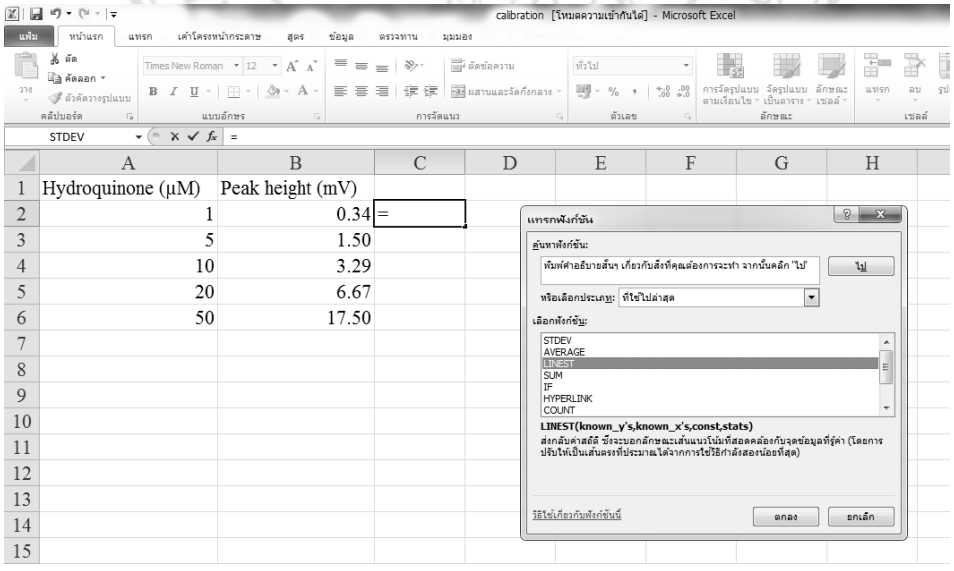
APPENDIX A

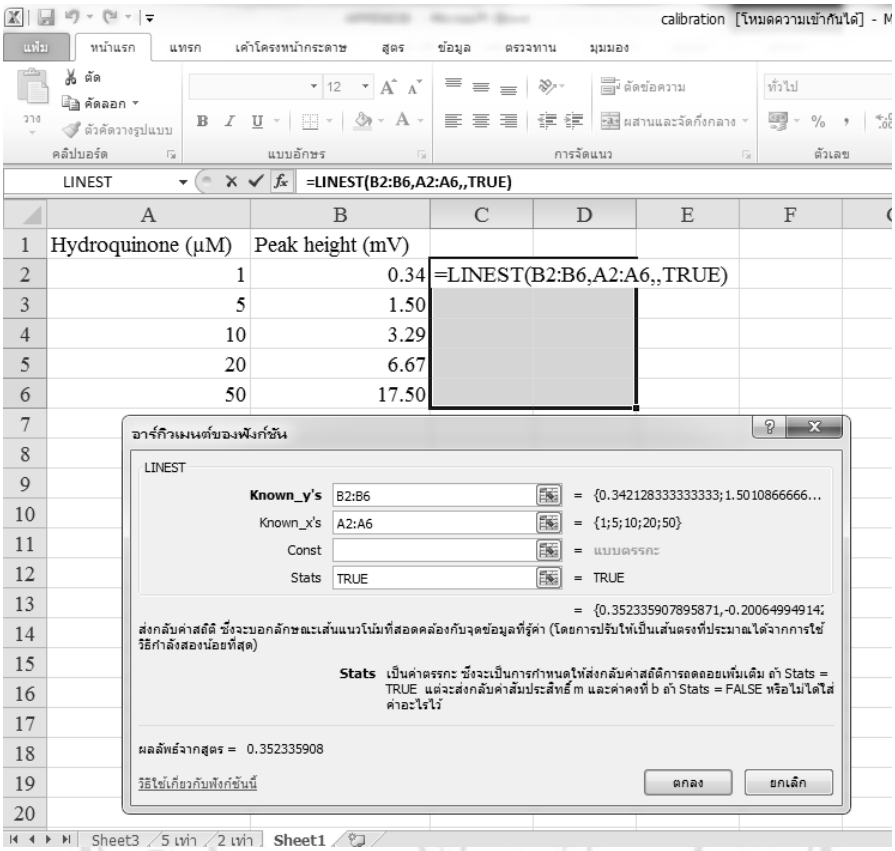
The Linest function for calculation of the limit of detection

