Medium and buffer preparation in Xcellerex XDM and XDUO 100 and 500 mixing systems

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Medium and buffer preparation in Xcellerex™ XDM and XDUO 100 and 500 mixing systems

This application note describes preparation of 1 M NaCl, 20 mM PBS, and HyClone™ HyCell™ CHO cell culture medium in Xcellerex single-use mixers for BioProcess™—XDM 100, XDM 500, XDUO 100, and XDUO 500. These procedures are applicable for the whole range of Xcellerex mixers. Cell culture medium preparation was followed by 0.1 µm filtration using ReadyCircuit™ single-use assemblies. Different impeller directions were tested for the NaCl and PBS preparation. Both NaCl and PBS were mixed to 95% homogeneity within 5 min for both mixer sizes. Medium preparation and filtration in XDM 100 and XDM 500 were performed using only single-use assemblies.

Introduction

Xcellerex single-use mixers (XDM and XDUO) are available in several different configurations. In terms of mixing capability, the XDM and XDUO are identical. XDUO, however, offers more powerful automation capabilities than XDM. The XDM mixers range in size from 50 to 1000 L, while XDUO mixers are available from 100 L to 2500 L. In common for all configurations is the robust mixing performance and ease of use. The mixers are designed for process development, commercial and clinical production of biopharmaceuticals, vaccines, and other biologics. Xcellerex mixers support upstream and downstream applications for preparation of buffer, media, product and intermediates, as well as other process fluids.

This application note describes how different solutions, prepared from both fine-grained, light cell culture powder and relatively heavy, crude salt solutions, are solubilized in the XDM/XDUO 100 and XDM/XDUO 500 single–use mixers, respectively. The experiments were designed to demonstrate the mixing performance of XDM and XDUO mixers for the full range of salt and powder types used in cell culture today and to evaluate potential differences in counterclockwise (CCW, up-pumping) and clockwise (CW, down-pumping) stirring.

NaCl and PBS were selected as a model system for mixing of sedimenting solids. NaCl in various concentrations is one of the most used salts in downstream buffers. PBS is a buffer commonly used in, for example, affinity chromatography and other downstream operations. Buffer and salt concentrations were selected in the higher range to mimic a worst-case scenario.

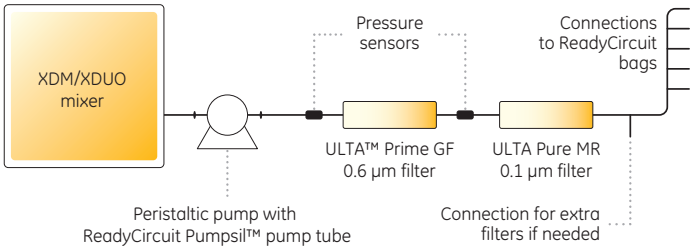

Fig 1. Setup for the filtration of the hydrated HyCell CHO medium.

Hydration of HyClone HyCell CHO, a commonly used medium for CHO cell cultivation, was also studied. HyCell CHO is light and will, unlike the salts, float on top of the liquid. All solids can aggregate hence efficient mixing is essential to obtain a homogeneous solution. A filtration step was incorporated in the study to complete the medium preparation (Fig 1).

Materials and methods

Preparation of salt solutions

The single-use mixer bag was installed in the mixer and filled with purified water to 90% of the nominal volume, that is, 90 L for the 100 L mixer and 450 L for the 500 L mixer. The impeller speed was set to 150 rpm. Both CW and CCW impeller directions were tested. Temperature was maintained at 20°C using a temperature control unit (TCU). Salts to give either 1 M NaCl or 20 mM PBS with 150 mM NaCl (pH 7.4) at the nominal volume were added through the powder port on top of the mixer bag. Conductivity was measured and the mixing time was assessed by calculating the time taken for mixing to 95% homogeneity (t_{m95}) in the conductivity step change.

Hydration of HyCell CHO medium

The single-use mixer bag was installed in the mixer and the medium was prepared according to the recommendations in the medium hydration protocol, see data file 29128610.

