Evaluation of mechanical stress on an oil-water emulsion system in magnetically levitated single-use centrifugal pumps

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1 Introduction

Mechanical stress can result in undesired cell damage, which is accompanied by qualitative and/or quantitative product loss [1-3]. The magnitude of the mechanical stress depends on the type of pump and the pump settings. For single-use technology based applications, peristaltic pumps, syringe pumps and diaphragm pumps are commonly used. However, these pumps generate high local mechanical stresses due to pulsating flows, pressure and compression of the pump tubing. Alternative pump types that reduce mechanical stress are required. The magnetically levitated Levitronix® centrifugal pump-systems enable pulsation-free transfer of fluids. They have the advantage of being bearingless and may be a suitable alternative to conventionally used peristaltic and diaphragm pumps. In order to evaluate pump related mechanical stress, shear stress investigations are carried out on shear sensitive fluids, for example cell suspensions or enzyme solutions [4, 5]. In addition to this, non-biological model emulsion systems allow mechanical stress to be evaluated under reproducible, cost-effective and time-saving conditions. In addition to this, the oil-water emulsion system makes detection easy and also has a comparable morphology to mammalian cells.

The results describe how the Sauter mean diameters of an oil-water emulsion system that is being pumped through a circuit are determined using inline endoscopy in combination with automatic image analysis (SOPAT GmbH, Germany). The Sauter mean diameter decreased with increasing mechanical stress and reached a steady state value at the end of the experiment. In order to estimate the mechanical stress in pumps the average value (measured Sauter mean diameter $d_{32,m}$) was used as a comparison criterion. The centrifugal-type PuraLev® multi-use (MU) and single-use (SU) pump series (Levitronix® GmbH, Switzerland), a peristaltic pump, and a 4-piston diaphragm pump were investigated.

2 Material and methods

Experimental setup

The investigations were performed using piped and non-piped pump setups (Fig. 1A, B). The piped setup enabled mechanical stress experiments at flow rates greater than 10 L min^{-1} to be performed. The cylindrical, baffled stirred tank was equipped with four baffles and three Rushton turbines, which were exclusively used to mix the surfactant. During the pumping process, the impellers were not used. The fluid was circulated by the pump in a closed loop. As shown in Fig. 1, the oil was added via a syringe port in front of the pump and was homogeneously distributed throughout the reactor by the pumping process. A flow meter (non-piped setup: clamp-on sensor, Levitronix® GmbH, Switzerland; piped setup: Presonic Flow 92, Endress+Hauser, Switzerland) and a single-use pressure sensor (SciLog BioProcessing Systems, USA) were integrated into the closed loop to monitor the volume flow rate and pressure for different pump settings. The investigations were varied by selecting different tubing configurations and by using a hose clamp in the non-piped setup or a hand-wheel valve in the piped setup (Freeflow Valve Assembly, Parker Hannifin Europe Sàrl, Luxembourg). It was found that the pressure loss in front of the pump was negligible, therefore pressure measurement was only required behind the pump. In order to record images of the emulsion drops inside the vessel the endoscope probe (SOPAT-VF; SOPAT GmbH, Germany) was equipped with a CCD camera. The experiments were carried out for different sizes of centrifugal multi-use and single-use PuraLev® pumps (PuraLev® 200MU/600MU, PuraLev® 200SU/600SU), which were then compared to a peristaltic pump and a 4-piston diaphragm pump (Fig. 2).

