Using order of magnitude calculations to extend student comprehension of laboratory data

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I previously published an Illuminations article concerning “challenge” questions that encourage students to think imaginatively with approximate quantities, reasonable assumptions, and uncertain information (2). This article has promoted some interesting discussion, which has prompted me to present further examples.

In our laboratory course, Scientific Methods in Biology, our students have used either of two different methods to measure light-dependent photosynthetic events in isolated thylakoid vesicles. In photosynthesis, absorbed photon energy drives a flow of electrons (e⁻), derived from water, through a sequence of molecular complexes associated with the thylakoid membrane of chloroplasts. At one point, plastoquinones within the thylakoid membrane become reduced by accepting electrons from photosystem (PS) II. As plastoquinones become reduced, they also accept protons from the outside membrane. When plastoquinones are re-oxidized, by transferring electrons to the next acceptor, cytochrome b₆f, protons are deposited on the inside of the thylakoid membrane. As a result, protons are transferred from the outside to the inside of the thylakoid vesicle, and this can be measured by observing the decline in pH in the thylakoid suspension bathing solution during light exposure (3) (see simplified diagram 1 below):

\[
\text{Light} \\
\text{Water} \rightarrow e^- \rightarrow \text{PS II} \rightarrow e^- \\
\rightarrow \text{plastoquinones} \rightarrow e^- \rightarrow \text{cytochrome b₆f} \ (1) \\

\text{protons outside} \\
\text{protons inside}
\]

In a second method using the synthetic electron acceptor oxidized 2,6-dichlorophenol indophenol (DCPIP), which becomes reduced by accepting electrons from plastoquinones, the rate of DCPIP reduction is derived by measuring the decline in the absorbance of DCPIP at 600 nm during light exposure (4) (see simplified diagram 2 below):

\[
\text{Light} \\
\text{Water} \rightarrow e^- \rightarrow \text{PS II} \rightarrow e^- \rightarrow \text{plastoquinones} \\
\rightarrow e^- \rightarrow \text{DCPIP} \ (2)
\]

In both cases, the activity is inhibited in a dose-dependent manner by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; the herbicide Diuron). DCMU binds to a site on PS II and prevents the transfer of electrons from PS II to plastoquinones. As a consequence, as the DCMU concentration is increased, fewer electrons are transferred to plastoquinones, so we observe fewer protons being translocated across the thylakoid membrane in the first assay method and decreasing rates of DCPIP reduction in the second method. At high DCMU concentrations, no electrons are transferred to plastoquinones, so no protons are translocated into thylakoid vesicles and no DCPIP reduction occurs (see simplified diagram 3 below):

\[
\text{Light} \\
\text{Water} \rightarrow e^- \rightarrow \text{PS II} \\
\rightarrow \text{DCMU} \ (3)
\]

The majority of students are content to collect the data, make the required calculations and graphs, and then move on. However, a few students are more engaged, and, for these students, I provide questions that challenge them to try to find if more can be discovered using the data that they obtain in class. In the case of the data obtained using different DCMU concentrations, the question is as follows: “Can these data be used to estimate the number of PS II units in an aliquot of thylakoid vesicles that contains 1 µg chlorophyll?” I tell them that they will need to make assumptions but that most of the calculations depend on basic chemistry.

One assumption is that the binding of just 1 DCMU molecule to 1 PS II unit is sufficient to block the transfer of electron from that PS II unit to the plastoquinones. If this is true, then the concentration of DCMU that generates a 50% reduction in the activity being measured can be used to estimate half of the number of PS II units present. When multiplied by 2, this gives an estimate of the total number of PS II units.

Calculations: Using typical data obtained in class, the calculation for the proton translocation assay with thylakoid vesicles suspended in 10 ml bathing medium is as follows.

Light-induced proton translocation into thylakoid vesicles is reduced by ~50% compared with controls at a DCMU concentration of ~4 µmol/l (3). Therefore, the quantity of DCMU in 10 ml thylakoid bathing solution is:

\[
4 \mu\text{mol}/l \times \frac{10 \text{ ml}}{1,000 \text{ ml/l}} = 4 \times 10^{-2} \mu\text{mol DCMU}
\]

In this assay, thylakoid vesicles were suspended in 10 ml bathing solution at a chlorophyll concentration of 3 mg/10 ml (= 3,000 µg chlorophyll):

\[
\frac{1.33 \times 10^{-5} \mu \text{mol DCMU/µg chlorophyll}}{4 \times 10^{-2} \mu\text{mol DCMU}} \div 3,000 \mu\text{g chlorophyll} = 1.33 \times 10^{-5} \mu\text{mol DCMU/µg chlorophyll}
\]
Multiply $1.33 \times 10^{-5} \text{mol/µg} = (1.33 \times 10^{-11} \text{mol/µg})$ by Avogadro’s number to derive the number of molecules of DCMU present:

\[
(1.33 \times 10^{-11} \text{mol/µg}) \times (6.022 \times 10^{23}) = 8 \times 10^{12} \text{ molecules of DCMU bound to } 8 \times 10^{12} \text{ PS II units}
\]

If this represents 50% of PS II units, then the total is:

\[
8 \times 10^{12} \times 2 = 1.6 \times 10^{13} \text{ PS II units/µg chlorophyll}
\]

**Calculation b.** Using typical data obtained in class, the calculation for the DCPIP reduction assay with thylakoid vesicles containing 20 µg chlorophyll suspended in 5 ml reaction mixture is as follows.

