The Chromatography Curriculum in a Writing-Intensive Course

Michelle L. Kovarik
Trinity College, Hartford CT
March 18, 2019

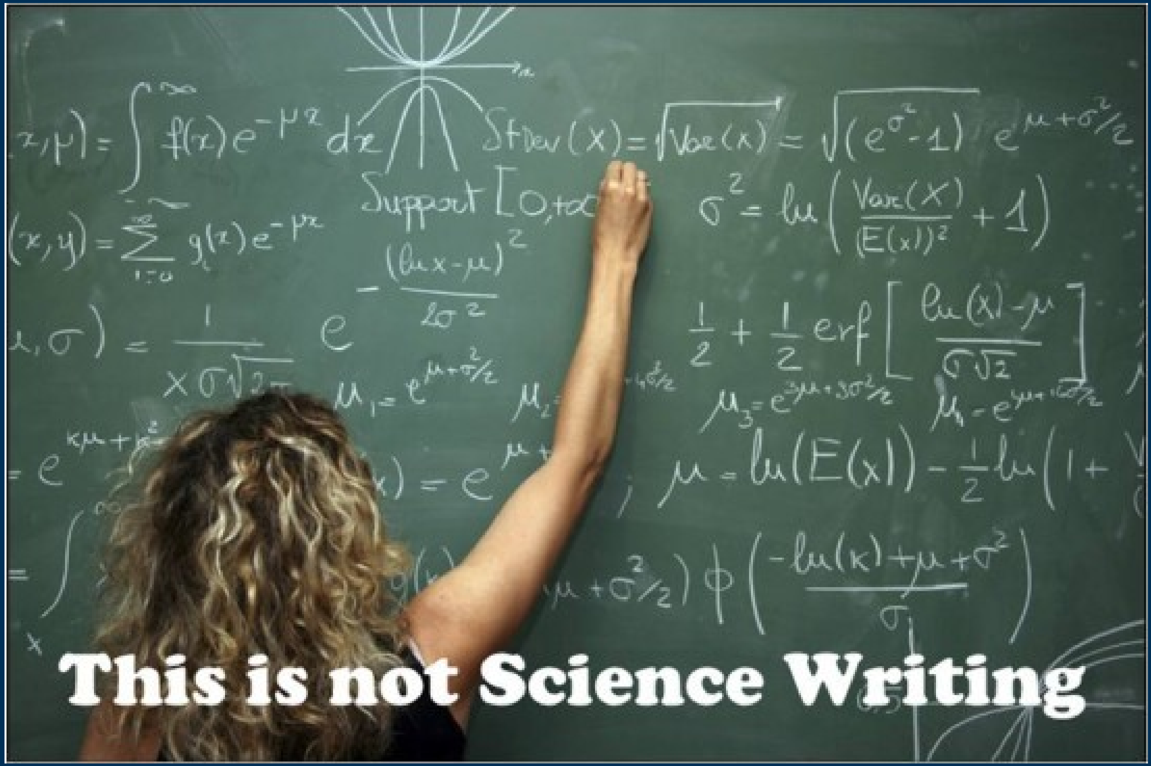


Image from http://www.nextscientist.com/science-writing-human/

Students need to learn how to write

Figure 1: Attributes Employers Seek on a Candidate's Resume

ATTRIBUTE	% OF RESPONDENTS
Communication skills (written)	82.0%
Problem-solving skills	80.9%
Ability to work in a team	78.7%
Initiative	74.2%
Analytical/quantitative skills	71.9%

Source: *Job Outlook 2019*, National Association of Colleges and Employers

We could do a better job teaching them

COMPETENCY	% OF EMPLOYERS THAT RATED RECENT GRADS PROFICIENT*	% OF STUDENTS WHO CONSIDERED THEMSELVES PROFICIENT**
Professionalism/Work Ethic	42.5%	89.4%
Oral/Written Communications	41.6%	79.4%
Critical Thinking/Problem Solving	55.8%	79.9%
Teamwork/Collaboration	77.0%	85.1%
Leadership	33.0%	70.5%
Digital Technology	65.8%	59.9%
Career Management	17.3%	40.9%
Global/Intercultural Fluency	20.7%	34.9%

Source: *Job Outlook 2018* (N=201 employing organizations) and *The Class of 2017 Student Survey Report* (N=4,213 graduating seniors), National Association of Colleges and Employers

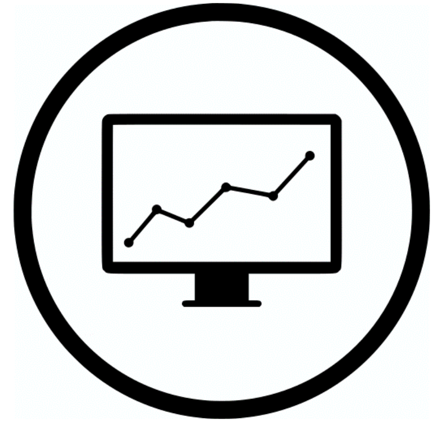
Analytical Chemistry at Trinity College

Lecture Topics	Lab Activities
Statistics and Figures of Merit	Determination of Vanillin in Vanilla Extract by LLE or SPE and UV-Vis Absorbance
Acid-Base Equilibria	Chromatography Rotations: HPLC Analysis of Chocolate GC-MS Analysis of Fatty Acids
Separations Science	Student-Proposed Projects

