SARTORIUS

Application Note #14

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Rapid and Scalable Buffer Preparation With Single-Use Magnetic Mixer

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Executive Summary

This application note presents a new approach to increase the speed and consistency of buffer preparation steps in bio manufacturing. The method combines ready to use buffer formulations from SAFC®, with scalable, high efficiency, single-use mixing systems provided by Sartorius Stedim Biotech. The mixing system is a key component of Flexact® BP, a configurable disposable solution for buffer preparation.

Three examples of large volume buffer preparation steps (Tris | Tris HCl | NaCl, citric acid | sodium citrate | NaCl, PBS), in nominal (×1) or concentrated (×20) forms are presented. The contained transfer of powdered buffer formulations into the high torque single-use mixing system enables a rapid dissolution and dispersion of the buffer powders in liquid for volumes of 50 L to 1,000 L. The performances of the single-use mixing system are characterized with quantitative (conductivity measurement) and qualitative (visual inspection) techniques. The proposed method and system provide seamless scale-up and consistent rapid buffer mixing for process development and GMP manufacturing.

Find out more: www.sartorius.com/en/products/fluid-management/mixing

Introduction

This application study presents the performances of a fully single-use mixing solution for the large scale preparation of three different buffers. The mixing technology selected for this application is Flexel® for Magnetic Mixer[1] with volumes of 50 L, 200 L and 1,000 L. The magnetic coupling of the impeller with the Magnetic Mixer Drive Unit enables a rotation speed up to 300 rpm, providing a powerful mixing of the buffer salts. The three buffer solutions prepared in this application study (Citric acid | sodium citrate, PBS and Tris buffer) are commonly used in biomanufacturing. Examples of applications using these buffers include storage and distribution of concentrated buffer, tangential flow filtration, pH re-equilibration, and final formulation.

The study will investigate the mixing performances for buffer preparation at different volumes of single-use bags (50 L, 200 L, 1,000 L), and at different transient concentrations of powders (16 g/L, 73 g/L, 224 g/L) before the final dilution.

200 L Flexel® with Magnetic Mixer Technology

[1] This product uses Pall patended Magnetic Mixer technology.
All information on patents can be found at www.Pall.com/patents.

Purpose of the Application Study

The purpose of this application study is to assess the performances of the Flexel® for Magnetic Mixer technology to dissolve the following powders used in the buffer preparation:

- TRI buffer: Citric Acid, Sodium Citrate, Sodium Chloride (final concentration = 14.4 g/L)
- Tris buffer: Tris, Tris HCl, Sodium Chloride (final concentration = 67.6 g/L)
- 20× PBS (final concentration = 191 g/L)

The mixing times are determined by conductivity and visual inspection of the solution in the Flexel® Bag for Magnetic Mixer. The usual procedure for a buffer preparation includes before final dilution the incorporation of the powders in the bag partially filled with WFI. For our study, the bags were respectively filled to 80% of the nominal volume for the concentrated PBS and 90% for the TRI and Tris buffers.

The actual concentrations of salts during the mixing phase before the final dilution are:

- TRI buffer: 16 g/L (fluid expansion due to the powders is negligible)
- Tris buffer: 73 g/L (density = 1.046 kg/L)
- 20× PBS: 224 g/L (density = 1.162 kg/L)

Then the final step of the process consists in the addition of water to achieve the expected buffer concentrations.

Materials and Methods

The list of materials and equipments used for this application is:

1. Standard Flexel® Bag for Magnetic Mixer (50 L: FMB114867, 200 L: FMB114893, 1,000 L: FMB114896)
2. Powder transfer bag 15 L (ref. FMA114008)
3. Palletank® for Lev Mixer and Magnetic Mixer[1] (50 L: FXC110820, 200 L: FXC110821, 1,000 L: FXC113384)
4. Magnetic Mixer Drive Unit, 230V, EU power cord (ref. LT-DU-006-EU)
5. Powder bag holder 200-400-650 L (ref. FXA114344)
6. SAFC® powders with the following formulations:
   TRI buffer (product number 44078 – dry powder packaging of 10 kg):
   - Citric acid anhydrous (0.04 g/L – 0.2 mM)
   - Sodium chloride (8.6 g/L – 147 mM)
   - Sodium citrate, 2 H_2O (5.8 g/L – 20 mM)

