Electronic Supplementary Material (ESI) for Analytical Methods.

MIcrofluidic Detection with Acoustic Spectroscopy (MIDAS) for Insulin Analysis

Charlie McIntosh, Karen Scida, and Sumita Pennathur
Department of Mechanical Engineering
University of California, Santa Barbara

Supplementary Information

A. CALCULATION OF STANDARD DEVIATION

In general, when performing some mathematical manipulation where A and B are combined in some way to form C (as in **Eq 1** below), the propagation of error is described by **Eq 2**.

$$C = f(A, B) \quad \textit{Eq. S1}$$

$$\sigma_C = \sqrt{\left(\frac{\partial C}{\partial A}\right)^2 * \sigma_A^2 + \left(\frac{\partial C}{\partial B}\right)^2 * \sigma_B^2} \quad \textit{Eq. S2}$$

For the case when fresh insulin is normalized by itself, we see that

$$C = \left(\frac{A}{A}\right) = 1 \quad \textit{Eq. S3}$$

for which the standard deviation is

$$\sigma_C = \sqrt{(0)^2 * \sigma_A^2} \quad \textit{Eq. S4a}$$

$$\sigma_C = 0 \quad \textit{Eq. S4b}$$

When the data from another sample is normalized by the fresh insulin data, we obtain the standard deviation in **Eq 6**.

$$C = \left(\frac{B}{A}\right) \quad \textit{Eq. S5}$$

$$\sigma_C = |C| * \sqrt{\left(\frac{\sigma_A}{A}\right)^2 + \left(\frac{\sigma_B}{B}\right)^2}$$ ***Eq. S6***

Therefore, we would obtain a plot where the normalized fresh insulin data is a uniform line at y=1 with zero standard deviation and some trace for the normalized sample data that had some large standard deviation. However, we wouldn't be able to tell from the graph what portion of the standard deviation came from the fresh insulin data and what portion came from the sample data. We calculated the standard deviation using **Eq S7** instead. Then when we plot the normalized intensities, each line has a standard deviation that directly relates back to the original data.

$$\sigma_{norm_{sample}} = \frac{\sigma_{sample}}{avg_{fresh}}$$ ***Eq. S7***

B. RAW DATA BEFORE NORMALIZATION

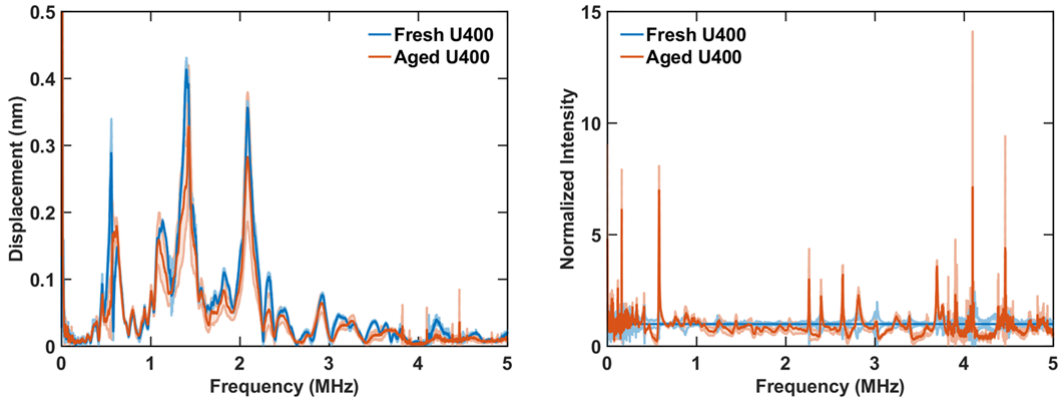

Figure S1. Comparison between two plots of the average and standard deviation for two samples of U400 insulin. ***Left:*** The raw data for fresh and aged U400 data. Differences between the two traces are difficult to distinguish. If instead, we normalized the traces with the fresh insulin data (***Right***), it becomes much easier to discern the differences.

