SARTORIUS

Application Note #15

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Scalable Buffer Preparation With Single-Use Lev Mixer Technology

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Executive Summary

This application note presents the results of buffer preparations using the Flexel® for Lev Mixer[1] technology. The method combines ready to use buffer formulations from SAFC®, with scalable, high efficiency, single use mixing systems provided by Sartorius Stedim Biotech.

The performances presented in this application note are achieved with the superconducting drive unit that enables a levitation of the impeller encapsulated into the Flexel® Bag. This technology is the alternative to the Flexel® for Magnetic Mixer[2] technology which is part 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 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 singleuse mixing system are characterized with quantitative (conductivity measurement) and qualitative (visual inspection) techniques.

As for the Flexel® for Magnetic Mixer technology, the Lev Mixer technology provides seamless scale up and rapid mixing for process development and GMP manufacturing of buffers at 16 g/L and 73 g/L. For the concentrated buffer at 224 g/L prepared in this study, the Flexel® for Magnetic Mixer technology is the preferred technology thanks to its higher rotation speed which minimizes the time for dissolution (refer to the related application note #14 for details).

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 used for this application study is the Flexel® for Lev Mixer with volumes of 50 L, 200 L and 1000 L. The levitated impeller enables a rotation speed up to 180 rpm, providing an efficient 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 at different volumes of single-use bags (50 L, 200 L, 1000 L), and at different concentrations of powders (16 g/L, 73 g/L, 224 g/L) before the final dilution.

Purpose of the Application Study

The purpose of this application study is to assess the performances of the Flexel® for Lev Mixer technology to dissolve the following powders used in the buffers 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 Lev Mixer.

The usual procedure for a buffer preparation requires the incorporation of the powders in the bag partially filled with WFI. For our study, the bags were respectively filled to 90% of the nominal bag's volume for the TRI and Tris buffer and 80% for the concentrated PBS.

The actual concentrations of salts for which mixing times are measured 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.

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

Materials and Methods

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

1. Standard Flexel® Bag for Lev Mixer (50 L: FXB111567, 200 L: FXB111420, 1000 L: FXB111569)
2. Powder Transfer Bag 30 L (ref. FMA114009)
3. Palletank® for Lev Mixer and Magnetic Mixer[1] (50 L: FXC110820, 200 L: FXC110821, 1000 L: FXC113384)
4. Superconducting drive unit, 230V, EU power cord (ref. LT-DBTL-007)
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 – 0.2mM)
   - Sodium chloride (8.6 g/L – 147mM)
   - Sodium citrate, 2 H2O (5.8 g/L – 20mM)

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

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

Method Used:

1. The buffer is prepared in standard Flexel® Bag for Lev 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 180 rpm to optimize the powders hydration and dispersion.
3. The powders are incorporated in the Flexel® Bag for Lev Mixer using either:
   - SAFC® packaging for dry powder,
   - or Sartorius Stedim Biotech 30 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.

