

Calibrating Response Curves for the Concentration of Melatonin Sulfate in Human Urine

Introduction

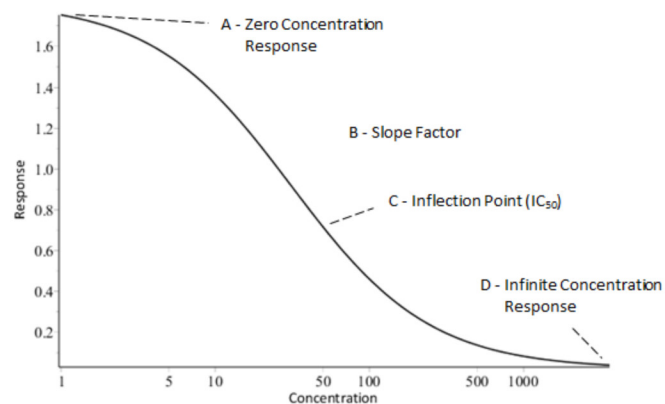
This application calibrates response curves against data from an immunoassay that records the concentration of melatonin in human urine and its optical density.

Melatonin is excreted by the pineal gland and regulates human circadian rhythms. Deviations outside of normal boundaries may indicate sleep disorders, depression, schizophrenia, or a range of other conditions.

Immunoassays typically generate sigmoidal response curves, with upper and lower asymptotes near the infinite and zero analyte concentrations.

This is accurately described by a four parameter logistic (4-PL) equation.

$$Y = D + \frac{A - D}{1 + \left(\frac{X}{C}\right)^B}$$



where

- Y is the response,
- D is the response at infinite analyte concentration,
- A is the response at zero analyte concentration,
- X is the analyte concentration,
- C is the inflection point on the calibration curve (the concentration that yields 50% activity of the assay)
- and B governs the slope of the curve.

A five parameter logistic equation includes an extra parameter G.

$$Y = D + \frac{A - D}{\left(1 + \left(\frac{X}{C}\right)^B\right)^G}$$

The additional parameter enables the fitting of asymmetric concentration-response data.

Experimental Data

> restart

This data represents the typical concentration of melatonin sulfate in human urine and is sourced from [this reference](#).