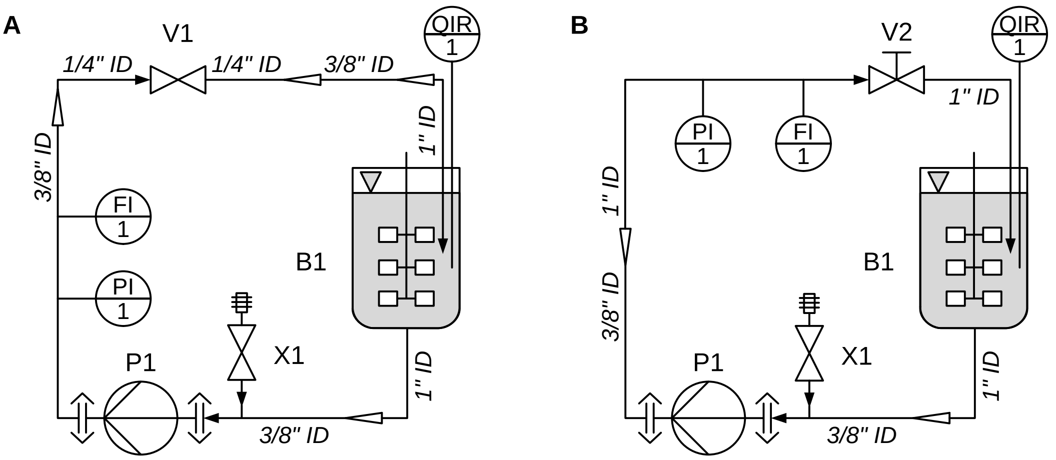

Fig. 1: Pump circuit for non-piped (A) and piped (B) setups using inline endoscopy as the measurement technique, (B1) storage vessel, (P1) pump, (V1) hose clamp, (V2) hand-wheel valve, (X1) syringe port, (QIR1) endoscope probe, (PI1) pressure sensor, (FI1) flow sensor. In the non-piped setup the inner tube diameter was only constricted from 1" to 3/8" in the pump circuit for the PuraLev® 200SU.

The preliminary investigations were performed for multi-use PuraLev® pumps (PuraLev® 200MU and PuraLev® 600MU), a peristaltic pump and a 4-piston diaphragm pump (= 3.4 L min^{-1} and 0.03, 0.30 and 0.61 bar) by using the non-piped pump setup. Mechanical stress investigations for single-use Levitronix® PuraLev® 200SU and PuraLev® 600SU pumps and in the counter parts were carried out at a constant flow rate of 3.4 L min^{-1} and 10 L min^{-1} and a pressure drop of 0.5 bar by using the piped pump setup.

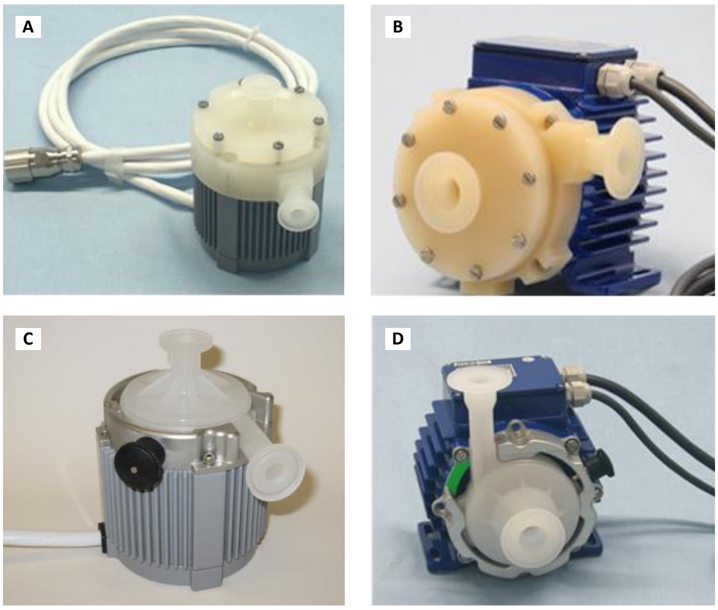

Fig. 2: Levitronix single-use PuraLev® pump series (A) PuraLev® 200MU, (B) PuraLev® 600MU, (C) PuraLev® 200SU, (D) PuraLev® 600SU.

Model system

All experiments were performed using a commercial oil-water emulsion, as described by Wollny [6]. Firstly, 5 L de-ionized water was poured into a stirred tank and mixed with a surfactant (Triton X-100; $c_{surfactant} = 0.18\ mL\ L^{-1}$; SigmaAldrich, USA). After complete dissolution of the surfactant, oil (Mobil EAL Arctic 22; $c_{oil} = 1.28\ mL\ L^{-1}$; Eberhart Schmierstoffe AG, Germany) was added via the syringe port and distributed by the pump that was being investigated (multi-use and single-use PuraLev® pump series; peristaltic pump, 4-pistion diaphragm pump).

Measurement and image analysis

For all experiments, an inline endoscope probe (SOPAT-VF, SOPAT GmbH, Germany) was positioned in the tank directly beneath the inlet in order to guarantee that individual drops were not recorded more than once, even at low flow rates. During the hour-long experiment, 50 images were recorded every minute. At least 300 drops were detected at each measurement point using SOPAT recognition software (SOPAT GmbH, Germany).

