

# Determination of the Limits of Detection for Acetamiprid Based on Gold Nanoparticle Aptasensor: Comparison Between Four-Parameter and Five-Parameter Logistics Equations

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## HISTORY

Received: 15<sup>th</sup> Aug 2022  
Received in revised form: 24<sup>th</sup> Nov 2022  
Accepted: 28<sup>th</sup> Nov 2022

## KEYWORDS

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

## ABSTRACT

Acetamiprid is a kind of broad-spectrum systemic pesticide that works on the nicotinic acetylcholine receptor. This chemical disrupts the transmission of a signal and causes a buildup of neurotransmitters, which leads to pests being paralyzed and eventually dying as a result. The calibration curve for the detection of acetamiprid utilizing a gold nanoparticle-based visual aptasensor showed a sigmoidal shape profile; hence, the 5-PL or 4-PL model should be used to fit the data rather than a linear model. The result of the error function analysis shows that the simpler 4-PL model is more reliable having smaller AICc,  $R^2$  and  $adjR^2$ , values whilst the other error functions such as RMSE, BIC and HQF, BF and AF values indicated that the 5-PL model shows that the 5-PL model was marginally superior to the 4-PL. As the 95% confidence interval overlap, the  $IC_{50}$  values were deemed not significantly different, and when this occur, based on Occam's razor, the model having a lower number of parameters, which was 4-PL, should be chosen instead. The 4-PL equation produced a value for the LOD of 0.159 mM, and the confidence interval for 95 percent of the results ranged from 0.132 to 0.177. According to the first study, the LOD was 3.81 mM, and the calculated LOD using 4-PL model with pooled standard deviations was much more sensitive. This indicates that utilizing only the linear portion of a sigmoidal curve to report the LOD values gave a less sensitive value than it should be.

## INTRODUCTION

Acetamiprid, a chemical used as an insecticide and manufactured by Aventis CropSciences under the brand names Assail and Chipco, is an organic compound belonging to the chloropyridinyl neonicotinoids class of insecticides. It is an odourless, systemic substance that spreads throughout plants to control sucking insects, mainly aphids, on a variety of crops including leafy vegetables, citrus fruits, pome fruits, grapes, cotton, cole crops, and ornamental plants. It is also effective against the larvae of the cherry fruit fly and is widely used in commercial cherry farming. In addition to its use in pest management, acetamiprid is also used to prevent flea infestations on cats and dogs in households. It is a -chloro-N-heteroaromatic compound with a chloropyridinyl group that makes it a neonicotinoid, similar to other neonicotinoids such as imidacloprid, nitenpyram, and thiacloprid, which all have a 6-chloro-3-pyridine methyl group but differ in the substituents attached to their acyclic or cyclic

moieties. It is a nicotinic agonist that stimulates the activity of nicotinic acetylcholine receptors (nACh-R) found in the post-synaptic dendrites of neurons in the brain, spinal cord, ganglia, and muscle junctions, causing hyperactivity, muscle spasms, and ultimately death [1–8]. Acetamiprid is highly toxic to insects but less toxic to mammals. It exists in two isomeric forms with E and Z configurations of the cyanoimino group and a range of stable conformers due to the rotation of single bonds in the N-pyridylmethylamino group, with the E-conformer being the more stable and active form.

Acetamiprid has a high potential for bioaccumulation and is highly toxic to birds and moderately toxic to aquatic organisms, with the potential to harm bird populations and other parts of the food chain if used excessively. However, the metabolites produced in honey bees after acetamiprid is absorbed are less toxic than those of other neonicotinoids, and acetamiprid has a shorter half-life of 25-30 minutes compared to 4-5 hours for some

other neonicotinoids. Despite this, some metabolites may still be present in honey bees after 72 hours, potentially posing a toxicological risk through chronic exposure to certain compounds. The Environmental Protection Agency (EPA) considers acetamiprid to be "only moderately toxic" to bees, but some media sources and the documentary *Vanishing of the Bees* have blamed neonicotinoids like acetamiprid for colony collapse disorder [1–8].

