Characterization of Xcellerex XDM 50 single-use mixer

Intellectual Property Notice: The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.

cytiva.com

CY13911-27May20-AN

Characterization of Xcellerex™ XDM 50 single-use mixer

This application note describes mixing and heating-cooling characterization data for the Xcellerex XDM 50 single-use mixer. A design of experiments (DoE) approach to liquid-liquid mixing was successfully applied to establish a model to predict the mixing time throughout the working range. Liquid-liquid mixing times as low as 14 s were observed at the nominal volume (50 L) and highest viscosity tested (20 cP). Further, in the solid-liquid mixing, PBS was mixed to 95% homogeneity (t_{m95}) in less than 20 s (50 L volume) while HyClone™ HyCell™ CHO powdered medium was mixed in less than 3 min (45 L volume). Heating of liquid from 5°C to 20°C and 20°C to 37°C was achieved within 1.2 h for all tested volumes (17, 33.5, and 50 L). Cooling from 37°C to 20°C and 20°C to 5°C was achieved within 1.3 h except cooling from 20°C to 5°C at the 17 L volume which took 2.1 h.

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 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.

The aim of this study is to give a detailed description of the physical characteristics of XDM 50 in terms of mixing and heating-cooling. The mixing properties of XDM 50 were investigated regarding both liquids and different types of solids. For mixing of liquids, a DoE approach was used to create a model predicting the mixing times across the working range. Model solutions such as HyCell CHO cell culture medium and phosphate buffered saline (PBS) were prepared in the mixer to show its ability to handle both light, fine-grained powders and relatively heavy, crude salts. Heating and cooling times for different temperature intervals were investigated for minimum, middle, and nominal liquid volumes (17, 33.5, and 50 L).

The characterization data presented in this application note is essential for optimizing the mixing or heating-cooling protocol of XDM 50 for bioprocess applications, and for effective scale-up.

Materials and methods

System setup

The mixer was equipped with an XDM 50 Plus bag and an Xcellerex temperature probe. A temperature control unit (TCU) was used to control the temperature of the liquid (Polyscience, 3 kW). External pH (ProMinent), temperature, and conductivity probes (Ahlborn) were used for logging of data via a data logger (Ahlborn).

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 the factors impeller speed, liquid volume, and viscosity were varied simultaneously. The factorial design was a central composite design.

The mixer bag was filled with liquid to the volume to be tested (17, 33.5, and 50 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. The pH was recorded at nine positions in the mixer for the 50 L volume (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. The number of probes was 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).

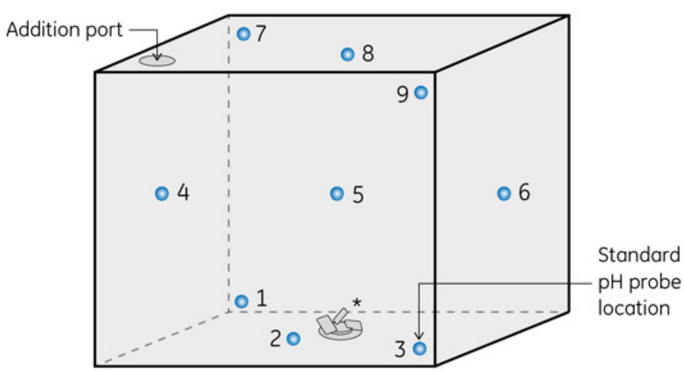

Fig 1. pH probe distribution in XDM 50 at 50 L. Probes 4 to 9 were lowered for testing at 33.5 L. At 17 L, probes 4 to 6 were lowered additionally and probes 7 to 9 were disconnected. *Note that the impeller is welded to the bag. The impeller design shown is for 100, 200, and 500 L bag sizes. The equivalent 50 L impeller is not shown.

Solid-liquid mixing

Mixing of solids was tested by preparing two different solutions: 20 mM PBS with 150 mM NaCl and HyCell CHO medium. The PBS salts are denser than water and sink to the bottom while the HyCell CHO medium is light and floats on top of the liquid; both solutions were of relatively high concentration. Thus, these solids represent two distinct, yet demanding, mixing scenarios.

For PBS, mixing was tested at 17 and 50 L (minimum and nominal working volumes) at 100 and 200 rpm. The temperature of the liquid was 20°C. Salts to produce a 20 mM PBS buffer with 150 mM NaCl at a pH of 7.4 were added through the funnel on top of the mixer bag while stirring in a clockwise (CW) direction. CW impeller direction creates a downward pumping mixing pattern. Conductivity was measured and the mixing time was assessed by calculating t_{m95} on the conductivity step change. The conductivity probe was placed in position 9 for tests at the nominal volume and it was lowered for the tests at the minimum volume (Fig 1).