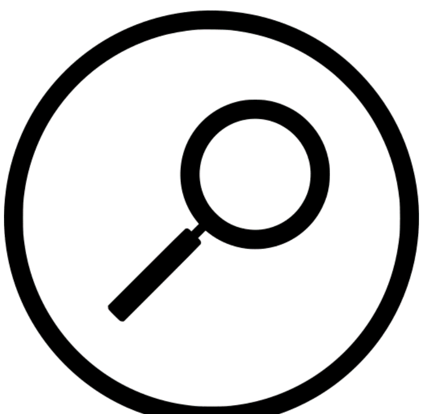

Writing-to-Learn
Interpretation of Authentic Data
Norms and Rhetorical Strategies
Capstone Lab Project

Writing-to-Learn

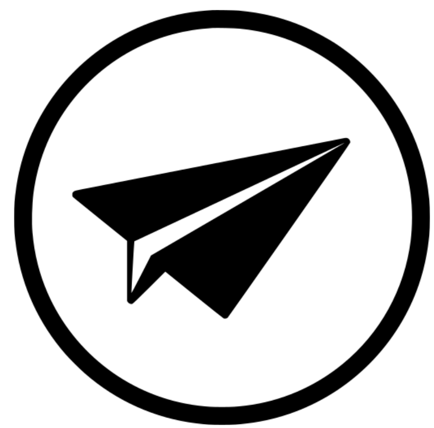
Interpretation of Authentic Data



Norms and Rhetorical Strategies



Capstone Lab Project



Writing-to-Learn: Annotated Problem Solving

Calculate the pH of 1.0 L of buffer composed of 0.10 M phenol and 0.050 M sodium phenolate after the addition 2.0 mmol of solid NaOH. The pK_a of phenol is 9.89.

Step 1: Stoichiometry

Where did that equation come from?

$$HA + OH^- \rightleftharpoons A^- + H_2O$$

$2\ mmol = 0.002\ mol$

$$[\text{HA}]_{after} = \frac{mol\ HA\ start - mol\ HA\ consumed}{Volume}$$

$$[\text{HA}]_{after} = \frac{0.1\ mol\ HA - 0.002\ mol\ HA}{1\ L} = 0.098\ M\ HA$$

How do we know this reaction goes to completion? In other words, why are we treating this like a stoichiometry problem?

$$[A^-]_{after} = \frac{mol\ A^-\ start + mol\ A^-\ formed}{Volume}$$

$$[A^-]_{after} = \frac{0.05\ mol\ A^- + 0.002\ mol\ A^-}{1\ L} = 0.052\ M$$

Step 2: Equilibrium

(Method A: Do an ICE Table)

Why are we using a different chemical reaction equation now than in Step 1?

	HA	+ *H₂O* ⇌	*A⁻*	+ H_3O^+
I	0.098	|	0.052	0
C	-x	|	+x	+x
E	0.098-x	|	0.052+x	x

Why is it OK to neglect +*x* and–*x* for A^- and HA, but not the *x* for H^+?

$$K_a = 10^{-9.89} = 1.3 \times 10^{10}$$

$$1.3 \times 10^{-10} = \frac{x(0.052 + x)}{0.098 - x} \approx \frac{0.052x}{0.098}$$

Useful Resource:
Student Writing in the Quantitative Disciplines: A Guide for College Faculty, by Patrick Bahls, Jossey Bass, 2012.

Writing-to-Learn: Annotated Problem Solving

Window cleaner contains ~7.5 mM aqueous ammonia. The pK_a of the ammonium ion is 9.24. If the cleaner is unbuffered, calculate its pH. In the right column, explain your strategy.

Solution	Explanation
Start by writing a reaction equation.	Why did you choose this equation?
Set up an ICE table.	Why is it necessary to use an ICE table for this problem?
Plug in to the expression for K_b.	Where did you get the value for K_b?
	Why use K_b and not K_a?
Solve for x.	What does x represent chemically?

Useful Resource:

Student Writing in the Quantitative Disciplines: A Guide for College Faculty, by Patrick Bahls, Jossey Bass, 2012.

