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Application Note

June 19, 2020

Keywords or phrases:
Flexsafe® Pro Mixer, Single-Use Mixing, Shear

Flexsafe® Pro Mixer

Low Shear Single-Use Mixing and Blending

Nicolas Voute, Myriam Lavie,*
Sartorius Stedim FMT S.A.S., ZI des Paluds, Avenue de Jouques – CS 91051, 13781 Aubagne Cedex

* Correspondence
E-Mail: myriam.lavie@sartorius-stedim.com

Abstract

Flexsafe® Pro Mixer is a unique single-use technology fitting all mixing applications from buffer and media preparations, downstream processes to final formulation. Flexsafe® Pro Mixer ergonomic design enables intuitive, modular and agile use to achieve fast installation and mixing operations. Additionally, the Flexsafe® film offers high standard quality attributes such as biocompatibility, integrity and supply network.

The effect of mixing systems on monoclonal antibodies and cells in downstream processing is a potential customer concern. Flexsafe® Pro Mixer preserves the quality of shear sensitive drug substances and drug products thanks to a levitating impeller combined with limited shear work.

The Flexsafe® Pro Mixer technology reduces the shear effects detrimental to product quality and prevents the generation of aggregates and particles that can reduce filtration performance.

This technical report gives performance data of the Flexsafe® Pro Mixer for shear stress in volume of 200 L and agitation speed 450 rpm. The maximum shear rate estimated by Computational Fluid Dynamic (CFD) study was found to be 9,350 s^{-1}, which is considerably lower than the level, 10^7~10^8 s^{-1} that was found to cause degradation of proteins (Thomas et Geer 2011) (Jaspen et Hagen 2006).

Find out more: www.sartorius.com/flexsafe-pro-mixer

Introduction

Use of magnetically driven, bottom-mounted mixers is becoming increasingly common during the mixing process in biopharma manufacturing because of the low risk of contamination, low shear stress and ease of use (Gikanga, et al. 2015).

Flexsafe® Pro Mixer technology combines speed and efficiency to deliver high performance mixing during powder dissolution, and a levitating impeller to preserve the drug during low shear blending applications (Sartorius 2019 a). The Pro Mixer technology eliminates the shear effects that can impact product quality and prevents the generation of aggregates and particles that can reduce filtration performance (Sartorius 2019 b).

Computational fluid dynamic is a numerical simulation of fluid motion. This technique provides useful information about fluid motion and flow patterns. The evaluation of mixing efficiency and vortex formation in Flexsafe® Pro Mixer was assessed by CFD and summarized in a technical Note "Flexsafe® Pro Mixer Computational Fluid Dynamics study" (Sartorius 2019). The present study is focused on shear profiles obtained with Flexsafe® Pro Mixer.

Mixing Technology

Flexsafe® Pro Mixer (50–1000 L) contains a central magnetic impeller assembly. The mixing technology principle is based on electromagnets in the motor drive interacting with fully encapsulated permanent magnet inside the mixing bag. The impeller is held in a cup that is welded to the bottom of the bag and the cup sits on the drive unit. When in use, the impeller is stabilized in a position away from the sides and bottom of the cup via magnetic coupling with the rotor magnet. There are no moving parts within the motor and no contact with the impeller as shown on Figure 1. Shear stress is limited to the cup region, the interface between the bag and the motor drive.

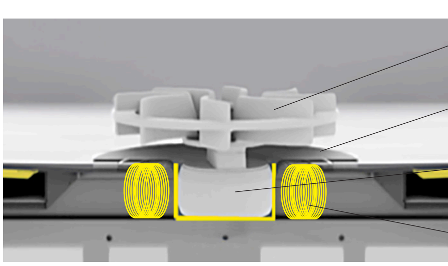

Figure 1: Mixing technology principle of Flexsafe® Pro Mixer

Theory

Shear stress is present in almost all bioprocesses and is known to play a role in protein aggregation and denaturation of proteins (Bee, et al. 2009) (Li, et al. 2019) (Tavakoli-Keshe, et al. 2014).

Cells and biomolecules, such as proteins and monoclonal antibodies, may be exposed to mechanical stress during processing in the biopharmaceutical industry. This stress can cause unfolding, aggregate or deterioration of the molecule or cell in question. Reference is usually made to mechanical stress from the use of the word shear. Thus, surface phenomena are also included in this term, such as the interaction of proteins with liquid-air interfaces and | or released metal particles. In short, it is a term used generically to refer to certain phenomena that occurs during mixing which could negatively affect a biopharmaceutical product (Bekard , Asimakis et Bertoli 2011) (Dreckmanna, et al. 2020) (Das, et al. 2020).

