

Remodelling of the Calibration Curve for the Potentiometric Sensing of Lamotrigine Based on Molecularly Imprinted Polymers Using the Four-Parameter Logistic Model

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HISTORY

Received: 5th Feb 2017
Received in revised form: 24th of March 2017
Accepted: 11th of April 2017

KEYWORDS

four-parameter logistic
Molecularly Imprinted Polymers
sigmoidal calibration curve
lamotrigine

ABSTRACT

A standard or a calibration curve allows the determination of an analyte concentration from an experiment. The calibration curve is constructed using a known concentration of analyte. The unidentified concentration of an analyte will then be determined utilizing this standard curve. Similar to immunoassay, ligand-binding interaction in Molecularly Imprinted Polymers (MIPs) also mimics immunoassay in that the calibration curve is sigmoidal in many cases. In a previous study an ion selective electrode based on molecularly imprinted polymers calibration curve for the detection of lamotrigine (LTG) showed a sigmoidal calibration curve but was not modelled according to a sigmoidal dose response model. Using the four-parameter logistic (4PL) equation, the calibration curve was remodelled according to this equation resulting in the calculated values of 19.87, -87.43, -4.614 and 0.6354 for the parameters a and d (maximum and minimum responses), Log EC₅₀ (value that produces a 50% signal response) and Hill slope (slope-like parameter or Hill coefficient). The use of the 4PL model gave a correlation coefficient value of 0.9985 indicating the model excellently fit the experimental data.

INTRODUCTION

A standard curve should mimic the true curve with very little contribution by experimental or measurement errors. To achieve this, ideally as many numbers of points as possible is needed and each point should have as many numbers of replicates if possible. If these requirements are fulfilled, then the standard curve will be very near to the true curve. However, in real life, this is not possible as the cost and time of running an experiment escalates as the number of points and replicates increases [1].

Standard curves can be linear or nonlinear and the best way to determine this can be easily done visually. However, when a discrimination cannot be made than the use of both kinds of curve fitting must be judged upon statistical evaluation. Often, when a curve appears non-linear, there is a need for a mathematical function that describes the curve. The descriptive function is known as a curve model that generally identifies a family of curves, each having one or more parameters. The raw data is then fitted to the curve through a modification of the curve model's

parameters to achieve an ideal fitting between experimental and calculated data, the latter is often represented by a line running through the experimental data [2].

Pure and lack-of-fit errors are two kind of errors that make a model does not fit the data. Pure errors are errors of random property and reflects uncertainty in the measurement and human fallibility [3]. Lack-of-fit errors are errors caused by the use of improper or "wrong" model. For example, using a linear regression to an otherwise non-linear data such as the Monod or Haldane models is an example of a lack-of-fit error. The remedy to pure error is to refine experimental handling and measurement whilst the remedy to lack-of-fit error is to change the model, but this must be based on statistical treatment or the use of a more recent technique—Monte Carlo simulations [4]. Numerous immunoassay standard curves are nonlinear in shape. To be precise, they are sigmoidal in shape and this kind of curve is usually model using a four or five parameter logistics curves [5]. In recent years the development of molecularly imprinted polymers or MIPs as an alternative to the fragile antibody often

used in immunoassays has emerged as a good contender. An emerging phenomenon is that the ligand-binding interaction in MIPs also mimics immunoassay—in that the calibration curve is sigmoidal [6–13]. This leads to several researchers employing the sigmoidal dose-response or four parameter logistics equation to fit the data [10,12,14,15] while other workers utilize a simpler but potentially incorrect linear assays. In a previous publication an ion selective electrode based on molecularly imprinted polymers calibration curve for the detection of lamotrigine (LTG) showed a sigmoidal calibration curve but was modelled according to a linear regression model, which sigmoidal dose response model

MATERIALS AND METHODS

Acquisition of Data

Data from the works of Sadeghi et al. [16] from figure 5 showing an ion selective electrode based on molecularly imprinted polymers calibration curve for the detection of lamotrigine (LTG). The data was processed using the software Webplotdigitizer 2.5 [17] which digitizes the scanned figure into comma separated data [18].