Mixing tests with HyCell CHO medium were performed according to the medium rehydration protocol (see data file 29128610), hence the medium powder was added to purified water at 90% of the final medium volume. The HyCell CHO medium was added to the mixer using a powder addition bag. Two volumes representing the minimum and maximum volumes of medium that can be mixed in XDM 50 were selected. The volumes used were 17 L (minimum volume) and 45 L (90% of nominal volume), which after addition of HyCell CHO medium and supplementary water corresponded to final media volumes of 18.9 and 50 L, respectively. Addition of media supplements and water to the final volume was not included in the mixing study. Impeller speeds that are typically deployed in bioprocessing applications were selected. Impeller speed was set to 100 and 200 rpm for the 17 and 45 L volumes, respectively. CCW impeller direction was used for both volumes. As for PBS mixing, conductivity was used to monitor the mixing and t_{m95} was calculated on the conductivity step change. The conductivity probe was positioned in position 9 (Fig 1), as in the PBS tests.

Heating-cooling

The heating-cooling properties of XDM 50 was tested at three different liquid volumes: 17, 33.5, and 50 L. The liquid consisted of 6 g/L NaCl in purified water. During testing, the impeller speed was constant at 125 rpm (CCW). The heating and cooling properties of XDM 50 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. 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 three different volumes: 17, 33.5, and 50 L and at three different viscosities: 1, 10, and 20 cP. The impeller speed was varied from 50 to 200 rpm. 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 6 and 28 s for the tested conditions (Fig 2). The mixing time decreased with decreasing volume and increasing impeller speed. Mixing was generally faster at the lower viscosities.

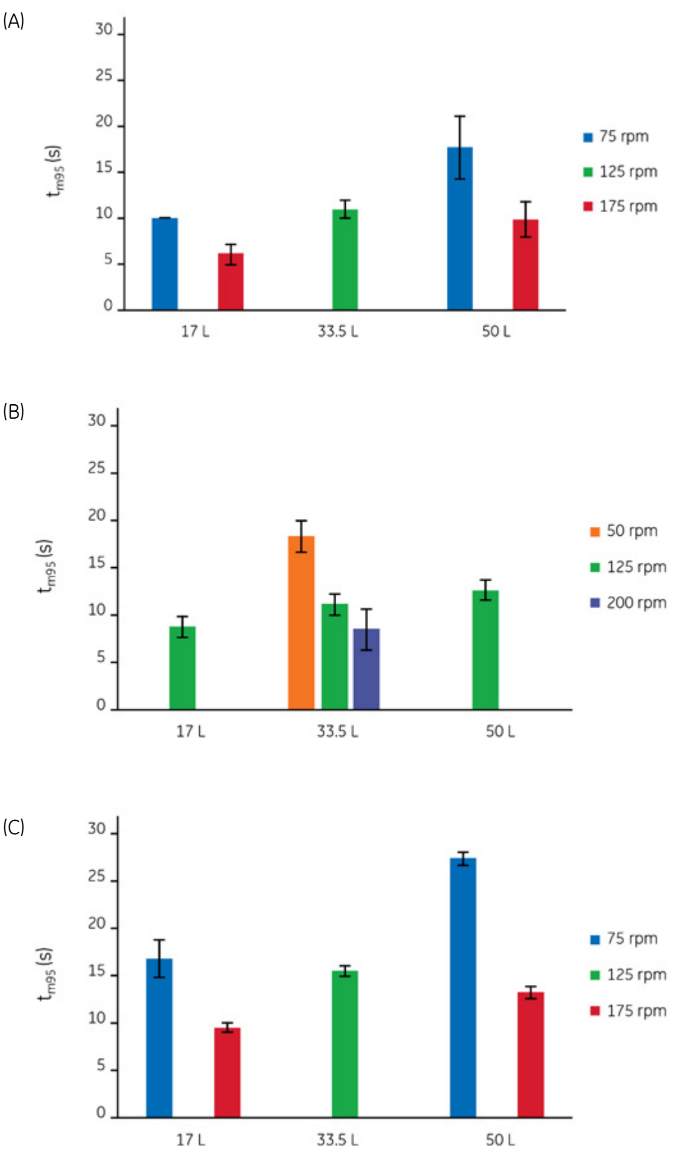

Fig 2. t_{m95} results from the liquid-liquid mixing study at (A) 1 cP, (B) 10 cP, and (C) 20 cP viscosities. Error bars display one standard deviation.