Shear stress is defined as a force per unit area, acting parallel to an infinitesimal surface element. Shear stress is primarily caused by friction between fluid particles, due to fluid viscosity.

Whenever there is relative motion of liquid layers, shearing forces exist that are related to the flow velocities. These forces, represented by shear stress, carry out the mixing process and are responsible for producing fluid intermixing, dispersing gas bubbles, and stretching | breaking liquid drops (Paul, Atiemo-Obeng et Kresta 2004). Shear stress: τ has pressure units because it is a force applied tangentially over a surface whereas shear rate: γ has s^{-1} or Hz as unit:

$$\tau = \frac{F}{A} = \mu\,\gamma = -\mu\,\frac{dv}{dy} \qquad \text{Eq. 1}$$

τ : Shear stress, F: Force, A: Surface area, μ: Dynamic viscosity,
γ: Shear rate, V: Velocity of the fluid along the boundary,
y: Height above the boundary, $\frac{dv}{dy}$: Gradient of velocity

Shear rates and speed are diverse in different parts of a mixing tank. As maximum fluid speed is normally found near the stirrer blades, commonly tip speed, maximum shear rate is high in that regions. Shear rate is linked to tip speed, and sometimes this could be used as a scale-up parameter.

In biopharma industry, in general, the fluids used are Newtonian, so the viscosity is constant for a given temperature.

Reynolds number represents a ratio between inertial and viscous forces and is defined, in agitation, as:

$$Re = \frac{pND^2}{\mu} \qquad \text{Eq. 2}$$

ρ: Fluid density, N: Speed of agitation, D: Agitator diameter,
μ: Dynamic viscosity

When $Re>10^4$ the flow is considered turbulent whereas $Re<10$ corresponds to laminar regime. In fluid dynamics turbulent flow is a fluid motion characterized by chaotic changes in pressure and flow velocity (Batchelor 2000).

For all practical purposes, the bulk fluid flow in mixing vessels containing rotating impellers is turbulent if the fluid viscosity is less than about 10 mPas. Turbulence is a state of fluid motion where the velocity fluctuates in time and in all three directions in space. These fluctuations reflect the complex layering and interactions of large and small structural elements, such as vortices, sheets, ejections, and sweeps of a variety of shapes and sizes (Paul, Atimo-Obeng et Kresta 2004).

A recent rigorous theoretical analysis (Sànchez, et al. 2006) shows that for turbulent flow the average shear rate γ in the fluid is a power law function of the rotational speed N of the impeller

$$y = k_s N \ \tfrac{3}{2} \qquad \text{Eq. 3}$$

γ: Shear rate, k_s: Constant of the mixer,
N: Speed of agitation

Shear work is defined as the time that a molecule or a cell is exposed to shear rate. It is a dimensionless parameter.

$$shear\ work = \gamma * t \qquad \text{Eq. 4}$$

γ: Shear rate, t: Time of exposure to shear rate

Shear rate is variable throughout the mixing system, consequently, a molecule may be subject to high or low shear at different times. All molecules will be subjected to the shear of the agitator, it is important to establish the maximum value of the shear rate even if this exposure time is very small.

Computational Evaluation of Shear Rate by Cfd CFD

The computational evaluation of Flexsafe® Pro Mixer system was performed using CFD (Computational Flow Dynamic) to understand the flow shear stress in a volume of 200 L and agitation speed 450 rpm.

The fluid flow was assumed to remain Newtonian throughout mixing with a viscosity of 1 cP.

Figure 2 shows the CFD simulation of the Flexsafe® Pro Mixer's shear profile of 200 L at 450 rpm. Red represents the maximum shear, of the order of $9.35 \cdot 10^3$ s^{-1} and in blue minimum values, of the order of 0.3 s^{-1}. It can be seen that the bottom of the bag remains blue, indicating that shear in this area is minimal. High shear rate can be observed adjacent to the blade in the regions where the impeller speed is high.