The Excel LINEST function was used to calculate the detection limit of the system which uses the least squares method to calculate the line of best fit for a supplied set of y- and x- values. The method can be followed by these steps.

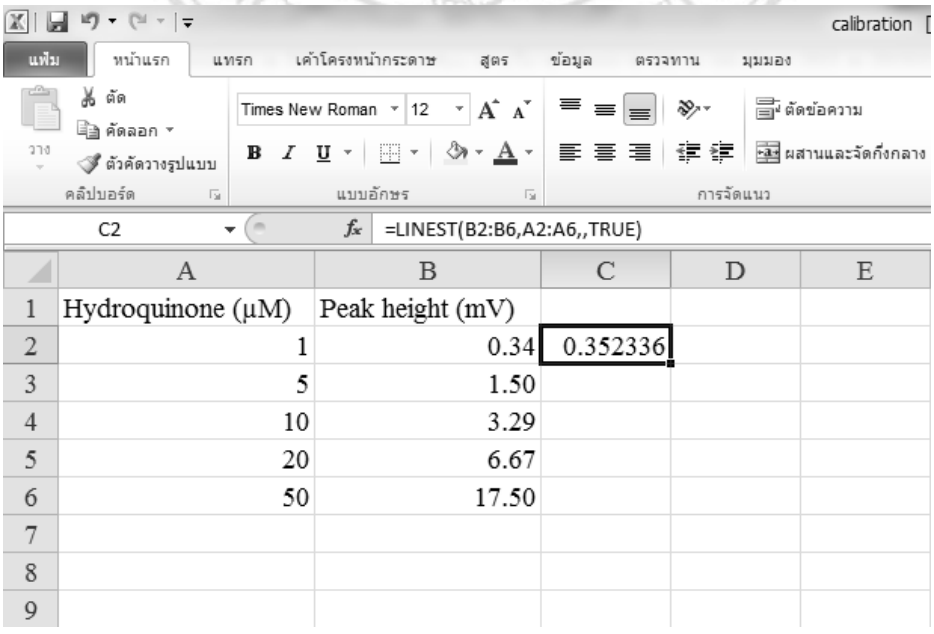
1. Type your data in two columns, one for the x variables (hydroquinone concentration, µM) and one for the y (peak height, mV) and Type = LINEST in the formula bar. It should look like:



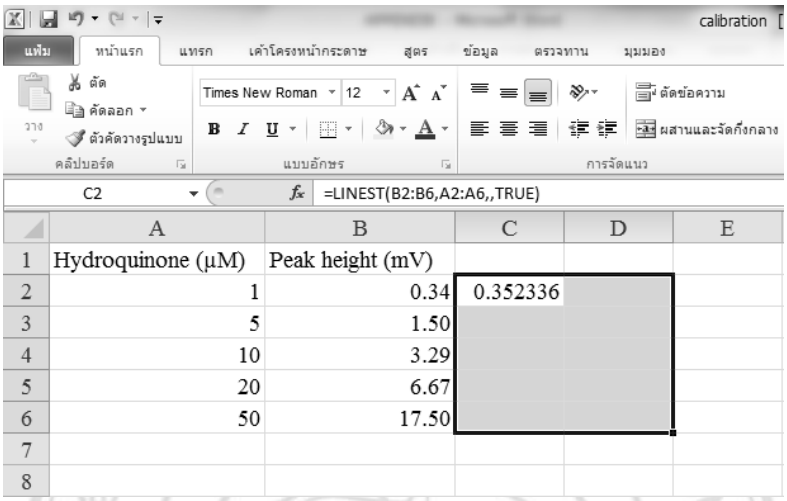
2. Click in the "known_x's and known_y's" dialog box, and select the cells containing the x and y values, respectively. Type in "TRUE" in the last dialog boxes. The Function dialog box should appear as below.