Writing-to-Learn: DEOI Lab Notebooks

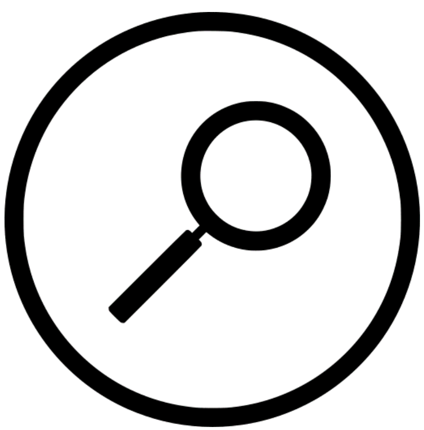
Decision	Explanation	Observation	Inference
Step 4. When the reaction period is over, let the mixture cool to room temperature. Test it with starch-iodide paper, and if the test is positive, add enough saturated sodium bisulfate solution dropwise to give a negative test.	What does bisulfate do? It is a reducing agent that can destroy any excess HOCl that remains after the reaction is over. Why use sodium bisulfate to get rid of HOCl? We cannot dispose of HOCl ourselves because it will react violently with the other solutions in the waste jar.	What do you see? We tested the mixture with starch paper and it turned purple. After adding sodium bisulfate and swirling the mixture the starch paper turned clear.	There is no HOCl present in the solution.

Reference and Useful Resource: Andrea Gay Van Duzor, "Using Self-Explanations in the Laboratory To Connect Theory and Practice: The Decision / Explanation / Observation / Inference Writing Method" *J. Chem. Educ.* **2016**, *93*, 1725-1730.

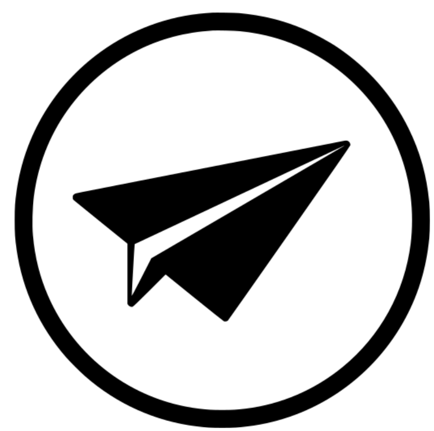
Writing-to-Learn

Interpretation of Authentic Data

Norms and Rhetorical Strategies



Capstone Lab Project



Interpretation of Authentic Data: Reading Assignments

In-Class Questions

Reading Assignment 3: B.Wei, D.S. Malkin, and M.J. Wirth, "Plate heights below 50 nm for protein electrochromatography using silica colloidal crystals," *Anal. Chem.* **2010**, *82*, 10216-10221.

1. When characterizing plate height for lysozyme in Figures 4 and 5, the authors determine the width of the peak in space rather than in time. They are able to do this because they are using a camera as a detector, but why do they need to do this? How does the width of the peak in space relate to the width of the peak in time? How does the detector contribute to plate height in these experiments?

2. In discussing Figure 6, the authors assert that the *A* and *C* terms of the van Deemter equation are negligible for their separations. In Figure 7 and the latter part of the Results & Discussion, the authors address whether the extremely low plate heights observed could be due to focusing rather than to the achievement of a diffusion-limited separation. Why would the *A* and *C* terms be negligible under the conditions used in this work? What evidence supports the authors' assertion that the efficiency of their separations is limited only by diffusion?

3. The authors specifically state that their goal for this work was not to achieve a practical method for protein separations. That would have been outside the scope of this paper because many practical considerations would need to be addressed before this type of packing could be made available in commercial columns. Imagine that an instrument manufacturer wants to use columns like these in a commercial HPLC instrument. What changes to the instrument and practical improvements in the column would be needed?

Useful Resources:

Analytical Sciences Digital Library (www.asdlib.org)

Kovarik, "Use of primary literature in the undergraduate analytical class," *Anal. Bioanal. Chem.* **2016**, *408*, 3045-3049.

Interpretation of Authentic Data:
Reading Assignments

Practice Exercises: Selectivity and Efficiency

These practice exercises are based on the article "Characterization of fullerene-modified silica as a complement to graphite-like phases for use in two-dimensional high performance liquid chromatography" by Tuan A. Tran, Ian Gibbs-Hall, Paul J. Young, Jonathan D. Thompson, and Dwight R. Stoll, *Analytical Chemistry*, **2013**, *85*, 11817-11825.[55]

This paper describes the use of fullerene molecules, also known as buckyballs, as a stationary phase for liquid chromatography. The performance of the fullerene-modified stationary phase (FMS) is compared to that of a more common C18 stationary phase and to two other carbon-based stationary phases, PGC and COZ.

1. Define selectivity, α, with words and an equation.

2. Explain how the choice of stationary phase affects selectivity.

3. Calculate the resolution of the nitrobenzene and toluene peaks in the top trace of Figure 2. (Estimate any values you need from the graph, and show your work.)

