Characterization of Xcellerex XDM and XDUO 1000 single-use mixers

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Characterization of Xcellerex™ XDM and XDUO 1000 single-use mixers

This application note describes mixing and heating-cooling characterization data for the Xcellerex XDM/XDUO 1000 single-use mixer. A design of experiments (DoE) approach to liquid-liquid mixing was successfully applied to establish a statistical model to predict the mixing time throughout the working range. Liquid-liquid mixing times as low as 130 s were observed at the maximum volume (1000 L) and highest viscosity tested (20 cP). Further, mixing of 10X phosphate-buffered saline salts was tested. Mixing to 95% homogeneity (t_{m95}) was reached within 3 min at 200 rpm. Heating of liquid from 5°C to 20°C and 20°C to 37°C was achieved within 3 h for all tested volumes (220, 610, and 1000 L). Cooling from 37°C to 20°C and 20°C to 5°C was achieved within 8 h for all tested volumes.

Introduction

Xcellerex single-use mixers (XDM and XDUO) are available in several configurations. In terms of mixing capability, XDM and XDUO mixers are identical. XDUO, however, offers more powerful automation capabilities compared with XDM. The XDM mixers range in size from 50 to 1000 L, while XDUO mixers are available from 100 to 2500 L. All configurations provide 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.

The aim of this study is to give a detailed description of the physical characteristics of XDM/XDUO 1000 in terms of mixing and heating and cooling. The mixing properties of XDM/XDUO 1000 have been investigated with regard to both liquids and solids. For mixing of liquids, a DoE approach was applied where liquid volume, viscosity, and impeller speed were varied to create a statistical model predicting the mixing time across the working range. In addition to liquid mixing, mixing of solids was investigated by preparation of a model solution, 10X PBS, from salts. Heating and cooling times for different temperature intervals were investigated for minimum, middle, and maximum liquid volumes (220, 610, and 1000 L).

The characterization data presented in this application note is essential for optimizing the mixing or heating-cooling protocol of XDM/XDUO 1000 for bioprocess applications, and for effective scale-up.

Materials and methods

System setup

The mixer was equipped with an XDM 1000 Plus bag and an Xcellerex temperature probe. A temperature control unit (TCU) was used to control the temperature of the liquid (Polyscience, 9 kW). External pH, temperature, and conductivity probes (Campbell Scientific) were used for logging of data via a data logger (Campbell Scientific).

Liquid-liquid mixing

The liquid-liquid mixing time was assessed by adding pulses of acid and measuring the pH change at different positions in the mixer (Fig 1). The tests were performed according to DoE where impeller speed, volume, and viscosity were varied simultaneously using a central composite design. Tests were performed in duplicate with the following exception: six replicates were run for 10 cP viscosity, 610 L volume with 125 rpm impeller speed.

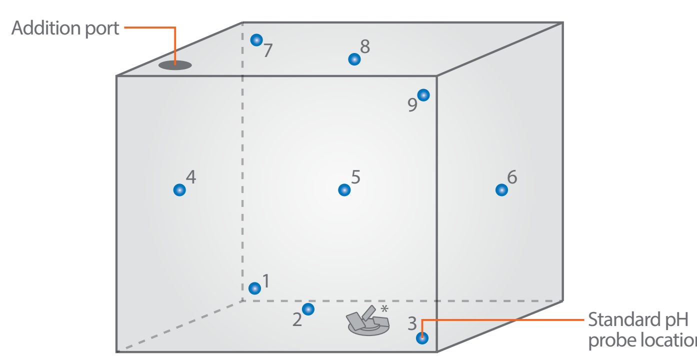

Fig 1. pH probe distribution in XDM/XDUO 1000 at 1000 L. Probes 4 to 9 were lowered for tests at 610 L. At 220 L, probes 4 to 6 were lowered and probes 7 to 9 disconnected.

