ALPHA MA SILK CALCULATOR (VERSION 2.0) GUIDE

The Excel File "Alpha MA silk calculator 2.0" allows calculating the value of the α^* parameter for major ampullate (MA) gland silk fibers. The α^* parameter classifies the MA fibers of any spider species by comparing the mechanical behavior of maximum supercontracted MA fibers of that species with a reference curve of maximum supercontracted *Argiope aurantia* MA fibers.

The details of the calculation can be found in R. Madurga et al., "Material properties of evolutionary diverse spider silks described by variation in a single structural parameter", Scientific Reports 6:18991 (2016) and in J. Garrote et al., "Application of the Spider Silk Standardization Initiative (S^3I) methodology to the characterization of major ampullate gland silk fibers spun by spiders from Pantanos de Villa wetlands (Lima, Peru)", Journal of the Mechanical Behavior of Biomedical Materials, 111: 104023 (2020).

Structure of "Alpha MA silk calculator 2.0"

The file is composed of three Excel sheets INPUT, Aux and Plot.

The **INPUT** sheet allows introducing the data and yields the value of the α^* parameter.

The file requires four sets of data to calculate the true stress-true strain curve of the MA fiber: length increment, ΔL (mm); Force, F(mN); Initial length, L_0 (mm); and initial area, A_0 (μm^2). It also allows introducing two additional data that are used for the comparison of the true stress-true strain curve of the MA fiber and the true stress-true strain curve of the *A. aurantia* reference curve: Value of stress for concurrence (MPa) and Stress range for calculating the slopes (MPa).

Before starting the introduction of the data, click on "Clear Input Data". The columns ΔL (mm) and F(mN) together with the Initial length L_0 and Initial Area A_0 are cleared out and ready for introducing the experimental data.

The column ΔL (mm) corresponds to the length increment of the fiber during the tensile test, i.e. if L is the instantaneous length of the fiber and L_0 the initial length:

$$\Delta L\ (mm) = L(mm) - L_0(mm)$$

It is important that the initial length, L_0, is such that no load is exerted on the fiber at that length, but forces build up immediately upon stretching.

The column F(mN) corresponds to the force exerted on the fiber at a given value of ΔL(mm). It is important that the initial value is F=0 mN, since this should be the value of the force at L_0.

The initial length of the fiber, L_0, is introduced in cell F5 in units of mm.

The initial cross sectional area of the fiber, A_0 (μm^2) is introduced in cell G5.

The value of stress for concurrence (MPa) is introduced in cell F8.

The stress range for calculating the slopes (MPa) is introduced in cell F10.

When all these data are introduced, the calculation starts upon clicking on the "Calculate" button.

Calculation of α*

The concurrence of both curves at the value of stress for concurrence is imposed by displacing the MA curve along the X (true strain axis). The value of this displacement corresponds to the value of the α* parameter for that curve. In general, higher values of the stress for concurrence yield more accurate results for the α* parameter.

The quality of the concurrence may be tested either visually or, if a quantitative parameter is required, by the comparison of the slopes of the true stress-true strain curve of the MA fiber and that of the true stress-true strain curve of the reference curve at the value of stress for concurrence.

Both slopes are obtained through a linear fitting of the curves using the least squares method in a region close to the value of stress for concurrence. The parameter "Stress range for calculating the slopes" allows varying the interval of points used for calculating the slopes. In general, it is assumed that a variation equal or less than 20% between both slopes indicates that the concurrence of both curves is adequate for the choice of the value of stress for concurrence.

The value of the α* parameter that results from the calculation is displayed in cell G13.

Aux and Plot sheets are intended to provide additional information, but its use is not necessary to obtain the α* parameter.

The **Aux** sheet displays:

The Engineering strain, e (column B) and the engineering stress, s (column C) calculated from the INPUT data and defined as:

$$e = \Delta L/L_0 \quad ; \quad s = F/A_0$$

The true strain, ε (column E) and the true stress, σ (column F) calculated from the INPUT data and defined as:

$$\varepsilon = Ln(L/L_o) = Ln(1+e) \quad ; \quad \sigma = F/A = s(1+e)$$

Where A is the instantaneous cross sectional area of the fiber. Calculation of true stress assumes that the volume remains constant during the deformation of the fiber, i.e. $A \cdot L = A_0 \cdot L_0$.

The true strain, ε, of the *Argiope aurantia* reference (column I) and the true stress, σ, of the *Argiope aurantia* reference (column J).

The **Plot** sheet shows the true stress-true strain curve calculated from the INPUT data and displaced with reference to the true stress-true strain *Argiope aurantia* reference. The value of

the α* parameter can be read off from this plot as the value at which the true stress-true strain curve of the sample touches the true strain, ε, axis. The stress range for calculation of the slopes is displayed as a segment on the reference curve.

Questions and comments on the ALPHA MA SILK CALCULATOR 2.0

Questions and comments on the ALPHA MA SILK CALCULATOR 2.0 can be sent to the address S3I@ctb.upm.es.