SOLUTION SESSION 3: MATRIX-DEPENDENT BEHAVIOR

Exercise 1.

1.a First, we take the end-point values of each measurement and correct them for translational matrix effect (by subtraction of the background signal):

Added analyte (µg/mL)	Sample 1 (AU)	**Sample1- offset (AU)**	Sample2 (AU)	**Sample2- offset (AU)**
0	11	8	8	5
10	17.4	14.4	12	9
20	23.8	20.8	16	13
30	30.2	27.2	20	17
40	36.6	33.6	24	21
50	43	40	28	25

Then we plot the data, determine the slopes of the curves (given in the graph below), and extrapolate to zero, from the extrapolated value we identify the real concentration of the pollutant in the sample, which is equal to an absolute value of the distance on x-axis from 0.

For sample 1:

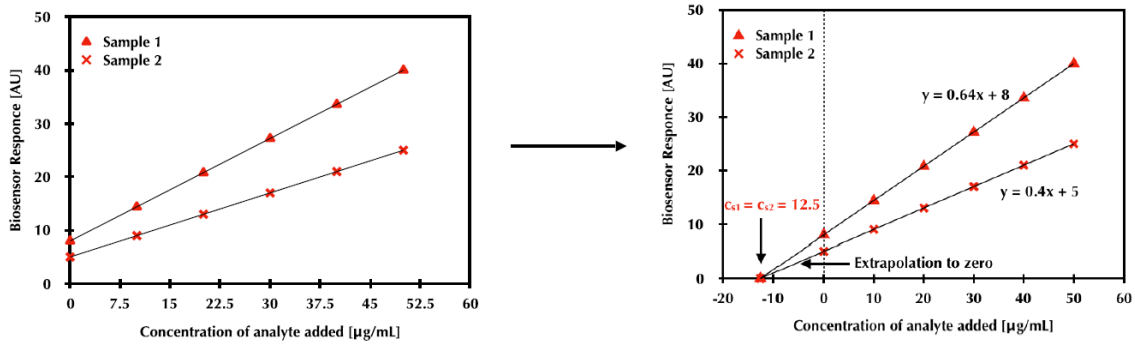
Slope = $\Delta y/\Delta x = (40-8)/50 = 0.64$; $y = 0.64x + 8$

$y = 0$: $x = -12.5$, concentration of pollutant is 12.5 µg/mL

For sample 2:

Slope = $\Delta y/\Delta x = (25-5)/50 = 0.4$; $y = 0.4x + 5$

$y = 0$: $x = -12.5$, concentration of pollutant is 12.5 µg/mL



1.b The concentration of the analyte (pollutant) is the same in both samples. The initial sample measurement had different measured values, due to a so-called matrix rotation effect. Because other constituents present in the test solution affect the measurement, thus samples from a different source will have different rotation effect. Using a standard addition method we can correct for this effect and determine the real concentration of the analyte.

1.c Matrix effects are important effects which need to be considered when biosensor or bioassay are developed. First, we need to consider different types of matrix: water, buffer, blood, urine, …, as our sensor will behave differently in a different kind of matrix. Second, differences between a matrix of the same type, water sample from lake near-by city and in the mountains will be distinct, which can influence our sensor or assay. Matrix effects are a result of variance in pH, salt concentrations and content of other molecules as phospholipids, carbohydrates, and metabolites in the sample. To avoid matrix effects except for standard addition method which is a rigorous approach, we can use calibration in matrix or dilution of samples in buffers.

Exercise 2.

2.a

A) Immunometric assays, also known as sandwich ELISAs, use two antibodies specific to the antigen to capture or "sandwich" antigens in the well for detection. Immunometric assays exhibit a direct correlation between antigen concentration and substrate response. The calibration curve is shown in Figure 1.

B) In a competitive immunoassay, the sample analyte is mixed with labelled analyte, which both and compete for a limited number of antibody-binding sites. Quantitative analysis can be achieved by determining the amount of labeled analyte that interacted at the binding sites. Therefore, with a fixed number of antibody sites, a smaller signal is expected when the ratio between the quantity of sample to the labeled analyte is large. In contrast, a larger signal is obtained when there is a small quantity ratio. Hence, the signal produced by the bound labeled analyte is usually inversely proportional to the amount of sample analyte. (Fowler, 2008) The calibration curve is shown in Figure 2.