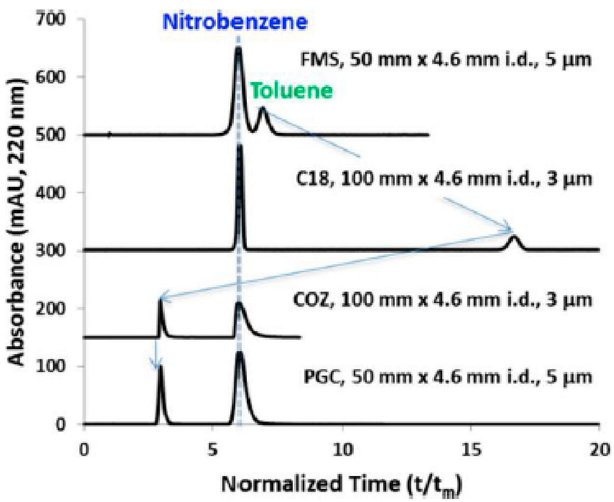


Figure 2. Normalized retention of toluene and nitrobenzene on a conventional C18 bonded phase compared to FMS, PGC, and COZ. Eluent compositions [acetonitrile/water (A/W) v/v] were adjusted to obtain a retention factor of about 6.0 for nitrobenzene in each case: FMS (23.5/76.5 A/W), C18 (35/65 A/W), COZ (43/57 A/W), and PGC (54/46 A/W). All other conditions were constant: flow rate 2 mL/min, column temperature 40 °C, and injection volume 1 µL.

Useful Resource:
Please contact me if you are interested in using these assignments!

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Interpretation of Authentic Data: Reading Assignments

4. In Figure 4, each stationary phase shows some negative correlation between plate count and retention factor. In other words, as *k*' increases, *N* decreases. Explain this relationship between *k*' and *N*.

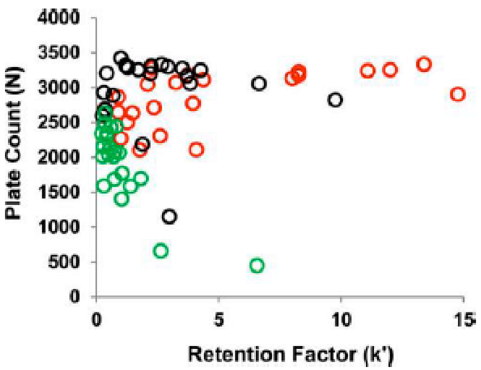

Figure 4. Column efficiency (N) vs retention factor (k′) for 22 nonionizable solutes on FMS (red), PGC (black), and COZ (green). Eluent compositions (acetonitrile/water, A/W) were adjusted to obtain k′ less than 15, which was achieved for most solutes as follows: FMS (30/70 A/W), PGC (60/40), COZ (80/20). Slightly different compositions were used for the most highly retained solutes. All columns were 50 mm × 4.6 mm i.d. and packed with 5 μm particles, except for COZ, which was packed with 3 μm particles. All other chromatographic conditions were constant: column length 5 cm, column i.d. 4.6 mm, flow rate 2 mL/min, column temperature 40 °C, and injection volume 0.5 μL.

5. Figure 5 shows retention factor data for 16 solutes used in the hydrophobic subtraction model (HSM), which is used to characterize how ionizable solutes will behave on a new stationary phase. (You will notice that many of the HSM solutes are weak acids and/or weak bases.) Which stationary phase (FMS or C18) is better at retaining the HSM solutes? Why is this the case?

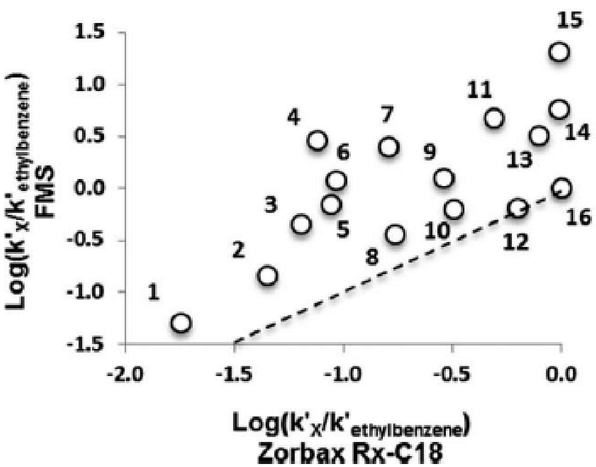

Figure 5. Retention of 16 HSM solutes relative to ethylbenzene on the FMS phase vs Zorbax Rx-C18. Data for the Rx-C18 phase were from Lloyd Snyder (personal communication). The dashed line has a slope of +1 and a y-intercept of 0. The R^2 value for this plot is 0.51. The Rx-C18 column was 150 mm × 4.6 mm i.d. with 5 μm particles, whereas the FMS column was 50 mm × 4.6 mm i.d. with 5 μm particles. All other chromatographic conditions were constant: mobile phase 50/50 ACN/60 mM potassium phosphate buffer at pH 2.8, flow rate 2 mL/min, column temperature 35 °C, and injection volume 10 μL.

Useful Resource:
Please contact me if you are interested in using these assignments!

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Interpretation of Authentic Data: Revision of Lab Report Guidelines

Question

What were the optimum conditions for the separation?

Evidence

- Include representative sample chromatograms (but not all of the raw data). Label the peaks in your chromatograms and ensure that all axes labels are legible.
- Include the graph of k' versus mobile phase composition.
- Discuss how the changes to mobile phase composition affected the overall separation and k' in particular.

- Report the mobile phase composition you chose to use for quantitative analysis, and justify your choice.

- Discuss how the changes to temperature affected the separation.

