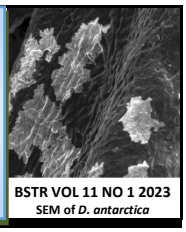
BSTR, 2023, Vol 11, No 1, 14-17
https://doi.org/10.54987/bstr.v11i1.831

HIBISCUS PUBLISHER

BIOREMEDIATION SCIENCE AND TECHNOLOGY RESEARCH

Website: http://journal.hibiscuspublisher.com/index.php/BSTR/index



Comparison between the Four-parameter and Five-parameter Logistics Equations in the Determination of the Limits of Detection of Ochratoxin A

Siti Noraini Bunawan[1] and Noor Azlina Masdor[1]*

[1]Biotechnology and Nanotechnology Research Centre, MARDI HQ, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.

*Corresponding author:
Noor Azlina Masdor,
Biotechnology and Nanotechnology Research Centre,
MARDI HQ,
Persiaran MARDI-UPM,
43400 Serdang,
Selangor,
Malaysia.
Email: azlina@mardi.gov.my

HISTORY

Received: 2nd of April 2023
Received in revised form: 27th June 2023
Accepted: 29th July 2023

KEYWORDS

Ochratoxin A
sigmoidal calibration curve
Four-parameter logistic equation
Five-parameter logistic equation
Error functions analysis

ABSTRACT

Ochratoxin A, a type of mycotoxin, was detected in plant sample matrices using the "optical waveguide lightmode spectroscopy" (OWLS) method. The calibration curve for the detection of ochratoxin A utilizing "optical waveguide lightmode spectroscopy" (OWLS) displayed a sigmoidal shape; hence, the 5-parameter logistics (5-PL) or 4-parameters logistics (4-PL) model should be used to fit the data rather than a linear model. Through the use of error function analysis and various functions such as Bias Factor, corrected Akaike Information Criterion (AICc), adjusted correlation coefficient (adjR^2), Hannan–Quinn information criterion (HQC), Root Mean Square Error (RMSE), Bayesian Information Criterion (BIC), and Accuracy Factor, the distinction between the 5-PL and 4-PL models is found to be inconsistent. The half maximal effective concentration (EC_{50}) confidence intervals overlapped, indicating no difference between the two methods; the 4-PL model was chosen because of its fewer parameters. The Limits of Detection (LOD) calculated using the 4-PL equation was 0.818 ng/mL, with a 95% confidence interval of 0.526-1.143 ng/mL. This value falls within the estimated range of 0.5 to 10 ng/mL found in the first investigation. Not only was the linear component of the data curve successfully represented by this study's use of the 4-PL model, but the model was also successful in representing the entire date curve.

INTRODUCTION

Ochratoxin A (OTA) is a kind of mycotoxin that has been linked to cancer and may be found in many foods, including cereals, dried fruits, wine, and coffee. Fungal species including *Aspergillus ochraceus, Aspergillus niger, Aspergillus carbonarius*, and *Penicillium verrucosum* are responsible for it. These species have distinct temperature and moisture requirements for optimal growth, and they can infect a wide range of products. Poor storage and improper drying procedures during agricultural production are common causes of contamination. Consumption of OTA is most common through food and drink, and it is not easily destroyed by conventional cooking processes. Multiple harmful consequences, including kidney damage, increased lipid peroxidation, an inability to synthesize macromolecules, immunotoxicity, and a halt in mitochondrial respiration, have been associated with its use in animals. It is also hypothesized to have a role in the development of human nephropathies such as chronic interstitial nephropathy (CIN) and Balkan endemic nephropathy (BEN) [1–5].

Due to its documented carcinogenicity in animal research, the International Agency for Research on Cancer has categorized OTA as a Group 2B probable human carcinogen; however, its human's carcinogenicity in humans is not confirmed. Low quantities of OTA-DNA adduct, not typical of genotoxic carcinogens, were identified in an evaluation of risk, and OTA tested negative in highly precise genotoxicity assays. The recommended maximum limits for OTA in different foods and drinks by worldwide health authorities have increased the public's awareness of OTA in recent years and may have an impact on the global marketability of these commodities. Unfortunately, the effects of OTA exposure through nutrition on the health of the general public are little understood [1,3,4,6,7].