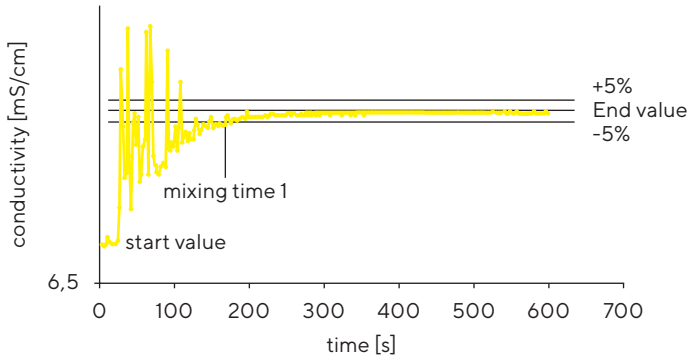

Fig. 1: General principle of mixing time determination via conductivity

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

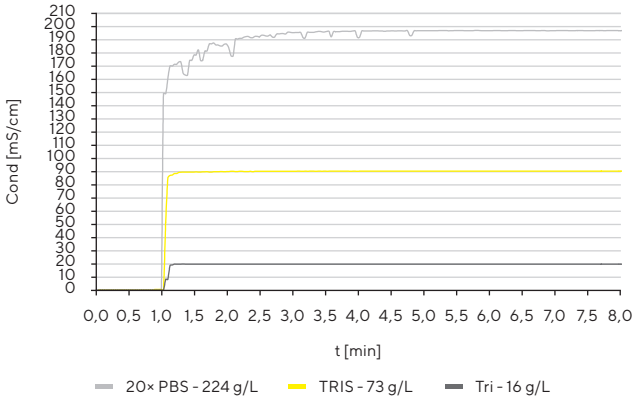

Fig. 2: Buffer preparations in 50 L Flexel® Bag for Lev Mixer

50 L Flexel® Bag for Lev Mixer: A single bucket liner from SAFC® was used to transfer the powders for each of the 3 buffers, thus explaining the continuous conductivity increase. A stable value was then quickly observed, indicative of the mixing completion.

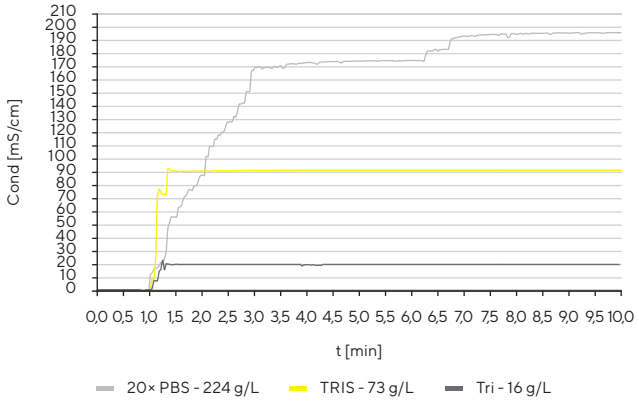

Fig. 3: Buffer preparations in 200 L Flexel® Bag for Lev Mixer

200 L Flexel® Bag for Lev Mixer: 2 separate Sartorius Stedim Biotech 30 L powder transfer bags were used to add the 38.3 kilos of PBS. The time for disconnection and connection of the 2nd powder transfer bag explains the stable period of conductivity observed between the 3rd and the 6th minute during the PBS addition. Two SAFC® bucket liners were used for the preparation of TRIS (13.5 kg) and a single one for 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.

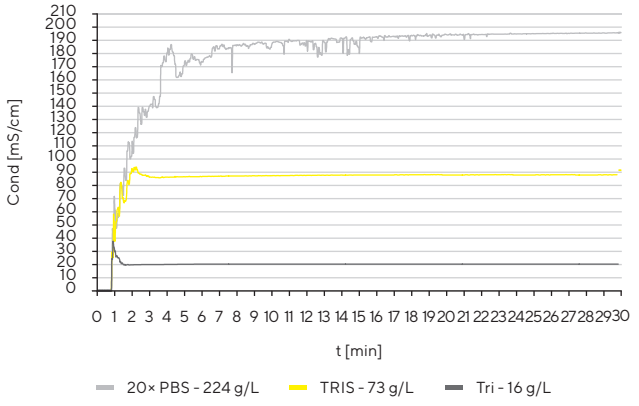

Fig. 4: Buffer preparations in 1000 L Flexel® Bag for Lev Mixer

1000 L Flexel® Bag for Lev 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. Again a stable value of conductivity was monitored at the mixing completion.