C. FRESH VS SHOOK INSULIN: Shaking after 7 hours

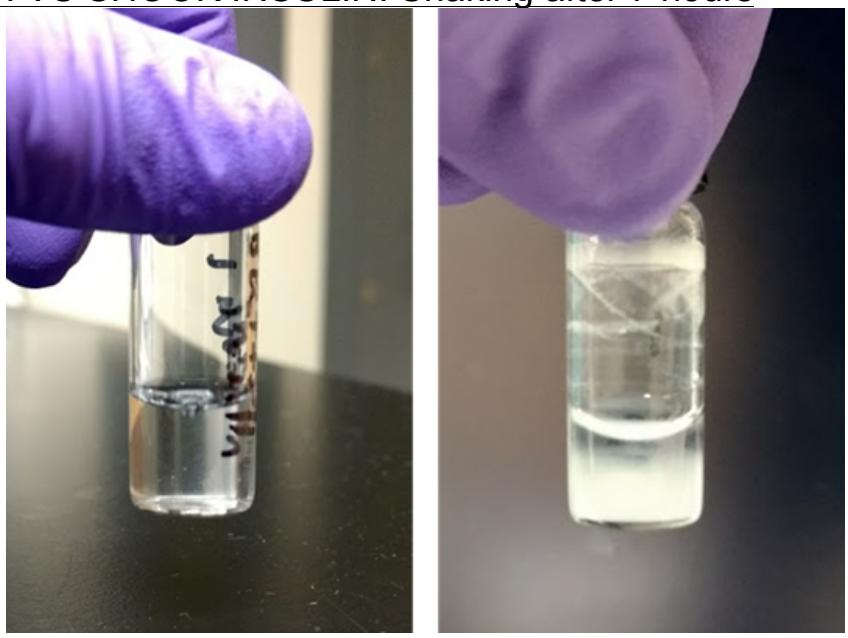

Figure S2. Sanofi U400 before (***Left***) and after (***Right***) shaking in an incubator for several hours. The aggregates in the artificially aged insulin (***Right***) settle in the solution. During shaking, some insulin dried to the vial, forming the residue in the top half of the vial. It is not surprising that the insulin aggregates settled because the density of insulin is higher (1.324 g/mL) than water [S1].

D. CONSISTENCY BETWEEN VIALS (Vial 1 avg vs Vial 2 & 3)

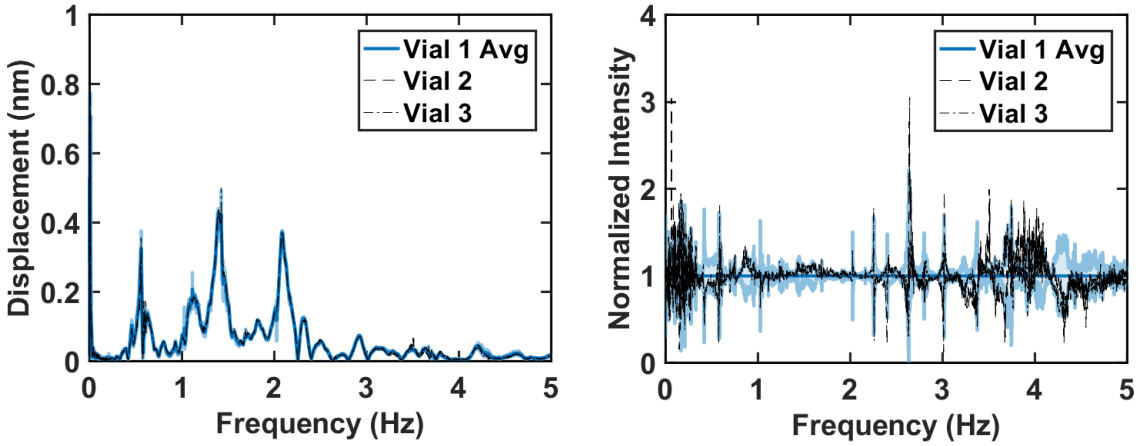

Figure S3. A comparison between the data collected from aliquots of one vial of fresh U100 insulin vs the data collected from single aliquots of two other vials of U100 insulin. The average of three aliquot measurements from vial 1 (fresh U100 insulin) are plotted with shaded error bars in blue. The single measurements for vials 2 and 3 are plotted as black dotted lines. In a few places, the vial 2 & 3 measurement fall just outside of one

standard deviation, though, at most frequencies, the other two lines do fall within one standard deviation.

E. Comparison of FIGURE 4A (pump insulin) WITH FIGURE 2C (aged insulin)

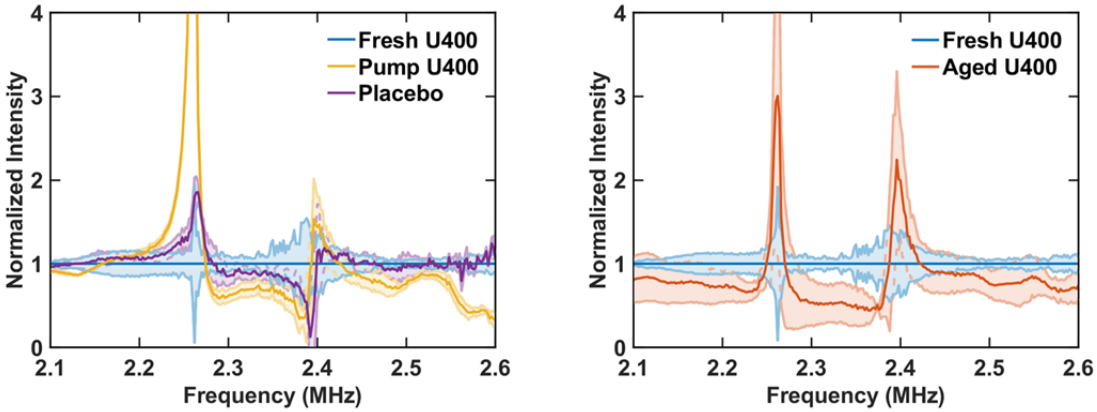

Figure S4. Compares **Figure 4A** and **Figure 2B** with the same axis limits. The trace for the pump insulin (***Left***) shows many similarities to the artificially aged insulin (***Right***), maybe indicating that the pump insulin is aggregating in a similar way

Reference:

[S1] Schiffter, Heiko, Jamie Condliffe, and Sebastian Vonhoff. "Spray-freeze-drying of nanosuspensions: the manufacture of insulin particles for needle-free ballistic powder delivery." *Journal of The Royal Society Interface* 7.Suppl 4 (2010): S483-S500.