Automatic image recognition was used to determine the drop size distribution. The drop sizes are expressed by the Sauter mean diameter d_{32} (Eq. 1), which is most commonly used as a representative diameter for particles in dispersions. After 50 minutes, the Sauter mean diameter d_{32} reached a steady state, therefore the average value of the Sauter mean diameters measured in the last 10 minutes (measured Sauter mean diameter $d_{32,m}$) was used as a comparison criterion to estimate the mechanical stress from the investigated pumps.

$$d_{32} = \frac{\Sigma\, d_p^3}{\Sigma\, d_p^2}. \qquad \text{Eq. 1}$$

3 Results

In all the experiments, the Sauter mean diameter d_{32} decreased over the pumping period and reached a steady state by the end of the experiment. As an example, the curve for the centrifugal PuraLev® 200MU pump at a flow rate of 3.4 L min^{-1} and at pressure drops of 0.03, 0.30 and 0.61 bar is shown in Fig. 3. The measured Sauter mean diameter decreased from $d_{32,0min}$ = 78 µm to $d_{32,60min}$ = 31 µm during the experiment for a pressure drop of 0.03 bar. Smaller drop sizes were determined for higher pressure drops as the rotation speed of the pump impeller increased ($d_{32,60min}$ = 15 µm at 0.30 bar; $d_{32,60min}$ = 11 µm at 0.61 bar). This indicates that the Sauter mean diameter is dependent on the mechanical stress exerted by the pump; the higher the mechanical stress, the smaller the Sauter mean diameter.

At least 300 drops were detected at each measurement point, guaranteeing statistical certainty. The highest deviations in the Sauter mean diameter were calculated for the PuraLev® 200MU ($d_{32,4min}$ ± 42 µm) and the PuraLev® 600MU ($d_{32,6min}$ ± 21 µm) for a pressure drop of 0.03 bar in the first few minutes of the experiment. These high deviations in the Sauter mean diameter indicate that the emulsion is inhomogeneous and are significantly influenced by larger drops (Eq. 1). The decrease in the Sauter mean diameter over time was mechanical stress dependent and in the last 10 minutes of the experiments the standard deviations were no greater than d_{32} ± 0.5 µm for both the PuraLev® 200MU and the PuraLev® 600MU. In contrast to the centrifugal pump types, the standard deviation for the Sauter mean diameters for the comparison pumps was below d_{32} ± 1.9 µm for pressure drops ranging from 0.03 to 0.61 bar. Consequently, the measured Sauter mean diameter is a suitable comparison criterion to evaluate mechanical stress from pumps (Fig. 3; boundary of the measured Sauter mean diameter $d_{32,m}$).

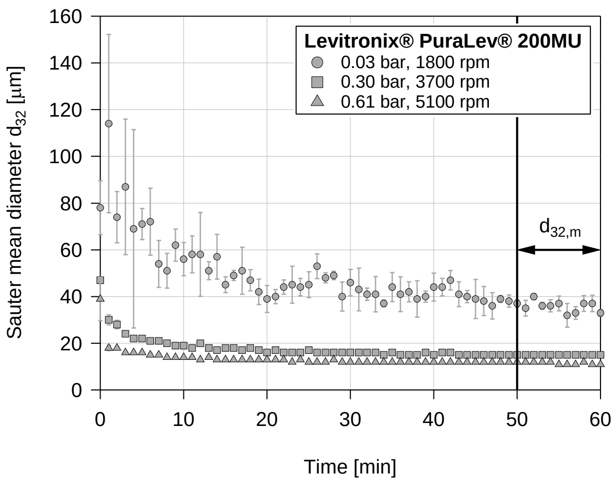

Fig. 3: Sauter mean diameter d_{32} for the PuraLev® 200MU at a flow rate of 3.4 L min^{-1} and for pressure drops of 0.03, 0.30 and 0.61 bar is shown as an example. The measured Sauter mean diameter $d_{32,m}$ was calculated for the last 10 minutes (boundary). The resulting standard deviation of the different Sauter mean diameters d_{32} (n ≥ 300) is shown.

In Fig. 4A, B the measured Sauter mean diameter $d_{32,m}$ of the multi-use and single-use PuraLev® pumps and the comparison peristaltic and 4-psiton diaphragm pumps are shown at constant flow rates of 3.4 L min^{-1} and 10 L min^{-1} and for pressure drops of 0.03, 0.30, 0.50 and 0.61 bar.