According to a 2002 report by the Environmental Protection Agency (EPA), acetamiprid poses low risks to the environment compared to other pesticides due to its rapid breakdown in soil and low potential for leaching into groundwater, although its degradation products may reach the groundwater but are not expected to be toxic. There have been two recorded cases of acute poisoning by ingestion of a pesticide mixture containing acetamiprid in humans attempting suicide, both of whom experienced nausea, muscle weakness, convulsions, and low body temperature. Symptoms such as muscle weakness may be similar to those caused by organophosphate insecticides, and the active component of acetamiprid that interacts with acetylcholine and nicotinic receptors may explain hypothermia and convulsions. While acetamiprid is toxic to humans at high doses, its toxicity to mammals is generally low [1–8]. The detection of these pesticides using biosensor technology is being intensely sought as biosensor technology is usually rapid and sensitive [9–15].

In the process of conducting ligand binding assays for biosensor works, researchers frequently ignore the sigmoidal profile that bioligand binding to target receptors frequently presents when the data is presented on a semi-log plot. Instead, they commonly convert the data to a linear form by making use of a log-log plot, which might bias the calculation of confidence intervals and distort the error structure [9–11,16]. However, there are several non-linear regression curve-fitting methods available, including cubic, exponential, quartic, quadratic, cubic spline, log-logit, a rectangular hyperbola (with and without a linear term), bi-rectangular hyperbola, bi-exponential, two-parameter exponential, Gaussian, two site competition, and Brain-Cousens. The sigmoidal profile is often best fit by the four-parameter logistic (4-PL) or five-parameter logistic (5-PL) equation [9–15]. The 4-PL function is similar to the linear logit-log model and is widely used in practice, although it has limitations when it comes to modelling asymmetric data. Similarly, the mass action model approximation, like the 4-PL, fails to account for asymmetry. The 5-PL model solves this problem by including a fifth parameter, which enables the adjustment of curve asymmetry. This makes the model more accurate. This model strikes a good balance between overparameterized models, which may fit the data closely but have a wide range of predictions, and under parameterized models, which have high lack-of-fit errors.

Overparameterized models may fit the data closely, but under parameterized models have a wide range of predictions. The 5-PL model may be easily fitted, and it is offered in a variety of commercial software packages including GraphPad and Origin. Both the 5-PL and the 4-PL models have been applied to the task of fitting a variety of dose-response curves generated by a variety of immunoassay and bioassay methods. Because it removes the lack-of-fit error that may occur when fitting these data with the 4-PL model, the 5-PL model is especially helpful for asymmetric sigmoidal dose-response data. This is because the lack-of-fit error may occur when fitting these data with the 4-PL model. The 5-PL model provides for the possibility of making adjustments to the magnitude of the asymmetry, as well as the location of the transition zone, the duration of the transition

region, and the total length of the transition zone. When dealing with asymmetric sigmoidal dose-response data, it might be challenging to construct a decent fit for functions that have fewer than five parameters [9–11,16,17].

The purpose of this study is to standardize data [1] that was obtained through the use of a gold nanoparticle-based visual aptasensor for the detection of acetamiprid and to calculate the Limits of Detection (LOD) by reshaping the data using a 4-PL model and a 5-PL model with the not usual  $x$  values not in their logarithmic forms. The data was collected for the detection of acetamiprid According to the findings of the study [1], a sigmoidal calibration curve is present in this kind of analysis; nevertheless, it did not fit into any of the sigmoidal models that were already in existence.

### Processing of Data

In this study, data from a published work [1] showing the calibration curve for acetamiprid in Figure 2 was used. The data was processed using Webplotdigitizer 2.5 software [18], which converts scanned figures into comma-separated data. This software has been widely used by researchers and is known for its reliability [19,20].

