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Limits of Detection Based on the Four-Parameter Logistic Model for *E. coli* Determined using a Fluorescent-based Sensor

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ABSTRACT

Because of the huge number of outbreaks, bacterial infection is becoming more common, which is compounded by the rise of antibiotic resistance. These issues have warranted the development of sensitive detection and therapeutic devices. A fluorescence-based detection of the pathogen *E. coli* is previously developed using a mini-emulsion technique to create fluorescent/electroactive poly(3-hexylthiophene) P3HT nanoparticles (NPs) stabilized with CTAB or cetyltrimethylammonium bromide, a quaternary ammonium salt forming CTAB-P3HT NPs. Limits of detection for the bacterium based on fluorescence spectroscopy is 5 CFU/mL. The curve showed a sigmoidal calibration curve but was not modelled according to any of the sigmoidal models available. The aim of this study is to use the Four-Parameter Logistic Model to determine the LOD value more accurately. The modelling exercise gave values of the parameters *a*, *d*, Log EC_{50} and Hillslope representing the maximum and minimum responses, value that produces a 50% signal response, and a slope-like parameter (Hill coefficient) of -0.01427 (DF/F_0), 0.6189 (DF/F_0), 3.473 and 0.413, respectively. The LOD value was 30 CFU/mL with the 95% confidence interval from 13 to 64 CFU/mL. The usage of the 4Pl model in this study was successful, since it was able to represent the entire date curve. The correlation coefficient values of 0.986 indicated good fitting of the experimental data to the 4PL model. The 4PL model has been found to be a good model in fitting the calibration curve for the detection of *E. coli*.

INTRODUCTION

Bacterial infection concerns are typically raised because of significant, generally high mortality outbreaks [1]. In addition, bacterial infections have become a serious worldwide health problem due to the rise of drug-resistant bacterial strains as a cause of heavy use of antibiotics [2]. There is thus a high relevance in dealing with a prompt diagnosis and effective alternate antibacterial therapy of pathogenic infections. Many researchers have been working on designing the next generation of antimicrobial detection samples for diagnostic and therapeutic applications (theragnostic antimicrobial) to solve this issue [3]. Fluorescence sensing is a good way to quickly and easily diagnose medicines. It is because a large number of fluorescence sensors with easy reading, no destruction, fast signal production and sensitive detection are available [4]. Beside its usage as fluorescent samples, several sophisticated fluorescent materials for biological applications including bacterial detection, discrimination and killing have been described as intelligent multi-tasking samples. In any assays where the calibration curve exhibits a sigmoidal pattern, the four-parameter logistic equation can fit the curves much better than linear regression using a double log transformed method [5,6]. A previous study used a simple mini-emulsion technique to create fluorescent/electroactive poly(3-hexylthiophene) P3HT nanoparticles (NPs) that is stabilised with CTAB or cetyltrimethylammonium bromide, a quaternary ammonium salt forming CTAB-P3HT NPs. The developed system was able to bind *E. coli* and this binding was detected by fluorescence quenching as well as changes in EIS. Limits of detection for the bacterium based on fluorescence spectroscopy and EIS are 5 and 250 CFU/mL, respectively. The curve showed a sigmoidal calibration curve but was not modelled according to any of the sigmoidal models available [7]. The objective of this study is the remodel the data using the standard 4-PL model and to determine the Limits of Detection (LOD) based on the standard method.

Acquisition of Data

Data from a previously published work [7] from figure 3(b) was processed using the software Webplotdigitizer 2.5 which digitizes the scanned figure into a comma separated data and has been utilized by many researchers and acknowledged for its reliability.

Four parameter logistic modelling

A non-linear regression using four-parameter logistic equations based on least square fitting [8] 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 response obtained (DF/F_0), x is the concentration of *E. coli* (log unit), a and d are the maximum and minimum responses (DF/F_0), respectively, Log EC_{50} is the value that produces a 50% signal response, and Hillslope is the slope-like parameter (Hill coefficient). Regression analysis was performed using the four-parameter logistic model and the PRISM programme (v 5.0) from www.graphpad.com. The limit of detection (LOD) was determined by multiplying three times the pooled standard deviation instead of the blank value of the lowest concentration of *E. coli* used. The error bars from figure 3(b) [7] was assumed to be standard deviation for three replicates as the authors did not indicate the identity of the error bars utilized in their work. These values were then interpolated from the sigmoidal dose-response 4-PL equation, and the associated *E. coli* concentration, including the confidence interval, was computed.

RESULT AND DISCUSSION

Normal curves based on ELISA are often nonlinear and sigmoidal in nature, and the easiest way to fit this type of curve is to use a standard four-parameter logistic (4-PL) or the seldom used five-parameter logistic (5-PL) model. [1]. The original data should therefore be matched to the 4-PL curve by modifying the curve model's parameters to obtain an optimum match between experimental and calculated data; the latter is frequently depicted by a line that runs thru the experimental observations. Although a patently sigmoidal profile was obtained [7], the authors did not resort to the 4PL model to fit the data and only report the LOD value without reporting the method utilized to calculate the LOD. **Fig.1** depicts a typical sigmoidal curve for the calibration curve based on the 4-PL equation. The result was a classic sigmoidal profile. An excellent correlation coefficient value of 0.986 was found, suggesting that the data was well fitted. The values of the parameters a, d, Log EC_{50} and Hillslope representing the maximum and minimum responses, value that produces a 50% signal response, and a slope-like parameter (Hill coefficient), were -0.01427, 0.6189, 3.473 and 0.413, respectively. The LOD value was 30 CFU/mL with the 95% confidence interval from 13 to 64 CFU/mL. This a bit higher than the LOD reported of 5.0 cells/mL but did not provide the confidence interval of the LOD value, which is critical for comparison purposes. The resulting LOD value from the 4PL modelling exercise demonstrates that the created approach is one order of magnitude less sensitive than the published LOD based on linear regression. Because LOD values should be determined using the 4-PL technique if the curve has a clearly sigmoidal profile, the LOD value derived using the 4PL modelling approach should be utilised to report the LOD value.

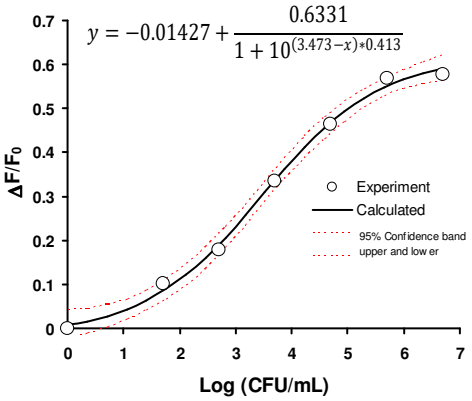

Fig. 1. Calibration curve for *E. coli* modelled according to the four-parameter logistic equation, and its 95% confidence band.

CONCLUSION

As the calibration curve has a sigmoidal profile, the 4PL model should be used to fit the data rather than a linear model, and the LOD value should be determined using the 4PL model. The usage of the 4Pl model in this study was successful, since it was able to represent the entire date curve rather than just the linear section of the curve. The LOD value was 30 CFU/mL (95% C.I. from 13 to 64 CFU/mL) was higher than the 5 CFU/mL reported in the original publication.

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