The mixer bag was filled with liquid to the volume to be tested (220, 610, and 1000 L). For the tests at 1 cP viscosity, the liquid consisted of 0.1 M NaCl in purified water. For tests at 10 and 20 cP, sucrose and NaCl were dissolved in water to generate a viscous liquid with a final NaCl concentration of 0.1 M. The impeller was set to rotate in a counterclockwise (CCW) direction giving an upward pumping mixing pattern. The temperature was controlled at 20°C. For pH change, acid (0.2 M HCl in purified water, 10 or 20 cP sucrose) was added at a ratio of 1:2667 for 1 cP and 1:1000 for 20 cP of the liquid volume in the mixer. The ratios were chosen to induce a pH step change of approximately 1 pH unit. For the tests at 1000 L volume, the pH was recorded at nine positions in the mixer (Fig 1). The probes were arranged to cover all areas where poor mixing could be expected to occur. The pH probes were connected to an external data logger for logging of data. Probe height and number were reduced as the liquid volume was lowered. The mixing time was assessed by calculating the time to reach 95% of the pH step change (t_{m95}). The slowest t_{m95} from each run was used for model calculations in the software MODDE™ (version 11.0.0.1717, MKS Umetrics AB).

Solid-liquid mixing

Mixing of solids was tested by preparing a standard solution with salts: 20 mM phosphate with 150 mM NaCl (10X PBS). The amount of NaH_2PO_4 (monohydrate) and Na_2HPO_4 (dihydrate) salts was calculated to give a pH of 7.4 at 20°C. A 10X solution was chosen to represent a higher solid content than typically encountered. Four conditions were tested: minimum and maximum volume at 100 and 200 rpm stirring rate. For each test the mixer bag was filled with purified water to the final volume, that is, 220 and 1000 L for minimum and maximum volume, respectively. The impeller speed was set to 100 or 200 rpm in clockwise (CW) impeller direction (downward pumping mixing pattern). The temperature was controlled at 20°C. The salts were added through a funnel connected to the powder port on top of the mixer bag. The mixing time was assessed by calculating t_{m95} on the conductivity step change. The conductivity probe was placed in position 3 (Fig 1).

Heating-cooling

The heating-cooling properties of XDM/XDUO 1000 were tested at three different volumes: 220, 610, and 1000 L. The mixer bag was filled with 6 g/L NaCl in purified water to the volume to be tested, and the impeller speed was set to 125 rpm (CCW). The heating and cooling properties of XDM/XDUO 1000 were assessed by measuring the time to reach 95% of the temperature step change (t_{95}) for four different temperature intervals: 5°C to 20°C, 20°C to 5°C, 20°C to 37°C, and 37°C to 20°C.

If using an XDUO mixer connected to an X-Station mobile control console, automatic temperature control with PID regulation is possible. However, for the results to be applicable for both XDM and XDUO mixers, a manual approach was used. The temperature setting on the TCU was controlled manually by setting the temperature to 10°C above (for heating) or below (for cooling) the intended set point and adjusting it to the intended set point when 95% of the step change had been reached. Temperature logging continued until it could be verified that the temperature stabilized at the intended set point. The temperature was logged using an external temperature probe and data logger.

Results and discussion

Liquid-liquid mixing

Liquid-liquid mixing was tested at 220, 610, and 1000 L at three different viscosities: 1, 10, and 20 cP. The impeller speed was varied from 75 to 175 rpm for 1 and 20 cP viscosities, and from 50 to 200 rpm for 10 cP viscosity. In Figure 2, results are shown from the probe position with the longest t_{m95} determined for each run, that is, the worst-case scenario.

The t_{m95} varied between 28 and 499 s for the tested conditions (Fig 2). The mixing time decreased with decreasing volume and increasing impeller speed.