Bioligand binding to targeted receptors typically displays a sigmoidal curve when plotted on a semi-log plot, although this fact is frequently overlooked by researchers conducting ligand binding tests. Log-log plots are commonly used instead, which can skew the error structure and bias confidence interval

calculation, but are nonetheless widely used [8–11]. Cubic, quartic, exponential, cubic spline, quadratic, log-logit, a rectangular hyperbola (with and without a linear term), a bi-exponential, a two-parameter exponential, a bi-rectangular hyperbola, a Gaussian, a two-site competition, and a Brain-Cousens method are all examples of non-linear regression curve-fitting methods. Logistic equations with four or five parameters are frequently the best fits to the sigmoidal profile. Although the 4-PL function has several restrictions when it comes to modeling asymmetric data, it is extensively utilized in practice because of its similarities to the linear logit-log model. Similarly, asymmetry is ignored in the mass action model assumption, just as it is in the 4-PL. The 5-PL model corrects for this problem by including a fifth parameter for curve asymmetry control.

Overparameterized models may fit the data tightly, but their predictions will be spread out across a large range. Underparameterized models will have large lack-of-fit errors. Commercial programs like GraphPad and Origin include the 5-PL model, makes fitting nonlinear curves easy. Countless dose-response curves from various immunoassay and bioassay techniques have been refitted using both the 5-PL and 4-PL models [8,10,12,13]. When fitting asymmetric sigmoidal dose-response datasets, the lack-of-fit error that can result when using the 4-PL model is eliminated by using the 5-PL model. The 5-PL model's tunable parameters include asymmetry intensity, transition zone location, transition region length, and transition zone duration. For asymmetric sigmoidal dose-response data, it is challenging to produce a good fit for functions with up to five parameters [8–11,14].

In order to standardize results from OWLS-based ochratoxin A detection, this study reshapes those results using both a 4-PL model and a 5-PL model to determine the respective limits of detection (LOD) [15] . The study [15] has demonstrated the presence of a sigmoidal calibration plot in such analyses, but these curves do not conform to any of the preexisting sigmoidal models.

Processing of Data

In this investigation, information used from a previously published paper by Adányi et al. [15] showing the calibration curve for Ochratoxin A in Figure 2 was used. Acquisition of the data was carried out using the Webplotdigitizer 2.5 software [16]. The output was in the form of comma-separated values (csv). This software is reliable and is widely used by researchers globally [17,18].

Four-parameter and five-parameter logistics models

The four- (**Eqn 1**) and five- (**Eqn 2**) parameter logistic equations [19] was utilized via a non-linear regression based on least square fitting as follows;

$$y = Top + \frac{(Bottom-Top)}{1+10^{(LogEC_{50}-x)Hillslope}} \qquad \text{Eqn. 1}$$

$$y = Top + \frac{(Bottom-Top)}{\left(1+10^{(LogEC_{50}-x)Hillslope}\right)^{S}} \qquad \text{Eqn. 2}$$

Where,

y is the mass (arbitrary unit),
Hillslope (Hill coefficient) is a slope-like parameter,
x is the concentration of Ochratoxin A (ng/mL),
S represents symmetry,
Log EC_{50} value represents the levels of ochratoxin A that produce a 50% signal response,
Top refers to the maximum responses and
Bottom refers to the minimum responses.

The models were fitted using the PRISM software (v 5.0) from www.graphpad.com. The pooled standard deviation was used to calculate the limit of detection (LOD) [8–10,14] instead of the null value or the weakest ochratoxin A concentration. The concentration was then extrapolated from these values using either the 4-PL or 5-PL sigmoidal dose-response equations.