Calibration curves for ligand binding assays (LBAs), which immunoassay are part of, are generally characterized by a nonlinear relationship between the response and concentration. For LBAs the typical calibration curve, when a semi-log scale is used, is sigmoidal in shape. This means that particular attention must be paid to the selection of appropriate algorithms for the fitting of the calibration curve data. LBAs rely upon the interaction of the analyte with a binding agent such as an antibody or a receptor component. The dynamic equilibrium nature of protein-protein interaction leads to a non-linear response in LBAs, which limits the concentration-response correlation at the upper and lower ends of the curve, resulting in plateaus and therefore an S-shaped curve. (Azadeh, 2017; Findlay, 2007)

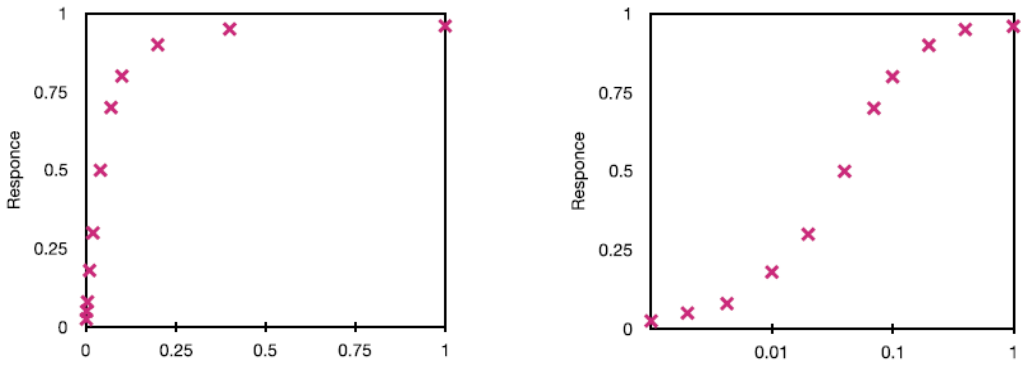


Figure 1: Sandwich assay calibration curves in linear and logarithmic scales.

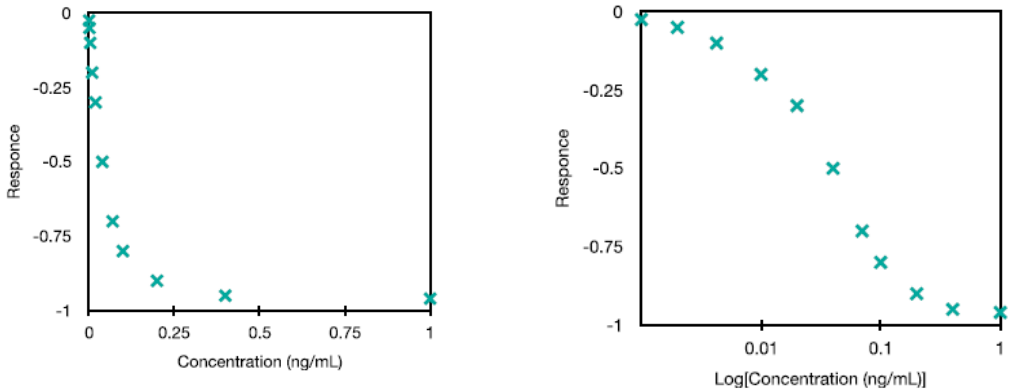


Figure 2: Competitive assay calibration curves in linear and logarithmic scales.

2.b Competitive assays are often used when an antigen is small and has only one epitope because only one antibody is required. These assays became popular because of their high sensitivity (i.e., the ability to detect small amounts of analyte); because a small amount of analyte is capable of producing a large output, thus compensate for background effects.

Competitive assays are less sensitive to matrix effects because part of the matrix effects results in the higher background and as we are in high output range, will not be influenced by this increase.