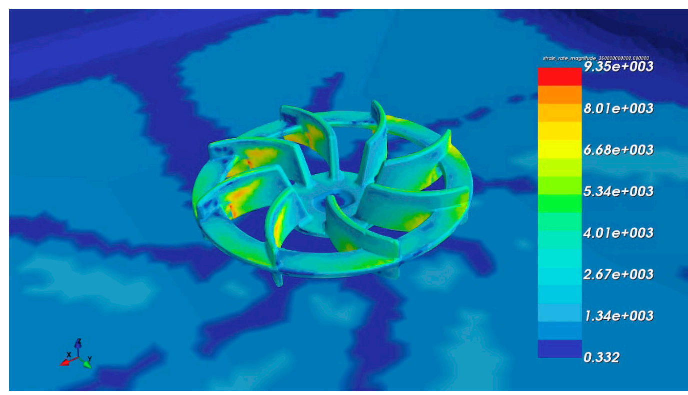

Figure 2: CFD simulation of the Flexsafe® Pro Mixer's shear profile of 200 L at 450 rpm

Figure 3 and Figure 4 represent simulations in the same conditions as Figure 2, but they correspond to iso-surfaces, that is, they are surfaces where the shear has a constant value. Figure 3 shows in yellow color, that only a very small fraction of the surface produces a shear rate of the order of $6.67 \cdot 10^3 s^{-1}$, about 70% of the maximum value. On the contrary, Figure 4 shows how most of the surface of the agitator exerts a shear rate of the order of $2.67 \cdot 10^3 s^{-1}$, about 25% of the maximum value.

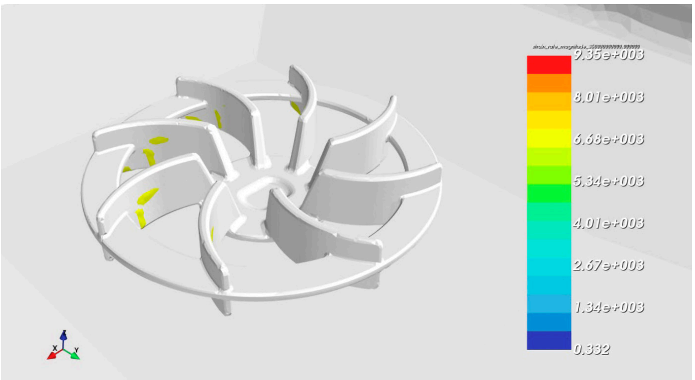

Figure 3: Iso-surface of shear rate > 70% of the maximum same conditions as Figure 1

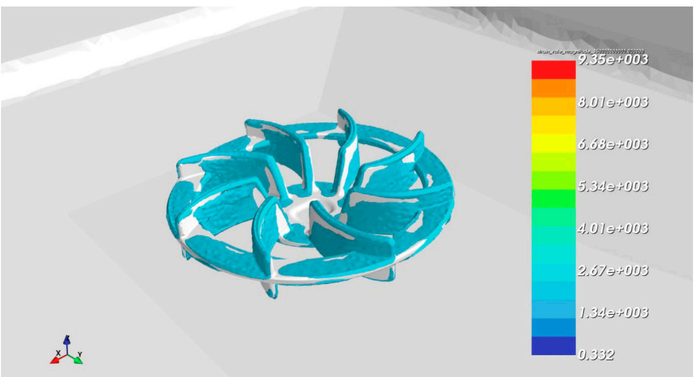

Figure 4: Iso-surface of shear rate < 25% of the maximum same conditions as Figure 1

Shear rate was also evaluated by CFD in the lower area of the cup. When in use, the impeller is stabilized in a position away from the sides and bottom of cup via magnetic coupling. Shear stress is limited to the cup region. Very low value was observed as indicated in Figure 5. Fluid circulation with no significant shear stress was found in the cup.

