KINETIC DETERMINATION OF SELENIUM BY VISIBLE SPECTROSCOPY

*VERSION 1.8*

I. BACKGROUND.

The majority of reactions used in analytical chemistry possess the following characteristics: (1) a large equilibrium constant and (2) fast kinetics. Reactions meeting these criteria either go to completion or reach equilibrium rapidly. In either case, a stable analytical measurement can be made soon after the reactants are mixed.

Quite often, a reaction has desirable analytical characteristics but its kinetics are slow enough that a prohibitive amount of time is required if the analyst must wait for the reaction to reach equilibrium. In such a case, it may be possible to obtain analytical information from the reaction without waiting for equilibrium to be established. An analysis based on measurements made before a reaction reaches equilibrium is termed a kinetic method.

In this experiment, the selenium-catalyzed reduction of methylene blue by sodium sulfide at pH 10.5 is followed by visible spectroscopy with a time-based measurement.

![Methylene Blue](image)

**Methylene Blue**

II. REAGENTS/MATERIALS.

Conditioner solution. Dissolve 6.25 g of Na₂EDTA·2H₂O, 0.10 g of FeCl₃, and 13 mL of triethanolamine in reagent water and dilute to 250 mL in a volumetric flask.

Alkaline sodium sulfide solution. Prepare a 50 mL aqueous solution that contains 2.4 g of Na₂S·9H₂O, 2.4 g of Na₂SO₃, and either 4.0 g of solid NaOH or 8.0 g of 50% (w/w) NaOH solution in a volumetric flask. Dissolve each reagent first rather than adding all solid material to the flask at once.

*Note:* The Na₂S·9H₂O contains a significant amount of hydrated water and some water may be present in the reagent bottle. Take care to use the solid material rather than the liquid.

Methylene blue (FW = 373.90) solution. Prepare a 0.07 mM aqueous solution in a 50 mL volumetric flask by dilution of the 0.7 mM stock solution (0.026 g in 100 mL) provided by the instructor.
Standard Se solutions (CAUTION: Se is a toxic element). From the 1000 ppm stock solution provided by the instructor, prepare a 2 ppm solution in a 100 mL volumetric flask. This will require a two-step dilution procedure. Dilute with distilled water. Prepare four calibration standards that span the 0.25-1.5 ppm range. Use 25 mL volumetric flasks for these standards.

Formaldehyde (36-38%) solution (provided). Keep this solution covered. (Caution: Lachrymatory!).

III. PROCEDURE.

A. The instructor will demonstrate the operation of the UV-Vis diode array spectrometer for a time-based data collection. Use distilled water as the spectroscopic blank. Determine $\lambda_{\text{max}}$ of methylene blue; obtain separate spectra for the other components of the reaction also. Plots of $A$ vs. $\lambda$ and peak table(s) should be obtained. Determine the best $\lambda$ to monitor the course of the reaction. You can specify a range of $\lambda$ over which to integrate.

B. Caution: Wear gloves during the handling of these solutions. Into the (clean and dry) cuvette, add (with a micropipettor) 400 µL of alkaline sulfide solution, 800 µL of conditioner solution, and 800 µL of formaldehyde. The following protocol will be executed for each of the four calibration standards and the unknown Se solutions. Three replicate runs should be made for each solution.

1. Set the data collection command on the instrument; add 800 µL of sample (standard or unknown) to the cuvette.

2. Add 800 µL of the 0.07 mM methylene blue solution to the cuvette and start the timer. Cap the cuvette, invert it several times, and place it in the sample compartment of the spectrometer. Start the data collection at the same time for each run (relative to the methylene blue addition). For example, wait until the timer reaches 15 seconds each time before pushing the “Execute” button. Record data at 0.5 second intervals until the absorbance for the run with the lowest concentration of Se reaches zero. This will typically occur between 1 and 3 minutes.

3. Printouts of absorbance (at a fixed $\lambda$) vs. $t$ should be obtained for each sample (obtain both a plot and a table of values).

IV. DATA TREATMENT.

A. Introduction.

Kinetic methods of analysis can be divided into two categories: (1) direct methods and (2) catalytic methods. Direct kinetic methods involve adding a reagent to the analyte and then monitoring the course of the subsequent reaction. The analyte is a reactant in the reaction that is monitored. In catalytic methods, the analyte is a catalyst that affects the rate of the reaction. Thus, the analyte is not a reactant in the reaction that is monitored. The selenium determination in this experiment is an example of a catalytic method.

Consider the case of a catalytic kinetic method based on the following reaction:

$$A \rightarrow P$$ (1)

where $A$ is the reactant and $P$ is the product. The rate constants of the forward and reverse
reactions are $k_1$ and $k_2$, respectively. The rate of the reaction can be expressed two ways:

$$\text{rate} = -\frac{d[A]}{dt} = \frac{d[P]}{dt} = k_1[A] - k_2[P]$$

(2)

where $d[A]/dt$ is the change in concentration with time. If the forward reaction has a large $K_{eq}$ so that the reverse reaction is negligible, this expression can be simplified to:

$$\text{rate} = -\frac{d[A]}{dt} = \frac{d[P]}{dt} = k_1[A]$$

(3)

Thus, we can monitor the disappearance of $A$ or the appearance of $P$ as a measure of the rate of reaction. For catalytic methods, the catalyst (i.e., the analyte) changes the rate constant of the reaction. Therefore the rate as a function of analyte concentration can be calibrated by using standards.

**B. Methods for Data Treatment.**

Three methods of data treatment are commonly used for kinetic analyses. Each method is based on the construction of a calibration model, employing a series of standard solutions. These three methods are described below for a catalytic method.