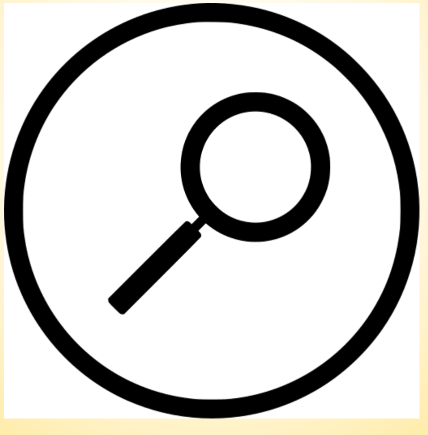
- Report the temperature you chose to use for quantitative analysis, and justify your choice.

Argument

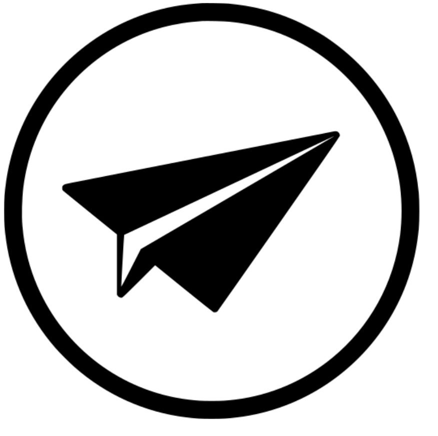
Writing-to-Learn

Interpretation of Authentic Data

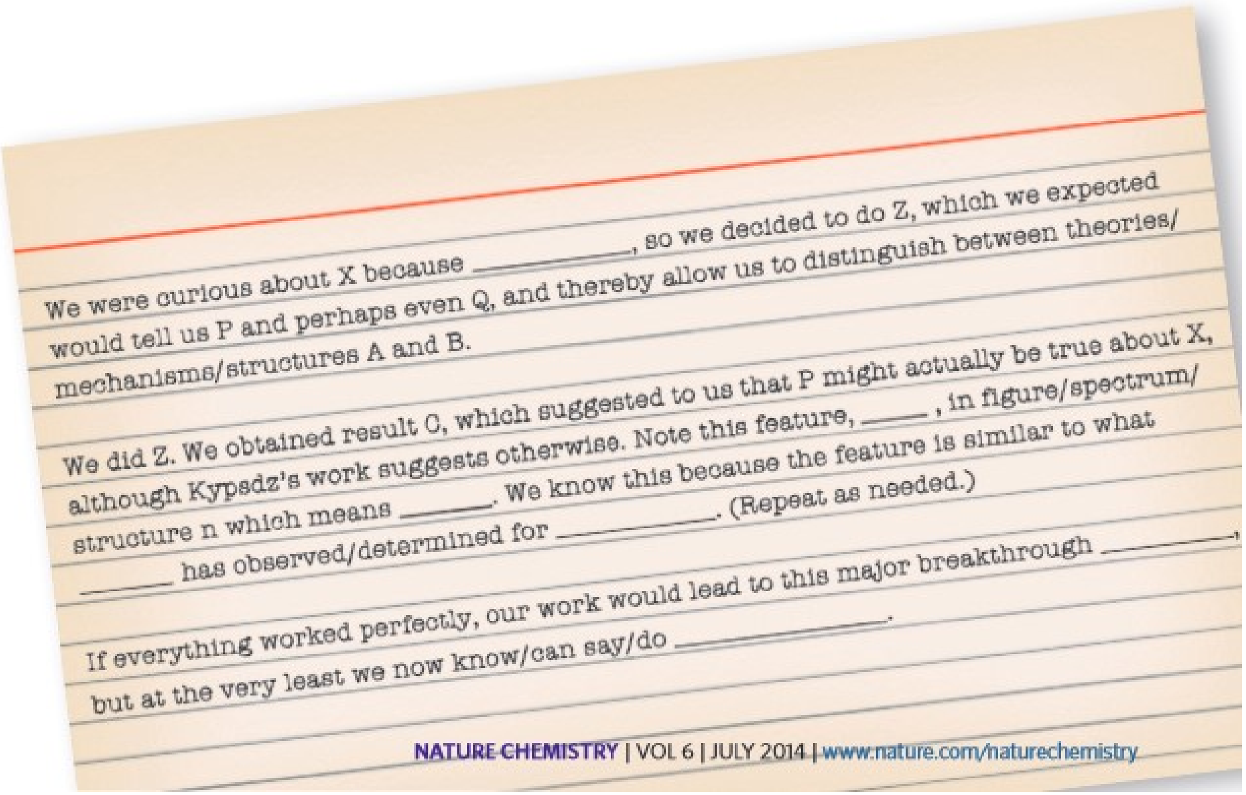
Norms and Rhetorical Strategies



Capstone Lab Project



Norms and Rhetoric:
Showing Students the Patterns


We were curious about X because ________, so we decided to do Z, which we expected would tell us P and perhaps even Q, and thereby allow us to distinguish between theories/mechanisms/structures A and B.

