

December 23, 2020

Flexsafe® Pro Mixer

The Fast, Flexible and Intelligent Solution for Alumina Gel Resuspension

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Abstract

Flexsafe® Pro Mixer is a unique single-use technology fitting all mixing steps from buffer and media preparations, downstream steps 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 standards quality attributes such as Biocompatibility, Integrity and Supply network.

This application study presents performance data of the 50 L and 200 L Pro Mixer for Alumina gel resuspension.

Alumina gel is commonly added to vaccine formulations as an adjuvant to enhance immune response. Alumina gel settles during storage. This settling behavior can make it challenging for mixing systems to re-suspend the gel and homogenize the formulation.

The performances of the single-use mixing system are assessed by determining the normalized settled gel height in samples taken at the top and bottom during resuspension after 2 week storage period.

Flexsafe® Pro Mixer is able to resuspend the solution in less than a minute.

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

Introduction

Since most highly purified recombinant antigens are poorly immunogenic, adjuvants are often required to increase the level and duration of protection induced by vaccines.

Aluminum-containing adjuvants, a successful and well-established adjuvant, have been used for over 90 years to enhance immune response to vaccines. Indeed, aluminum adjuvants are effective with many of the various vaccine antigens in currently licensed vaccines.

The purpose of this application study is to assess the performances of the single-use Pro Mixer to homogeneously resuspend alumina gel, also known as aluminum hydroxide gel, after storage and settling, and more broadly its suitability for gel resuspension and viscous liquid-liquid mixing applications. The purpose is also to perform it under low shear mixing conditions to ensure its adequacy for downstream and final formulation applications.

The notation for alumina gel concentration can be based on aluminum oxide (Al_2O_3) content, aluminum hydroxide ($\text{Al}(\text{OH})_3$) content, aluminum (Al^{3+}) content, or some combination of the 3. It is possible to convert between all three using the chemical equations below.

• $\text{Al}_2\text{O}_3 + 3\text{H}_2\text{O} \rightarrow 2\text{Al}(\text{OH})_3$.
Hydrated aluminum oxide becomes aluminum hydroxide.

• $\text{Al}(\text{OH})_3 \rightarrow \text{Al}^{3+}_{(\text{aq})} + 3\text{OH}^{-}_{(\text{aq})}$.
Dissociation of aluminum hydroxide into constituent ions.

A worst-case concentration of 4.6 mg/mL Al^{3+} was chosen to test the Flexsafe® mixing system.

The solution is prepared in a Flexsafe® bag for Pro Mixer 50 L and 200 L. The bags are stored for at least 2 weeks in order to let the gel settle down. The solution is then mixed at low speed and sampled in Falcon tubes at the same time through the bottom and top of the bag. Samples are stored again for 2 weeks and the normalized settled gel height is determined. The values representing 95% and 105% of the final normalized gel height are calculated. The 95% resuspension time is determined as the time when both the top and bottom normalized gel heights enter between 95% and 105% of the final normalized settled gel height and stay between those boundaries.

Materials and Methods

Materials

Consumable

- Standard Flexsafe® Bags for Pro Mixer (50 L and 200 L) including drain valve
- Alumina gel from SPI Pharma
- Sodium Chloride
- Deionized water
- 15 mL Falcon tubes

Equipment

- Palletank for Mixing
- Pro Mixer drive unit
- Serological pipette
- Peristaltic pump
- Bench scale and floor scale

Method

1. Pre-test to determine the settling duration: The gel cloud height was recorded daily for 2.5 weeks. A best-fit exponential-decay curve was generated from the cloud height data. This curve was used to predict how much further the gel would settle if it were allowed to settle for more time.