The bag was filled with water to 90% of the nominal volume. Agitation was set to 150 rpm with CCW impeller direction. The set point on the temperature control unit was adjusted to keep the liquid at 25°C. HyCell CHO powdered medium was added through the powder addition port on top of the mixer bag to a concentration of 25.4 g/L of the nominal volume. pH and conductivity responses were recorded and samples for analysis of osmolality were taken. After the powder medium had dissolved, 1 g/L of Pluronic™ F-68 was added and allowed to dissolve for 15 to 20 min. At this point, 2.2 g/L of sodium bicarbonate was added and left to dissolve for 30 min. When all media components were completely dissolved, pH was measured and adjusted with 5 M NaOH to a pH between 7.0 and 7.4. Water was added to give final volumes of 100 and 500 L in the XDM/XDUO 100 and 500 mixers, respectively. The HyCell CHO medium was mixed for another 20 min to ensure a homogeneous solution.

Filtration of the hydrated HyCell CHO medium

ReadyCircuit assemblies with filters were connected to the mixer bag (Fig 1). The ReadyCircuit assemblies contained two NFF filters in series: ULTA Prime GF capsule assembly (0.6 µm × 6" [152 mm] filter cartridge) with ULTA Pure MR (0.1 µm × 6") for XDM/XDUO 100; and ULTA Prime GF capsule assembly (0.6 µm × 10" [254 mm]) with ULTA Pure MR (0.1 µm × 10") for XDM/XDUO 500.

For XDM/XDUO 100, the feed flow was set to 1.7 L/min and 8.0 L/min for XDM/XDUO 500. The flow was set to give a filtration time of approximately 1 h. The pressure was monitored to ensure that a pressure of 0.1 MPa (1 bar, 14.5 psi) was not exceeded. ReadyCircuit bags of varying sizes were used to collect the filtered medium.

Results and discussion

NaCl and PBS mixing

The NaCl and PBS mixing was tested at 150 rpm and with both CW and CCW impeller directions. Mixing to t_{m95} of 1 M NaCl as well as the 20 mM PBS, 150 mM NaCl buffer was achieved in less than 1 min in XDM/XDUO 100 (Fig 2). In XDM/XDUO 500, 95% mixing of the both solutions was achieved within 4.5 min (Fig 3). No significant difference was seen in mixing time for CW and CCW impeller directions for XDM/XDUO 100 (Fig 4). For XDM/XDUO 500, the CW impeller speed was approximately 2 min faster ($p < 0.05$) than for CCW when mixing PBS. The longer mixing times in XDM/XDUO 500 compared with XDM/XDUO 100 were caused by the larger liquid volume and the larger amount of salt added. Pretesting showed that mixing time is dependent on salt addition time, which is longer in XDM/XDUO 500 due to the larger amount of salt needed.

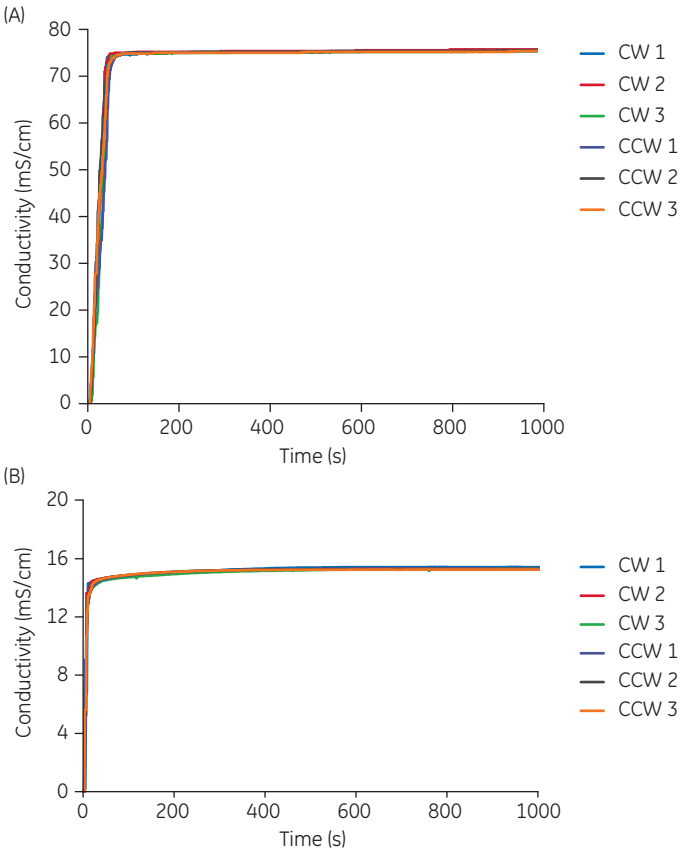

Fig 2. Conductivity curves for (A) NaCl and (B) PBS mixing in XDM/XDUO 100.

