

Characterization of a Single-Use Bioreactor for Pharmaceutical Applications for the Mixing of Non-Newtonian Fluids

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In the pharmaceutical industry, single-use bioreactors (SUBs) are commonly employed due to their versatility and reduced risk of contamination between batches. Their applications range from vaccine production to the blending of formulated products. These formulations often exhibit non-Newtonian rheological behaviors which strongly affects mixing and whose behavior is not completely understood yet. For this study, we use a 50 L cubic tank, designed to mimic the geometry of a commercial SUB mixer. The shape and size of the vortex formed at the top of the vessel were monitored and measured using optical techniques. This study aims to investigate the effects of fluid level and agitation speed on energy consumption during mixing of shear-thinning fluids. Carboxymethylcellulose (CMC) solutions were examined in the SUB, and their rheological properties were characterized using a rheometer. The overall goal of this work is to enhance understanding of the mixing process for shear-thinning fluids by identifying optimal operating conditions based on agitation speed, filling level, and fluid rheology. Our results indicate that the rheology of the solution strongly affects the shape of the vortex and the trends of power per unit volume versus filling heights is affected drastically by the changes in rheology as well. These findings could contribute significantly to the development of more efficient and sustainable pharmaceutical processes.

1. Introduction

In the pharmaceutical industry, the efficient mixing of formulated liquid products is critical for processes such as homogenizing miscible liquids, dispersing immiscible phases, and dissolving solids (Dreher et al., 2014). A key challenge lies in ensuring containment and sterilization, operations that are complex and costly (Alberini et al., 2024). Single-use bioreactors (SUBs) are increasingly utilized in biopharmaceutical processes, such as the cultivation and production of vaccines, monoclonal antibodies, and therapeutic proteins (Gikanga et al., 2015; Ton et al., 2023). They offer significant cost savings by eliminating the need for cleaning and sterilization, while minimizing contamination risks, making them ideal for multiproduct facilities. Despite their higher initial cost, SUBs are more economical in the long term (Alberini et al., 2024). Optimizing SUBs is particularly important for non-Newtonian formulations, as their rheology influences bioreactor performance, especially in terms of energy consumption, the distribution of dispersed phases, and mixing times. Since the viscosity of non-Newtonian fluids varies with shear rate, effective design must account for the system's hydrodynamics and the rheological properties of the medium. These factors have a direct impact on mixing efficiency and product quality (de Boulard & Kienle, 2022). Moreover, in SUBs, high shear rates can damage microorganisms, while "dead zones" created by low mean velocities may lead to byproduct accumulation. Therefore, the design of agitation systems must carefully consider not only power input but also other factors such as cavern formation around impellers and material sedimentation (Bernemann et al., 2024, de Boulard & Kienle, 2022). This study investigates the effect of fluid level and agitation speed on energy consumption (Alberini et al., 2023; Ascanio et al., 2004; Delbridget et al., 2023) during the mixing of shear-thinning non-Newtonian fluids.

Rheological measurements were conducted to characterize the behavior of various solutions, along with torque and power measurements to assess motor energy consumption under different conditions. Additionally, vortex area measurements were performed to explore the relationship between vortex size and area introduction. The objective of this work is to develop tools for quantifying the power per unit volume across different working volumes of the same reactors. Additionally, it aims to identify the conditions under which a vortex is present or absent, in order to prevent air entrapment within the tank during the manufacturing process of a liquid product.