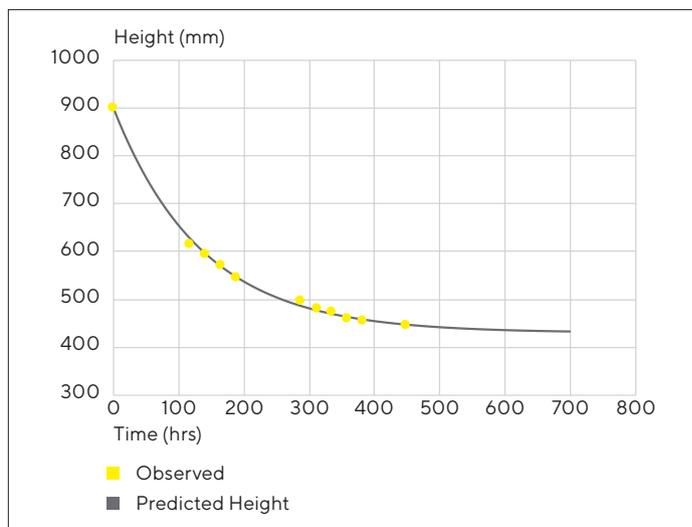


Fig 1: Alumina gel cloud height versus settling time

- The curve had extremely good fit with the experimental data points.
- After 2 weeks of settling, waiting longer would not result in significantly higher settling height. Thus, 2 weeks of settling time was deemed well representative time of longer storage period and was chosen for the experiment.

2. Alumina gel suspension is prepared in standard Flexsafe® Bags for Pro Mixer according to table 2 to obtain a final solution at 4.6 mg/mL Alumina gel concentration (Al³⁺) and 150 mM NaCl. The solution is mixed during the filling phase at the speed given in table 3.

Bag Volume	50 L	200 L
DI water (L)	35.65	142.6
NaCl (kg)	0.2	0.8
Alumina gel (kg)	14.65	58.60

Table 1: Recipe for Alumina gel suspension preparation

The Alumina gel is transferred into the bag via peristaltic pumping. While doing this operation, the alumina gel is kept homogenized.

3. Once all of the aluminum gel has been added, the suspension continues to be mixed at the same speed for 30 minutes, then mixing is stopped.

4. The Pallettank is stored for 2 weeks to allow the gel to settle.

5. After a period of 2 weeks of settling has elapsed, solution is resuspended at the speed indicated in table 3. Samples of 15 mL are collected in Falcon tubes simultaneously from both the top port, with a serological pipette and the bottom port, through the drain valve every 20 seconds after the start of the mixing during the first 600 seconds and additional time points at 1200 seconds and 1800 seconds.

Bag Volume (L)	Pro Mixer Speed (rpm)	
	During filling	During resuspension
50	300	115
200	500	150