Statistical analysis

Statistical tests allow for the comparison of models with varying amounts of parameters to determine whether or not there is a statistically significant distinction in terms of the fitness of the models. The adjusted coefficient of determination (R^2), Root-Mean-Square Error (RMSE), corrected Akaike Information Criterion (AICc), bias factor, and accuracy factor (BF, AF) were applied to the same set of experimental data. The RMSE, which accounts for the penalty for the number of parameters, was calculated using Eqn 3, where n is the number of experimental data, p is the number of parameters, Ob_i is the experimental data, and Pd_i is the value predicted by the model. [20].

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(Pd_i - Ob_i)^2}{n-p}} \qquad \text{(Eqn. 3)}$$

The R^2 or coefficient of determination was used to find the best linear regression model. However, the R^2 statistic is not useful for making comparisons between models with varying numbers of parameters, as is the case in nonlinear regression. To get around this problem, the quality of the nonlinear models was determined using an adjusted R^2 (adjR^2). S_y^2 is the total variance of the y-variable, and RMS is the residual mean square, in the adjusted R^2 formula. (Eqns. 4 and 5).

$$Adjusted\ (R^2) = 1 - \frac{RMS}{S_Y^2} \qquad \text{(Eqn. 4)}$$

$$Adjusted\ (R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)} \qquad \text{(Eqn. 5)}$$

Using the Akaike Information Criterion, a number of statistical models can be compared for a certain set of experimental data (AIC). For data sets with many parameters or few data point values, the alternative, AICc (the corrected AIC), is recommended [21]. The AICc was calculated based on the following Eqn. 6.

$$AICc = 2p + n1n\left(\frac{RSS}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{n-p-2}$$
(Eqn. 6)

Differences in the number of parameters and the degree of fitting between two models can be learned via AICc. When comparing models, the lowest AICc value would represent the optimal fit [21]. The Bayesian Information Criterion is another statistical method based on information theory (Eqn. 7). The number of parameters is punished more harshly by this error function than it is by AIC [22].

$$BIC = n.\ln\frac{RSS}{n} + p.\ln(n) \qquad \text{(Eqn. 7)}$$

Another error function strategy grounded in information theory is the Hannan-Quinn information criterion (HQC) (Eqn. 8). In contrast to the AIC, the HQC is highly consistent due to the inclusion of the *ln ln n* factor in the equation [23];

$$HQC = n \times ln\frac{RSS}{n} + 2 \times p \times ln(\ln n) \qquad \text{(Eqn. 8)}$$

Both BF and AF were utilized to evaluate the reliability of the models. For perfect agreement between projected and observed values, the Bias Factor should be set to 1. A fail-safe model is indicated if the Bias Factor (as given in Eqn. 9) is larger than 1, and a fail-negative model if it is less than 1. If Accuracy is below 1, then the prediction is not as precise as it could be (Eqn. 10).

$$Bias\ factor = 10\left(\sum_{i=1}^{n} log\frac{(Pd_i/Ob_i)}{n}\right) \quad \text{(Eqn. 9)}$$

$$Accuracy\ factor = 10\left(\sum_{i=1}^{n} log\frac{|(Pd_i/Ob_i)|}{n}\right) \quad \text{(Eqn. 10)}$$

RESULT AND DISCUSSION

Nonlinear, sigmoidal standard curves are often found in ligand-receptor binding assays. To fit these curves, the 4-PL model or, less frequently, the 5-PL model is utilized [24]. Fitting the raw data to the 4-PL curve, which is frequently depicted by a line running through the experimental data, allows one to modify these models to get a good fit between experimental and computed data. Previous studies found a sigmoidal profile, but the researchers only employed linear regression to explain the data. This led to the following equation: lg $y = 0.042x + 0.16$, with an R^2 of =0.995 [15]. The detection limit was reported to be between 0.5 and 10 ng/mL. Calibration curves obtained with the 4-PL equation and the 5-PL equation plotted side-by-side on the same graph (**Figs. 1** and **2**), respectively. The generated sigmoidal profile was typical, and the value of 0.996 for the correlation coefficient suggested a satisfactory fit.