**Derivative Method**

Experimental conditions are established so that $[A]$ remains essentially constant (i.e., $[A]_0 = [A]_t$, where $[A]_0$ is the concentration initially and $[A]_t$ is the concentration at any time $t$). Equation 3 above can now be written:

$$\text{rate} = -\frac{d[A]}{dt} = k_1[A]_0$$

(4)

that is, the rate is directly proportional to the rate constant. Assuming a linear relationship between the catalyst and the rate constant, we can write:

$$-\frac{d[A]}{dt} \text{ or } \frac{d[P]}{dt} = [\text{analyte}] \cdot \text{constant}$$

(5)

The initial slopes of the kinetic plots are proportional to analyte concentration. The derivative method will not be used in your analysis.

**Fixed Time Method**

The loss of reactant at a fixed time $t$ for two different rates $k_1$ and $k_2$ is

$$[A]_{k1} = - k_1[A]_0 t \text{ and } [A]_{k2} = - k_2[A]_0 t$$

(6)

$$\Delta = [A]_{k1} - [A]_{k2} = - (k_1-k_2) [A]_0 t \text{ or } \Delta A = (k_1-k_2) \cdot \text{constant}$$

(7)

If $k_2 = 0$ (such as for a blank), then

$$\Delta A = k_1 \cdot \text{constant} = [\text{analyte}] \cdot \text{constant'}$$

(8)

If $k_2 \neq 0$, then a linear correction is required.

*Calibration model.* For each of a series of standards, measure a quantity that is proportional to $[A]$ (such as its absorbance) vs. time. From these data, select a time ($t$) that provides the greatest sensitivity (i.e., greatest change in the measured quantity) across the
calibration standards. Make a calibration model of the measured quantity that is proportional to \([A]_t\) (e.g., absorbance at time \(t\)) vs. concentration of standard.

**Unknown.** Collect \([A]\) vs. time data for the unknown. Employing \([A]_t\) (i.e., absorbance at time \(t\)) for the unknown, use the calibration model to compute the unknown concentration.

**Variable Time Method**

Equation 3 above is a differential equation that can be solved by substituting the mass balance expression \([P] = [A]_0 - [A]\), integrating both sides, and evaluating the integral from time \(= 0\) to \(t\). This produces the following expression:

\[
[P]_t = [A]_0(1 - e^{-kt}) \quad (9)
\]

where \([P]_t\) is the concentration of \(P\) at a specific time \((t)\). One can thus measure the time \((t)\) that it takes for \([P]\) to reach a certain value. In certain cases, this measurement scheme is easier to automate, as you can monitor the measurement of \([P]\) and then use this measurement to control the experiment. The problem with this approach, however, is that equation 9 does not specify a linear relationship between \([A]_0\) and \(t\). To overcome this problem, the following approximation is used:

\[
e^{-kt} \approx 1 - kt \quad (10)
\]

This approximation is good for small \(t\). Substituting this expression into equation 9 yields

\[
[P]_t \approx [A]_0(kt) \quad (11)
\]

For measuring a sample at two different times (after \(t_0\)), the reaction of sample 1 is described by

\[
\Delta[P]_1 \approx [A]_{01}(kt_1 - kt_0) \approx [A]_{01}k(t_1 - t_0) \quad (12)
\]

while the reaction of sample 2 is described by

\[
\Delta[P]_2 \approx [A]_{02}(kt_2 - kt_0) = [A]_{02}k(t_2 - t_0) \quad (13)
\]

With \([A]_0\) effectively constant for a catalytic assay and the catalyst again affecting the rate constant,

\[
\Delta[P]_1 \approx [A]_0 k_1 \Delta t_1 \quad (14)
\]

and

\[
\Delta[P]_2 \approx [A]_0 k_2 \Delta t_2 \quad (15)
\]

where we define \(\Delta t_n = t_n - t_0\). By measuring the same \([P]\), we can set equations 14 and 15 equal to each other and get

\[
[A]_0 k_1 \Delta t_1 \approx [A]_0 k_2 \Delta t_2 \quad (16)
\]

\[
k_1 \Delta t_1 \approx k_2 \Delta t_2 \quad (17)
\]

Assuming a linear relationship between the analyte concentration and the rate,

\[
[\text{analyte}] = \text{constant} \cdot 1/\Delta t \quad (18)
\]
Calibration model. For each of a series of standards, measure [A] (i.e., a measurement quantity such as absorbance that is proportional to [A]) vs. time. From these data, select a fixed [A] (i.e., a fixed value of absorbance) that provides the greatest sensitivity across the calibration standards and find times that correspond to this [A]. Make a calibration model of 1/t vs. concentration of standard.

Unknown. Collect [A] vs. time data for the unknown. Employing the same [A] (i.e., the same absorbance value), find the corresponding time. Use the calibration model curve to compute the unknown concentration.

V. CALCULATIONS

1. Tabulate the mean, standard deviation, and 95% confidence interval for all absorbance or time data used in the generation of calibration models.

2. Absorbance vs. t plots obtained from the spectrometer should be included in the report.

3. Calibration plots with error bars should be included for the fixed time and variable time data analysis methods. For each model, tabulate the computed slope, intercept, standard error of estimate, and r².

4. Compute the concentration of your unknown using each calibration model. For each model, use each replicate measurement separately to compute a concentration for the unknown and then report a mean value, standard deviation, and 95% confidence interval.

5. In your discussion section, compare the data analysis methods. In which method do you have the most confidence?

VI. REFERENCES