# S-factor Optimization for MFI using Lumetics LINK

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## INTRODUCTION

Distinction of silicone oil vs. non-silicone oil particulates in protein formulations is important both in terms of accurately detecting protein aggregation during stress/stability, as well as for the study of protein-silicone oil interactions. Strehl et al reported a novel multi-parametric S-factor application for such distinction, using a ProteinSimple MFI 4100 flow microscopy system with a 400 µm cell.

However, the S-factor Cut-off function permitting distinction between silicone oil and proteinaceous particulate is dependent both on the optics/image processing of the flow microscopy system utilized, as well as the optical properties of the sample (delta RI between fluid and particulate). Therefore, time-consuming offline data processing must be performed for S-factor optimization in each instance.

This work investigates the application of a new automated data management and analysis technique towards S-factor filter optimization for the MFI 4200/5200 optical set-point, and the development of an optimization process that may be easily replicated.

## INSTRUMENTS

Data presented in this poster were collected on ProteinSimple MFI 4200 and 5200 instruments in manual mode, equipped with a 100µm flow cell.

## METHODS

A well formulated monoclonal antibody was mixed with silicone pooled from buffer filled glass BD syringes incubated for 2 days at 50ºC.

Mixtures of protein and silicone oil were produced in the following silicone oil/protein ratios: 100/0%, 95/5%, 85/15%, 70/30%, 30/70%, 5/95%, and 0/100%. Each ratio was analyzed in triplicates. The LINK (*Lumetics Inc.*) data management and analysis platform was then used to:

- Visualize the particle size distribution for each sample, and remove outlier measurements with an assignable cause (e.g. schlieren lines or air entering the flow cell).
- Assess morphological parameter distinction between silicone oil and protein as a function of particle size, to estimate the most impactful S-factor definition.
- Calculate S-factor parameters for each particle measured by the MFI system, and overlay the protein/silicone oil S-factor distributions in particle size segments from which the S-factor Cut-off functions may be determined.
- Define silicone oil and protein sub-populations based on the S-factor Cut-off functions for both the MFI 4100 (400µm cell) optimization (*Strehl et al.*), and the newly determined MFI 4200/5200 (100µm cell) optimization presented here.
- Estimate the MFI 4100 and MFI 4200/5200 S-factor optimization filter accuracy for particles >= 5µm, qualitatively based on particle images and quantitatively based on mixture concentrations.

## RESULTS

### MFI 4200/5200 Silicone Oil vs. Protein Distinction by Morphological Parameter

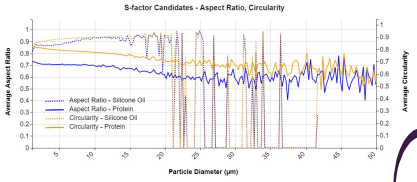



Figure 1 **Aspect Ratio and Circularity** exhibit good separation for sizes <20µm. There is poor separation >20µm due to low silicone oil counts and extrinsic particles.

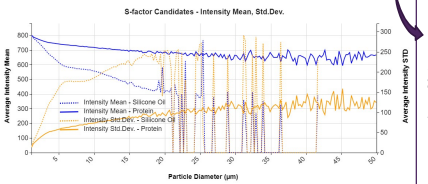



Figure 2: **Intensity Mean and St Dev** exhibit good separation values for Intensity Mean and are higher for protein than silicone oil.

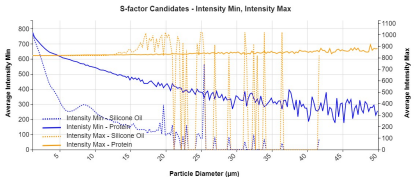



Figure 3: **Intensity Min** exhibits good separation for particles < 20µm. Intensity Max exhibits poor separation.

