# Mechanical stress exerted on proteins by magnetically levitated single-use centrifugal pumps

**Katharina Blaschczok[1], Christian Löffelholz[1], Ina Dittler[1], Nicolai Lehmnann[1], Stephan C. Kaiser[1], Dieter Eibl[1], Pascal Bösch[2], Reto Schöb[2]**

[1] Zurich University of Applied Sciences, Life Sciences and Facility Management, Institute for Biotechnology, Biochemical Engineering and Cell Cultivation Technique, Wädenswil, Switzerland

[2] Levitronix® GmbH, Zurich, Switzerland

## 1 Introduction

In the pharmaceutical industry, membrane or peristaltic pumps are most frequently used for fluid transfer in upstream and downstream processing steps. As an alternative, Levitronix® GmbH has developed novel single-use pumps which are optimally suited for biopharmaceutical applications. These pulsation-free and bearing-less centrifugal pumps with replaceable plastic pump heads prevent leakage and extend the operating range of single-use pumps to higher flow rates [1]. In order to prove their applicability for biological and shear sensitive fluids, investigations with protein solutions were performed.

The evaluation of mechanical stress on proteins is particularly relevant for downstream processing. In order to investigate the influence of different pump types on the protein quality, chicken egg white lysozyme was chosen as a model protein. This 14.3 kDa (129 amino acids) enzyme has already been used for protein refolding studies after mechanical treatment [2, 3, 4]. To detect changes in the protein, enzyme activity and particle size measurements were carried out.

## 2 Materials and methods

### Experimental set-up

The experimental set-up (see Fig. 1) consisted of three identical 3.5 L vessels (Chemap AG, Switzerland). The respective pumps were connected to the bottom drain. The fluid was transferred back into the vessel from the pump outlet via a port in the vessel lid. Air was prevented from being absorbed into the liquid by means of a dip tube. In the closed pump circuit, consisting of C-Flex tubes (374-375-3, Saint-Gobain, Frankreich; 190-945-001, Cole-Parmer Instrument Company, USA) with an internal diameter of 3/8″, sensors for pressure (single-use pressure sensor SciPres 080-695PSX-5, SciLog, Inc., USA) and flow (clamp-on ultrasonic flow sensor, Levitronix® GmbH, Switzerland) measurements were integrated. The vessels were maintained at 42 °C using a double jacket.