2. Material and Methods

The schematic of the rig used in this study is shown in Figure 1a. A cubic vessel with a 50 L volume, matching the dimensions of the Flexel Levmixer (square base measuring 395 mm x 395 mm, T x T), was employed. Key modifications to the original vessel include the replacement of disposable plastic walls with plexiglass to ensure optical transparency and the incorporation of a standard shaft connection to the motor for precise measurement of impeller power consumption, instead of the magnetic levitation system. For agitation, a 3D-printed replica of the original impeller with a diameter of 126 mm was used, fabricated using a Guider II s 3D printer (Flashforge, China). The motor (2) is connected to a variable frequency driver for precise impeller speed control. Although commercial mixers employ a magnetic stirrer for agitation, our replica incorporates a shaft, enabling precise torque measurements using strain gauge torque meter. A Kistler torque sensor (1) type 4502A0.5HA, with a measurement range of 0.5 Nm is used. Measurements were taken at various stirring speeds (ranging from 70 to 190 rpm, which are standard operating conditions for such systems) and at different tank filling levels, adhering to standard operating procedures in the pharmaceutical industry.

For this work three filling heights (H_L) are investigated, 10, 20 and 30 cm for the characterization of the power consumptions using three different CMC dispersion solutions.

Such solutions provide three distinct rheological behaviors which are described in Figure 1b. Such rheological characterizations were repeated 3 times for each fluid and the statistics of such investigations are reported in Figure 1b. The rheological characterizations were achieved using an Anton Paar rheometer equipped with a concentric cylinder. The flow curve analysis was performed imposing a shear ramp from 0.1 up to 100 s^{-1} . Generally, the consistency index (k) increases with the increase of the concentration of the CMC and n (power law exponent) decreases. Those solutions were chosen to investigate the effect of the increase of the viscous behavior of the fluid (increase of k) and the effect of the increase of the shear thinning behavior (decrease of n). For a fixed filling level, 10 cm, and varying the rheology of the mixed fluid with the CMC concentration, the shape and size of the formed vortex was measured using a digital camera (3) (2048x2048 pixels).