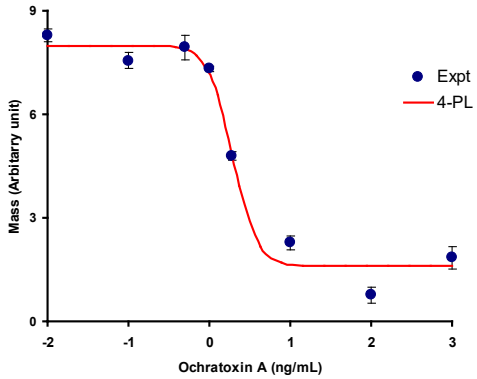


Fig. 1. Ochratoxin concentration vs calibration curve for its measurement A modeling done in accordance with the logistic equation using four parameters.

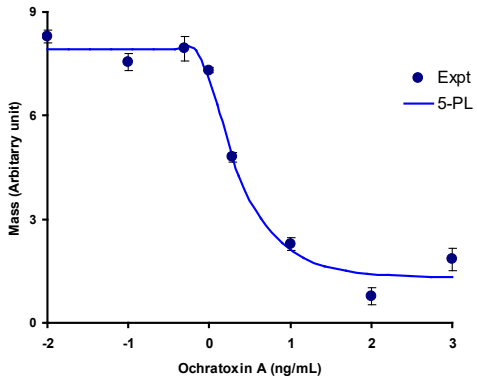


Fig. 2. Ochratoxin concentration vs calibration curve for its measurement A modeling done in accordance with the logistic equation using five parameters.

With lower AICc, BIC, and HQF values, the simpler 4-PL model is more trustworthy, whereas the superior 5-PL model was shown to have higher RMSE, R^2, adjR^2, BF, and AF values in the error function analysis. To settle this issue the EC_{50} values for both models are compared. The EC_{50} value for the 4-PL model was 1.940 ng/mL (95% confidence interval or C.I. of 1.695 to 2.750) while the 5-PL model shows an EC_{50} value of 2.038 ng/mL (95% C.I. of 1.694 to 2.525). As the 95% confidence interval overlap, the EC_{50} values were deemed not significantly different [25]. When this happens, the model with fewer parameters is preferred according to Occam's razor [19].

Table 1. Error function analysis of the 4-PL and 5-PL models.

Model	p	RMSE	R^2	adR^2	AICc	BIC	HQC	BF	AF
4-PL	4	0.602	0.978	0.948	34.35	-5.34	-7.80	1.04	1.18
5-PL	5	0.575	0.985	0.947	89.31	-6.30	-9.37	1.03	1.14

The LOD was calculated to be 0.818 ng/mL using the 4-PL equation, with a 95% confidence interval of 0.526 to 1.143. This falls within the estimated range of 0.5 to 10 ng/mL found in the first investigation. The 4-PL method is preferred for calculating the LOD value when a curve has a sigmoid profile. This is so because this strategy yields more precise results. Therefore, the LOD value arrived at by 4-PL modeling should be used for reporting.

R^2

In this study, rather than making use of the more common R^2 statistic, we report on the application of an adjusted coefficient of determination, which is abbreviated as adjR^2. This is because the standard coefficient of determination, known as R^2, does not take into account the number of parameters that an equation possesses. This is the reason why this occurs. As a consequence of this, it is not possible for it to provide an appropriate reflection of a comparison of models that contain different numbers of parameters. R^2 is referred to as "the coefficient of multiple determination," and it assesses "the proportion of the variation in the dependent variable that can be explained by variations in the independent variables when all of those variations are taken into account." [26].

In order to make up for this shortcoming, a new word designated as adjusted R^2 (adjR^2) was developed. Adjusted R^2 differs from standard R^2 in that it takes into account the total number of occurrences as well as variables that are contained within the model. Increasing the number of variables in a model will always result in an increase in the regular R^2 value, irrespective of whether or not the model's technical characteristics will be upgraded in the future.