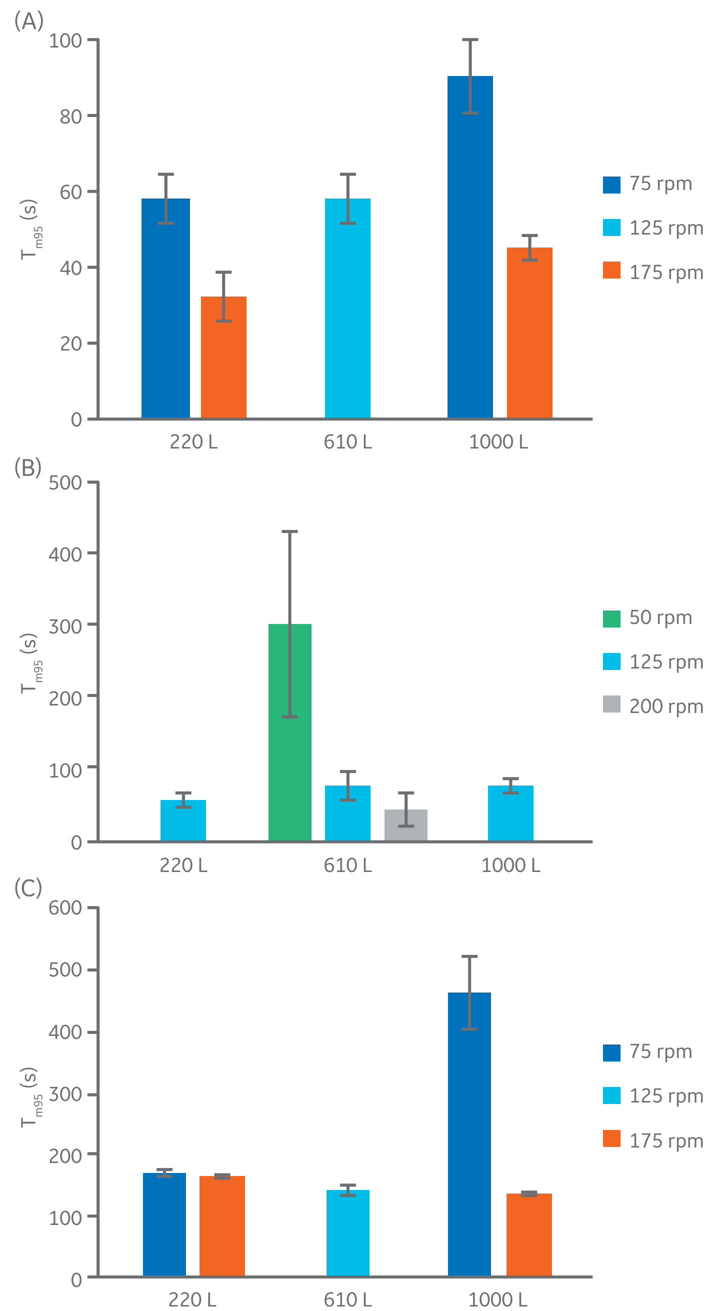

Fig 2. t_{m95} results from the liquid-liquid mixing study at (A) 1 cP, (B) 10 cP, and (C) 20 cP viscosities. Error bars display one standard deviation.

Mixing times for typical conditions (1–10 cP mixtures, 75–200 rpm, various volumes) are all completely homogeneous within 2 min. Only under extreme conditions (slow impeller, high viscosity, large volume) would mixing take up to 10 min to complete.

The resulting t_{m95} from the slowest probe positions were evaluated using the statistical software MODDE. A multiple linear regression (MLR) model was created from the central composite test design. Liquid volume, impeller speed, and viscosity were evaluated at a 95% significance level. The model fit was judged by the fraction of variation of the response explained by the model (R^2) and the fraction of variation of the response that can be predicted by the model (Q^2, Table 1). The overall fit of the model to the data is good with an R^2 value of 0.841. How well the model can predict new data points is also acceptable with a Q^2 value of 0.680. Q^2 values greater than 0.1 indicate a significant model. The model showed a good fit with acceptable R^2 and Q^2 and high reproducibility (Table 1). Altogether this means that the model is adequate and can be used for predictions of t_{m95} within the design space.