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

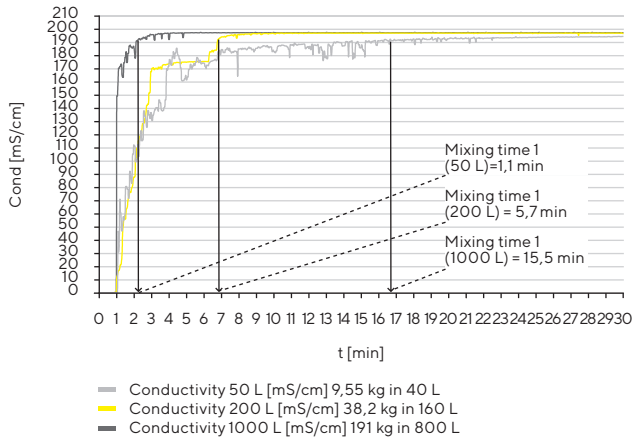

Fig. 5: PBS 20× buffer preparation in Flexel® Bag for Lev Mixer

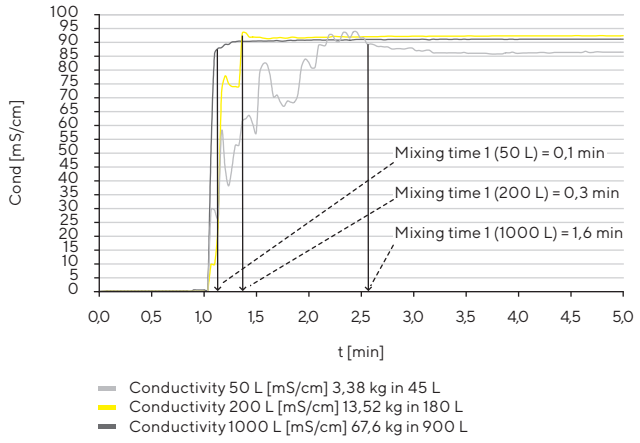

Fig. 6: TRIS buffer preparation in Flexel® Bag for Lev Mixer

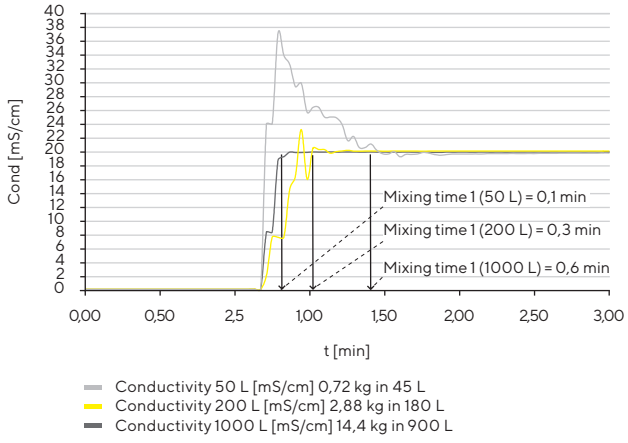

Fig. 7: Sodium citrate | citric acid | NaCl buffer preparation in Flexel® Bag for Lev Mixer

Bag Volume [L]		50	200	1000
Powders Nature				
Citric Acid Sodium Citrate NaCl (16.0 g/L)	Based on conductivity	< 1 min	< 1 min	< 1 min
	Based on visual inspection	< 5 min	< 5 min	< 5 min
Tris Tris HCl NaCl (73.0 g/L)	Based on conductivity	< 1 min	< 1 min	< 1–2 min
	Based on visual inspection	< 5 min	8 min	14 min
20× PBS (224.0 g/L)	Based on conductivity	1–2 min	5–6 min(*)	15–16 min
	Based on visual inspection	10 min	32 min	74 min

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

* 5-6 minutes including 3 minutes for Sartorius Stedim Biotech's exchange of powder bag.

General Comments:

- The mixing times reported in this study include the transfer time of the multiple Sartorius Stedim Biotech Powder Transfer Bag (for the 200 L scale experiment) or SAFC® bucket liners (50, 200 and 1000 L experiments) into the mixing bag assembly.
- For each volume of Flexel® Bag, the conductivity of the solutions reaches a stable value in a few minutes. However, some fine particles of salt can still be visually observed in the solution. The agitation at 180 rpm was maintained until the solution became totally clear. This visual control is facilitated by the large windows of the Palletank®.
- With respective mixing times below 2 minutes (by conductivity) or 14 minutes (by visual observation), a rapid dissolution of the salts was observed with:
  - the TRIS (50 L, 200 L, 1000 L)
  - the Sodium Citrate, Citric Acid (50 L, 200 L, 1000 L)
  - the 20× PBS (50 L).
- The worst-case conditions identified with the 20× PBS in the 200 L and 1000 L Flexel® Bags for Lev Mixer require extended times for complete powder dissolutions. Nevertheless a stable value of the conductivity and a complete dissolution were achieved like with the lower concentrations and volumes.

To the mixing time 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 efficient mixing of the Flexel® with Lev Mixer Technology. For the concentrated buffer 20× PBS prepared in large volumes (200 L and 1000 L), the Magnetic Mixer Technology is recommended to optimize the mixing times thanks to the higher rotation speed (300 rpm with Magnetic Mixer versus 180 rpm with Lev Mixer).
- The contained processing conditions with the closed Powder Transfer Bag docked onto the sterile Flexel® Bag for Lev Mixer are favourable to maintain low bioburden and to reduce to 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 1000 L.

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