3. Click on "Finish." The formula bar should then appear as below,

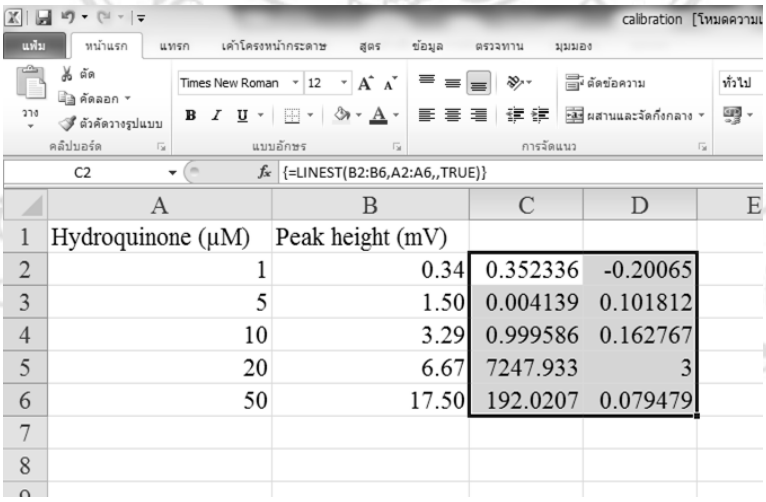


4. Highlight a block of cells (where it will give you the results) 5 rows and 2 columns. It should look like:



	A	B	C	D	E
1	Hydroquinone (µM)	Peak height (mV)			
2	1	0.34	0.352336		
3	5	1.50			
4	10	3.29			
5	20	6.67			
6	50	17.50			
7					
8					

5. Hit F2 and then hit ctrl + shift + enter all at the same time and Excel will calculate the linear regression. Each of the cells in the 5X2 block contains a regression parameter. Your results should look like these:



	A	B	C	D	E
1	Hydroquinone (µM)	Peak height (mV)			
2	1	0.34	0.352336	-0.20065	
3	5	1.50	0.004139	0.101812	
4	10	3.29	0.999586	0.162767	
5	20	6.67	7247.933	3	
6	50	17.50	192.0207	0.079479	
7					
8					

6. The meaning of each cell is arranged as:

	C	D	
Slope (m) →	0.352336	-0.20065	← Intercept of y (b)
Standard error of m →	0.004139	0.101812	← Standard error of b
R^2 →	0.999586	0.162767	← Standard deviation of y
F-test statistic →	7247.933	3	← Degree of freedom
Regression ss →	192.0207	0.079479	← Residual sum of square

7. The limit of detection can be calculated as below,

$$LOD = ((b + 3S_b) - b)/m$$

$$LOD = (-0.20 + 3(0.10) - (-0.20))/0.35$$

$$LOD = 0.86\ \mu M$$

Therefore, limit of detection is 0.86 µM.

APPENDIX B

Summary on laccase enzyme

Introduction and occurrence of laccase

Laccase (oxygen oxidoreductase, EC 1.10.3.2) is copper-containing oxidase enzymes that catalyzes the monoelectronic oxidation of a wide variety of organic and inorganic substrates, including phenols and their derivatives, aromatic amines and ascorbate coupled to a four electron reduction of dioxygen to water. Laccase is most widely distributed in a wide range of higher plants, microorganisms and fungi as well as in bacteria. Laccase in plants have been identified in trees, cabbages, beets, apples, potatoes and various other vegetables. Most common laccase producers are the wood rotting fungi such as *Trametes hirsute*, *Trametes villosa*, *Trametes gallica* and *Trametes versicolor* which was used in this work as shown in Figure 5.1.

Figure 5.1 *Trametes versicolor*.

