Determination of reaction stoichiometry by applying the Job’s method and digital image processing for precipitation reactions

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Students’ Handout
Determination of reaction stoichiometry

**Aim:** Understanding the concept of the experimental determination of reaction stoichiometry by applying the Job’s method and a precipitation reaction.

## 1 Introduction

When a new reaction is explored or a new product is yielded in a chemical synthesis, primary importance is dedicated to define the reaction stoichiometry both for theoretical and economical purposes. If reactants $R_1, R_2, \ldots, R_n$ produce products $P_1, P_2, \ldots, P_m$, then the overall reaction can be described as

$$r_1R_1 + r_2R_2 + \cdots + r_nR_n = p_1P_1 + p_2P_2 + \cdots + p_mP_m.$$  

Being aware the reaction stoichiometry means that we know the ratio of the above $r_1, r_2, \ldots, r_n, p_1, p_2, \ldots, p_m$ stoichiometric coefficients. For simple conceptualization, performance, and evaluation, aqueous reactions of

$$r_1R_1 + r_2R_2 = pP$$  

are studied in student laboratory courses.

For its visualization capabilities, the most frequently selected experimental method is the Job’s method (also called the method of continuous variation or of isomolar series). The method relies on applying reactant stock solutions of equal molar concentrations ($[R_1]_0 = [R_2]_0$) and mixing them in different volume ratios while keeping the total volume constant. This way the sum of reactant concentrations ($[R_1]_0 + [R_2]_0$) is also constant, and the product concentration at the end of the reaction ($[P]_\infty$) will be a function only of the volume ratio applied. $[P]_\infty$ reaches its maximum value if the two reactants are present exactly in the stoichiometric ratio. We obtain informative graphs when $[P]_\infty$ is plotted as a function of the mole fraction of one of the reactants (e.g., $x_{R_2}$; Fig. 1).

If the reaction is quasi irreversible, the $[P]_\infty - x_{R_2}$ function depicts a sharp breaking point as the intersection point of the lines fitted through the points located on the left and right sides of the maximum mole fraction ($x_{\text{max}}$), as seen for three different stoichiometric ratios in Fig. 1a. However, if the reaction is reversible and not dominantly shifted to any extremum, a smooth transition is observed instead of the sharp breaking point (Fig. 1b). In this scenario, the points close to the two extrema are linearly fitted and extrapolated to obtain $x_{\text{max}}$. Observe in Fig. 1b, that the extrapolated $x_{\text{max}}$ may significantly differ from that obtained

![Figure 1: Schematic Job’s plots for irreversible (a) and reversible (b) reactions of different stoichiometric ratios: $r_1 : r_2$ = 1 : 1 (black bullets), 3 : 1 (red triangles), and 1 : 2 (green diamonds). Dashed vertical lines in Part: a correspond to the mole fractions ($x_{\text{max}}$) where the product concentration is maximal for a given reaction stoichiometry; the same dashed lines are also added in Part: b to illustrate the uncertainty of the experimentally determined value if the same stoichiometry would belong to a reversible reaction.](image-url)
for an irreversible reaction having the same stoichiometric ratio. For this reason, we will apply an other method for evaluation. From the Job’s plots the reaction stoichiometry can be determined. By definition,

\[ x_{R_2} = \frac{n_{R_2}}{n_{R_1} + n_{R_2}} \]

where \( n \) is the amount of substance, and thus \( x_{max} = \frac{r_2}{r_1 + r_2} \) from which

\[ \frac{r_2}{r_1} = \frac{x_{max}}{1 - x_{max}}. \]  

(2)

The stoichiometric coefficients \( r_1 \) and \( r_2 \) are the smallest possible integers for which eq. (2) holds.

2 Concept

In order to determine the reaction stoichiometry, it is not necessary to know the absolute amount or concentration of the product. It is sufficient to measure a quantity being proportional to one of those descriptors, and reveal the trend how this quantity changes with the composition of the reactant solution. In many cases, the absorbance of a solution is followed by a spectrophotometer. If the constituent ions of a sparingly soluble salt are separately contained by two reactant solutions with an appropriately high concentration (i.e., above the solubility product), the solid phase (chemical precipitate) sooner or later will appear upon mixing the reactant solutions. In most cases this precipitate sediments to the bottom of the container. If the reactions of various reactant compositions are performed in identical test tubes, the height of the precipitate column is proportional to the amount of precipitate. Therefore, it can be applied to prepare \([P]_\infty - x_{R_2}\) plots and determine the reaction stoichiometry. In this practice, since daily life of chemists and chemical engineers often requires the use of digital image processing and data treatment methods, we will obtain the height of the precipitate columns and the stoichiometric coefficients by such procedures.