The largest measured Sauter mean diameter of $d_{32,m}$ = 36 µm was obtained for a pressure drop of 0.03 bar with the PuraLev® 200MU (Fig. 4A). Under the same conditions, the PuraLev® 600MU delivered a similar drop size of $d_{32,m}$ = 34 µm. In contrast to the PuraLev® MU series, the comparison pumps exhibited a smaller measured Sauter mean diameter $d_{32,m}$ of 19 µm for the 4-piston diaphragm and 10 µm for the peristaltic pump, indicating greater mechanical stress in the emulsion system. In general, a decrease in the measured Sauter mean diameters was observed for the PuraLev® 200MU, the PuraLev® 600MU and the 4-piston diaphragm pumps as mechanical stress increased. In contrast, the peristaltic pump showed a measured Sauter mean diameter of $d_{32,m}$ = 10 µm for all pressure settings, indicating that the mechanical stress remained the same.

After being able to successfully determine that the multi-use PuraLev® pumps are suitable for shear sensitive applications, the single-use Levitronix® pumps were also evaluated. As shown in Fig. 4B, the measured Sauter mean diameters for the PuraLev® 200SU and PuraLev® 600SU are very similar (deviation ≤ 14 %) to the peristaltic and 4-piston diaphragm pumps at a flow rate of 3.4 L min^{-1} and a pressure drop of 0.5 bar. The largest measured Sauter mean diameter of $d_{32,m}$ = 13 µm was obtained at a flow rate of 10 L min^{-1} with the PuraLev® 200SU, and the smallest measured Sauter mean diameter ($d_{32,m}$ = 9 µm) with the 4-piston diaphragm pump. The fact that the bearingless PuraLev® SU pumps result in higher measured Sauter mean diameters, indicates that there is reduced hydrodynamic stress compared to the peristaltic and 4-piston diaphragm pumps. Comparing the multi-use and single-use PuraLev® pump series at 3.4 L min^{-1} showed that the measured Sauter mean diameters of the single-use PuraLev®SU pumps and the 4-piston diaphragm pump at 0.5 bar are approximately equal to those of the multi-use PuraLev®MU and 4-piston diaphragm pumps at 0.61 bar. The highest deviation (about 17 %) was observed for the peristaltic pump at a flow rate of 3.4 L min^{-1} and at a pressure drop of 0.61 bar.

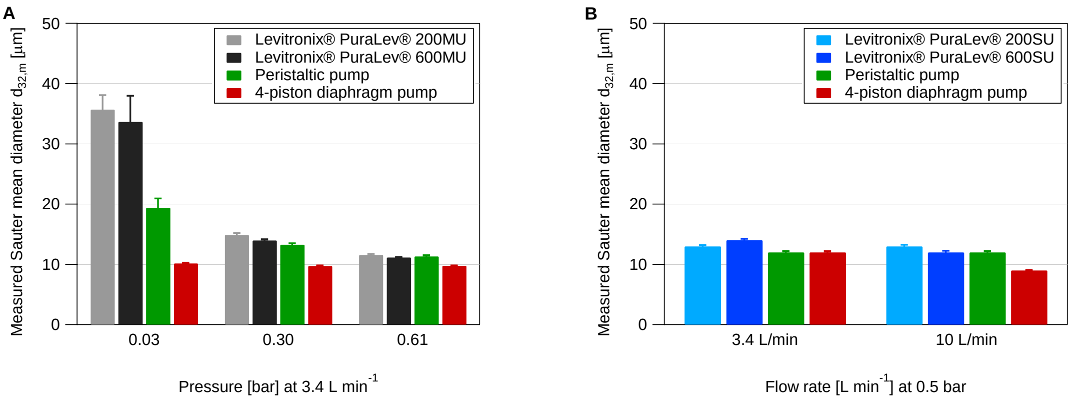

Fig. 4: Measured Sauter mean diameter $d_{32,m}$ of the multi-use and single-use centrifugal pumps and the comparison peristaltic and 4-piston diaphragm pumps. (A) Measured Sauter mean diameter for the multi-use PuraLev® 200MU/600MU and their counterparts at 3.4 L min^{-1} and pressure drops of 0.03, 0.30 and 0.61 bar, (B) measured Sauter mean diameter for the single-use PuraLev® 200SU/600SU and their counterparts at 10 L min^{-1} and a pressure drop of 0.5 bar. The resulting standard deviation of the measured Sauter mean diameters $d_{32,m}$ (n = 10) is shown.

4 Conclusion

The investigation showed that multi-use and single-use centrifugal pumps are characterized by up to 59 % larger Sauter mean diameters than their counterparts for comparable operational conditions. This indicates that hydrodynamic stress is lower in the PuraLev® MU/SU pumps. Levitronix® pumps are therefore a suitable alternative to commercially used peristaltic and 4-piston diaphragm pumps in the biopharmaceutical industry.

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