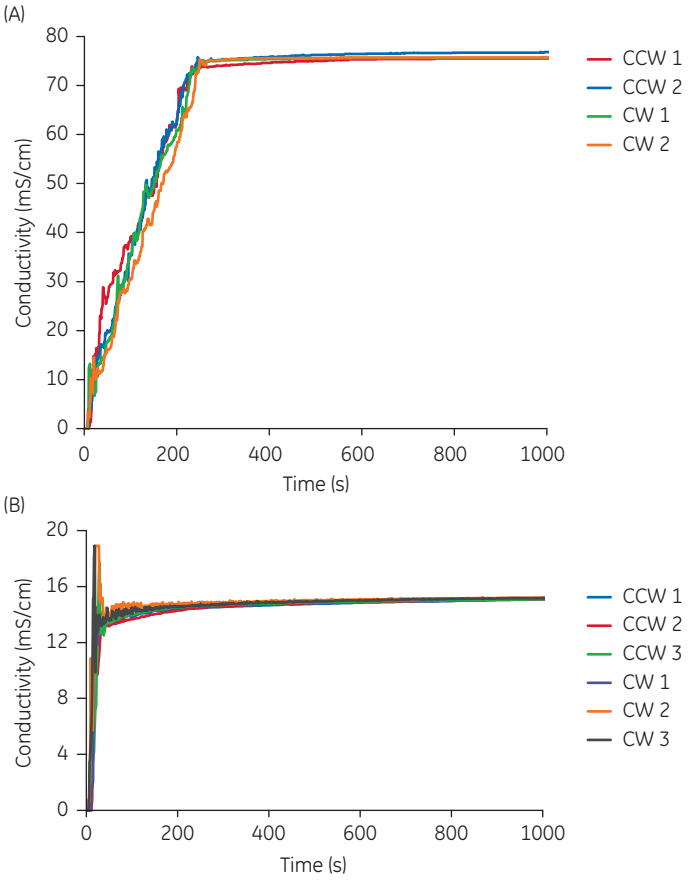

Fig 3. Conductivity curves for mixing of (A) NaCl and (B) PBS in XDM/XDUO 500.

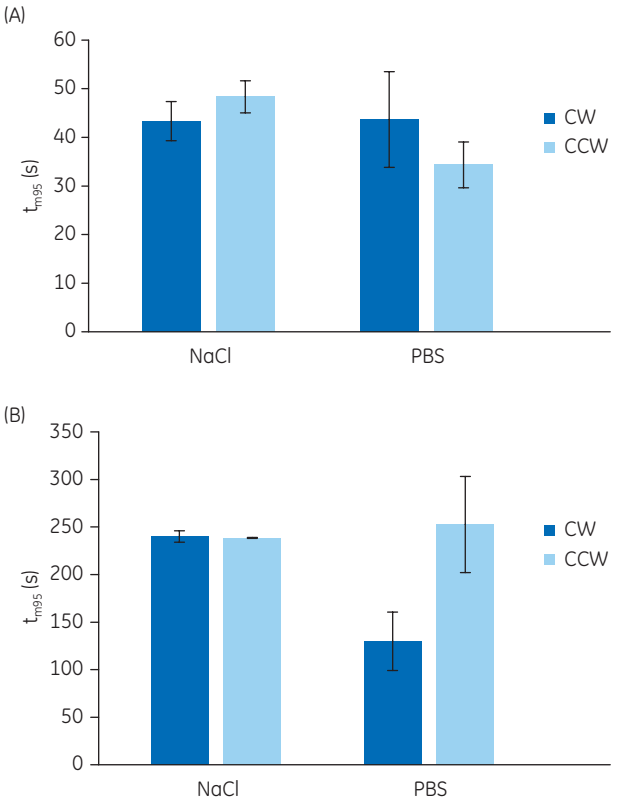

Fig 4. Bar charts showing mean values of t_{m95} for the CW and CCW impeller direction in the mixing of NaCl and PBS in (A) XDM/XDUO 100 and (B) XDM/XDUO 500. The impeller speed was 150 rpm.