### MFI 4200/5200 S-factor Filter Development

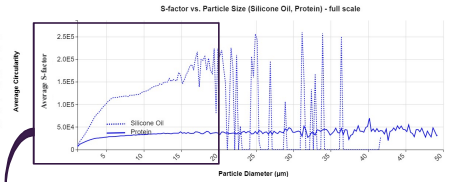
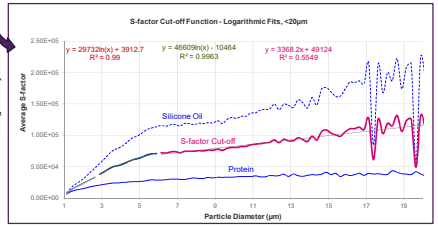



Figure 4: MFI 4200/5200 S-factor application (S-factor = Aspect Ratio * Circularity * Intensity Max * Intensity STD)




The S-factor Cut-off function represents an estimate of the mid-point between the average S-factor for the Silicone Oil vs. that of Protein, as a function of particle size. This function may then be utilized to generate and report the silicone oil or protein populations based on whether a given particle is above or below its S-factor Cut-off value. The S-factor Cut-off function for both the previously reported MFI 4100 and the newly generated MFI 4200/5200 are as follows:

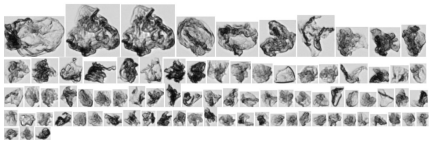
**MFI 4100 (400 µm cell)**
2.13 µm–4.38 µm: 36375*ln(x)-23108)
4.63 µm–10.88 µm: 38767*ln(x)-27428)
11.13 µm–400 µm: 72000

**MFI 4200/5200 (100µm cell)**
1.125 µm–2.875 µm: 29732* LN(ECD)+3913
2.875 µm–6.125 µm: 46609*LN(ECD)-10464
6.125 µm–400 µm: 3368*(ECD)+49124

Figures 5, 6: New S-factor Cut-off function required for MFI 4200/5200

### Qualitative and Quantitative S-factor Filter Performance

**Protein-Only, MFI 4100 (400 µm cell) Strehl et al filter**
Protein misclassified as silicone oil: 6,959 of 54,381 particles >=5 µm, or **12.8%**

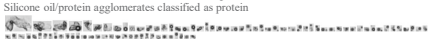


**Protein-Only, MFI 4200/5200 optimized filter**
Protein misclassified as silicone oil: 903 of 54,381particles >=5 µm, or **1.7%**

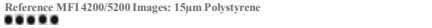
**Silicone-Only, MFI 4100 (400 µm cell) Strehl et al filter**
Silicone/extrinsic particles misclassified as protein: 94 of 9,117 particles >=5 µm, or **1.0%**

**Silicone-Only, MFI 4200/5200 optimized filter**
Silicone/extrinsic particles misclassified as protein: 197 of 9,117 particles >=5 µm, or **2.2%**

**85% Silicone/15% Protein, MFI 4200/5200 optimized filter**
Silicone oil/protein agglomerates classified as protein



**Reference MFI 4200/5200 Images: 15µm Polystyrene**



| Formulation | # Meas. | Protein % | Silicone Oil % | Particle Conc. >=5um (#/ml) | Expected Protein (#/ml) | MFI 4100 (400µm cell): Protein Conc. (#/ml) | MFI 4100 (400µm cell): Deviation from Expected | MFI 4200/5200: Protein Conc. (#/ml) | MFI 4200/5200: Deviation from Expected | Expected Silicone Oil (#/ml) | MFI 4100 (400µm cell): Silicone Oil Conc. (#/ml) | MFI 4100 (400µm cell): Deviation from Expected | MFI 4200/5200: Silicone Oil Conc. (#/ml) | MFI 4200/5200: Deviation from Expected |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Protein | 3 | 100% | 0% | 54,381 | 54,381 | 47,422 | -12.8% | 53,478 | -1.7% | 0 | 6,959 | | 903 | |
| Silicone Oil - 5% | 3 | 95% | 5% | 50,802 | 48,262 | 44,341 | -8.1% | 49,368 | 2.3% | 2,540 | 6,461 | 154.3% | 1,434 | -43.6% |
| Silicone Oil - 30% | 3 | 70% | 30% | 43,386 | 30,370 | 33,786 | 11.2% | 38,505 | 26.8% | 13,016 | 9,600 | -26.2% | 4,880 | -62.5% |
| Silicone Oil - 70% | 3 | 30% | 70% | 27,102 | 8,131 | 14,170 | 74.3% | 17,259 | 112.2% | 18,972 | 12,932 | -31.8% | 9,843 | -48.1% |
| Silicone Oil - 85% | 3 | 15% | 85% | 20,559 | 3,084 | 7,635 | 147.7% | 9,344 | 203.0% | 17,475 | 12,924 | -26.1% | 11,216 | -35.8% |
| Silicone Oil - 95% | 3 | 5% | 95% | 12,912 | 646 | 2,486 | 284.9% | 3,249 | 403.3% | 12,267 | 10,426 | -15.0% | 9,664 | -21.2% |
| Silicone Oil | 3 | 0% | 100% | 9,117 | 0 | 94 | | 197 | | 9,117 | 9,023 | -1.0% | 8,920 | -2.2% |