### Four- and five-parameter logistics equations

A non-linear regression using the four- (Eqn 1) and five- (Eqn 2) parameter logistic equations with the  $x$  values not in the usual logarithmic forms [21] were utilized to fit the curve based on least square fitting as follows;

$$y = Bottom + \frac{(Top-Bottom)}{1 + \left(\frac{IC_{50}}{x}\right)^{Hillslope}} \quad \text{Eqn. 1}$$

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

Where,

In this study, the mass (arbitrary unit) is represented by  $y$ , and the concentration of acetamiprid ( $\mu\text{M}$ ) is represented by  $x$ . The top and bottom refer to the maximum and minimum responses in mass, respectively. The  $IC_{50}$  value represents the levels of acetamiprid that produce a 50% signal response, and the Hillslope (Hill coefficient) is a slope-like parameter. The  $S$  parameter represents symmetry. The models were fitted using the PRISM software (v 5.0) from [www.graphpad.com](http://www.graphpad.com). The limit of detection (LOD) was determined based on the pooled standard deviation [9–11,17] rather than the blank value or the lowest concentration of acetamiprid used. These values were then interpolated using the 4-PL or 5-PL sigmoidal dose-response equations to determine the corresponding concentration.

### Statistical analysis

To determine if there is a significant difference in terms of fitness among models with varying numbers of parameters, statistics functions such as 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. [22].

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

To determine the validity of the models, both BF and AF were used. The Bias Factor should be set to 1 to achieve a correlation of 1 between the predicted and observed values. If the Bias Factor (as shown in Equation 4) is greater than 1, it indicates a fail-safe model, and if it is less than 1, it indicates a fail-negative model. If Accuracy is less than 1, it means that the prediction will be less accurate (Eqn. 5).

$$\text{Bias factor} = 10 \left( \sum_{i=1}^n \log \frac{(Pd_i/Ob_i)}{n} \right) \quad (\text{Eqn. 4})$$

$$\text{Accuracy factor} = 10 \left( \sum_{i=1}^n \log \frac{|(Pd_i/Ob_i)|}{n} \right) \quad (\text{Eqn. 5})$$

In linear regression, the best fitting model was determined by  $R^2$  or coefficient of determination. However, in nonlinear regression, the  $R^2$  does not give a comparative analysis where the number of parameters between models is different. To overcome this, adjusted  $R^2$  was used to calculate the quality of the nonlinear models. In the adjusted  $R^2$  formula,  $S_y^2$  is the total variance of the y-variable and RMS is Residual Mean Square (Eqns. 6 and 7).

$$\text{Adjusted } (R^2) = 1 - \frac{RMS}{S_y^2} \quad (\text{Eqn. 6})$$

$$\text{Adjusted } (R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)} \quad (\text{Eqn. 7})$$

Various statistical models can be evaluated for a given range of experimental data using the Akaike Information Criterion (AIC). Alternatively, AICc (the corrected AIC) should be used for data sets with numerous parameters or a few data point values. [23]. The AICc was calculated based on the following Eqn. 8.

$$AICc = 2p + n \ln \left( \frac{RSS}{n} \right) + 2(p+1) + \frac{2(p+1)(p+2)}{n-p-2} \quad (\text{Eqn. 8})$$

The AICc gives information about the differences that exist between the two models in terms of the number of parameters and the fitting. The AICc value that is the smallest possible would suggest the model that best fits the data [23]. A further information-theory-based approach to statistics is the Bayesian Information Criterion (Eqn. 9). The number of parameters is punished more harshly by this error function than it is by AIC [24].