Reaction mechanism

The laccase enzyme catalyzes the oxidation of substrates by removing one electron per time and generates free radicals which can be polymerized. The enzyme stores electrons of individual oxidation reactions and in active site totally reduced state contains a total of four electrons, thus, the enzyme can transfer these electrons to oxygen molecular to form water.

The active site with copper binding residues of laccase enzyme is showed in Figure 5.2. T1 copper (Cu) is the primary electron acceptor and at least one from both electrons required for reducing the site T3, acceptor of an electronic pair, comes from this site. The reaction rate of T1 Cu is the limit step of the total reaction rate. The T2 Cu is necessary for the aerobic oxidation of reduced site T3, in addition it allows site T3 to act like a two electron acceptor. The role of the T2 Cu maybe it participates in the transference of one of the electrons required to reduce site T3.

In the catalytic mechanism of laccase enzyme, it has been suggested that the T2 Cu stabilizes an intermediary in the reduction of O_2 to H_2O indicating that T2 Cu is part of the O_2 reduction site in the enzyme. It has been suggested that enzyme inhibition at an elevated pH value, is due to the formation of a copper T2-OH^- complex. This copper ion cannot allow the reduction of site T3 until the OH^- has been turned into a water molecule. At low pH values, one of the water molecules formed in the reduced enzyme re-oxidation seems to be united to site T2 Cu [Octavio, 2006].

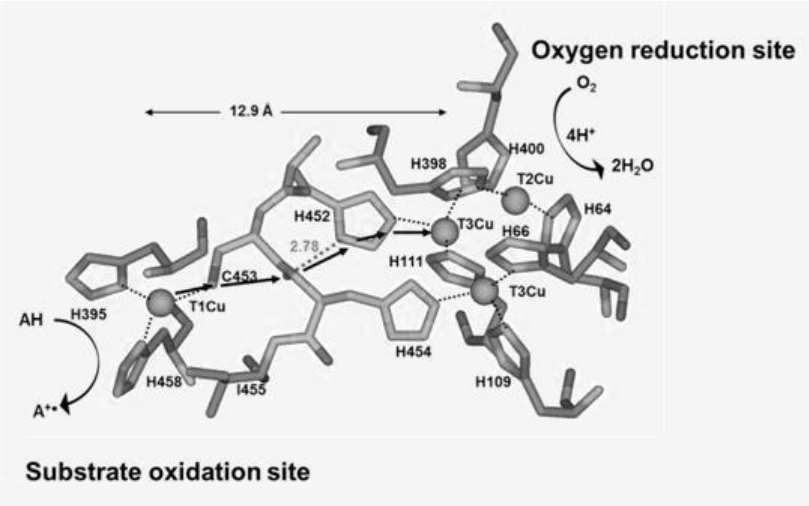


Figure 5.2 Copper binding residues of laccase enzyme.

Substrate specificity of laccase

Laccase is remarkably non-specific due to their reducing substrates and it has broad substrate specificity towards aromatic compounds containing hydroxyl and amine groups, phenolic compound. K*m* values are similar for the co-substrate dissolved oxygen about 5–10 M, but V*max* varies with the source of laccase 50–300 M/s. The turnover is heterogeneous over a broad range depending on the source of enzyme and substrate/type of reaction [Madhavi, 2009].

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Experience

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2014 J. Upan, P. Reanpang, O. Chailapakul, and J. Jakmunee, "Development of simple screen printed electrodes for application in electrochemical sensor for determination of hydroquinone by flow injection amperometric system (Poster presentation)," The 19th International Conference on Flow Injection Analysis (ICFIA), 30 November – 5 December, 2014, Fukuoka, Japan

2015 J. Upan, P. Reanpang, O. Chailapakul, and J. Jakmunee, "Hydroquinone biosensor based on simple screen printed electrodes modified with nanomaterial for determination of hydroquinone by flow injection amperometric system (Poster presentation)," TRF Seminar Series in Basic Research CVIII, 14 January, 2015, Chiang Mai, Thailand