3 Experimental

1. Place 17 identical test tubes (20 ml) next to each other in an appropriate stand and mark them from 1 to 17.

2. The reactions are to be performed with two unknown reactants (\( R_1 \) and \( R_2 \)). By a pipette, measure 1 ml of \( R_1 \) stock solution into the first test tube. For each coming test tube, increase the volume by 0.5 ml, so that the last test tube (#17) contains 9 ml of \( R_1 \) stock solution. Afterwards, using a clean pipette, measure the volumes of \( R_2 \) stock solution into the test tubes in a way that each tube contains 10 ml solution (consider the solution volumes additive).

3. Manually and rigorously shake the test tubes. Precipitation sets in immediately upon the contact of the reactant solutions. To ensure appropriate crystal growth and sedimentation, shake the test tubes second time approx. 1 min after the first shaking. Let the precipitate settle down in rest.

4. After 90 mins sedimentation time, take a photo of the system with your cellphone by paying attention to the following:

- Test tubes should be vertical and nothing should hide the precipitate columns;
- To achieve better contrast, place a black background behind the test tubes; avoid glittering;
- Scale bar must be clearly seen on the picture;
- Take the photo in front of and in plane with the tube stand; avoid twisting;
- If possible, take a single photo which depicts all test tubes at the same time (do not show the entire test tubes, precipitate columns must be focused). Alternatively, take two photos and make sure that the scale bar is visible on both;
- Check the scale bar on the photo, it should not be blurry when zooming in.
4 Evaluation

1. Hand in the photo(s) together with the calculations.

2. Determine the height of the precipitate column ($h / \text{mm}$) by digital image processing for each test tube (reactant solution composition); see Appendix for a step-by-step image processing guide. Tabulate your results according to Table 1. To calculate $x_{R_2}$ for the reaction mixtures, consider that no need to know the molarity of the stock solutions. Since $[R_1]_0 = [R_2]_0$, only the dilution must be taken into account.\(^1\)

<table>
<thead>
<tr>
<th>No. test tube</th>
<th>$x_{R_2}$</th>
<th>$h / \text{mm}$</th>
<th>$h'$</th>
</tr>
</thead>
</table>

3. Plot the $h - x_{R_2}$ data pairs; do not forget axis labels and units.

4. To determine the reaction stoichiometry, look for the mole fraction ($x_{\text{max}}$) where the height of the precipitate column is maximal, i.e., find the maximum position of the function described by the $h - x_{R_2}$ data set. This might not be equivalent with the solution composition of any test tube. To find $x_{\text{max}}$, numerically differentiate the data set with a spreadsheet editor, e.g., MS Excel (i.e., compute the difference quotients, $h' = \frac{\Delta h}{\Delta x_{R_2}}$ for each measured point, which has lower and upper neighbors; see Fig. 2). Tabulate the difference quotients in Table 1 and plot the $h' - x_{R_2}$ data sets in the previous graph; use separate y-axis if needed.

5. The difference quotient is positive for increasing precipitate heights, while it is negative for decreasing tendency. In the region where, based on the cellphone image, the maximum height of the precipitate column is expected, look for the two $h' - x_{R_2}$ data pairs, where the different quotient changes sign. To do so, apply linear interpolation and calculate $x_{\text{max}}$ (i.e., where $h' = 0$).

6. With the aid of eqn. (2) and $x_{\text{max}}$, find the stoichiometry of the reaction.

\[ \frac{dy}{dx} = tg \gamma \equiv tg \alpha = \frac{\Delta y}{\Delta x} \]

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\[ \Delta y = y_{i+1} - y_i \]

\[ \Delta x = x_{i+1} - x_i \]

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\[^1\] Since $x_{R_2} = \frac{n_{R_2}}{n_{R_2} + n_{R_1}} = \frac{c_{R_2} \cdot V_{R_2}}{c_{R_1} \cdot V_{R_2} + c_{R_1} \cdot V_{R_1}}$, where $c_{R_1} = c_{R_2}$ due to experimental design, the equation simplifies and mole fractions can be calculated as dilution.
Questions

1. Explain the Job’s method in 3 – 5 sentences.
2. Sketch a Job’s plot for 2 : 1 stoichiometry expecting (a) irreversible and (b) equilibrium reaction.
3. Explain the meaning of $x_{max}$ and its relation to $r_1$ and $r_2$ in an $r_1R_1 + r_2R_2 = pP$ type reaction.
4. Explain in 3 – 5 sentences, how you can determine $x_{max}$ from the precipitation column height measurements.
5. The maximum of a Job’s plot is located at $x_{max} = 0.25$. Calculate the stoichiometric coefficients of the reactants for an $r_1R_1 + r_2R_2 = pP$ type reaction.
6. We measured 7.00 ml of $R_1$ and 3.00 ml of $R_2$ stock solutions into a test tube. The molarity of the stock solutions is equal. Express the composition of the reactant solution according to the mole fraction of the second reactant, $x_{R_2}$.
7. From the experiments, you obtained the mole fraction–precipitate column height data set presented in the table below. By applying numerical differentiation, calculate the slope of the $h–x_{R_2}$ function a) either for the first or for the last point of the data set (two-point formula), and b) for an arbitrarily selected mole fraction excluding the two above (three-point formula).