The average difference in t_{m95} for the fastest and slowest probe position for each run was 6 s, indicating that mixing is fast and efficient throughout the whole mixer volume.

The resulting t_{m95} from the slowest probe position of each run was evaluated using the statistical software MODDE. A multiple linear regression (MLR) model was created from the central composite test design. The investigated factors: 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). Values close to 1.0 for both R^2 and Q^2 indicate a good model with excellent prediction power. Q^2 values greater than 0.1 indicate a significant model.

The model showed a good fit with high R^2 and Q^2 and a residual standard deviation of 1.78 (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

Statistical parameter	Value
R^2 (Model fit)	0.90
Q^2 (Model fit prediction)	0.83
RSD (Residual standard deviation)	1.78

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

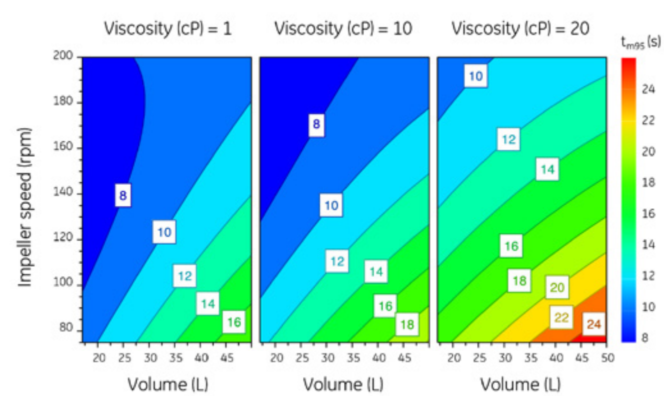

Fig 3. t_{m95} for liquid-liquid mixing. Contour plot of the statistical model, produced in MODDE software.

Solid-liquid mixing

Mixing to t_{m95} was achieved within 3 min for PBS at 100 rpm impeller speed (Fig 4) for both the 17 and 50 L volumes. Mixing time was significantly faster at 200 rpm with mixing times of less than 20 s. At 200 rpm, little difference was seen in mixing time for the 17 and 50 L volumes.

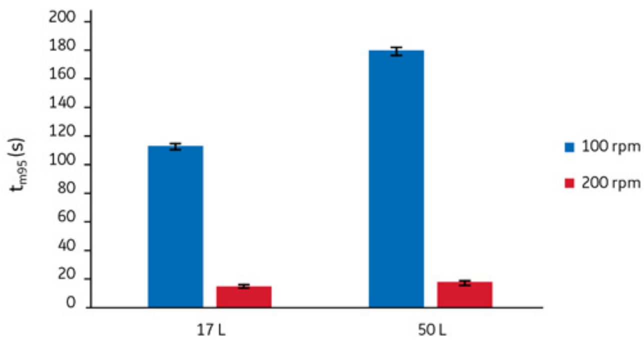

Fig 4. t_{m95} results from the PBS mixing at CW impeller direction. The error bars display one standard deviation.

To demonstrate the mixer's capability of mixing light powders such as cell medium, HyCell CHO medium was mixed using CCW impeller direction (Fig 5 and 6). Average t_{m95} was less than 5 min for both the 17 and 45 L volumes.

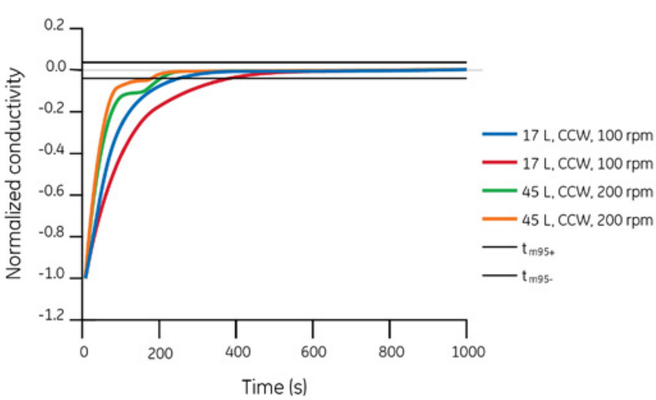

Fig 5. Normalized conductivity plots for the mixing of HyCell CHO medium. The gray lines indicate the interval for t_{m95}. The tests at 45 L were run at 200 rpm and the tests at 17 L were run at 100 rpm.