Table 2: Pro Mixer Speed setting during filling and resuspension phases

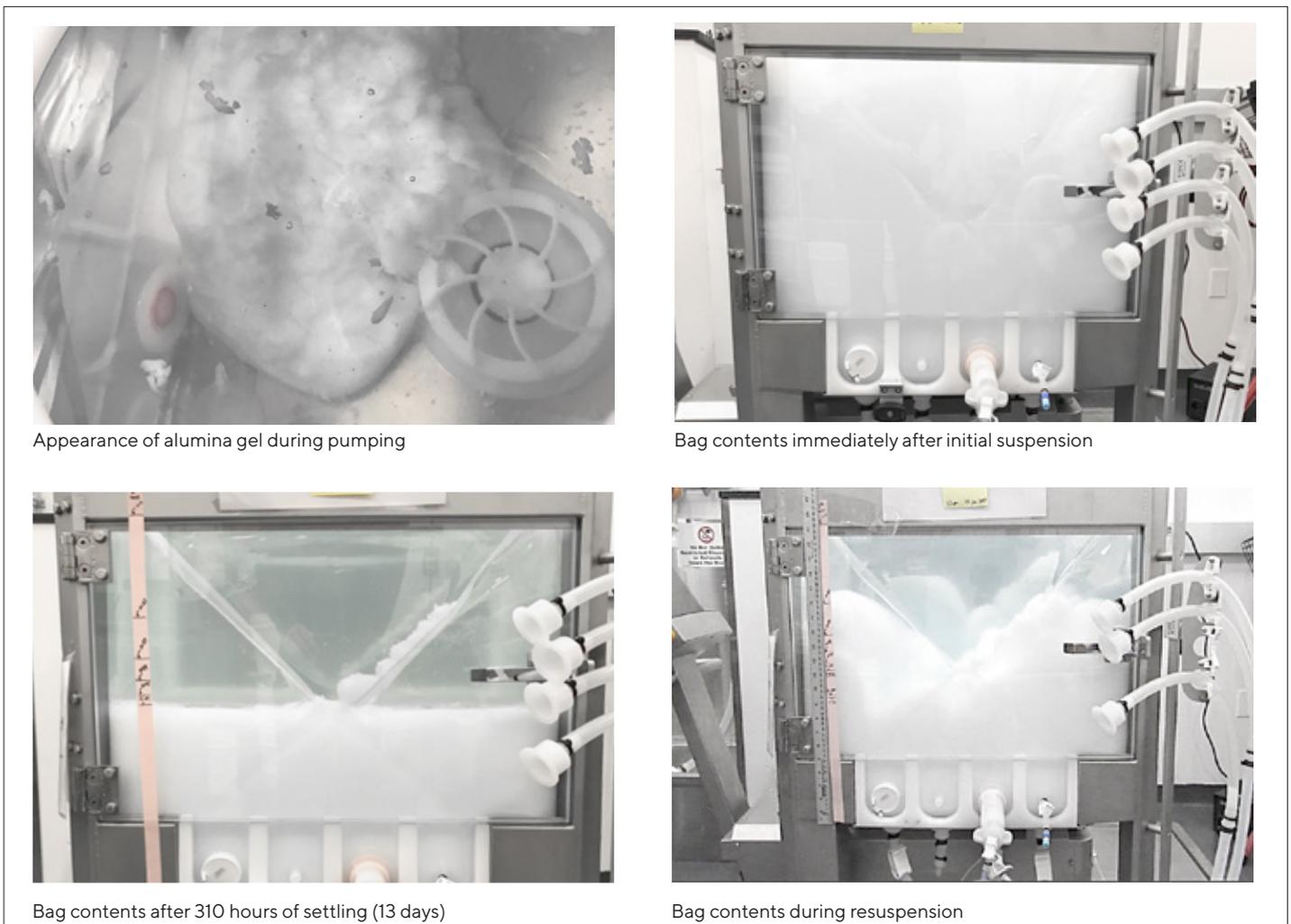


Fig 2: Alumina gel settling and suspension during pre-test performed at 1000 L

Results

6. Samples are then analyzed. All the Falcon tubes are shaken simultaneously and stored for 2 weeks to allow the gel to settle in the tubes. After this 2 week period, a ruler with 1mm resolution is used to determine the settled gel height in each sample and each sample tube is weighed to account for variations in sample volumes. The gel height is normalized using the formula:

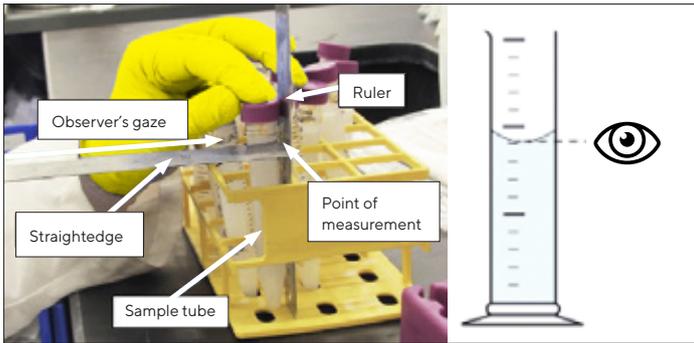
$$\text{Normalized Gel Height } \left(\frac{\text{mm}}{\text{g}} \right) = \frac{\text{Settled Gel Height (mm)}}{\text{Total (g)}}$$

$$95\% \text{ of final settled Height} = \frac{(\text{Settled HeightTop, 1800 s} + \text{Settled HeightBottom, 1800 s})}{2} \times 0.95$$

$$105\% \text{ of final settled Height} = \frac{(\text{Settled HeightTop, 1800 s} + \text{Settled HeightBottom, 1800 s})}{2} \times 1.05$$

Results are presented in a graph including the top normalized gel height, the bottom normalized gel height, a line representing 95% of the final normalized gel height and a line representing 105% of the final normalized gel height.