   Tris buffer (product number 44077 – dry powder packaging of 10 kg):
   - Sodium chloride (58.44 g/L – 1 M)
   - Tris (6.06 g/L – 50 mM)
   - Tris HCl (3.15 g/L – 20 mM)

   Concentrated 20× PBS (product number 44079 – dry powder packaging of 10 kg):
   - Potassium chloride (4 g/L – 54 mM)
   - Potassium phosphate, monobasic, anhydrous (4 g/L – 29 mM)
   - Sodium chloride (160 g/L – 2.7 M)
   - Sodium phosphate, dibasic, anhydrous (23 g/L – 162 mM).
7. Conductivity sensor: WTW InoLab Cond 740i

Method Used:

1. The buffer is prepared in standard Flexel® Bags for Magnetic Mixer filled with deionised water to 80% of the final volume for the concentrated PBS, and 90% of the final volume for the Tris and TRI buffers.
2. Impeller speed is set to the maximum speed of 300 rpm to optimize the powders hydration and dispersion.
3. The powders are incorporated in the Flexel® Bags for Magnetic Mixer using either:
   - SAFC® packaging for dry powder,
   - or Sartorius Stedim Biotech 15 L powder transfer bag for a contained transfer to the mixing bag assembly.
4. Two mixing times are monitored from the addition of buffer powders:
   4.1 "mixing time 1" is determined from the conductivity signal as follows:
   The "mixing time 1" corresponds to the time when 95% of the final value is reached and when all next measurements stay within a 5% tolerance (see Fig. 1).

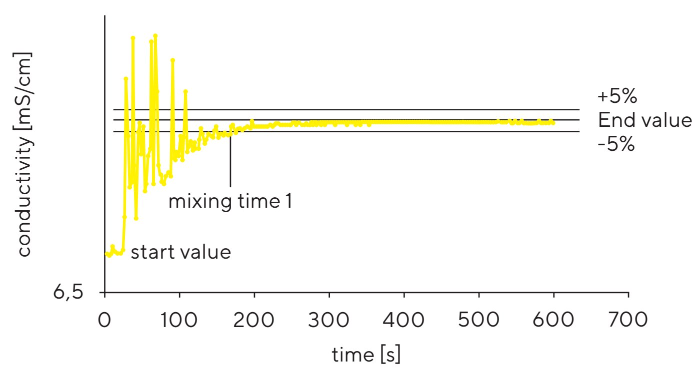

Fig. 1: General principle of mixing time determination via conductivity

   4.2 "mixing time 2" is determined by a visual inspection. The "mixing time 2" corresponds to the time when all suspended particles are visually dissolved.

Results and Discussions

1. Mixing performances vs. volume of buffer preparation

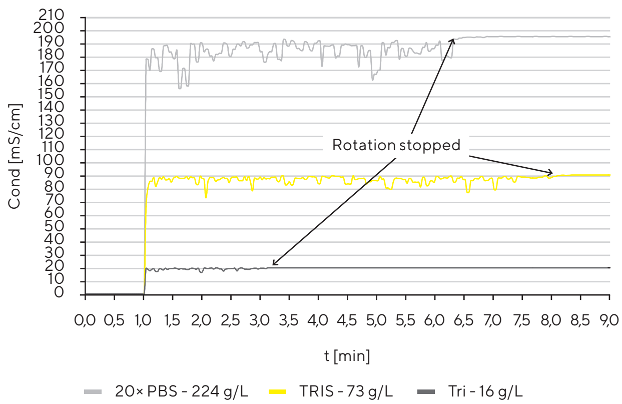

Fig. 2: Buffer preparations in 50 L Flexel® Bag for Magnetic Mixer

50 L Flexel® Bag for Magnetic Mixer: the vortex at 300 rpm in the 50 L bag volume resulted in the generation of air bubbles that interfered with conductivity measurement with air trapped in the conductivity cell. The conductivity was not unwavering even though mixing was completed. A stable value of the conductivity could be observed only at lower impeller rotation speed. Therefore mixing time cannot be precisely defined using the conductivity signal. The mixing time is at least as good as with the larger scale.