$$BIC = n \ln \frac{RSS}{n} + k \ln(n) \quad (\text{Eqn. 9})$$

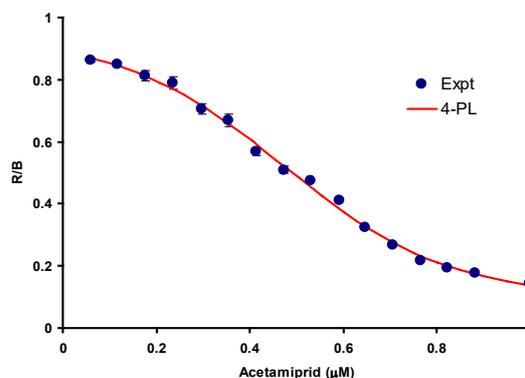
The Hannan–Quinn information criterion, often known as the HQC, is an additional error function approach that relies on the information theory (Eqn. 10). In contrast to the AIC, the HQC exhibits a high level of consistency because the equation contains the  $\ln \ln n$  term. [25];

$$HQC = n \times \ln \frac{RSS}{n} + 2 \times k \times \ln(\ln n) \quad (\text{Eqn. 10})$$

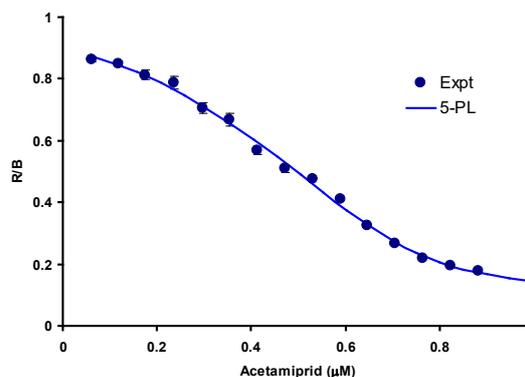
## RESULT AND DISCUSSION

In ligand-receptor binding experiments, nonlinear, sigmoidal standard curves are frequently observed; the 4-PL model or, less commonly, the 5-PL model is typically utilized in order to fit these curves [26].

By fitting the raw data to the 4-PL curve, which is frequently represented as a line running through the experimental data, these models can be modified to achieve a good fit between the calculated and experimental data. This can be done in order to achieve a good fit between the experimental and calculated data. In earlier studies, a sigmoidal profile was seen, but a linear regression model was utilized instead. This led to the formulation of the equation  $y = 0.0027x + 1.6936$ , and the  $R^2$  value came out to be =0.9984 [1]. The detection limit was reported to be between 0.5 and 10  $\mu\text{M}$ . The sigmoidal curve obtained using the 4-PL equation for the calibration curve, as well as the 4-PL and 5-PL equations on the same graph, are shown in **Figs. 1** and **2**. The sigmoidal profile obtained was typical, and the correlation coefficient value of 0.996 indicated a good fit.



**Fig. 1.** Acetamiprid concentration vs calibration curve for its measurement A modelling was done following the logistic equation using four parameters.



**Fig. 2.** Acetamiprid concentration vs calibration curve for its measurement A modelling was done following the logistic equation using five parameters.

The result of the error function analysis shows that the simpler 4-PL model is more reliable having smaller AICc,  $R^2$  and  $\text{adj}R^2$  values whilst the other error functions such as RMSE, BIC and HQF, BF and AF values indicated that the 5-PL model shows that the 5-PL model was marginally superior to the 4-PL. To settle this issue the  $\text{IC}_{50}$  values for both models are compared. The  $\text{IC}_{50}$  value for the 4-PL model was 0.5603  $\mu\text{M}$  (95% confidence interval or C.I. of 0.5299 to 0.6025) while the 5-PL model shows an  $\text{IC}_{50}$  value of 0.228  $\mu\text{M}$  (95% C.I. of 0.1064 to 0.5761). As the 95% confidence interval overlap, the  $\text{IC}_{50}$  values were deemed not significantly different [27], and when this occurs, based on Occam's razor, the model having a lower number of the parameter should be chosen instead [21].