The 95% resuspension time is determined as the time when both the top and bottom normalized gel heights fall between 95% and 105% of the final normalized settled gel height and stay between those boundaries.



Use a ruler to determine settled gel height. It can be helpful to use a straightedge oriented parallel to the observer's gaze as a measurement aid.

Rules for liquid height measurement: eyes are directly in front of the liquid level (not on an angle); the measurement is taken from the bottom of the meniscus (not the top).

The Flexsafe® Pro Mixer system was able to achieve a complete homogenization of high concentrated 4.6 mg/mL alumina gel in 50 L and 200 L in less than 1 minute at low-shear speed.

In both cases, for 50 L and 200 L (see fig.2 and fig. 3), both the "top" and "bottom" values of the samples are staying within $\pm 5\%$ of the well-mixed result after less than a minute of resuspension, allowing to have a completely homogenized solution very quickly despite the use of a low speed rotation. This is confirmed by the additional points at 1200 and 1800 s that show a very high level of homogeneity.

We also observed that the system was efficient from the very start. After settling, the lower layer of the alumina gel can be very dense and viscous and thus more difficult to mix, especially with the impeller positioned at the bottom, but no issues were observed with mixing start-up, proving once again the Flexsafe® Pro Mixer performances.



50 L Alumina gel resuspension (4.6 mg/mL)

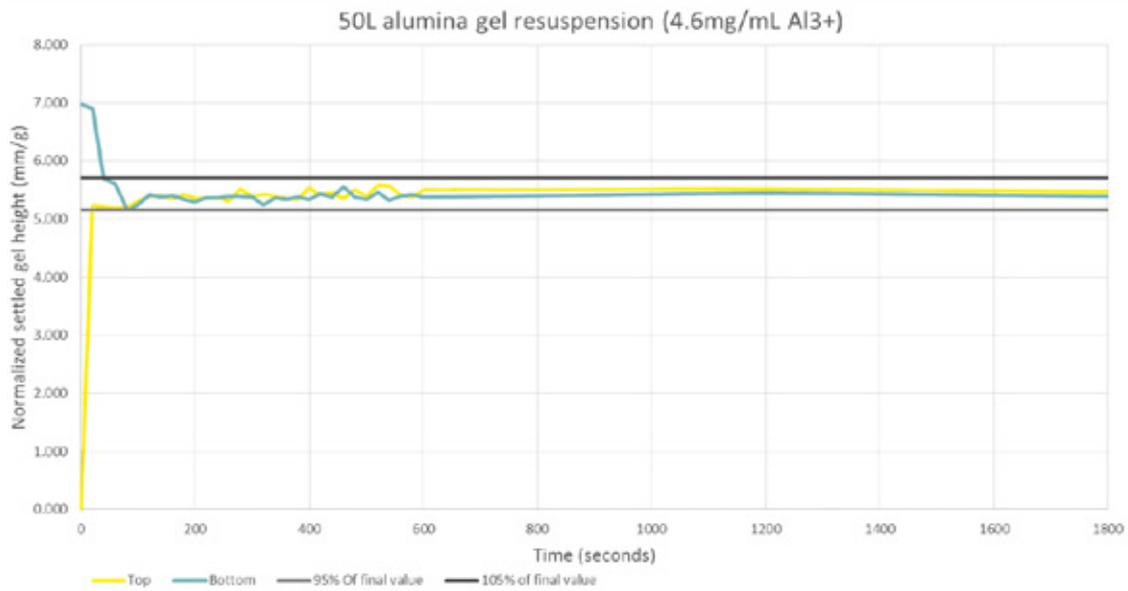


Fig. 3: Alumina gel resuspended in less than a minute at a low speed of 115 rpm in 50 L Flexsafe® Bag for Pro Mixer