Figures 7-13: Optimization of an S-factor filter cut-offs for the MFI 4200/5200 significantly reduced protein misclassification errors.

## DISCUSSION

The S-factor developed for the MFI 4100 by Strehl et al. identified the most effective morphological parameters for silicone oil vs. protein distinction to be Aspect Ratio, Circularity, Intensity Max, and Intensity STD. Analysis using LINK for the MFI 4200/5200 found that Intensity Max was not particularly effective, and Intensity Mean and Intensity Min should be considered. However, the standard deviation associated with other morphological parameters was larger, necessitating further investigation regarding their suitability.

The optical differences of the MFI 4100 (400 µm cell) used by Strehl et al., relative to the MF 4200/5200 and possible differences in delta RI of particulate vs. carrier fluid, required new logarithmic fits to estimate the S-factor Cut-off function. Application of the MFI 4100 optimized S-factor to MFI 4200/5200 measurements, was compared to the application of the MFI 4200/5200 optimized S-factor. When considering the protein-only samples, application of the MFI 4100 optimized S-factor resulted in 12.8% of the protein misclassified as silicone oil. This error was reduced to 1.7% upon application of the S-factor optimization for the MFI 4200/5200.

The samples containing 100% silicone oil suspension resulted in similar misclassification errors for both optimizations: 1.0% and 2.2% respectively, with the majority of the misclassified particles being extrinsic particles or silicone "doublets".

With respect to the silicone oil/protein mixtures, similar results were observed for both optimizations. As the percentage of silicone oil increased, the quantity of protein reported became larger relative to the expected theoretical amount based on mixing ratios. Upon visual assessment of particle images in LINK, this is attributed largely to agglomerates of protein and silicone oil.

## CONCLUSION

In order to more accurately distinguish silicone oil droplets from proteinaceous particles in a protein formulation, Flow Microscopy S-factor Cut-off functions must be developed for the specific flow microscopy instrument/model utilized, and for the optical properties of the particulate/fluid system. An S-factor optimization process has been developed using the LINK data management analysis software package, offering a practical and efficient method for protein formulation characterization improvements.

## FUTURE WORK

1. S-factor optimization involving different S-factor definitions (e.g. Intensity Mean, Intensity Min)
2. S-factor optimization of silicone in solutions with different refractive properties (such as high viscosity)
3. Comparisons of silicone from different sources
4. Comparisons of the effect of surfactant on silicone droplets
5. Analysis of droplet distribution in pure silicone (no protein)

## REFERENCES

Discrimination Between Silicone Oil Droplets and Protein Aggregates in Biopharmaceuticals: A Novel Multiparametric Image Filter for Sub-visible Particles in Microflow Imaging Analysis; René Strehl & Verena Rombach-Riegraf & Manuel Diez & Kamal Egodage & Markus Bluemel & Margit Jeschke & Atanas V. Koulov, 2011

How subvisible particles get invisible Relevance of refractive index for protein particle analysis; Sarah Zölls1,2, Manuel Gregoritza1,2, Ruedeeporn Tantipolphan1, Michael Wiggenhorn1, Gerhard Winter2, Wolfgang Frieß2, Andrea Hawe

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