Table 1. The liquid-liquid mixing t_{m95} model's statistical parameters

Parameter	Value
R^2 (Model fit)	0.841
Q^2 (Model fit prediction)	0.680
Reproducibility	0.902

The model for liquid-liquid mixing t_{m95} is visualized as a contour plot in Figure 3. The model predicts impeller speed and viscosity as significant factors affecting the mixing time.

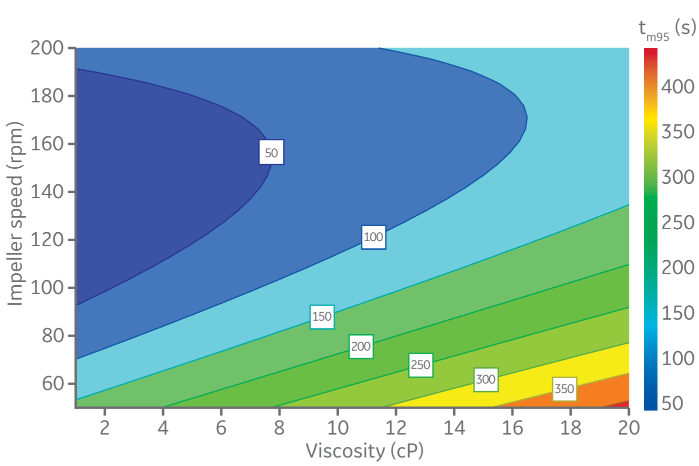

Fig 3. t_{m95} for liquid-liquid mixing. Contour plot of the statistical model, produced in MODDE software. Data is shown for 610 L volume.

Solid-liquid mixing

Mixing times to t_{m95} were less than 10 min for all tests (Fig 4). The pH was within expected values of 7.4 ± 0.2. t_{m95} was reached faster with the 200 rpm impeller speed compared with the 100 rpm speed. At lower impeller speeds, the smaller volumes reached t_{m95} sooner than the larger volumes did. Unexpectedly, at higher impeller speeds, the larger volumes reached t_{m95} sooner than the smaller volumes did.

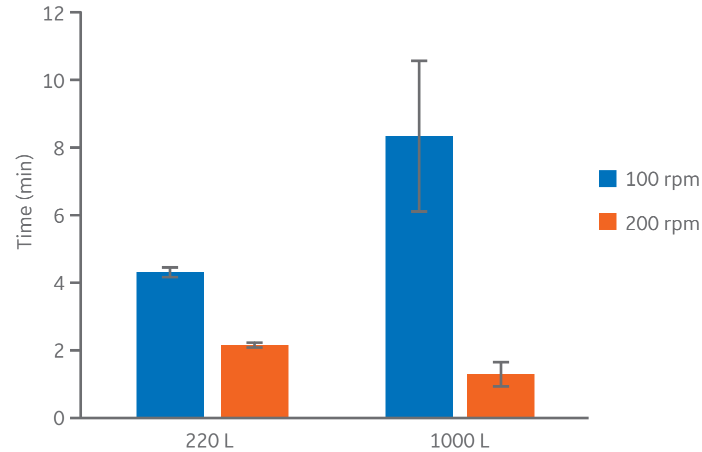

Fig 4. Solid-liquid mixing, time to 95% final conductivity (t_{m95}). Error bars display one standard deviation.

Heating-cooling

The manual temperature control was efficient, and the intended set point was reached as depicted (Fig 5). Heating was faster than cooling. t_{95} for the heating intervals, 5°C to 20°C and 20°C to 37°C, was 2.4 h or less for all tested volumes (Fig 6). Cooling time was proportional to volume. t_{95} for cooling from 37°C to 20°C temperature interval varied between 2.5 and 4.3 h. Cooling times for 20°C to 5°C varied between 4.5 and 7.6 h.