200 L Alumina gel resuspension (4.6 mg/mL)

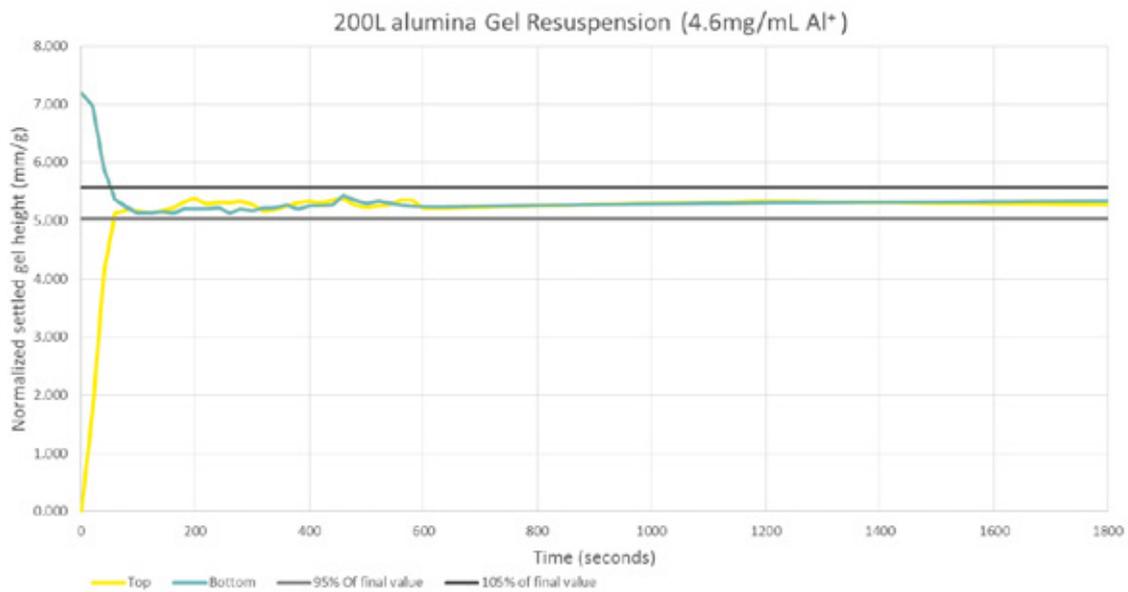


Fig.4: Alumina gel resuspended in less than a minute at a low speed of 150 rpm in 200 L Flexsafe® Bag for Pro Mixer

Summary of the Flexsafe® Pro Mixer Mixing Performances

Solution	Typical Application	Gel Characteristics	Resuspension Time and Rotation Speed per Volume	
			50 L (115 rpm)	200 L (150 rpm)
Alumina gel at 4.6 mg/mL Al ³⁺	Vaccines' adjuvants	Thick, viscous paste at room temperature. Insoluble in water. Settle over time.	< 1 minute	

Table 3: Alumina gel characteristics and resuspension performances with Pro Mixer System

Conclusion

Flexsafe® Pro Mixer is a unique single-use technology platform fitting all mixing steps from buffer and media preparations, downstream steps to final formulation in 50 L, 100 L, 200 L, 400 L, 650 L and 1,000 L volumes.

Despite the fact that the alumina gel is settling down quickly when stored, forming a viscous paste, the Flexsafe® mixing system was able to resuspend the solution homogeneously, in less than a minute. The low speed ensures preservation of shear sensitive preparations and minimize foaming.

Flexsafe® Pro Mixer ergonomic design enables intuitive, modular and agile use to achieve fast installation and mixing operations. Thanks to its high efficiency, the Flexsafe® Pro Mixer system was able to achieve very short resuspension time even in worst case conditions (gel concentration, settling time and low speed mixing), making the final steps of vaccine manufacturing, quick and easy to perform.



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