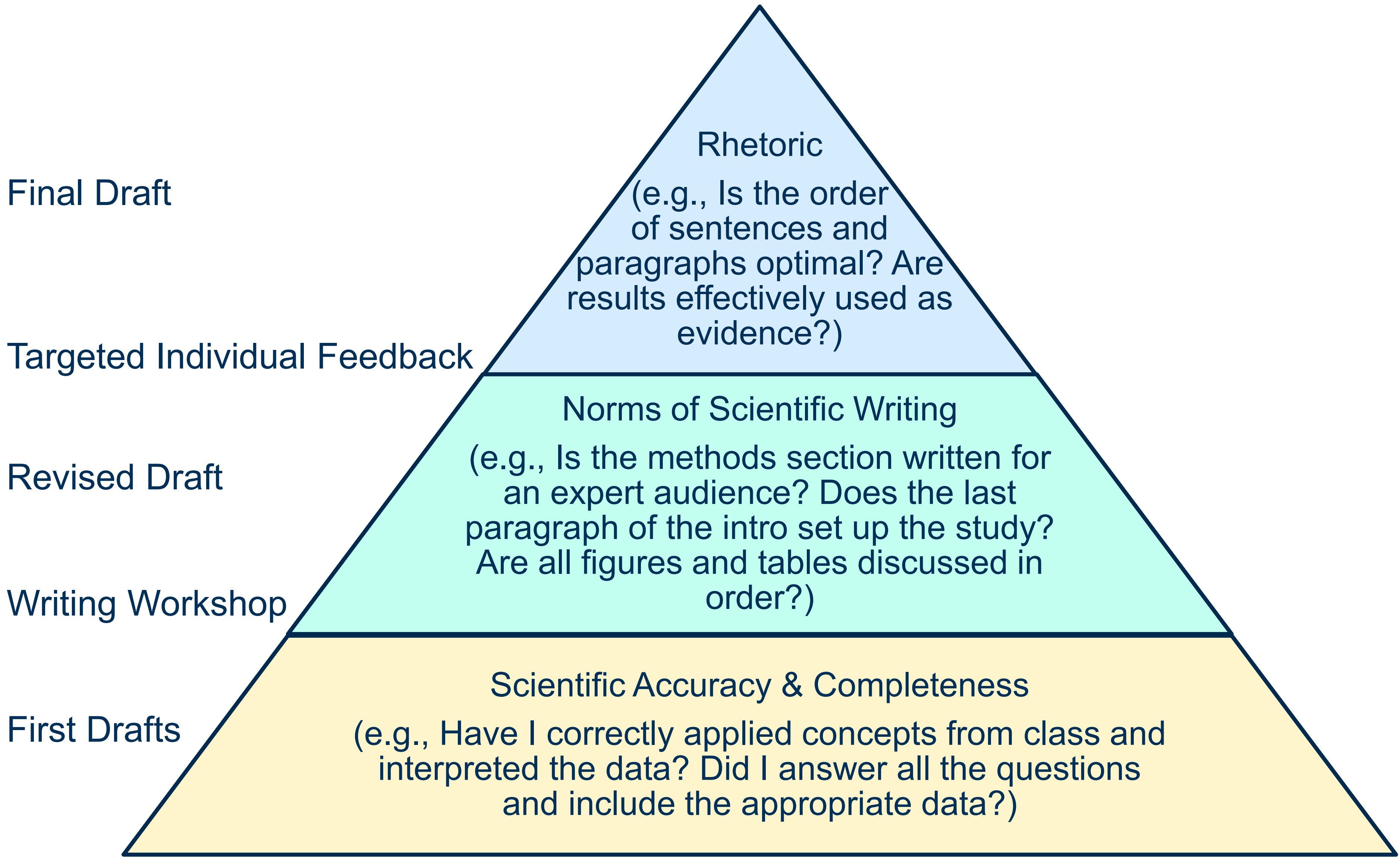
We did Z. We obtained result C, which suggested to us that P might actually be true about X, although Kypsdz's work suggests otherwise. Note this feature, ____ , in figure/spectrum/structure n which means ______. We know this because the feature is similar to what ______ has observed/determined for ________. (Repeat as needed.)

If everything worked perfectly, our work would lead to this major breakthrough ________, but at the very least we now know/can say/do ____________.

NATURE CHEMISTRY | VOL 6 | JULY 2014 | www.nature.com/naturechemistry

Reference and Useful Resource:
Michelle Francl, "The write stuff" *Nature Chemistry*, **2014**, *6*, 555-556.

Norms and Rhetoric:
Drafting and Revision Process



Norms and Rhetoric:
Workshop Activities

Level	Task	Description
1	Listing	Display of important terms
2	Definition	Brief or extensive explanation of a word or concept
3	Seriation	Ordered list or description of a procedure
4	Classification	Application of specific categories to specific data
5	Summary	Identification of important facts or ideas in a text
6	Comparison/Contrast	Listing/analysis of similarities and differences
7	Analysis	Breaking down a complex idea into its constituent parts
8	Academic/Scientific Argument	Use of facts and theories to support a proposition

Reference and Useful Resource: Jeffrey Kovac and Donna W. Sherwood, "Writing in Chemistry: An Effective Learning Tool," *J. Chem. Educ.* **1999**, *76*, 1399-1403.