According to Hair et al., the coefficient of determination is considered accurate once it has been adjusted to account for both the size of the sample and the number of factors that can be considered independent. When there are more independent variables accounted for in a model, the coefficients of determination will nearly always show an upward trend. However, the modified coefficient of determination might go down if the additional independent variables do not adequately describe the phenomenon in question, or if there aren't enough degrees of freedom to begin with.

This statistic is particularly helpful for comparing equations due to the fact that the total number of independent variables and the total number of participants of the sample may both be different from one another [27]. Confidence intervals around an expected value can be calculated using the standard error of estimate (SEE). SEE quantifies the extent to which actual results

differ from those predicted. The statistical sampling distribution's standard deviation, as calculated by calculating the sample standard deviation of means. The standard error of the mean, for instance, is computed by using the sample standard deviation of means. Standard error of estimate (SEE) is a measure of how much one sample differs from another in terms of the value of a test statistic [27]. "the anticipated distribution of predicted values that would occupy multiple samples of the data" is the definition of a normal distribution, which is comparable to the standard deviation of a variable around its mean [28].

CONCLUSION

In conclusion, the sigmoidal patent was observed in the calibration curve for ochratoxin A detection using OWLS, and either the 5-PL or the 4-PL model should be used to match the data rather than a linear model. The findings of the experiment confirmed this. Using error function analysis with functions like AICc, HQC, BIC, RMSE, adjR^2, Bias Factor, and Accuracy Factor to distinguish between the 5-PL and 4-Pl models yields equivocal results. Overlapping confidence ranges for EC50 values suggested that the two methods were not distinguishable statistically. Therefore, the 4-PL model was chosen because it requires fewer parameters overall. This investigation showed that the 4-PL model accurately predicted the full curve, rather than just the linear portion. The linear element has historical significance since it provides a quick and easy method of assessing the sensitivity of newly developed biosensor technology. In addition, the linear part is often more practical for field applications that call for fast and easy evaluation.