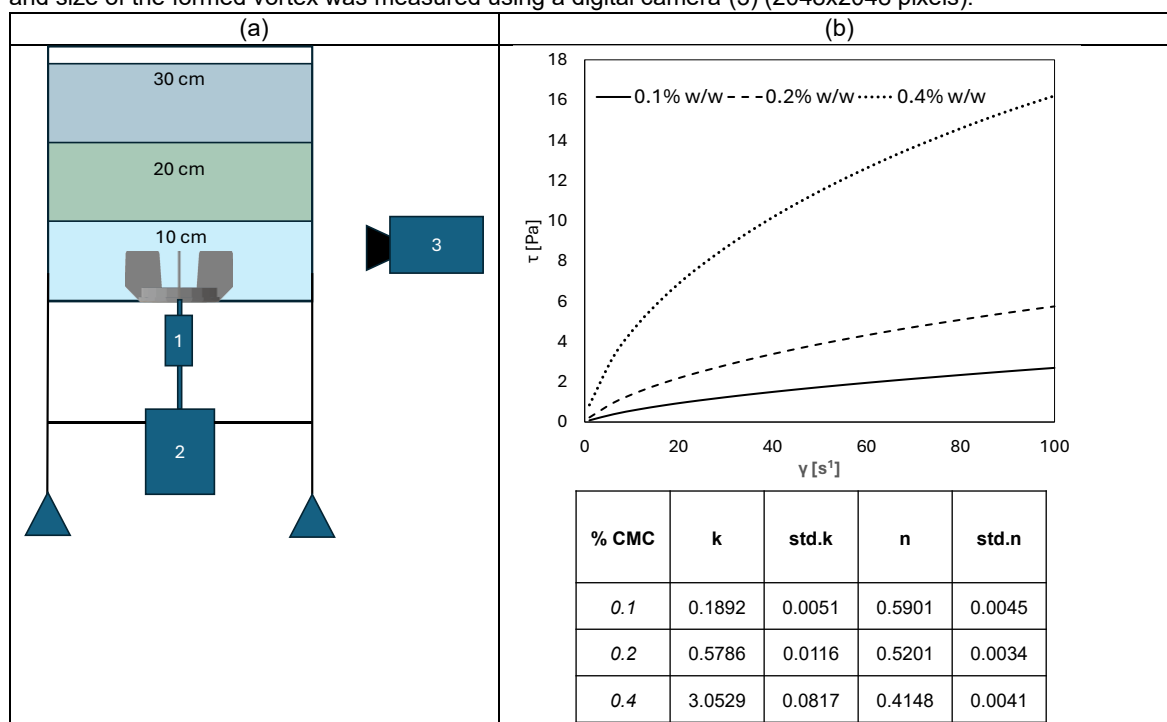


Figure 1: (a) Experimental rig and measurement techniques: 1) torque sensor, 2) electrical motor connected to the impeller with a shaft for a mechanical agitation, 3) digital camera to capture vortex shape. b) Rheological characteristic curves of the fluids used for the experimental campaign.

3. Results

Figure 2 presents the results of the torque measurements for the three CMC solutions at two impeller speeds, 90 rpm and 150 rpm.