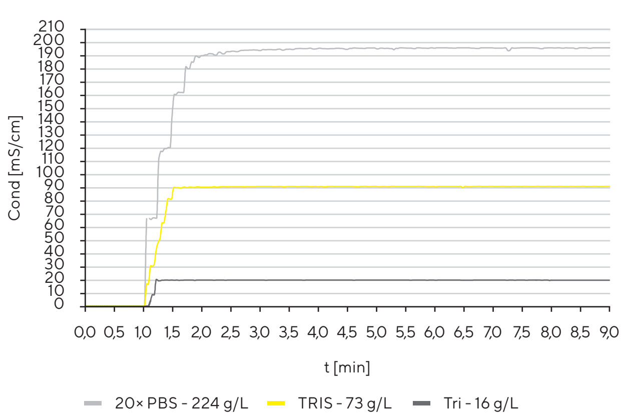

Fig. 3: Buffer preparations in 200 L Flexel® Bag for Magnetic Mixer

200 L Flexel® Bag for Magnetic Mixer: 4 SAFC® bucket liners were used to transfer 38.2 kg of PBS into the mixing bag as shown by the multiple steps on the conductivity signal for PBS buffer preparation (see Fig. 3). A single Sartorius Stedim Biotech powder transfer bag was used for the preparation of Tris (13.5 kg) and sodium citrate | citric acid (2.9 kg), which is reflected by continuous increase of conductivity signal. A stable plateau conductivity value was then quickly achieved for the three buffers, indicative of the mixing completion.

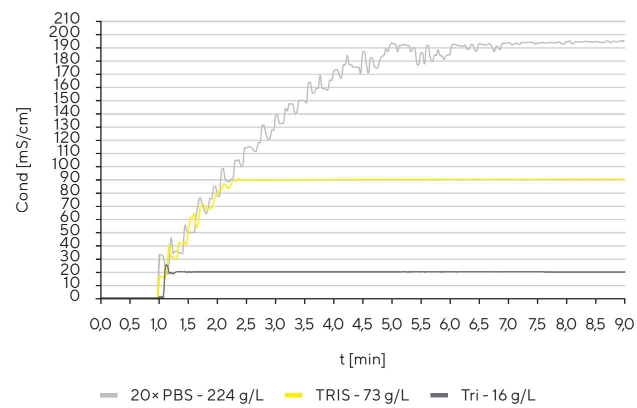

Fig. 4: Buffer preparations in 1,000 L Flexel® Bag for Magnetic Mixer

1,000 L Flexel® Bag for Magnetic Mixer: 19 SAFC® bucket liners were used to transfer 191 kg of PBS, 7 liners for the Tris (66.7 kg) and a single one for the sodium citrate | citric acid (14.4 kg). The multiple powder addition steps are visible on the conductivity graph (see Fig. 4). Again a stable value of conductivity was monitored at the mixing completion.