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

Model	p	RMSE	$R^2$	$adjR^2$	AICc	BIC	HQC	BF	AF
4-PL	4	0.017	0.996	0.995	-110.13	-123.04	-125.97	1.00	1.05
5-PL	5	0.016	0.997	0.996	-107.06	-124.53	-128.19	1.00	1.02

The value of 0.159  $\mu\text{M}$  was obtained for the LOD through the application of the 4-PL equation, and the confidence interval for 95 percent of the data varied from 0.132 to 0.177. The limit of detection (LOD) was determined to be 3.81  $\mu\text{M}$  in the first investigation, while the LOD that was estimated using 4-PL was shown to be significantly more sensitive. When a curve unmistakably displays a sigmoid profile, the 4-PL method of computing the LOD value is the one that should be used. This is due to the fact that this procedure produces more accurate results. As a consequence of this, the LOD value that was established through the application of 4-PL modelling ought to be employed for the objectives of reporting.

In this study, rather than employing the more common  $R^2$  statistic, we report on the utilization of an adjusted coefficient of determination, which is abbreviated as  $adjR^2$  for short. This is because the standard coefficient of determination, known as  $R^2$ , does not take into account the number of parameters that an equation has. 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 also referred to as "the coefficient of multiple determination," and it determines "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," among other things [28]. In order to remedy this deficiency, a brand-new word that has been given the designation of adjusted  $R^2$  ( $adjR^2$ ) has been established. The adjusted  $R^2$  differs from the standard  $R^2$  in that it takes into consideration the total number of occurrences in addition to the variables that are included in the model. In contrast, the standard  $R^2$  just takes into account the variables themselves.

When the number of variables in a model is increased, the regular  $R^2$  value will always increase as a direct consequence of this change, regardless of whether or not the model's specifications are improved as a direct result of this change. 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. The coefficient of determination will almost invariably increase in proportion to the number of independent variables that are included in a model. However, if the additional independent variables don't provide enough of an explanation or if the number of degrees of freedom is too low, the adjusted coefficient of determination can go down. This statistic is highly helpful when comparing equations that involve a variety of numbers of independent variables or sample sizes, as both of these elements could vary. This is because this statistic takes into account the fact that both of these components might differ.

The standard error of the estimate is a statistical measure that can be utilized in the construction of confidence intervals around an expected value. It is often referred to by its acronym, SEE. The standard error of estimate (SEE) is a measurement of the variation in the projected values. It is computed using the sample standard deviation of the means, and it is a representation of the statistical sampling distribution's standard deviation. When calculating the standard error of the mean, for instance, the sample standard deviation of the means is what is utilized as the

basis for the calculation. According to Hair and his colleagues, the standard error of estimate (SEE) is a useful indication that can be used to determine the extent to which the value of a test statistic can vary from one sample to another [29]. "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 [30].

## CONCLUSION

In conclusion, the calibration curve for the detection of acetamidrid using a gold nanoparticle-based visual aptasensor demonstrated the sigmoidal pattern, and rather than using a linear model to fit the data, either the 5-PL or the 4-PL model should be employed instead. The findings of the experiment led to this conclusion. The results of employing error function analysis with functions such as AICc, HQC, BIC, RMSE,  $adjR^2$ , Bias Factor, and Accuracy Factor to try to discern between the 5-PL model and the 4-PL model are unclear. Because the confidence ranges for the  $IC_{50}$  values were so close to one another, it was clear that the two methodologies were not statistically distinguishable from one another. As a result of this, the 4-PL model was chosen since it had a smaller total number of parameters than the other models. This analysis indicated that the 4-PL model was successful in forecasting the entire curve, as opposed to simply modelling the linear component of the curve as would be done in a more traditional modelling approach. The linear element is essential because it provides a quick and easy method for testing the sensitivity of a newly developed biosensor technology. This is one of the reasons why it is so vital. In addition, the linear part is generally more practicable for field applications, which typically require a straightforward and speedy evaluation. On the other hand, the sigmoidal models 4-PL and 5-PL encompass the entire range of the response data; hence, it is recommended that these models be employed instead.

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