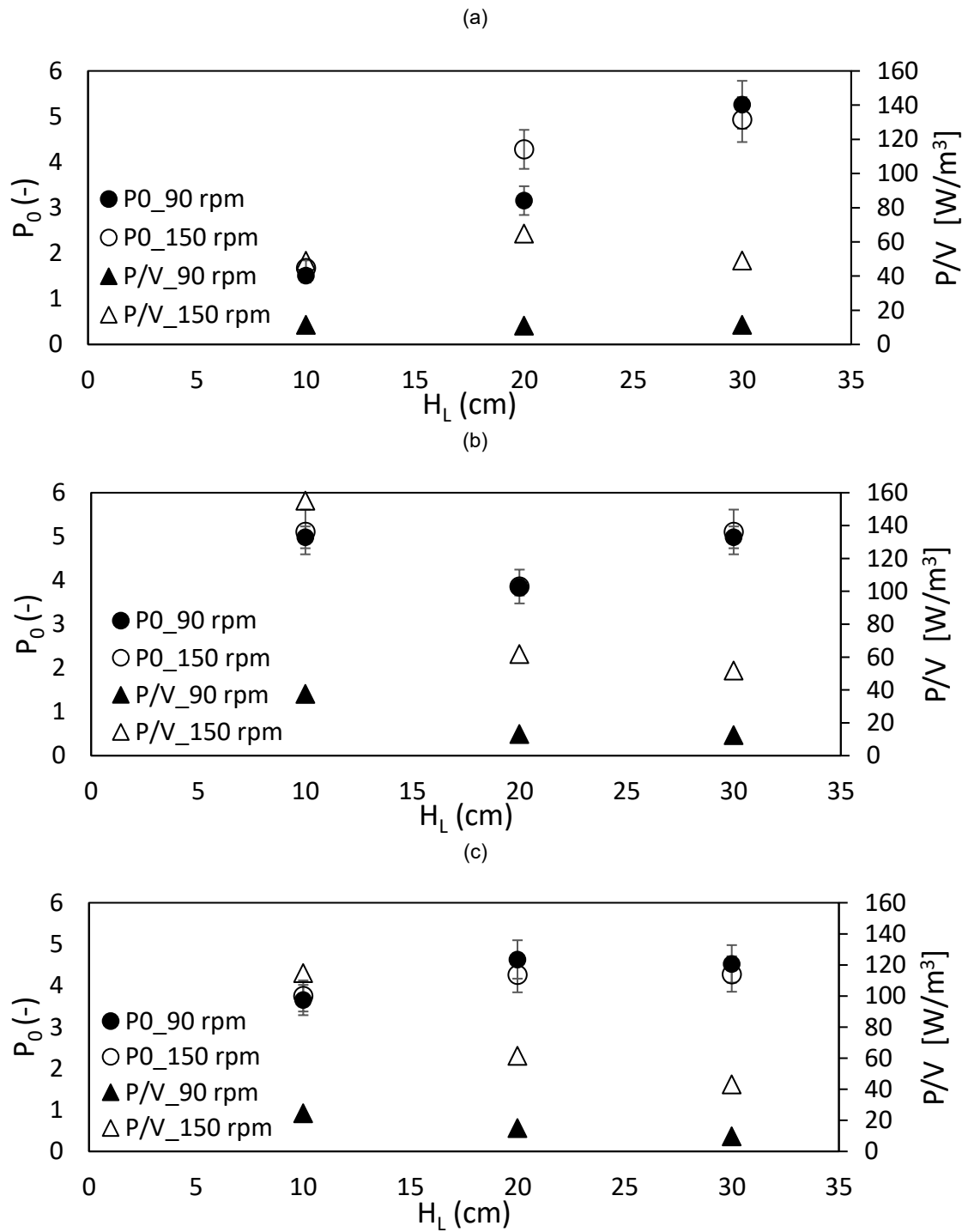


Figure 2: Power number (Po) and power per unit volume (P/V) at different filling heights for the different fluids: specifically for the polymer dispersions a) 0.1% w/w CMC, b) 0.2% w/w CMC, c) 0.4% w/w CMC.

These results are analyzed using the Metzner–Otto correlation, which evaluates the average shear rate around the impeller. The shear rate is calculated by multiplying the impeller speed by a constant specific to the impeller, as follows:

$$\gamma_{avg} = k_s N \quad \text{eq.1}$$

Given that the impeller used is relatively uncommon in the literature, but is primarily a radial impeller, we adopted a constant $k_s=11$, which is typical for impellers of this geometry. Using this constant, we estimate that the average shear rate for the two impeller speeds falls within the range of 15 to 30 s^{-1} . This estimate enabled us to calculate the average apparent viscosity (μ_a) of the fluid around the impeller and, consequently, the Reynolds number for our system, where:

$$Re = \frac{\rho N D^2}{\mu_a} \quad \text{eq.2}$$

and

$$\mu_a = \frac{\tau(\gamma_{avg})}{\gamma_{avg}} \quad \text{eq.3}$$

Where the $\tau(\gamma_{avg})$ is the shear stress for the specific solution at the average shear rate which is extrapolated from the rheological data.

This led to the consideration of three distinct ranges of apparent viscosity, based on the two impeller speeds and different fluid compositions. Specifically, the ranges were as follows: 0.06–0.05 Pas for the 0.1% w/w CMC solution, 0.13–0.10 Pas for the 0.2% w/w CMC solution, and 0.35–0.27 Pas for the 0.4% w/w CMC solution. Corresponding Reynolds numbers for these apparent viscosities were 380–770, 170–370, and 60–140, respectively. When comparing these results with those previously reported by Alberini et al. (2024) using water, we observe distinct differences in the mixing behavior due to the increased viscosity of the fluids. Specifically, Figure 2a presents the results for the 0.1% CMC solution. For both impeller speeds, the power consumption (P_o) increases with the fluid height, whereas in the case of water, a peak in P_o was observed at a filling height of approximately 20 cm (Alberini et al., 2024). This discrepancy can be attributed to the difference in the fluid dynamic regime: water behaves in a fully turbulent regime, while the 0.1% CMC solution operates in a transitional regime. As a result, the power per unit volume (P/V) remains relatively consistent across different filling heights for both impeller speeds. This behavior can be explained by the pumping characteristics of the impeller. At lower apparent viscosities, the impeller's inlet pumping capacity increases with the fluid height, but unlike the water case, this does not lead to the formation of recirculation loops at higher filling heights (around 20 cm). Instead, the fluid primarily circulates around the impeller, with minimal movement near the tank walls, as visually observed during the experiment.