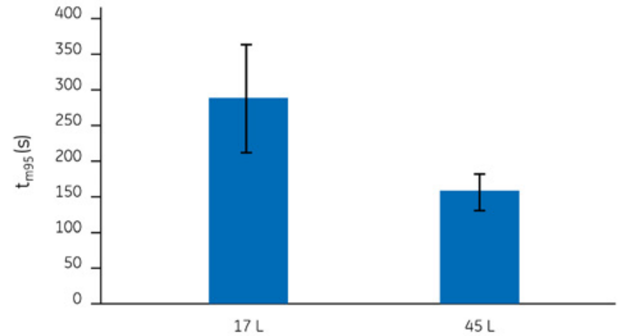

Fig 6. t_{m95} for the HyCell CHO medium mixing at CCW impeller direction. The error bars display one standard deviation.

Heating-cooling

The manual temperature control showed fast heating and cooling times and the intended set point was reached with little or no overshoot/undershoot in temperature (Fig 7). The t_{95} for the heating and cooling intervals was less than 1.3 h for all tested conditions, except for cooling from 20°C to 5°C at 17 L (Fig 8). Cooling from 20°C to 5°C at 17 L had a t_{95} of 2.1 h. Heating from 5°C to 20°C and 20°C to 37°C took approximately 1 h for all tested volumes. The minimum volume, 17 L, had the longest heating and cooling times amongst all tested volumes. This can be explained by the lower heat transfer area-to-volume ratio at 17 L compared to the other volumes.

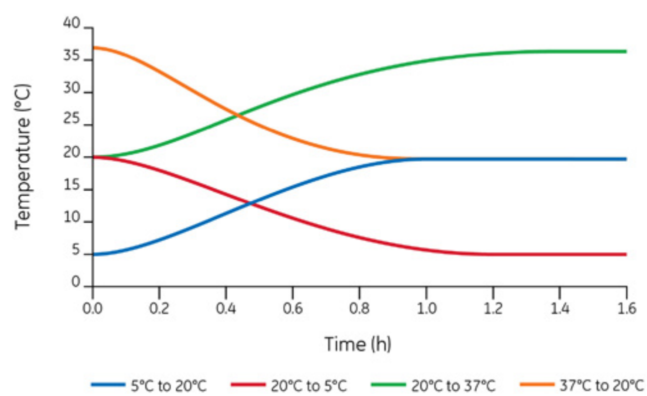

Fig 7. Heating and cooling curves at 50 L.

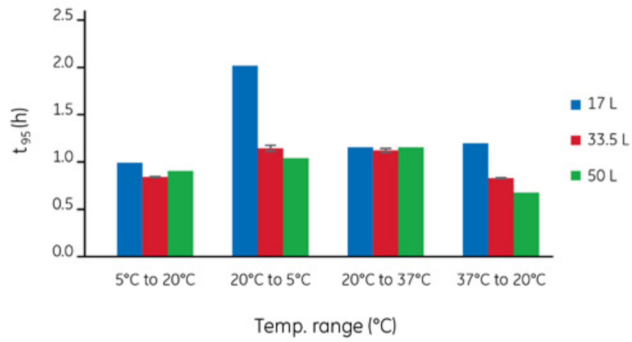

Fig 8. t_{95} results from the heating-cooling tests for different temperature ranges. The error bars display one standard deviation.

Conclusions

This characterization study demonstrates the ability of Xcellerex XDM 50 single-use mixer in the preparation and handling of solutions in multiple applications and conditions. Robust liquid-liquid mixing times as low as 14 s were observed at the highest volume and viscosity tested. The mixing was efficient throughout the complete mixer volume. We successfully used a DoE approach to liquid-liquid mixing and created a model to predict the mixing time within the operating range. In the solid-liquid mixing, effective t_{m95} mixing of PBS was achieved within only 20 s at 200 rpm for both the 17 L and 50 L volume, while HyCell CHO medium was mixed in under 3 min for the highest volume (45 L) tested. Heating and cooling of liquid was generally achieved within 1.3 h except for cooling from 20°C to 5°C at 17 L, which was somewhat longer (2.1 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
XDM 50 Plus bag	888-0351-C
HyClone HyCell CHO powdered medium	SH30933.03

Related documents	Product code
Performance guide: Characterization of Xcellerex XDM and XDUO single-use mixers	29237251
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.

GE Healthcare Bio-Sciences AB
Björkgatan 30
SE-751 84 Uppsala
Sweden

gelifesciences.com/xcellerex

GE Healthcare UK Ltd., Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Dharmacon Inc., 2650 Crescent Dr, Lafayette, CO 80026, USA
GE Healthcare Bio-Sciences Corp.,100 Results Way, Marlborough, MA 01752, USA.
HyClone Laboratories Inc., 925 W 1800 S, Logan, UT 84321, USA
GE Healthcare Japan Corp., Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

29237878 AB 02/2017