<table>
<thead>
<tr>
<th>$x_{R_2}$</th>
<th>0.9</th>
<th>0.8</th>
<th>0.7</th>
<th>0.6</th>
<th>0.5</th>
<th>0.4</th>
<th>0.3</th>
<th>0.2</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$h$/mm</td>
<td>3.2</td>
<td>5.9</td>
<td>7.7</td>
<td>11.6</td>
<td>15.0</td>
<td>22.4</td>
<td>22.5</td>
<td>13.4</td>
<td>6.5</td>
</tr>
</tbody>
</table>
Appendix:
Digital image processing

The digital photo of the experiment is to be processed by the freely available ImageJ software in order to
determine the height of the precipitate columns.

1. Download and extract the .zip file into a selected location; link: https://imagej.nih.gov/ij/download.html

2. When a vertical line is drawn along the precipitate column, the software displays its grayscale profile. By numerically differentiating this profile, the end points of the precipitate column can be determined, since a sharp change marks the transition from the background to the precipitate, and vice versa. Expecting a dark background and a bright precipitate, the latter one is characterized by higher grayscale value. The minimum and maximum of the differentiated grayscale profile denotes the end points of the precipitate column. ImageJ uses a macro to differentiate the grayscale profiles which can be downloaded here for free: https://github.com/MontpellierRessourcesImagerie/imagej_macros_and_scripts/wiki/MRI_Plot_Tool

Open the plot_tools.ijm file at the website of the macro (Getting Started chapter) and copy its whole content into Notepad, save it as derivative.txt. Copy this file into ImageJ macros/toolsets folder. If needed, copy as administrator. Run ImageJ, click on icon in the toolbar and select the derivative macro. The icon, inviting the macro appears in the toolbar. By right-clicking on the icon, inactivate show zero crossings option.

3. Open the experimental image(s) (File/Open) and correct any deviation from the horizontal plane; Image/Transform/Rotate menu (Fig. 3a). Set Angle to 0.0 and select Preview. Increase the value of Grid lines until they are numerous enough to judge whether, e.g., a certain part of the test tube holder or the test tube itself is in parallel with the grid lines. Set Angle (± value) until the parallel alignment is satisfactory, then select OK.

4. To set scale, zoom in (Ctrl+scroll) to the mm-paper fixed on the bottom section of the tube stand. Select straight line from the tool bar ( ). By holding Shift key and moving the mouse, draw a line along the mm-paper which crosses several main grids of the mm-paper (more reliable calibration needs longer measured distance). Pay attention to that the line passes in between two grid lines of the mm-paper. Plot the gray scale profile along the horizontal line (Ctrl+K, see 3b).

The deep minima correspond to the main grid lines of the mm-paper, the shallower minima belong to the mm scale. Select two minima located far from each other, and read out the x coordinates by mousing over them. Make a note of those data and close the gray scale profile window. Note: Closing
the window is not optional before moving to the next step. Perform the calibration in Analyze/Set Scale menu with the aid of the above coordinates and by knowing the factual distance between them (i.e., between the selected grid lines). Type the difference of the x coordinates in Distance in pixels bracket (122 – 6 = 116 in case of Fig. 3b, red circles in Fig. 3b). In the present case, we wrote 10 for Known distance, since two consecutive main grid lines of the mm-paper were selected, which are separated by 10 mm. Set mm for Unit of length and select OK. After this calibration, if a distance is measured on the digital photo, we will obtain the result in factual size instead of pixel. Double-check the success of the calibration. For example, draw a 1 cm long line along the mm-paper and press Ctrl+M to show the results of the measurement. In the length column of the table, a number close to 10 should appear which is the length of the line in mm. If not, repeat the calibration. If ImageJ is closed and re-opened, or a photo taken from different position is imported, new calibration is required.

5. After setting up the scale, navigate to the first test tube by icon. Draw a vertical line along the test tube by holding Shift key, as shown in Fig. 4a. Display the gray scale profile along the vertical line (Ctrl+K), and differentiate it by clicking on ; a graph similar to Fig. 4b will appear. The height of the precipitate columns is obtained as the difference of the x coordinates of the minimum and maximum positions seen in the plot. (If those extrema are not well-recognized, attempt to draw a new vertical line near the first one.) Make a note of the height of the precipitate (h/mm) in the spreadsheet used to evaluate the results, close the plots, and continue with the next test tube.

Figure 4: Determination of the precipitate column height by digital image processing: close-up of the experimental image (a) and the derivative of the gray scale profile (b).