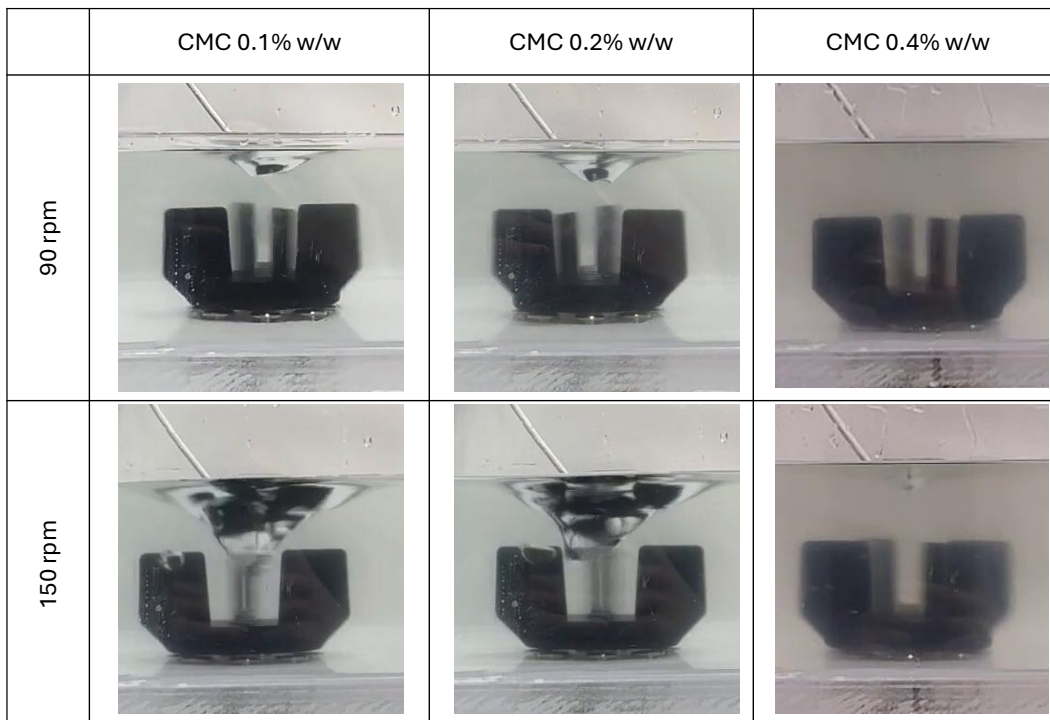


Figure 3: Snapshots of vortex formation at given impeller speed and CMC concentration.

As the apparent viscosity increases, the power consumption (P_o) becomes more stable and is less influenced by the filling height, regardless of impeller speed. This can be explained by the fact that the fluid's mixing zone, where the fluid moves most vigorously, remains largely confined to the volume directly occupied by the impeller. As the filling height increases, the mixing zone does not expand significantly, leading to nearly constant power consumption. Consequently, power per unit volume (P/V) decreases with an increase in the total volume. It can be speculated that as the volume grows, the size of the dead zones also increases, since the mixing zone remains constant while less fluid is effectively stirred.

Figure 3 presents selected snapshots of vortex formation for the different investigated fluids, captured at two impeller speeds (90 and 150 rpm). Due to the square base of the tank, the vortex forms with an inclined axis, causing its shape to vary depending on the position. These changes are minimal at lower impeller speeds, but become more pronounced at higher speeds, as indicated by the error bars in Figure 4. The error bars were calculated by analyzing the vortex shape and area from 10 different images for each analyzed case.

Increasing the apparent viscosity of the solution the vortex formation is delayed at higher speed and the increase of apparent viscosity makes the shape of vortex more uniform at different positions of the impeller as it is shown in Figure 4 comparing same impeller speeds and different solutions.