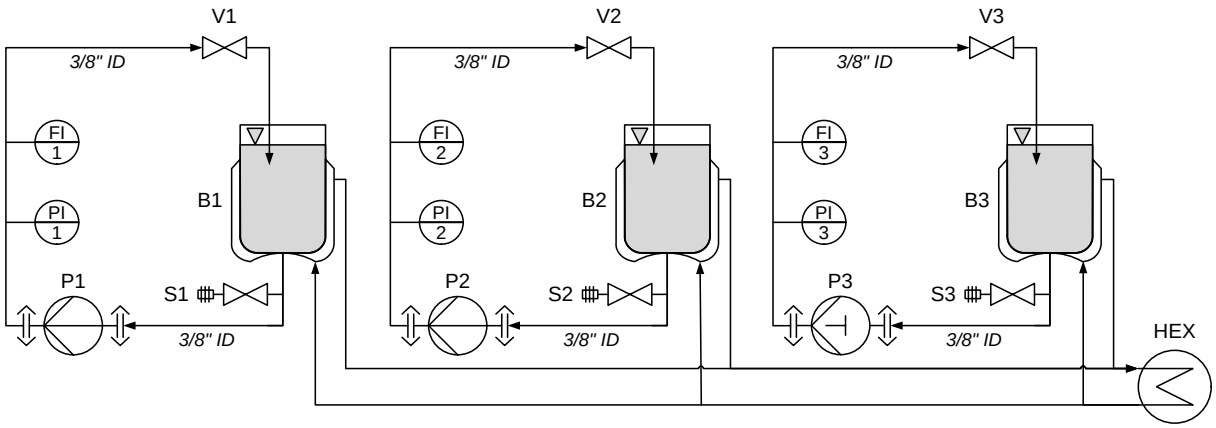


**Fig. 1: Schematic of the experimental set-up for the evaluation of mechanical stress in PuraLev® 200SU (P1), PuraLev® 600SU (P2) and 4-piston diaphragm pumps (P3). (B1, B2, B3) Vessels, (FI1, FI2, FI3) flow sensors, (PI1, PI2, PI3) pressure sensors, (S1, S2, S3) sampling port, (HEX) thermostat. Hose clamps (V1, V2, V3) were installed if needed, depending on the operating pressure.**

The centrifugal PuraLev® 200MU, 200SU and 600SU pumps from Levitronix® (see Fig. 2) were compared with positive displacement pumps (4-piston diaphragm and peristaltic). The mechanical stress on the model protein was evaluated by pumping 2 L lysozyme solution (1 g $L^{-1}$ lysozyme (L6876) in 66 mM phosphate buffer $KH_2PO_4$ (P5655), both from Sigma-Aldrich Corporation, USA) in a closed loop for 4 days in the preliminary experiments and 2 days in the main experiments.

In a preliminary study, a multi-use PuraLev® 200MU was compared with a 4-piston diaphragm pump and a peristaltic pump. The flow rate was set to 10 L $min^{-1}$ (peristaltic pump 8 L $min^{-1}$) and the pressure to 1 bar for the investigations to determine the decrease in enzyme activity. Additionally, mechanical stress was also investigated in a stirred tank reactor, which are commonly used in biotechnological production processes. The 1 L glass bioreactor was equipped with a rushton turbine and operated with a rotational stirring speed of 375 rpm. The main investigations were carried out with single-use PuraLev® 200SU and 600SU pumps and a 4-piston diaphragm pump for identical operating conditions of 10 L $min^{-1}$ and 0, 0.5 and 1 bar. In addition to enzyme activity measurements, particle sizes were also determined.

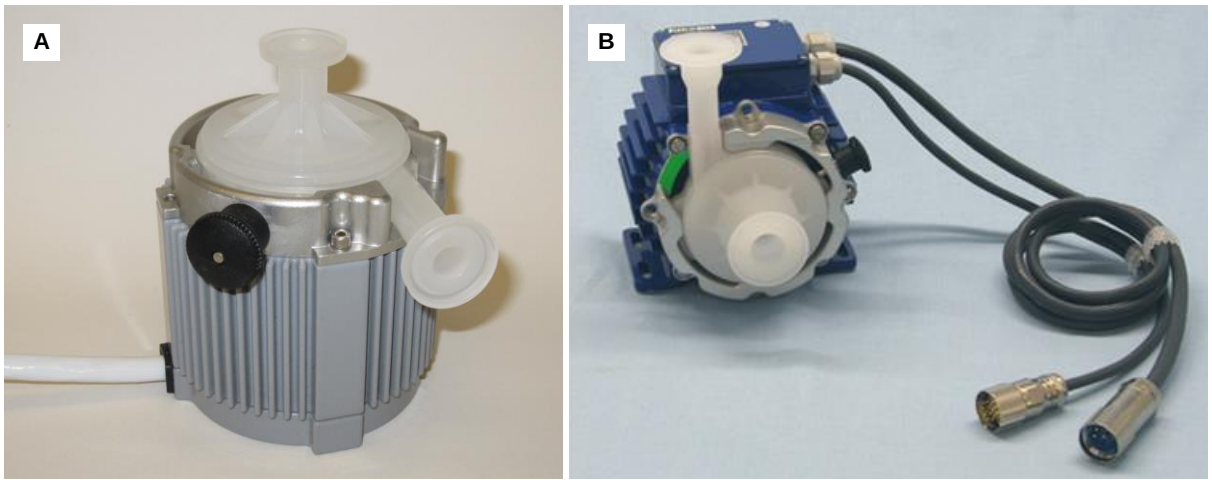


**Fig. 2: Levitronix® PuraLev® centrifugal pumps. (A) PuraLev® 200SU, (B) PuraLev® 600SU.**

## Analysis

Samples were periodically withdrawn from the vessels and cooled down on ice in order to prevent continuing stress as a result of the increased temperature in the pump circuit. To evaluate the mechanical stress caused by the different pumps, enzyme activity measurements were performed. A decrease in lysozyme activity was expected as mechanical stress increased. Dynamic light scattering (DLS) was used to detect the aggregation of lysozyme molecules.

