

GInaFit

A user guide

March 2015

An illustration of how to model bacterial inactivation curves using GInaFit v1.6, a freeware add-in for Microsoft® Excel designed for bridging predictive modelling approaches and end-users in the food industry or research groups.

Further documentation can be found in Geeraerd *et al.* (2005).

GInaFit can be downloaded from
<http://cit.kuleuven.be/biotec/ginafit.php>



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1. Produce the survival curve that relates the log-concentration of heat-injured cells (\log_{10} of N, expressed in CFU.mL⁻¹) with duration of the treatment (time in minutes).

2. Determine the shape of the bacterial inactivation curve using key descriptive features using the diagram on Figure 1, a flow chart of the procedure to assess the shape of the survival curve. The description starts with the decline phase: is the decline phase linear? If so, does the curve have a shoulder; if not, does it have a tail? The survival curve will be categorised as one of the ten possible shapes described and numbered by Geeraerd *et al.* (2005).

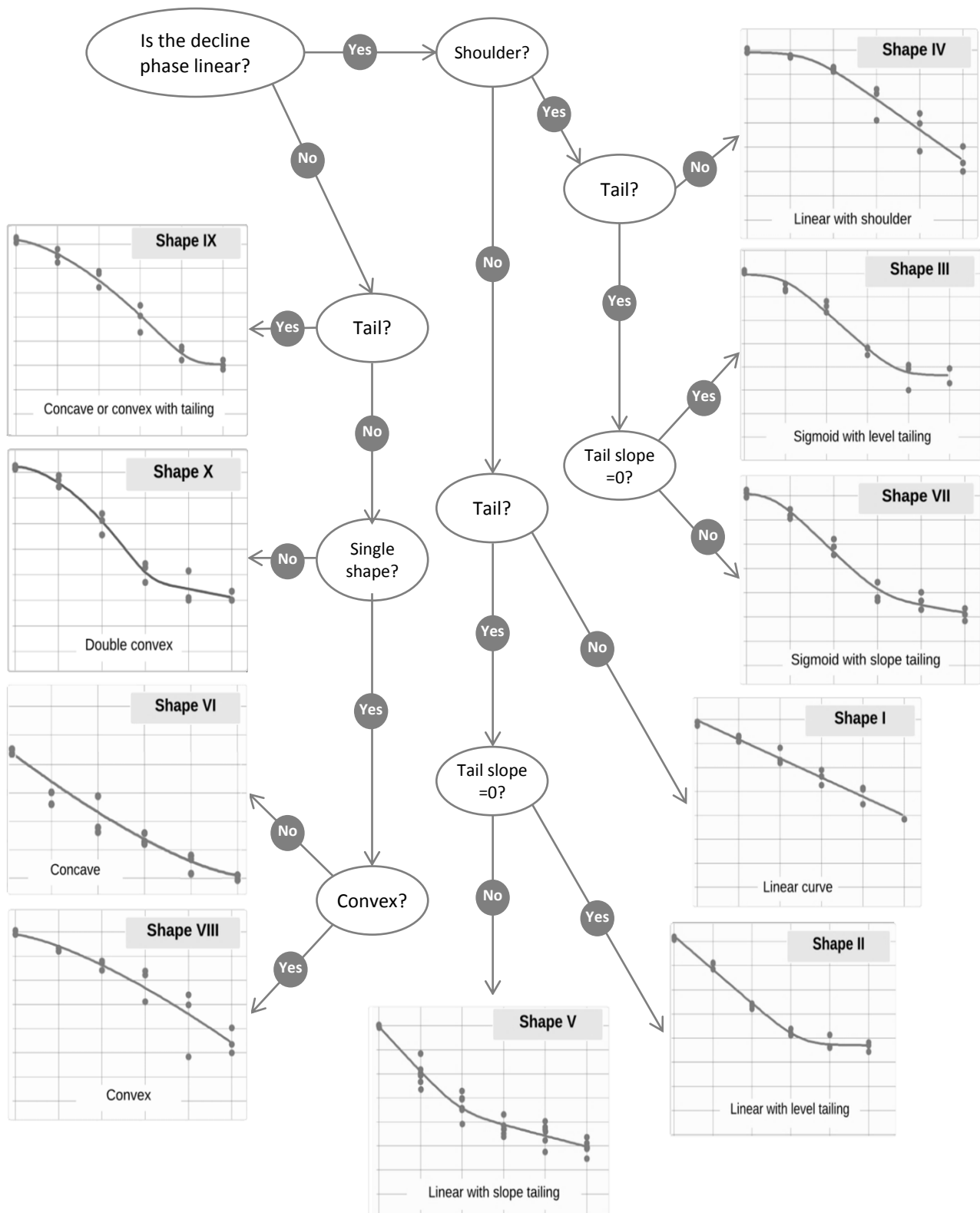


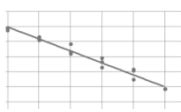
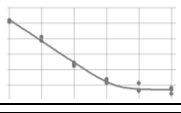
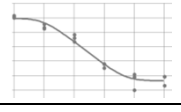
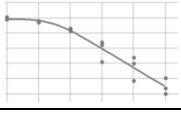
Figure 1: determine the shape of the bacterial inactivation curves using key descriptive features.