Four parameter logistics modelling

A non-linear regression using four-parameter logistic equations based on least square fitting [19] was utilized to fit the curve as follows;

$$y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{-(\text{LogEC}_{50} - x) * \text{Hillslope}}}$$

where y is the absorbance obtained (E (mv)), x is the concentration of lamotrigine (log unit), a and d are the maximum and minimum responses (E (mv)), respectively, Log EC_{50} is the value that produces a 50% signal response and Hillslope is the slope-like parameter (Hill coefficient). Regression analysis using the four-parameter logistics model was calculated using the PRISM software (v 5.0) available from www.graphpad.com.

RESULTS

Data obtained from the digitized version of the standard curve for lamotrigine (LTG) was then remodeled using the sigmoidal 4PL model. The result shows visually good fitting (Fig. 1). The correlation coefficient obtained for the regressed data was good at 0.998.

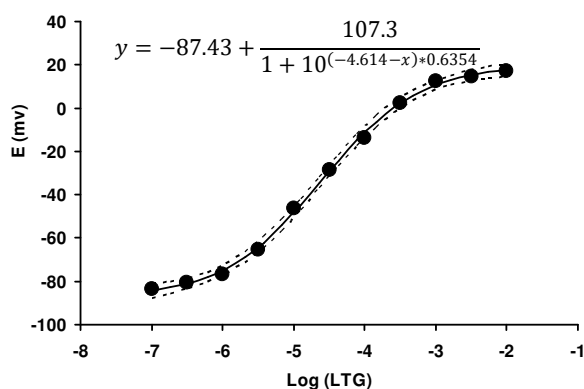


Fig. 1. Calibration curve for the ion selective electrode based on molecularly imprinted polymers for the detection of lamotrigine as modelled using the four-parameter logistic model (solid line) and its 95% confidence band (dotted lines).

The value of the curve parameters is shown in the form of the four-parameter logistic equation as follows;

$$y = -87.43 + \frac{107.3}{1 + 10^{(-4.614 - x) * 0.6354}}$$

The calculated value according to the four-parameter logistic equation were 19.87, -87.43, -4.614 and 0.6354 for the parameters a , d , Log EC_{50} and Hillslope, respectively. The limit of detection (LOD) was unable to be calculated as the data have no visible error bars that can be attributed to either the standard deviation or standard errors and the author also did not mention any reference to the use of these indicators of pure errors.

DISCUSSION

Molecularly imprinted polymers (MIPs) are polymer-based materials that have been touted as alternatives to bio-based receptors with excellent properties including stability, selectivity, recyclability, uniformity and economics of production. MIPs can be design at the macro or nanoscale. As have been mentioned previously, numerous researchers opted for the simple approach of linearizing and otherwise nonlinear standard curve.

The results from the four-parameter logistics modelling exercise is an evidence for the nonlinearity of the ion selective electrode based on molecularly imprinted polymers calibration curve for the detection of lamotrigine (LTG). In the original publication, the calibration curve is patently non—linear and the authors reported a linear correlation coefficient (R^2) of 0.9956 that is likely to cover only the linear portion of the sigmoidal curve. The major output from this remodelling exercise is that sigmoidal dose-response model will give a better fitting to the whole range of the data for the curve instead of the linear portion. In addition, with appropriate measure of pure error in the form of standard deviation or standard error for each data point, this will allow for the determination of Limits of Detection, which is three times the standard deviation of the blank value.

ACKNOWLEDGEMENT

The author wishes to acknowledged grants received from Universiti Putra Malaysia (High Impact Grant No UPM/700-1/2/GPPI/2017/9531900) and from the Malaysian Higher Education (Malaysia) under the Fundamental Research Grant Scheme (FRGS, Grant No FRGS/1/2017/TKO5/UPM/02/11)

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