### Activity measurement

Determination of lysozyme activity is based on first order enzyme kinetics during the

$$\textit{Micrococcus lysodeikticus}\ \text{(intact)} \xrightarrow{\text{lysozyme}} \textit{Micrococcus lysodeikticus}\ \text{(lysed)}$$

reaction. During the reaction, decreasing turbidity of the bacteria suspension due to the lytic action of lysozyme was recorded using a photometer (Ultrospec 3000 pro, Amersham / GE Healthcare Life Sciences, Sweden) at 450 nm. The enzyme activity was calculated by means of the (negative) extinction curve, since activity is higher the faster the turbidity decreases.

As the reaction is heavily dependent on temperature, the measurement was carried out at a constant temperature of 25 °C. A 0.015 % w/v lyophilized *Micrococcus lysodeicticus* suspension (M3770, Sigma-Aldrich Corporation, USA) in 66 mM phosphate buffer ($KH_2PO_4$) was used as a substrate. For evaluation purposes, the measured values were normalized to the initial value (= 100 %) in order to provide a better comparison.

### Dynamic light scattering

Dynamic light scattering was used to determine aggregate formation caused by the pumps. Using this laser-based method the size of particles suspended in liquids can be measured based on Brownian motion, which states that larger particles move faster than smaller ones. DLS measures particle velocity using scattered light by registering fluctuations in light intensity. From the resulting diffusion coefficient, software calculates the hydrodynamic diameter of the particles (diameter of a sphere with the same diffusion coefficient as the particle).

The measurement was performed using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK), which displays the particle size distribution as histograms. For evaluation purposes, the hydrodynamic diameter of the most frequent particle size was determined for every sample. Since Brownian motion is heavily depends on temperature and viscosity, all measurements were carried out at a constant temperature of 20 °C.

## 3 Results

For both the PuraLev® 200MU and the single-use PuraLev® pumps, no significant change in enzyme activity could be observed for any of the pressure conditions tested. This was confirmed for the PuraLev® 200SU and 600SU pumps by particle size measurement (see Fig. 3 A, C and D). The dynamic light scattering showed constant particle sizes in the nanometer range (3.6 ± 0.2 nm) for both single-use PuraLev® pumps.

In contrast to the centrifugal Levitronix® pumps, the enzyme activity when using the 4-piston diaphragm pump started to decrease for all pressure conditions immediately after starting the experiment and declined to zero within 34 to 43 h (see Fig. 3 A and B). Furthermore, the particle size strongly increased within the first 10 h up to 3.8 µm at 0.5 bar (see Fig. 3 B). Afterwards, because the aggregate size exceeded the measurement range of the Zetasizer (0.3 nm to 10 µm), reliable measurements were no longer possible,.

For the comparison system, a 1 L stirred bioreactor with a Rushton turbine, no impact on lysozyme activity was observed at 375 rpm over 4 days (see Fig. 3 A). Likewise, there was no apparent effect caused by the peristaltic pump during the first 36 h of pumping. Only after a duration of 48 h was a decline in protein activity measured, resulting in a relative activity of 81 % of the initial value after 4 days (see Fig. 3 A).