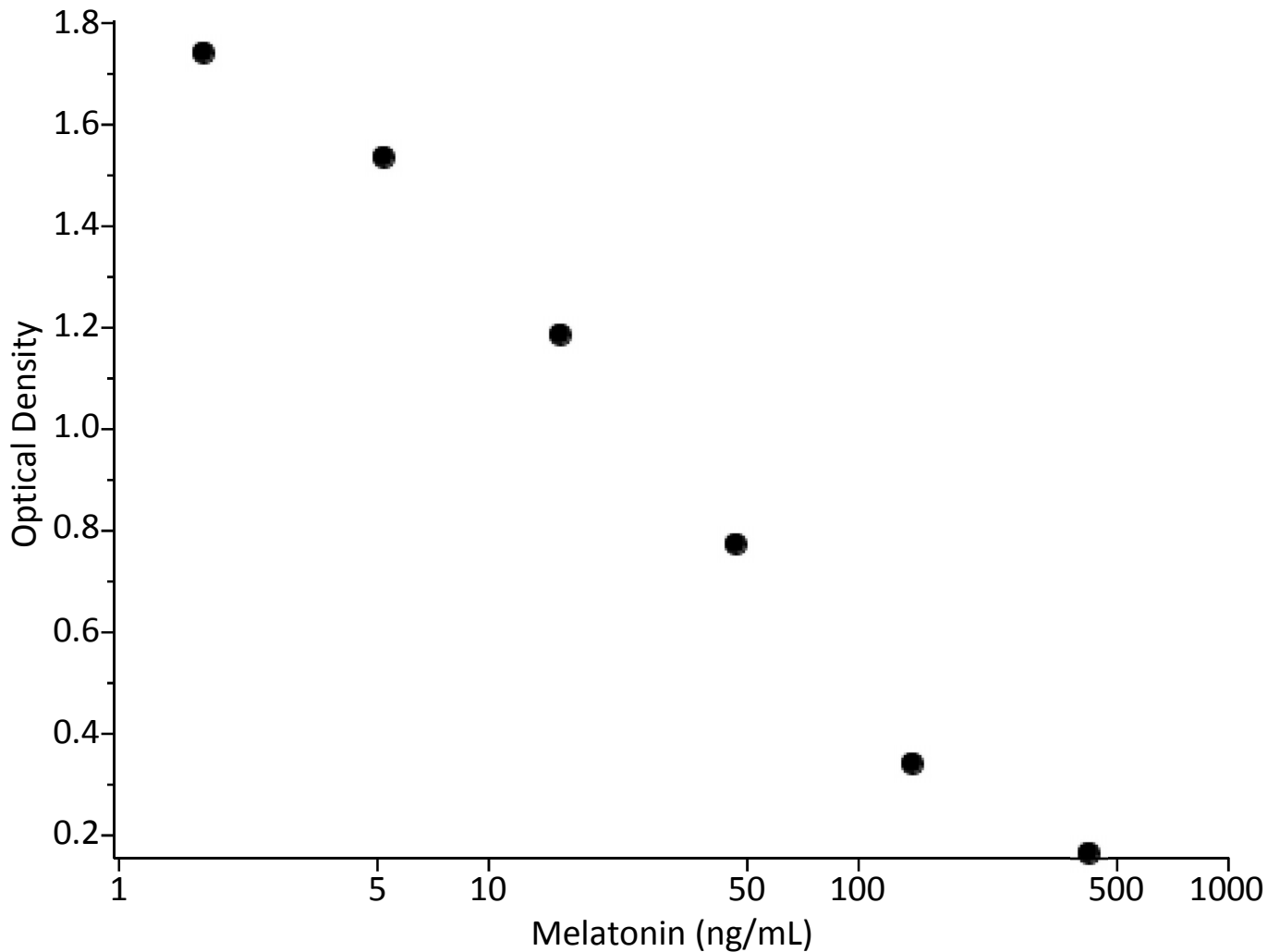
Melatonin Sulfate concentration

> X := [0.0, 1.7, 5.2, 15.6, 46.7, 140.0, 420.0] :

Mean optical density

> Y := [1.805, 1.741, 1.536, 1.185, 0.773, 0.341, 0.164] :

> p1 := dataplot(X, Y, style = point, symbol = solidcircle, symbolsize = 15, axesfont = [Calibri], size = [600, 400], axis[1] = [mode = log], view = [1 ..1000, default], labels = ["Melatonin (ng/mL)", "Optical Density"], labeldirections = [horizontal, vertical], labelfont = [Calibri], color = black) :
plots:-display(p1);



4-Parameter Logistic Model

> $\text{Response_4PL} := D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$:

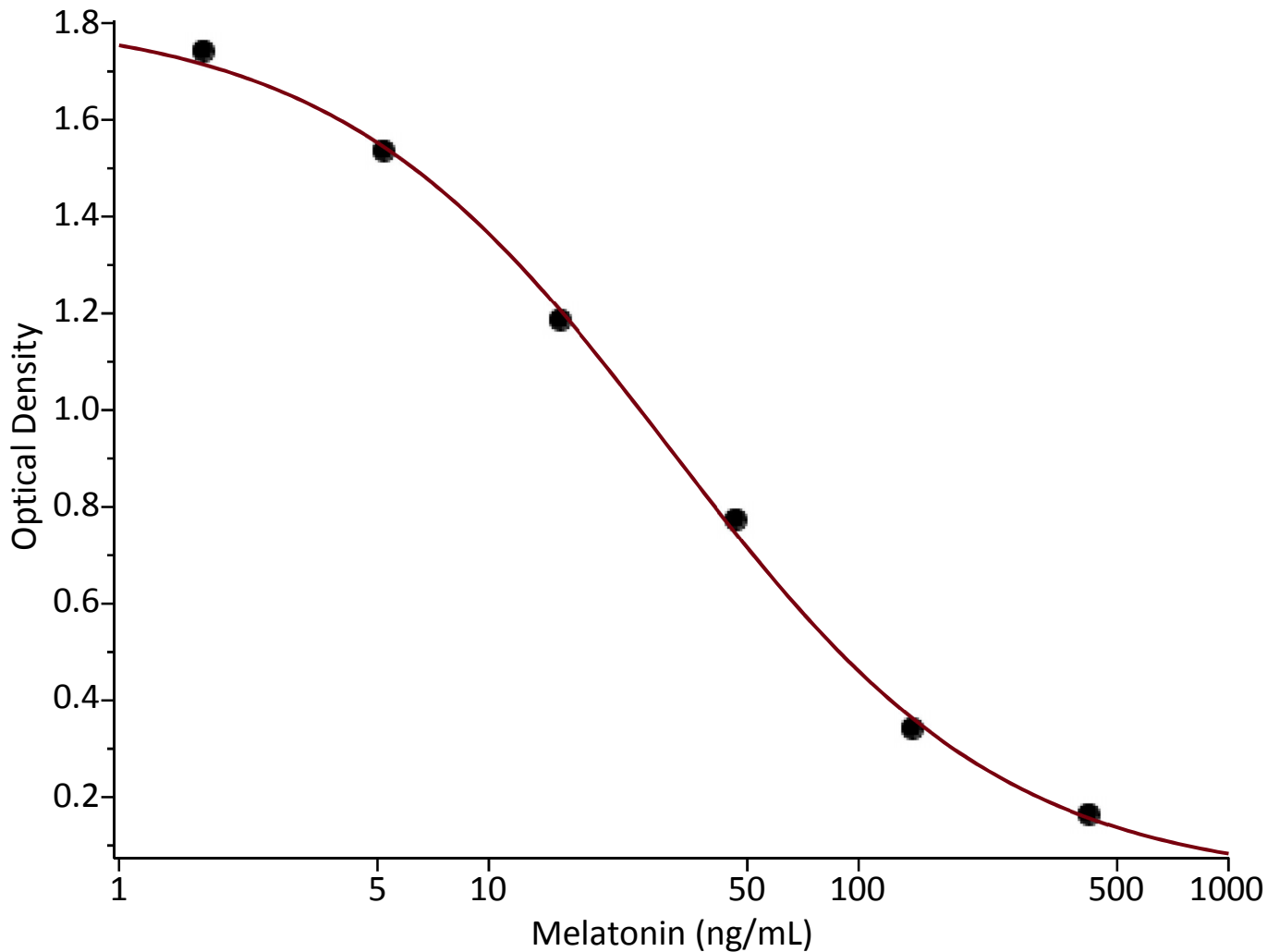
> `res_4PL := Statistics:Fit(Response_4PL, X, Y, x, initialvalues = { A = 1, D = 1, B = 1, C = 1 }, output = solutionmodule) :`

> `pars_4PL := res_4PL:-Results(parametervalues)`

`pars_4PL := [A = 1.81802944410037, B = 0.962163254997407, C = 30.9242632275591, D = 0.0218286774365395]`

> `p2 := plots:-semilogplot(eval(Response_4PL, pars_4PL), x = 1 ..1000) :`

> `plots:-display(p1, p2, size = [600, 400])`



5-Parameter Logistic Model

> Response_5PL := $D + \frac{A - D}{\left(1 + \left(\frac{x}{C}\right)^B\right)^G}$:

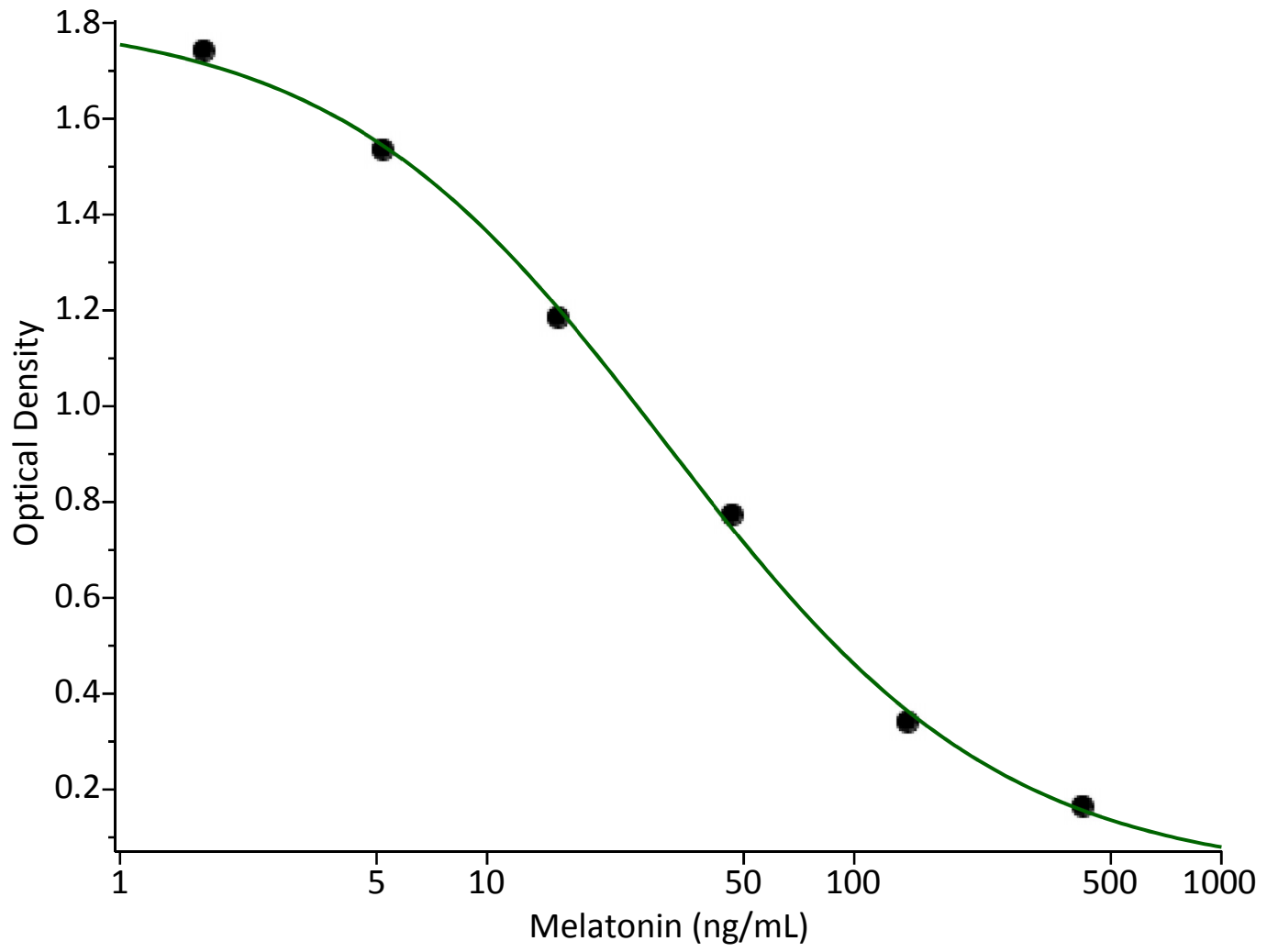
> res_5PL := Statistics:Fit(Re(Response_5PL), X, Y, x, initialvalues = {A = 1, D = 1, B = 1, C = 1, G = 1}, output = solutionmodule) :

> pars_5PL := res_5PL:-Results("parametervalues")

pars_5PL := [A = 1.81746973518297, B = 0.972864409187924, C = 28.7077081165721, D = 0.0122415148949829, G = 0.943235788340732]

> p3 := plots:semilogplot(eval(Response_5PL, pars_5PL), x = 1..1000, color = "DarkGreen") :

> plots:display(p1, p3)



>