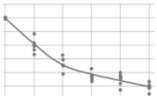
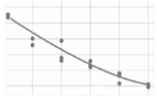
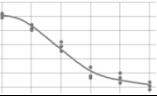
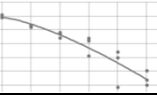
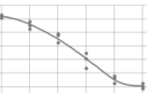
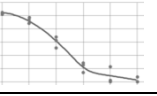
The curve can take one of ten shapes: linear (Shape I), linear with tailing (Shape II), sigmoidal-like (Shape III), linear with a preceding shoulder (Shape IV), biphasic (Shape V), concave (Shape VI), convex (Shape VIII), convex or concave with a tail (Shape IX), double convex or concave (Shape X).

Inactivation curves used for illustration are triplicates of repeated measurements of the concentration ($\log\text{CFU}\cdot\text{ml}^{-1}$) of heat-injured cells of various strains of *Campylobacter* over time (minutes).

3. Select candidate model(s) : Identify one or several model types relevant to the shape of the survival curve and available in GlnaFIT.

Table 1: Candidate models per type of inactivation shape.

Shape	Applicable models in GlnaFIT	GlnaFIT menu item	Implications/assumptions from model structure	Cited literature in GlnaFIT
Shape I Linear 	Log-linear regression	#1	<ul style="list-style-type: none"> • Traditional first-order inactivation kinetics equation • Assumes all cells have equal sensitivity and inactivation depends on random chance of receiving lethal treatment Note: these models can produce survival curves that replicate the classical Log-linear regression depending on the underlying response, but may not be the most appropriate choice for modelling the data	Bigelow and Esty, 1920
	Models by Geeraerd <i>et al.</i> , 2000	#2		Albert & Mafart, 2005 Anonymous, 2000 Cerf, 1977
	Weibull + tail	#3		Cole <i>et al.</i> , 1993
	Biphasic model	#4		Geeraerd <i>et al.</i> , 2000
Shape II Biphasic, level tailing 	Log-linear + tail	#2	Traditional first-order inactivation kinetics equation with added tailing parameter	Geeraerd <i>et al.</i> , 2000
	Biphasic model	#4	Assumes one initially major subpopulation, that is more sensitive to stress (initial decline), and one minor subpopulation that is more resistant to stress (tail)	Cerf, 1977
Shape III Sigmoidal, level tailing 	Log-linear + shoulder + tail	#2	Traditional first-order inactivation kinetics equation with added parameters for tailing and shoulder	Geeraerd <i>et al.</i> , 2000 Greenacre <i>et al.</i> , 2003 Marquenie <i>et al.</i> , 2003 Mossel <i>et al.</i> , 1995
Shape IV Linear, shoulder 	Log-linear + shoulder	#2	Traditional first-order inactivation kinetics equation with added shoulder parameter	Geeraerd <i>et al.</i> , 2000 Mossel <i>et al.</i> , 1995

Shape	Applicable models in GlnaFIT	GlnaFIT menu item	Implications/assumptions from model structure	Cited literature in GlnaFIT
Shape V Biphasic, slope tailing 	Biphasic model	#4	Assumes one initially major subpopulation, that is more sensitive to stress (initial steep constant decline), and one minor subpopulation that is more resistant to stress (final, smoother constant decline)	Cerf, 1977
Shape VI Concave 	Weibull	#3	<ul style="list-style-type: none"> • Shape parameter ($p < 1$) describes the concave shape • Non-autonomous model, <i>i.e.</i> D varies with time 	Mafart <i>et al.</i> , 2002 Peleg & Cole, 1998 Van Boekel, 2002
Shape VII Sigmoidal, slope tailing 	Biphasic + shoulder	#4	<ul style="list-style-type: none"> • Most complex shape • Combines biphasic model and shoulder parameter 	Geeraerd <i>et al.</i> , 2005 Whiting, 1993
Shape VIII Convex 	Weibull	#3	<ul style="list-style-type: none"> • Shape parameter ($p < 1$) describes the convex shape • Non-autonomous model: D varies with time 	Mafart <i>et al.</i> , 2002 Peleg & Cole, 1998 Van Boekel, 2002
Shape IX Convex or concave, tailing 	Weibull + tail	#3	<ul style="list-style-type: none"> • Shape parameter describes the convex/concave shape • Non-autonomous model, <i>i.e.</i> D varies with time 	Albert & Mafart, 2005 Albert & Mafart, 2003
Shape X Double convex 	Mixed Weibull	#3	Assumes one initially major subpopulation, that is more sensitive to stress (first wave), and minor subpopulation that is more resistant to stress (second wave)	Coroller <i>et al.</i> , 2006