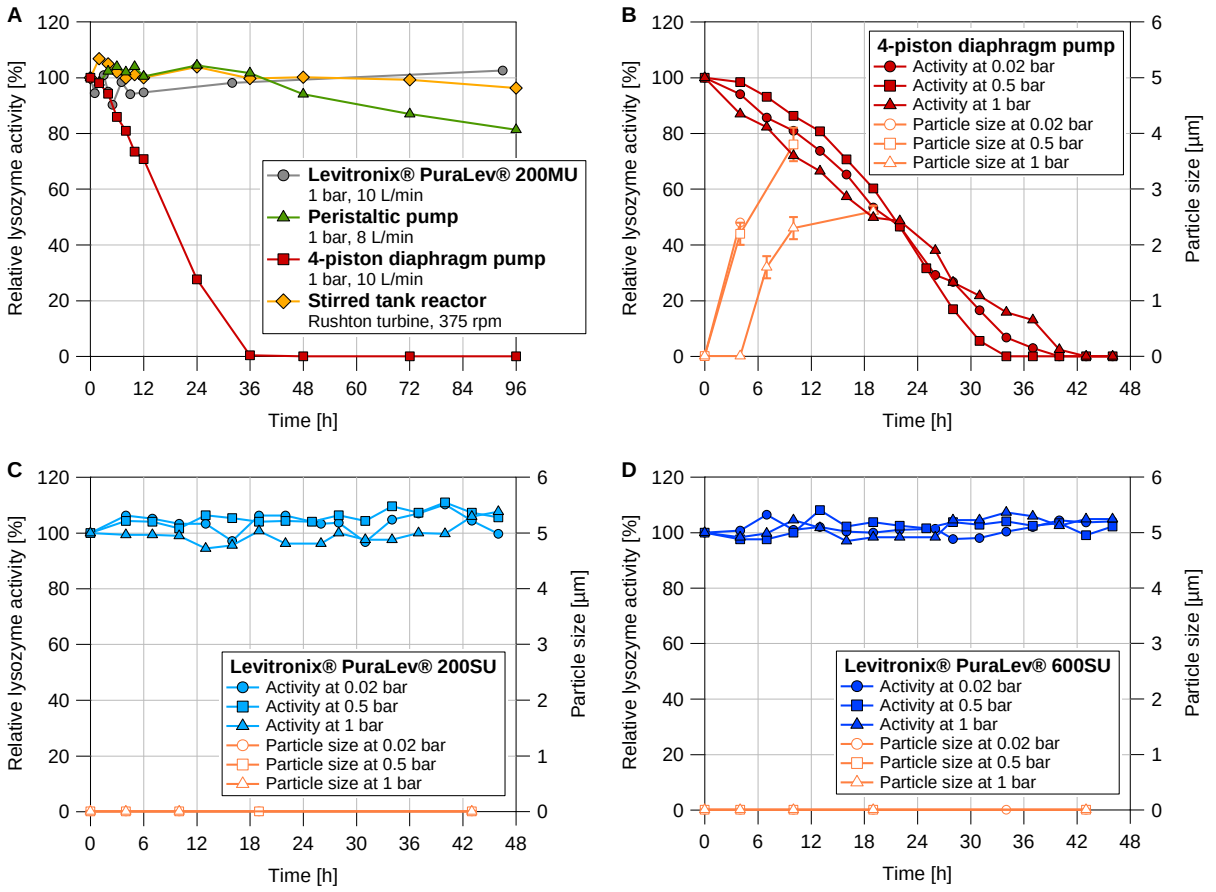


**Fig. 3:** **Results of the activity and DLS measurements. (A) Preliminary experiment with the Levitronix® PuraLev® 200MU, the peristaltic pump, the 4-piston diaphragm pump and the stirred bioreactor. Main investigations with the (B) 4-piston diaphragm pump, (C) Levitronix® PuraLev® 200SU and (D) Levitronix® PuraLev® 600SU.**

The changes in the protein were also visible for the positive displacement pumps (4-piston diaphragm and peristaltic pumps) as a result of the increasing turbidity in the lysozyme solution over time. Using the 4-piston diaphragm pump as an example (see Fig. 4 B and C), it can be seen that the intensity of the turbidity and the amount of sediment increases continuously up to 36 h, from which point it remains constant, which correlates with the results from the activity measurements. In contrast, the lysozyme solution from the PuraLev® centrifugal pumps and the stirred bioreactor remained clear (see Fig. 4 A by the example of the PuraLev® 200MU).