Medium hydration

pH, conductivity, and osmolality were recorded during the medium hydration (Fig 5). As for the NaCl and PBS mixing, solubilizing of the medium powder was faster in XDM/XDUO 100 compared with XDM/XDUO 500 due to the smaller volume. This causes a shorter mixing time because of the liquid volume being smaller, but also because the addition time of the powder is faster. The medium was prepared according to the HyCell CHO medium hydration protocol (see data file 29128610) and supplementary Pluronic F-68 and $NaHCO_3$ were added in addition to NaOH for pH adjustment (Fig 5).

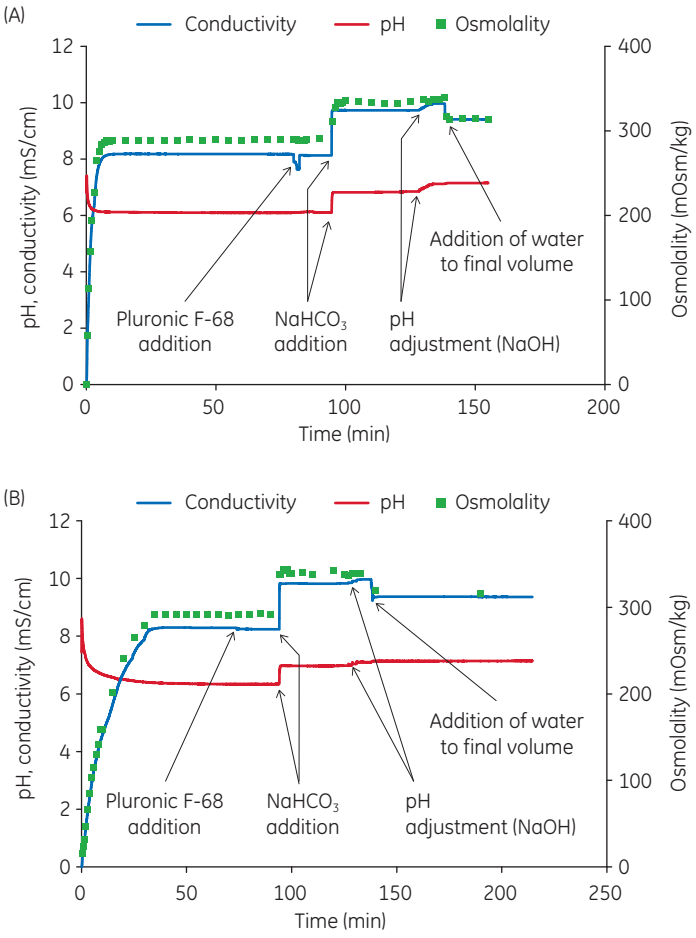

Fig 5. Conductivity, pH, and osmolality curves from the hydration of HyCell CHO medium in (A) XDM/XDUO 100 and (B) XDM/XDUO 500.

After the medium was hydrated, it was filtered. During the filtration, pressure was measured at two positions, before and after the prefilter. The pressure curves can be seen in Figure 6. An increase in pressure over time is seen, which is to be expected due to buildup of material on the filter area. To avoid pressures above 0.1 MPa (1 bar, 14.5 psi), the flow rate was lowered gradually in XDM/XDUO 500.

Hydration of HyCell CHO medium was achieved within 2.6 h, for both 100 L and 500 L mixers. Normal flow filtration of the medium was achieved within 1.1 h. Using this protocol, medium hydration and filtration can be performed in less than 4 h both for XDM/XDUO 100 and XDM/XDUO 500.

