sartorius stedim biotech

Scalable buffer preparation with single-use LevMixer® technology

Application Note

#14
#15
#16
#17
#18

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Executive Summary

This application note presents the results of buffer preparations using the Flexel® for LevMixer®[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 (x1) or concentrated (x20) 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 50L to 1,000L. The performances of the single-use mixing system are characterized with quantitative (conductivity measurement) and qualitative (visual inspection) techniques.

As for the Flexel® for Magnetic Mixer technology, the LevMixer® technology provides seamless scale up and rapid mixing for process development and GMP manufacturing of buffers at 16g/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).

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 LevMixer® with volumes of 50L, 200L and 1000L. 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 (50L, 200L, 1000L), 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 LevMixer® 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)
- 20x PBS (final concentration = 191 g/L)

The mixing times are determined by conductivity and visual inspection of the solution in the Flexel® Bag for LevMixer®.

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)
- 20x 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] LevMixer® is a trademark of Pall Corporation and this product uses Pall patented LevMixer® technology.

[2] This product uses Pall patented Magnetic Mixer technology.

All information on patents can be found at Pall.com/patents.

Materials and methods

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

1. Standard Flexel® Bag for LevMixer® (50L: FXB111567, 200L: FXB111420, 1000L: FXB111569)
2. Powder Transfer Bag 30L (ref. FMA114009)
3. Palletank for LevMixer® and Magnetic Mixer[1] (50L: FXC110820, 200L: FXC110821, 1000L: FXC113384)
4. Superconducting drive unit, 230V, EU power cord (ref. LT-DBTL-007)
5. Powder bag holder 200-400-650L (ref. FXA114344)
6. SAFC® powders with the following formulations:
   TRI buffer (product number 44078 – dry powder packaging of 10kg):
   - 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 10kg):
   - Sodium chloride (58.44 g/L – 1M)
   - Tris (6.06 g/L – 50mM)
   - Tris HCl (3.15 g/L – 20mM)

   Concentrated 20x PBS (product number 44079 – dry powder packaging of 10kg):
   - 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 LevMixer® 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 LevMixer® using either:
   - SAFC® packaging for dry powder,
   - or Sartorius Stedim Biotech 30L 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.

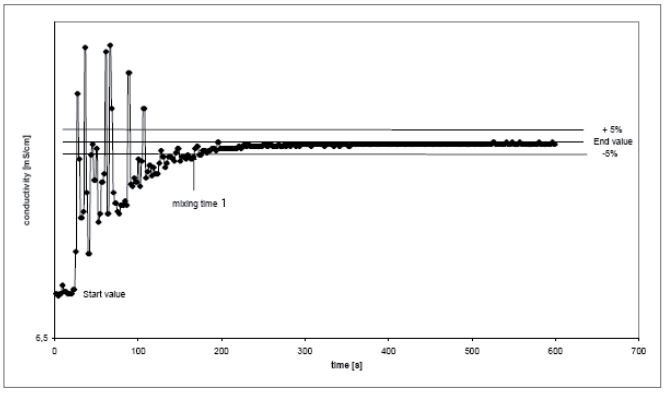

Fig. 1: General principle of mixing time determination via conductivity

   - 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

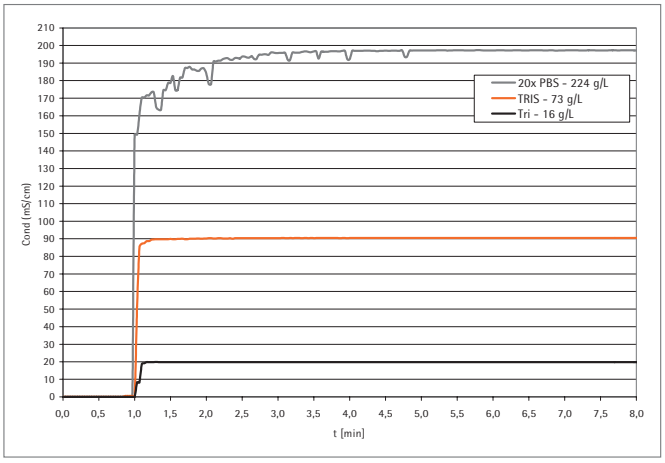

Fig. 2: Buffer preparations in 50L Flexel® Bag for LevMixer®

50L Flexel® Bag for LevMixer®: 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.

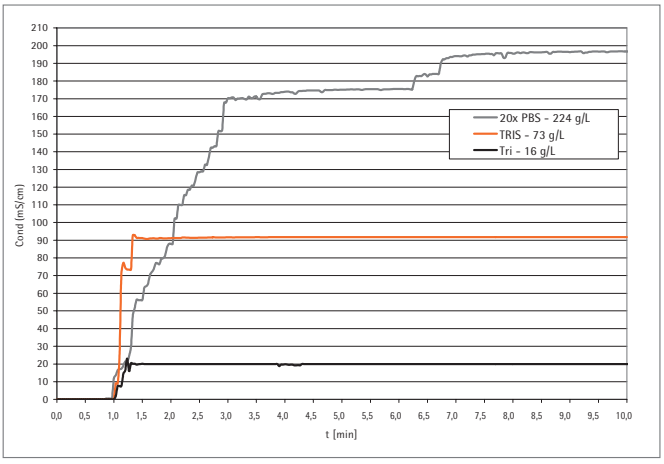

Fig. 3: Buffer preparations in 200L Flexel® Bag for LevMixer®

200L Flexel® Bag for LevMixer®: 2 separate Sartorius Stedim Biotech 30L 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.