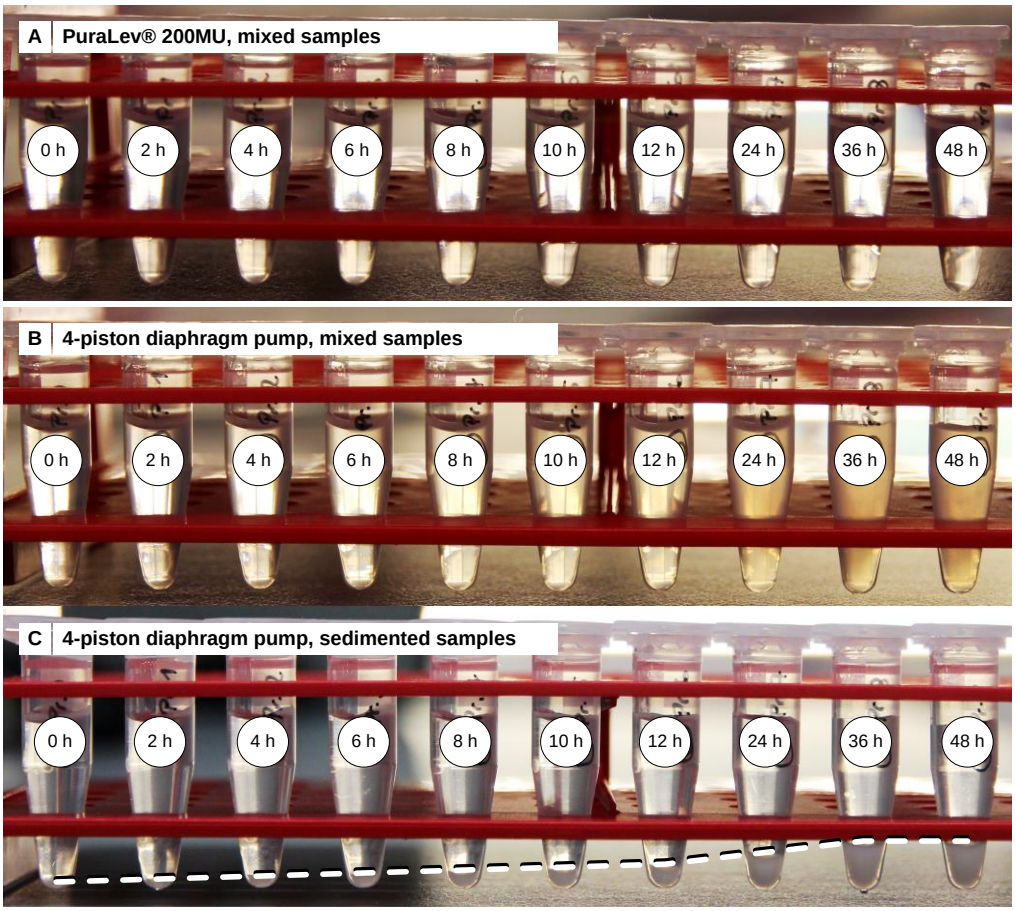


**Fig. 4:** **Lysozyme solution turbidity over a 48 h pumping duration, for the PuraLev® 200MU (A) and the 4-piston diaphragm pump mixed samples (B) and settled samples (C).**

## 4 Conclusion

The investigations demonstrate that the Levitronix® PuraLev® 200MU, 200SU and 600SU centrifugal pumps have no impact on the model protein lysozyme, whereas the 4-piston diaphragm pump and the peristaltic pump caused the protein to be modified or damaged, resulting in one case in massive loss of activity. Consequently, due to their bearing-less design, the PuraLev® centrifugal pumps have a significant advantage in protein purification compared to other commonly used industrial pumps.

## References

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