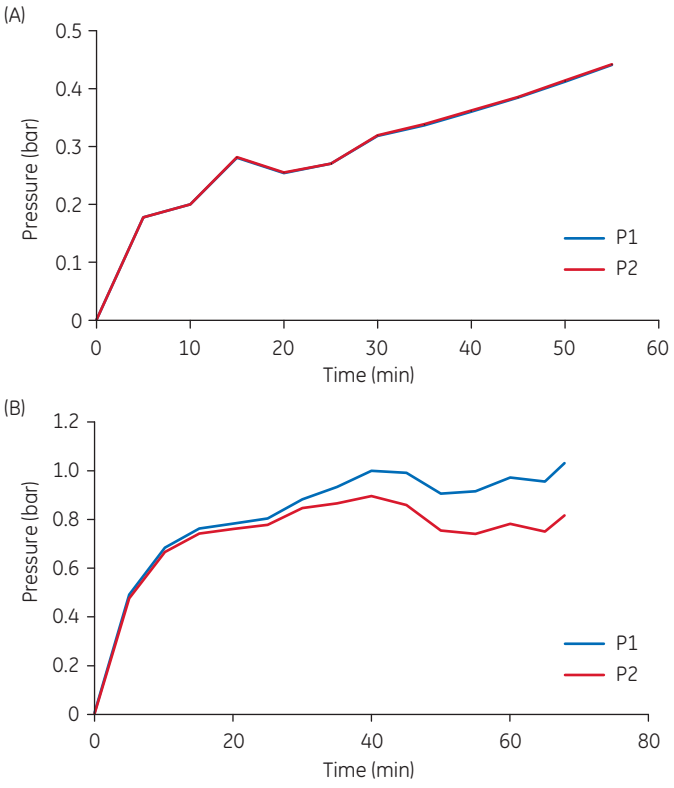

Fig 6. Pressure trend with data sampling every 5 min in (A) XDUO 100 and (B) XDUO 500. Curve P1 displays the inlet pressure and P2 the pressure before the final cartridge from the filtration of HyCell CHO medium.

Conclusions

This study demonstrates that the Xcellerex mixers enable fast and efficient mixing for upstream and downstream applications such as buffer and cell culture media preparation. The medium preparation is easy and quick to perform in both XDM/XDUO 100 and XDM/XDUO 500. The mixers are easily integrated with ReadyCircuit assemblies to perform a complete medium preparation solution—from hydration to filtration and storage—using an entire circuit of single-use products.

Ordering information

Product	Product code
Xcellerex XDM-T Jacketed Stainless Steel Mixer	29054862
Xcellerex XDUO-T Jacketed Stainless Steel Mixer	29054863
XDM 100 Plus Bag	888-0154-C
XDM 500 Plus Bag	888-0156-C
HyClone HyCell CHO powder medium	SH30933.03
ULTA Prime GF capsule assembly, 0.6 µm × 10" (254 mm)	12410069
ULTA Pure MR capsule assembly, 0.1 µm × 10" (254 mm)	29096798
ULTA Prime GF capsule assembly, 0.6 µm × 6" (152 mm) tubing	12410068
ULTA Pure MR capsule assembly, 0.1 µm × 6" (152 mm)	29096794
ReadyCircuit Sensor Assembly w SciLog Pressure Sensor, 0.375" (9.53 mm) × 6" (152 mm) RSRM, 1 pk	28979471
ReadyCircuit Jumper T Manifold, 6 ports, 6" (152 mm) of 0.375" (9.53 mm) tubing, 1 pk	12410182
ReadyCircuit Jumper Y Manifold, 3 ports, 6" (152 mm) of 0.375" (9.53 mm) tubing, 1 pk	12410190
ReadyCircuit Jumper Tube Set, 3 ft (914 mm) of 0.5" (12.7 mm) tubing, 1 pk	12410121
ReadyCircuit Jumper T Manifold, 4 ports, 6" (152 mm) of 0.5" (12.7 mm) tubing	12410175
ReadyCircuit Jumper Tube Set, 19" (483 mm) of Pumpsil 0.375" (9.53 mm) × 0.563" (14.3 mm)	28979432
ReadyCircuit 1 L Hanging/pillow bags/2-D pillow bag[1]	12410218
ReadyCircuit 3-D bag assemblies (for use with ReadyKart)[1]	12410206

[1]Bags available with a range of ports and volumes depending on the volume of sample to be filtered.

For more information on Xcellerex XDM and XDUO mixing systems, please contact your local sales representative.

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