REFERENCES

1. Kuiper-Goodman T, Scott PM. Risk assessment of the mycotoxin ochratoxin A. Biomed Environ Sci BES. 1989 Sep;2(3):179–248.
2. Bondy GS, Pestka JJ. Immunomodulation by fungal toxins. J Toxicol Environ Health B Crit Rev. 2000;3(2):109–43.
3. Abid S, Hassen W, Achour A, Skhiri H, Maaroufi K, Ellouz F, et al. Ochratoxin a and human chronic nephropathy in Tunisia: is the situation endemic? Hum Exp Toxicol. 2003 Feb 1;22(2):77–84.
4. Duarte SC, Pena A, Lino CM. Human ochratoxin a biomarkers--from exposure to effect. Crit Rev Toxicol. 2011 Mar;41(3):187–212.
5. Haighton LA, Lynch BS, Magnuson BA, Nestmann ER. A reassessment of risk associated with dietary intake of ochratoxin A based on a lifetime exposure model. Crit Rev Toxicol. 2012 Feb;42(2):147–68.
6. Munkvold GP, Arias S, Taschl I, Gruber-Dorninger C. Chapter 9 - Mycotoxins in Corn: Occurrence, Impacts, and Management. In: Serna-Saldivar SO, editor. Corn (Third Edition) [Internet]. Oxford: AACC International Press; 2019 [cited 2022 Nov 20]. p. 235–87. Available from: https://www.sciencedirect.com/science/article/pii/B9780128119716000097
7. Yazid SNE, Ng WJ, Selamat J, Ismail SI, Samsudin NIP. Diversity and Toxigenicity of Mycobiota in Grain Corn: A Case Study at Pioneer Grain Corn Plantations in Terengganu, Malaysia. Agriculture. 2021 Mar;11(3):237.
8. Padrilah SN, Masdor NA. Limits of Detection Based on the Four-Parameter Logistic Model for E. coli Determined using a Fluorescent-based Sensor. J Environ Microbiol Toxicol. 2021 Jul 31;9(1):1–2.
9. Masdor NA. Detection Limit of the Four-Parameter Logistic Model for the Quantitative Detection of Serum Squamous Cell Carcinoma Antigenin Cervical Cancer Based on Surface Plasmon Resonance Biosensor. J Environ Microbiol Toxicol. 2021 Dec 31;9(2):30–2.
10. Masdor NA. Determination of the Detection Limit of the Detection of GMO in Food Using the Isothermal Solid-Phase Recombinase Polymerase Amplification on Microfluidic DVDs. Asian J Plant Biol. 2021 Dec 31;3(2):17–9.
11. Masdor NA. Determination of the detection limit using the four-parameter logistic model for the double-antibody sandwich ELISA for the rapid detection of Bacillus cereus in food. J Environ Microbiol Toxicol. 2017 Jul 31;5(1):12–3.
12. Uba G, Yakasai HM, Abubakar A. Limits of Detection Determination of Aflatoxin B1 using the Optical Waveguide Lightmode Spectroscopy via the Four-Parameter Logistic Model. Bioremediation Sci Technol Res. 2022 Dec 31;10(2):40–4.
13. Masdor NA. Detection Limit Determination using the Four-Parameter Logistic Model for the Ultrasensitive Detection of Vibrio cholerae DNA with Polystyrene-coacrylic Acid Composite Nanospheres. Bull Environ Sci Sustain Manag E-ISSN 2716-5353. 2021 Dec 31;5(2):1–4.
14. Holstein CA, Griffin M, Hong J, Sampson PD. Statistical method for determining and comparing limits of detection of bioassays. Anal Chem. 2015 Oct 6;87(19):9795–801.
15. Adányi N, Levkovets IA, Rodriguez-Gil S, Ronald A, Váradi M, Szendrő I. Development of immunosensor based on OWLS technique for determining Aflatoxin B1 and Ochratoxin A. Biosens Bioelectron. 2007 Jan 15;22(6):797–802.
16. Rohatgi A. WebPlotDigitizer. http://arohatgi.info/WebPlotDigitizer/app/ Accessed June 2 2014.; 2015.
17. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Mathematical modelling of the degradation kinetics of *Bacillus cereus* grown on phenol. J Environ Bioremediation Toxicol. 2014;2(1):1–5.
18. Khare KS, Phelan Jr FR. Quantitative comparison of atomistic simulations with experiment for a cross-linked epoxy: A specific volume–cooling rate analysis. Macromolecules. 2018;51(2):564–75.
19. Motulsky HJ, Ransnas LA. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J Off Publ Fed Am Soc Exp Biol. 1987;1(5):365–74.
20. Wayman M, Tseng MC. Inhibition-threshold substrate concentrations. Biotechnol Bioeng. 1976;18(3):383–7.
21. Głuszcz P, Petera J, Ledakowicz S. Mathematical modeling of the integrated process of mercury bioremediation in the industrial bioreactor. Bioprocess Biosyst Eng. 2011;34(3):275–85.
22. Kass RE, Raftery AE. Bayes Factors. J Am Stat Assoc. 1995 Jun 1;90(430):773–95.
23. Burnham KP, Anderson DR. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer Science & Business Media; 2002. 528 p.
24. Masdor NA, Altintas Z, Tothill IE. Sensitive detection of *Campylobacter jejuni* using nanoparticles enhanced QCM sensor. Biosens Bioelectron. 2016;78:328–36.
25. Schenker N, Gentleman JF. On judging the significance of differences by examining the overlap between confidence intervals. Am Stat. 2001;55(3):182–6.
26. Schroeder LD, Sjoquist DL, Stephan PE. Understanding Regression Analysis: An Introductory Guide. Second edition. Los Angeles: SAGE Publications, Inc; 2016. 120 p.
27. Hair J, Money A, Page M, Samouel P. Research methods for business. Chichester: John Wiley & sons Ltd; 2007. 448 p.
28. Figueiredo D, Júnior S, Rocha E. What is R2 all about? Leviathan-Cad Pesqui Polútica. 2011 Nov 16;3:60–8.