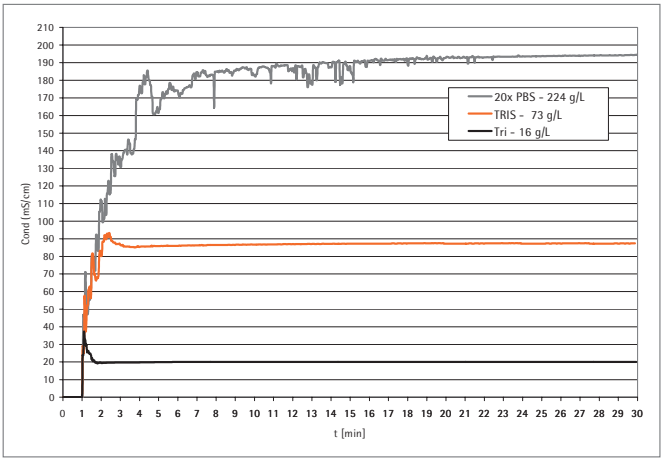

Fig. 4: Buffer preparations in 1000L Flexel® Bag for LevMixer®

1000L Flexel® Bag for LevMixer®: 19 SAFC® bucket liners were used to transfer 191kg of PBS, 7 liners for the Tris (66.7kg) and a single one for the sodium citrate | citric acid (14.4kg). 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

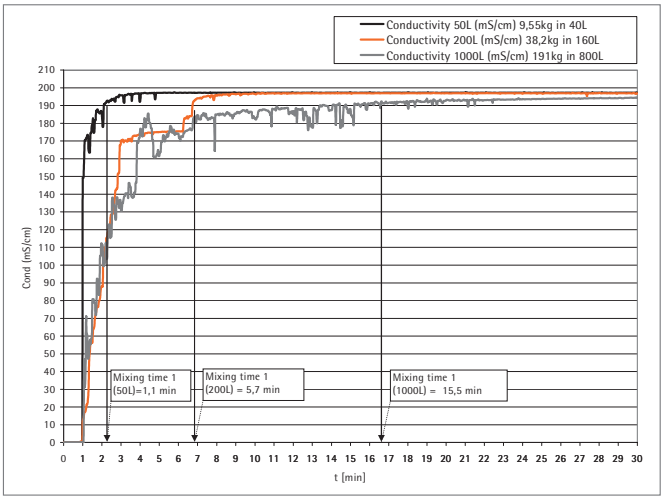

Fig. 5: PBS 20x buffer preparation in Flexel® Bag for LevMixer®

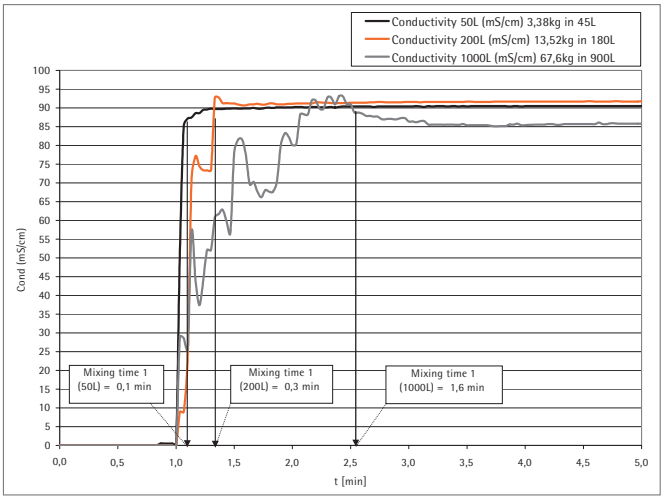

Fig. 6: TRIS buffer preparation in Flexel® Bag for LevMixer®

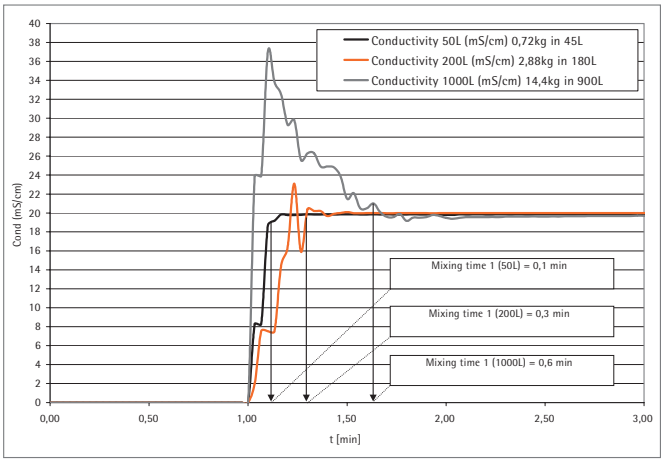

Fig. 7: Sodium citrate | citric acid | NaCl buffer preparation in Flexel® Bag for LevMixer®

Bag Volume (L) / Powders Nature		50	200	1000
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
20x 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 200L scale experiment) or SAFC® bucket liners (50, 200 and 1000L 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 (50L, 200L, 1000L)
  - the Sodium Citrate, Citric Acid (50L, 200L, 1000L)
  - the 20x PBS (50L).
- The worst-case conditions identified with the 20x PBS in the 200L and 1000L Flexel® Bags for LevMixer® 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 LevMixer® Technology. For the concentrated buffer 20x PBS prepared in large volumes (200L and 1000L), 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 LevMixer®).
- The contained processing conditions with the closed Powder Transfer Bag docked onto the sterile Flexel® Bag for LevMixer® 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 50L, 100L, 200L, 400L, 650L and 1000L.

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