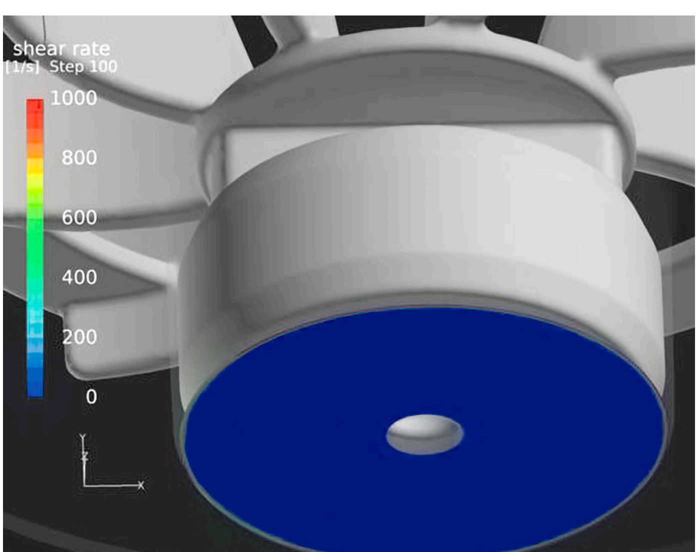

Figure 5: CFD simulation of the Flexsafe® Pro Mixer's shear profile in the lower part of the cup for a volume of 200 L at a speed of 450 rpm

Discussion

Table 1 summarizes literature findings on shear rates or shear works for different conditions and for different technologies.

Technology	Conditions	Shear rate (s^{-1}) or shear work	Reference
Mixing processes	Biopharmaceutical industrial conditions	Relatively low shear rates of about 50 s^{-1} for up to several hours	(Bee, et al. 2009)
Pipe flow	Biopharmaceutical industrial conditions	Relatively low shear rates of about 2000 s^{-1} for up to several seconds	(Bee, et al. 2009)
Self-lubricated pumps (piston or rotary lobe)	piston velocity of 1,000 mm/s and a low clearance of 0.05 mm	20,000 s^{-1} to a small fraction (less than 1%) of the pumped solution	(Bee, et al. 2009)
Specific lobe pump	Large self-lubricated clearance	16,000 to 20,000 s^{-1}	(Gomme PT 2006)
Cross-flow filtration	Biopharmaceutical industrial conditions	Shear rates of 1,000–10,000 s^{-1} for a few seconds	(Bee, et al. 2009)
Final filling operation	Dispensing a protein solution into vials through a 20-gauge by 10 cm needle at a rate of 0.5 mL/s	Shear rate of 20,000 s^{-1} for about 50 ms	(Wilkes 2006)
Capillary flow	Laminar flow	$1,5.10^5$ to $2,5.10^5$ s^{-1}	(Bee, et al. 2009)
Peristaltic pump	Pump head occlusion	Not applicable Shear sensitivity is evaluated by hemolysis of blood cells	(Cole-Parmer 2020)

Table 1: Comparative table of shear rate in biopharmaceutical technology

Therapeutic protein formulations are exposed to shear stress during their commercial production as shown in Table 1. The magnitude and duration of shear exposure, in the absence of the air-water interface is unlikely to cause protein aggregation or denaturation in processing operations of the biopharmaceutical industry (Bee, et al. 2009).

Medical peristaltic pumps are often used for dialysis, infusions, dosing, and drug delivery, as these pumps allow low steady flow rates, low shear rates, and the easy exchange of sterile tubing (Falk, Tom et Thomas 2019).

However Cole-Parmer demonstrated, using hemolysis of blood cells to evaluate the shear sensitivity, that motor speed is very dominant in a pump's ability to maintain cell viability during recirculation as well as pump head occlusion (Cole-Parmer 2020). Substantial loss of cell viability is observed with a flat design of the roller and high motor speed.

Conclusion

A maximum shear rate of 9,350 s^{-1} is estimated by Computational fluid dynamic (CFD) for Flexsafe® Pro Mixer 200 L and 450 rpm.

This value is considerably lower than the level, 10^7~10^8 s^{-1} that was found to cause degradation of proteins.

The evaluation was performed in worst case conditions in term of velocity for shear sensitive product.

For shear sensitive product a lower agitation speed will be used (lower than 450 rpm) resulting in a lower shear rate (lower than 9,350 s^{-1}) due to the power law equation between shear rate and agitation speed.

Nomenclature

CFD: Computational Fluid Dynamic
τ : Shear stress
F: Force
A: Surface area
μ: Dynamic viscosity
V: Velocity of the fluid along the boundary
y: Height above the boundary
$\frac{dv}{dy}$: Gradient of velocity
γ: *Shear rate*
t: Time of exposure to shear rate
ks: Constant of the mixer
N: Speed of agitation
ρ: Fluid density
D: Agitator diameter

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Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For further contacts, visit
www.sartorius.com

DIR: 2617030-000-00
Status: 07 | 2020