The rate of photoreduction of DCPIP by isolated thylakoid vesicles is reduced to ~50% of that seen in controls at a DCMU concentration of ~0.1 µmol/l (4). Therefore, the quantity of DCMU in the 5 ml reaction mixture is:

\[
0.1 \text{µmol/l/1} \times \frac{5 \text{mL}}{1000 \text{mL/L}} = 5 \times 10^{-4} \text{ µmol DCMU}
\]

\[
0.5 \times 10^{-4} \text{ µmol DCMU} = 2.5 \times 10^{-3} \text{µmol DCMU/µg chlorophyll}
\]

Multiply $2.5 \times 10^{-5} \text{µmol/µg} = (2.5 \times 10^{-11} \text{mol/µg})$ by Avogadro’s number to derive the number of molecules of DCMU present:

\[
(2.5 \times 10^{-11} \text{mol/µg}) \times (6.022 \times 10^{23}) = 1.5 \times 10^{13} \text{ molecules of DCMU bound to } 1.5 \times 10^{13} \text{ PS II units}
\]

If this represents 50% of PS II units, then the total is:

\[
1.5 \times 10^{13} \times 2 = 3 \times 10^{13} \text{ PS II units/µg chlorophyll}
\]

The value of $1.6 \times 10^{13} \text{ PS II units/µg chlorophyll}$ from **calculation a** is ~53% of $3 \times 10^{13} \text{ PS II units/µg chlorophyll}$, the value from **calculation b**. These values fall comfortably within an order of magnitude.

Finally, to see if these huge numbers are reasonable, one can make a purely back-of-the-envelope calculation using estimates derived from published sources. Both PS I and PS II contain antenna complexes composed of pigment molecules, including chlorophylls, that absorb light energy. The questions is as follows: “How many reaction centers associated with both PS I and PS II are present per microgram of chlorophyll in an isolated thylakoid suspension?” We will assume that the ratio between PS I and PS II is 1:1.

The molar mass of chlorophyll a is 893.49 g/mol and that for chlorophyll b is 907.47 g/mol. Thus, we assume 900 g/mol for the molar mass of chlorophylls in the following calculation:

\[
: 1 \text{ mol chlorophyll weighs } 900 \text{ g}
\]

One mole of chlorophyll contains $6.022 \times 10^{23} \text{(Avogadro’s number)}$ molecules of chlorophyll.

\[
: 1 \text{ chlorophyll molecule weighs } 900 \text{ g} + 6.022 \times 10^{23} = 1.5 \times 10^{-21} \text{g}
\]

Published estimates of the number of chlorophyll molecules per reaction center in PS I and PS II range from ~100 to ~500, so, we assume 300 chlorophyll molecules/reaction center.

\[
: 1 \text{ reaction center weighs } 300 \text{ chlorophyll molecules } \\
\times 1.5 \times 10^{-21} \text{g} = 4.5 \times 10^{-19} \text{g}
\]

\[
: 1 \text{ µg chlorophyll contains } 1 \times 10^{-6} \text{g} + 4.5 \times 10^{-19} \text{g} = 2.22 \times 10^{12} \text{ reaction center}
\]

If the ratio between PS I and PS II is 1:1, then there are:

\[
2.22 \times 10^{12} \text{ reaction centers } \div 2 = 1.11 \times 10^{12} \text{ PS II units/µg chlorophyll}
\]

This value is about one order of magnitude from the values in **calculations a** and **b** using laboratory data.

The value of Fermi estimates is that they “encourage students to reason creatively with approximate quantities and uncertain information” (5). Whether or not the assumptions are accurate is of secondary importance in order of magnitude calculations. As long as the assumptions are in the right ballpark, such calculations are of considerable heuristic value.

There is a good opportunity for peer teaching when students collaborate to solve these types of problems, but if they need guidance a dialectical approach is very useful because the students are already aware of the basic chemistry needed to find an answer. The dialectical (Socratic) approach, a “method of seeking knowledge by question and answer” (6), is useful as “The matters that are suitable for treatment by the Socratic method are those as to which we already have enough knowledge to come to a right conclusion but we have failed, through confusion of thought or lack of analysis, to make the best logical use of what we know” (6). Consequently, simple questions like “What is a mole?” and “What does a mole represent numerically?” can elicit a Eureka response when students suddenly realize how they might proceed.

Students who have attempted these questions report that they enjoyed the challenge and that, using data obtained in the laboratory as a starting point, their understanding of the implications of what had been measured was enhanced. Some were pleased to find that what they had learned in chemistry was useful in a biological context. All, including me, were impressed with the magnitude of the answers. I had expected the numbers to be large but had not expected them to be in the trillions.

In making curriculum recommendations for programs in Molecular Biology and Biochemistry, the American Society for Biochemistry and Molecular Biology provided a list of skills that students should acquire during their undergraduate programs. Among these are “The ability to dissect a problem into its key features” and “The ability to think in an integrated manner” (1). Questions such as those presented here provide students with practice in developing these important skills.

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DISCLOSURES

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