Norms and Rhetoric:
Workshop Activities

1. Put the subject and the verb as close together as possible.
2. Each unit of discourse should make a single point.

The smallest of the URF's (URFA6L), a 207-nucleotide (nt) reading frame overlapping out of phase the NH_2-terminal portion of the adenosinetriphosphatase (ATPase) subunit 6 gene <u>has been identified</u> as the animal equivalent of the recently discovered yeast H^+-ATPase subunit 8 gene.

The smallest of the URF's <u>is</u> URFA6L, a 207-nucleotide (nt) reading frame overlapping out of phase the NH_2-terminal portion of the adenosinetriphosphatase (ATPase) subunit 6 gene; **it** <u>has been identified</u> as the animal equivalent of the recently discovered yeast H^+-ATPase subunit 8 gene.

The smallest of the URF's (URFA6L) <u>has been identified</u> as the animal equivalent of the recently discovered yeast H^+-ATPase subunit 8 gene.

<u>Reference and Useful Resource</u>: Gopen and Swan, "The Science of Scientific Writing," *American Scientist*, **1990**, *78*, 550-558.

Norms and Rhetoric:
Workshop Activities

3. Important information should be in the *stress position* at the end of the sentence.
4. The **topic position** should provide transition and context by presenting "old" information.

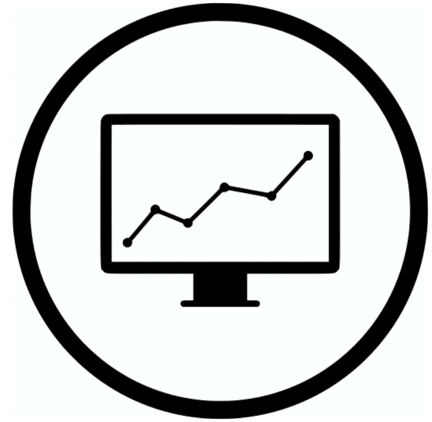
The rates at which tectonic plates move and accumulate strain at their boundaries are *approximately uniform*. Therefore, in first approximation, **one** may expect that large ruptures of the same fault segment will occur at *approximately constant time intervals*. If **subsequent main shocks** have *different amounts of slip across the fault*, then **the recurrence time** may vary, and **the basic idea of periodic mainshocks** must be modified.

The rates at which tectonic plates move and accumulate strain at their boundaries are *roughly uniform*. Therefore, **nearly constant time intervals** (at first approximation) would be expected **between large ruptures of the same fault segment**. [However?], **the recurrence time** may vary; **the basic idea of periodic mainshocks** may need to be modified if subsequent mainshocks have *different amounts of slip across the fault*.

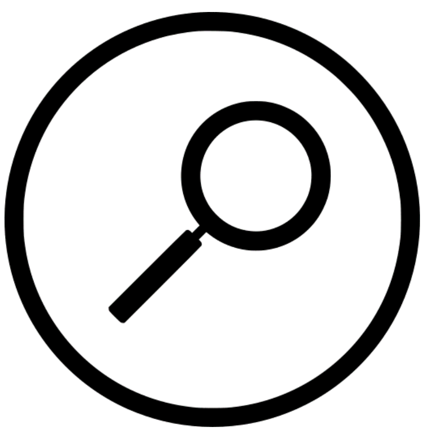
Reference and Useful Resource: Gopen and Swan, "The Science of Scientific Writing," *American Scientist*, **1990**, *78*, 550-558.

Writing-to-Learn

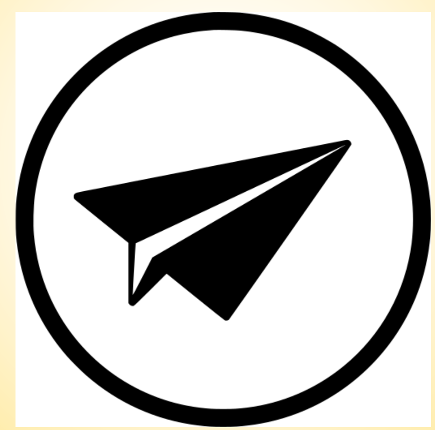
Interpretation of Authentic Data



Norms and Rhetorical Strategies



Capstone Lab Project



Capstone Project: Student-Designed Experimentation

Each student submits 3-5 ideas in the form of "How much X is in Y?"

↓

Instructor selects a subset of feasible potential projects.

↓

Students are sorted into groups based on their interest in projects.

↓

Each group submits a joint proposal including statement of significance, proposed methods, and budget.

↓

Students spend 4 weeks in lab collecting data.

↓

Individual students submit their own final formal report.