4. Run and assess with GlnaFiT: See Geeraerd *et al.* (2012) manual for general installation and use. Develop relevant model(s); the software will test for mis-uses (*e.g.* when applying a model with tailing on data not having a tail, or when the number of parameters is too high in comparison with the number of data points). Model outputs include indicators of:

Goodness of fit:

The Root mean sum of squared error (RMSE) quantifies the goodness of fit for both linear and non linear models via the difference between predicted and observed values. Best fit indicated when this value is close to zero.

Precision:

calculate SD, the standard deviation of data.

- RMSE >> SD: model not capturing trend, too flexible (overfitting, following noise)
- RMSE << SD: model not capturing trend, not flexible enough (underfitting, not following signal)

Variation explained:

R-square adjusted coefficient of determination is the proportion of variation in the inactivation curve that is explained by the model, with penalization for irrelevant parameters (overfitting). Best fit indicated when this value is close to 1.

5. Interpretation and inferences: alongside numeric model outputs, inferences can be drawn using:

Shape of the curve and associated model type may vary within and between bacterial strains, be influenced by stress intensity (frequently concave may become convex or sigmoidal), physiological state of cells, phase of growth (exponential or stationary phase), pre-stress conditions *etc.* In addition to the elements for interpretation in Table *pp4-5*, also consider:

Shoulder effect suggests initial resistance to stress (see Albert & Mafart, 2005)

Tailing effect can suggest varying levels of resistance, for instance due to mixed populations, clumping, protective effect of the suspension medium

Parameter estimates can provide insights into destructive mechanisms. resistance to stress, residual cell concentration, treatment efficiency *etc.* Literature provides various illustration and theoretical background for model parameters (*e.g.* Coroller *et al.*, 2006; Xion *et al.*, 1999; Buchanan *et al.*, 1993 on t_{4D}); elements for interpretation are summarised in Table 2 for the model parameters in GlnaFit.

Table 2: Elements for interpretation of parameters involved in GlnaFit models.

Parameter	Model	Focus for interpretation
S_I Duration of shoulder effect	Log-linear with shoulder Log-linear with shoulder and tail Biphasic with shoulder and tail	Time before decrease: initial resistance to stress
k_{max} First order inactivation rate constant	Log-linear with shoulder Log-linear with tail Log-linear with shoulder and tail	Speed of decrease per time unit (constant, after shoulder and/or before tail)
	Biphasic with tail Biphasic with shoulder and tail	K_{max1} and K_{max2} for speed of decrease for initially major and minor populations (constant, after shoulder and/or before tail)
N_0 Initial inoculum concentration	Log-linear with shoulder Log-linear with tail Log-linear with shoulder and tail Weibull Weibull with tail Mixed Weibull Biphasic with tail Biphasic with shoulder and tail	Initial inoculum concentration (similar to population size in constant volume)
N_{res} Starting point of tail	Log-linear with tail Log-linear with shoulder and tail Weibull with tail	Residual cell concentration after stabilisation at the end of the decrease
δ Time to first log-reduction of first subpopulation	Weibull Weibull with tail	Treatment lethality, close to the classical D -value
	Mixed Weibull	δ_1 and δ_2 for first and second subpopulation; subpopulation ₁ is more sensitive to stress than subpopulation ₂ when $\delta_1 < \delta_2$
p Shape of inactivation curve	Weibull Weibull with tail Mixed Weibull	$p < 1$ for convex, $p > 1$ for concave
α Fraction of first subpopulation remaining in total population	Mixed Weibull	α is defined as the logit of f and is equivalent to $\alpha = \log_{10}(N_{01}/N_{02})$, and the α value then is close to the graphic difference between $\log_{10}(N_0)$ and the logarithm of the population size where the inflection is observe
f Fraction of initially major subpopulation	Biphasic with tail Biphasic with shoulder and tail	Major subpopulation is the least resistant of both
t_{4D} logcycles of reduction	Automatically reported for data sets covering at least 4 decimal reductions	Treatment lethality: time needed for a 4log reduction of N_0 . While relevance of the classical D -value is restricted to log-linear curves; t_{4D} applies to log-linear and non-log linear survival curves.

6. Further Reading

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