lev Mixer includes volumes of 50 L, 100 L, 200 L, 400 L, 650 L and 1000 L offering full scalability for such process steps.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For further contacts, visit
www.sartorius.com

Publication No.: SL-1076-e | Order No.: 85032-537-18 | Status: 09 | 10 | 2021