Capstone Project:
Student-Designed Experimentation

Trinity College Chemistry

PROJECT REPORT

HPLC Analysis of Catechin Content Difference between Freshly-Brewed Green Tea and Bottled Green Tea

N.S. Andersen,[a] K. N. Reardon[b] and M. A. Rednor[c]

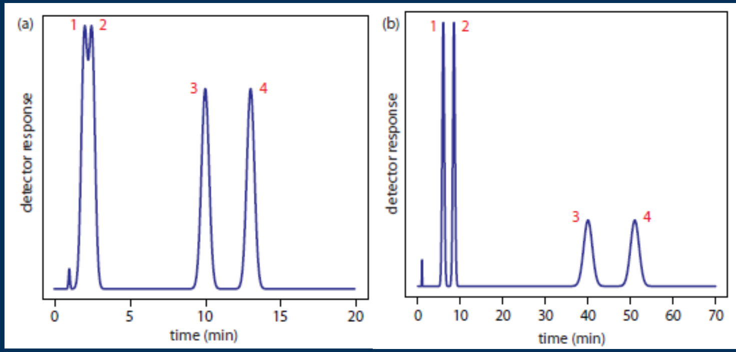
CHEM 311
Analytical Chemistry
Fall 2018

Green tea has numerous health benefits including prevention of cancer, cardiovascular disease, and type II diabetes as well as decreasing inflammation and obesity. These benefits have been attributed to the catechins in tea, which are bioactive compounds and powerful antioxidants. The catechins in green tea were isolated in this experiment using HPLC. The chromatographic separations were carried out using a Grace C18 column (15 cm x 4.6 mm x 5 µm) with an oven temperature of 40°C, detector UV 223 nm and flow rate of 1.000 mL/minute. The mobile phase consisted of solvent A, 10 mM sodium phosphate (pH 2.6), and solvent B, acetonitrile, in a 93:7 ratio. The freshly brewed green tea was found to have a significantly higher catechin content than the bottle green tea.

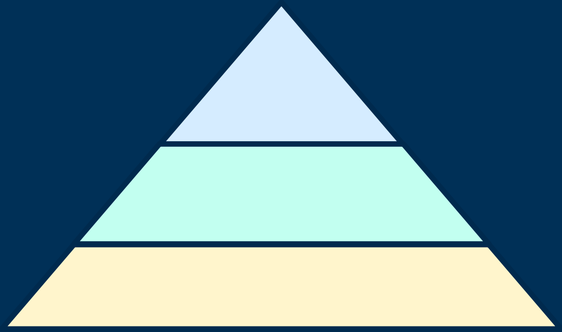
Student work shared with permission.

Conclusions / Take-Away Messages

- Chromatography theory and practice provide a dense, rich topic for student writing.



D.T. Harvey



- Best outcomes were attained by scaffolding assignments and providing multiple opportunities for revision and practice.

- Don’t be afraid of teaching writing to chemistry students!

Acknowledgments & References

Trinity College Writing Center & Writing Fellows Program

esp. Tennyson O'Donnell, Irene Papoulis, Erin Frymire, and Nick Merino

Chem 311 students of Trinity College

Research Corporation for Scientific Advancement

Patrick Bahls, *Student Writing in the Quantitative Disciplines: A Guide for College Faculty*, Jossey-Bass, 2012.

Andrea Gay Van Duzor, "Using Self-Explanations in the Laboratory To Connect Theory and Practice: The Decision / Explanation / Observation / Inference Writing Method" *J. Chem. Educ.* **2016**, *93*, 1725-1730.

Michelle Francl, "The Write Stuff" *Nature Chemistry*, **2014**, *6*, 555-556.

Jeffrey Kovac and Donna W. Sherwood, "Writing in Chemistry: An Effective Learning Tool," *J. Chem. Educ.* **1999**, *76*, 1399-1403.

Gopen and Swan, "The Science of Scientific Writing," *American Scientist*, **1990**, *78*, 550-558.

Barbara E. Walvoord and Virginia Johnson Anderson, Chapter 8: "Making Grading More Time-Efficient" in *Effective Grading: A Tool of Learning and Assessment*, Jossey-Bass, 1998.

Week of	Lecture	Lab	Assignment Due (8 am)
Sept 4	Sampling & Sample Prep	Sampling	
Sept 11	Accuracy, Precision, Statistical Testing	Sample Preparation	Sampling Lab Spreadsheet
Sept 18	Figures of Merit	Calibration	Sample Prep Write-Up
Sept 26	Calibration Methods	Effect of pH	Calibration Write-Up
Oct 2	Solubility Equilibria	Writing Workshop	pH Write-Up
Oct 9	Ionic Strength & Activity	Trinity Days – No Lab	Vanillin Introduction
Oct 16	Acid-Base Equilibria	Chromatography 1A	Vanillin Full Report
Oct 23	Acid-Base Equilibria	Chromatography 1B	Project Proposal
Oct 30	Buffers	Chromatography 2A	Chromatography 1 Write-Up
Nov 6	Titrations, ISEs	Chromatography 2B	Revised Vanillin Report
Nov 13	Selectivity	Project Lab (Week 1)	Chromatography 2 Write-Up
Nov 20	Efficiency	Project Lab (Week 2)	Project Introduction
Nov 27	GC	Project Lab (Week 3)	Figure & Question Outline
Dec 4	HPLC and CE	Project Lab (Week 4)	Project Methods