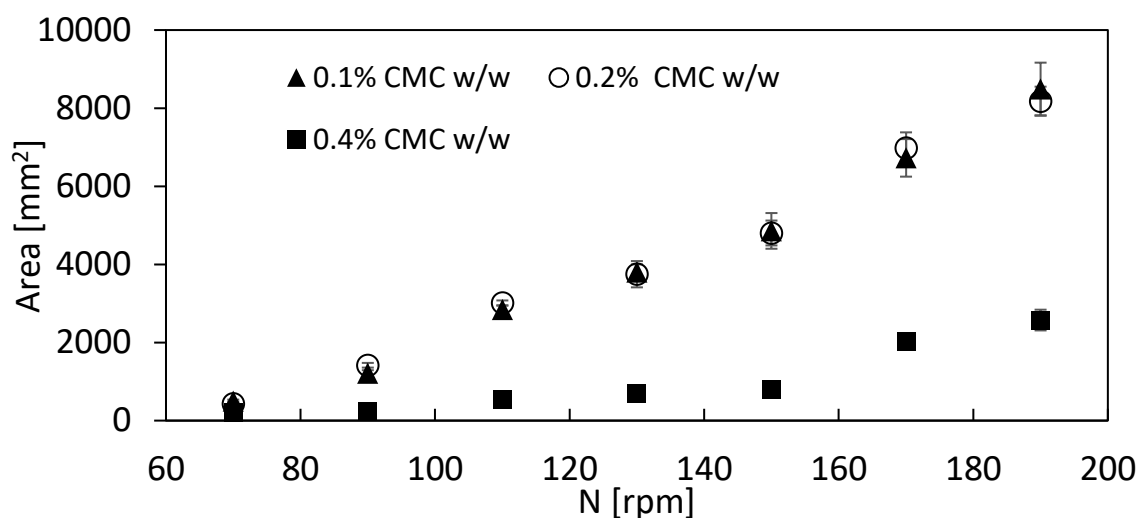


Figure 4: Area of vortex at different impeller speeds for the three CMC solutions.

4. Conclusions

This study provides valuable insights into the mixing dynamics of shear-thinning fluids in single-use bioreactors (SUBs), which are commonly employed in the pharmaceutical industry. The research focused on understanding how fluid rheology, agitation speed, and filling height influence energy consumption and vortex formation in a 50 L cubic tank, designed to replicate the geometry of a commercial SUB mixer. The rheology of the fluid plays a significant role in power consumption during mixing. As the apparent viscosity of the CMC solutions increased, the power consumption became more stable and less dependent on the filling height, irrespective of impeller speed. The shape and size of the vortex were strongly influenced by the apparent viscosity of the fluid. At higher viscosities, vortex formation was delayed and the vortex shape became more uniform across different impeller positions. This indicates that higher viscosity fluids are less prone to generating complex flow patterns, such as recirculation loops, which are more common in water or lower viscosity solutions. The study revealed that, as the apparent viscosity of the fluid increased, the effect of filling height on power consumption and vortex shape diminished. This is likely because the mixing zone, which remains confined to the region around the impeller, does not significantly expand with increasing fluid height. As a result, power per unit volume (P/V) decreased with increasing tank volume, while the formation of dead zones in the tank also grew, limiting effective mixing at higher volumes. The results from this study are particularly relevant for optimizing mixing processes in pharmaceutical applications, where uniform mixing is essential for the quality and consistency of products such as vaccines and therapeutic proteins. The understanding of how rheological properties impact mixing efficiency and energy consumption in SUBs can help in the design of more efficient bioreactor systems, leading to reduced operational costs and improved process sustainability.

Nomenclature

CMC – Carboxymethylcellulose	SUB – Single Use Bioreactor
H_L – Filling height, cm	T – Tank diameter
k – Consistency index, Pas	V – Volume, m ³
k_s – Metzner Otto constant	w – weight
n – Power law index	γ_{avg} – average shear rate, s ⁻¹
N – Impeller speed, revolution per second	γ – shear rate, s ⁻¹
Po – Power Number	τ – shear stress, Pa
P – Power, W	μ_a – apparent viscosity, Pas
rpm – revolution per minute	
std – standard deviation	

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