2. Mixing performances vs buffer type – determination of the mixing times

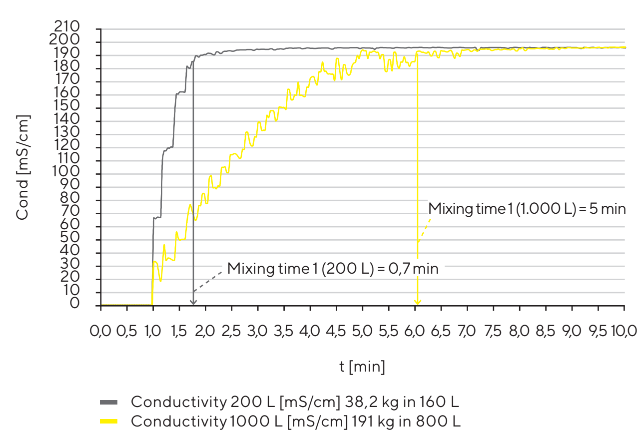

Fig. 5: PBS 20× buffer preparation in Flexel® Bag for Magnetic Mixer

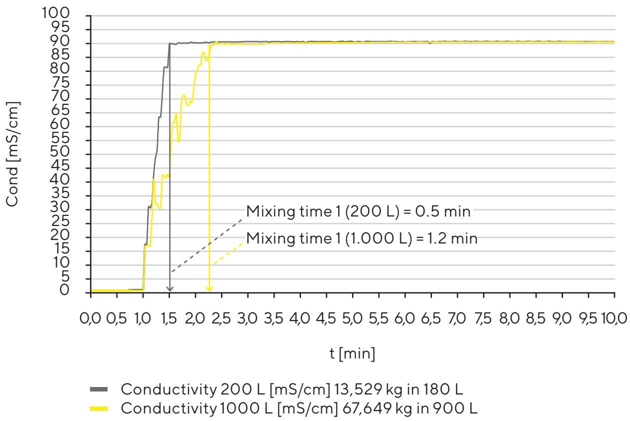

Fig. 6: TRIS buffer preparation in Flexel® Bag for Magnetic Mixer

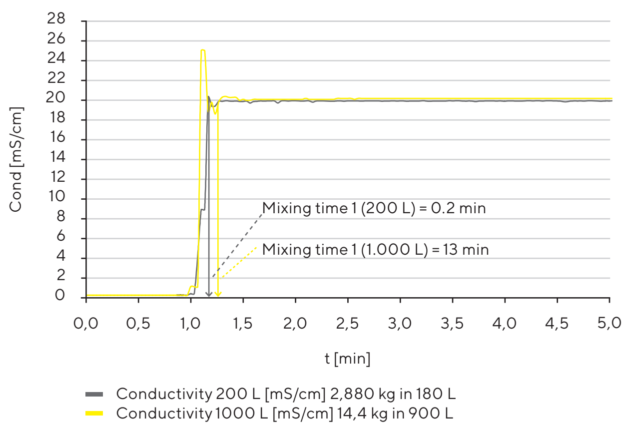

Fig. 7: Sodium citrate | citric acid | NaCl buffer preparation in Flexel® Bag for Magnetic Mixer

200 L Palletank® for Magnetic Mixer equipped with the powder transfer bag for the mixing trial

Bag Volume [L]		**50**	**200**	**1000**
Buffer powders				
Citric Acid Sodium Citrate NaCl (16.0 g/L)	Based on conductivity		0,2 min	0,3 min
	Based on visual inspection	< 5 min	< 5 min	< 5 min
Tris Tris HCl NaCl (73.0 g/L)	Based on conductivity		0,5 min	1,2 min
	Based on visual inspection	< 5 min	6 min	10 min
20× PBS (224.0 g/L)	Based on conductivity		0,7 min	5 min
	Based on visual inspection	13 min	14 min	20 min

Fig. 8: Overview on mixing times via conductivity and visual inspection

* conductivity values not stable at 50 L due to presence of air bubbles around the conductivity cell (strong vortex)

General Comments:

- The mixing times reported in this study include the transfer time of the multiple Sartorius Stedim Biotech powder transfer bags (for the 200 L scale experiment) or SAFC® bucket liners (50 and 1,000 L experiments) into the mixing bag assembly.
- A rapid dissolution of the powder salts was observed for the three buffers thanks to the strong mixing torque at 300 rpm (see Fig. 5-7).
- For the 200 L and 1,000 L volume, the conductivity of the solutions reaches a stable value in few minutes. However, some fine particulates of salt can still be visually observed in the solution. The agitation at 300 rpm was maintained until the solution became totally clear. This visual control is facilitated by the large windows of the Palletank®.
- For all cases a 20× increase in buffer preparation volumes (50 L to 1,000 L) only leads to a 2× increase in the mixing times.
- The worst-case buffer preparation condition (1,000 L 20× PBS with mixing of 191 kg of salts in 800 L of water) resulted in a mixing time of 20 min.
- To the mixing times presented in Fig. 8, additional time for equipment set up and water filling to 80% as well as time for filling after mixing from 80 to 100% to reach final volume and concentration should be taken into consideration for total process time calculation.

Conclusion

- Large volume buffer solutions are quick and easy to prepare using the combination of ready to use buffer formulations and the high efficiency mixing of the Flexel® with Magnetic Mixer Technology.
- The contained processing conditions with the closed powder transfer bag docked onto the sterile Flexel® Bag for Magnetic Mixer are favourable to maintain low bioburden and to reduce the minimum exposure of the operator to chemicals.
- The platform provides a single-use scalable buffer preparation capability with a range of Flexel® Bags including volumes of 50 L, 100 L, 200 L, 400 L, 650 L and 1,000 L.
- Flexel® for Magnetic Mixing system integrated in a Flexact® BP system provides monitoring and automation capability to better control all the operations of a buffer preparation step.

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