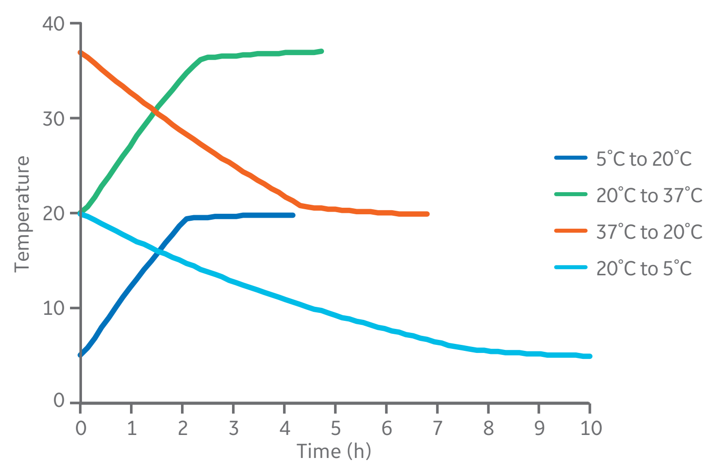

Fig 5. Heating and cooling curves at 1000 L volume.

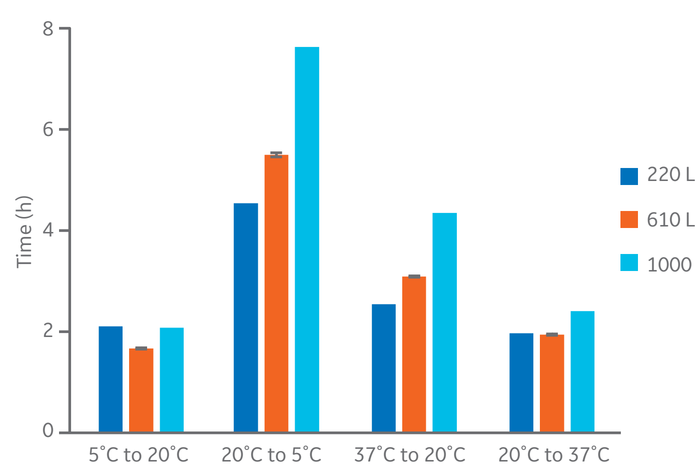

Fig 6. t_{95} results from the heating-cooling tests for different temperature ranges. The error bars for 610 L volume display one standard deviation (n=3).

Conclusions

This characterization study demonstrates the ability of Xcellerex XDM and XDUO 1000 single-use mixers in the preparation and handling of solutions in multiple applications and conditions. Robust liquid-liquid mixing times as low as 130 s were observed at the maximum volume and viscosity tested. The mixing was efficient throughout the complete mixer volume.

A DoE approach to liquid-liquid mixing was successfully used and a model created to predict the mixing time within the operating range. In the solid-liquid mixing, effective mixing to 95% homogenity of PBS was achieved within 3 min at 200 rpm impeller speed. Heating of liquid was achieved within 2.4 h for all tested volumes and temperature intervals. Cooling times varied between 2.5 and 7.6 h.

The results of these studies should aid in the implementation of single-use mixers in new facilities and help in process optimization and scale-up.

Ordering information

Product	Product code
Xcellerex XDM-T Jacketed Stainless Steel Mixing System	29054862
Xcellerex XDUO-T Jacketed Stainless Steel Mixing System	29054863
XDM 1000 Plus Bag	888-0157-C

Related documents

Document	Product code
Performance guide: Mixing and heating-cooling characterization of Xcellerex XDM and XDUO single-use mixers	29237251
Application note: Characterization of Xcellerex XDM 50 single-use mixer	29237878
Application note: Characterization of Xcellerex XDM and XDUO 100 single-use mixers	29242783
Application note: Characterization of Xcellerex XDM and XDUO 200 single-use mixers	29242788
Application note: Characterization of Xcellerex XDM and XDUO 500 single-use mixers	29242789
Data file: Xcellerex XDM Mixer	29048367
Data file: Xcellerex XDUO Mixer	29048366
Data file: Xcellerex XDUO 2500 Mixer	29153543

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

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