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In the Classroom

The Evolution of a Laboratory Syllabus for Quantitative Analysis

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The charm of this syllabus for the instructor was in the ease and objectivity of the grading.

In this paper, we describe the evolution of a laboratory syllabus for a course in Quantitative Analysis. Over the past two decades, the syllabus has changed from one having individual students do mostly "wet" chemical analyses on commercial unknowns to one having groups of students do instrumental analyses on "real" samples. We describe, in some detail, the current laboratory syllabus, which requires students to calibrate volumetric equipment, to determine the active ingredients in Dristan using UV-visible absorption spectroscopy, to determine calcium levels in over-the-counter tablets using both atomic absorption spectroscopy and an ion-selective electrode , to determine lead in wine bottle caps using differential pulse polarography, to measure pK_a values for sulfonated naphthols using UV-visible absorption spectroscopy and to determine caffeine and

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aspartame in common beverages using HPLC. Group work is emphasized, and written reports are required. Students have responded quite positively to the current syllabus, especially to the use of “real” samples.

Analytical chemistry has a long history at Union College. As early as 1857, the college catalog lists a Department of Analytical Chemistry, and in that same year the college offered a laboratory course in analytical chemistry, which is thought to be the first such offering on an American college campus. Currently, the college, which has an undergraduate enrollment of 2000, has two analytical chemists in its ten tenure-track chemistry lines. Two courses in analytical chemistry are offered for the chemistry major: Quantitative Analysis (Chem 40), which typically enrolls 20–25 students per year, and Chemical Instrumentation (Chem 142), which typically enrolls 6–12 students per year. In both courses students are in the laboratory for six hours per week for a total of 60 hours in a ten-week term.

While much has been written about the need to update laboratory syllabi for introductory and organic chemistry courses, it can be argued that no syllabus has been more static than that which exemplifies the prototypical laboratory for Quantitative Analysis. In this paper we describe changes in a laboratory syllabus for Quantitative Analysis over the past two decades, as well as discuss the philosophy driving these changes. We also describe in some detail the present syllabus for this course.

Until the early 1980s, Introductory Chemistry was the only prerequisite for Chem 40. The syllabus at this time included an acid-base titration of carbonate using hydrochloric acid, a redox titration for iron using dichromate, a complexometric titration for calcium using EDTA, a gravimetric procedure for sulfate using barium, the determination of copper by both electrodeposition and DC polarography, and the determination of calcium by ion-selective electrode. All determinations involved the use of commercial (Thorn Smith) unknowns. A student’s grade on a given determination depended exclusively on how close her or his reported value came to the listed value for the commercial unknown.

The charm of this syllabus for the instructor was in the ease and objectivity of the grading. Moreover, many students developed good laboratory habits and confidence in

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their laboratory skills. There were, however, several serious problems and deficiencies with this syllabus.

- There was too much emphasis on classical techniques, causing the laboratory to be more "ritualistic" than reflective of modern analytical chemistry.
- Commercial unknowns have limited appeal for students (as well as for instructors).
- There was little opportunity for students to interact and no opportunity for them to improve their writing skills.
- There were waste-disposal problems with some of the laboratories (barium, dichromate).

By the late 1980s the syllabus had changed to address some of these shortcomings. The gravimetric procedure and the redox and complexometric titrations were dropped, thereby eliminating the two worst waste-disposal problems. A spectrophotometric procedure for the analysis of P_2O_5 in commercial phosphate rock unknowns was added, which produced heavy metal (vanadium and molybdate) wastes. In addition, two project-type laboratories, performed by students working in groups of two or three, were added to the syllabus. In the first of these, entitled "The Titration of Multifunctional Bases," students determined the complete titration curve (using 0.2 M HCl) of a solid unknown that could contain one of the following: CO_3^{2-}, a mixture of CO_3^{2-} + HCO_3^-, PO_4^{2-} or a mixture of PO_4^{3-} + HPO_4^{2-}. While a goal of this experiment was to quantitate the analyte(s), the primary role of this experiment was to give students a better understanding of acid-base equilibria by having them compare calculated and measured pHs along the titration curve. A full description of this experiment has been published [1].

The second project involved the analysis of a "real" sample. Students were given the option to choose among several such analyses, some examples of which are given below, and were required to submit written reports for each of these project labs.

- Iron in Vitamin Tablets by Visible Absorption Spectroscopy [2].
- Acetylsalicylic Acid in Aspirin Tablets by Fluorescence Spectroscopy [3].
- Lead in Drinking Water by Differential Pulse Polarography with Anodic Stripping.

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The syllabus has continued to evolve throughout the decade of the 1990s. This evolution was aided by the additional requirement that students must have completed at least one term of organic chemistry (almost all students have taken two terms) to enroll in Chem 40. What follows is a description of the present Chem 40 syllabus, which we believe adequately addresses the failings of the traditional Quantitative Analysis Laboratory syllabus.

The Current Laboratory Syllabus for Chem 40

1. The Care and Feeding of Volumetric Equipment

This laboratory requires three 3-hour laboratory periods.

There are several general goals for this experiment:

- to acquaint the student with the proper use of volumetric equipment.
- to demonstrate how such equipment can be calibrated.
- to demonstrate the number of significant figures to include in calculations requiring volumetric measurements with this equipment.
- to introduce statistical tests which allow for the evaluation of the accuracy and precision of measurements with this equipment.
- to introduce students to the use and limitations of Beer's Law.

In the course of this experiment each student calibrates the following volumetric equipment by measuring the mass of water each delivers or contains at a given temperature:

- a volumetric flask (50- or 100-mL).
- a 1-mL Class A glass transfer pipet.
- a 100- to 1000-μL digital pipet at its **maximum** and **minimum** settings.
- a 10- to 100-μL digital pipet at its **maximum** setting **only**.

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a 1- to 10-mL digital pipet (Eppendorf Maxipettor) at its **maximum** and **minimum** settings using two types of pipet tips (short Maxitip P and long Maxitip S).

A real advantage of this experiment is the ease with which students can make repeat measurements. As a consequence, they can do *Q* tests to eliminate outliers and then to determine mean values, standard deviations, and relative standard deviations from their "acceptable" replicates. With these data, they can make comparisons of accuracy and precision, as well as make decisions based on t tests. In their laboratory report discussion section, we ask students to use their statistical data to answer the following questions.

1. Are the reported (mean) values for the volumes of your volumetric flask and transfer pipet within the tolerances listed in the text?

2. How do the accuracy and precision of a 1-mL measurement compare using the glass transfer pipet with those values obtained using the 1000-µl digital pipet?

3. How do the accuracy and precision of a 100-µL measurement compare using the 1000-µl digital pipet at its lower limit with these values obtained using the 100-µL digital pipet at its upper limit? Do these data suggest a guideline that you might propose when a choice of pipets exists to provide a given volume?

4. Does the type of pipet tip affect the accuracy and precision of volume measurements with the Maxipettor? Any guidelines lurking in these data?

5. How many significant figures should be reported for each of these volume measurements?

In the second part of this experiment, we ask student groups (2–3 students/group) to prepare a thymol blue (TB) solution in triplicate for absorbance measurements using an assigned volume of a Stock TB solution (9.0×10^{-4} M TB in 0.010 M NaOH). Students add the assigned volume (250 to 2000 µL) and 1.00 mL of 1.2 M HCl to 10 mL volumetric flasks, dilute to the mark, and measure the absorbances of their solutions at 394 nm and 544 nm using an Hewlett-Packard 8452A UV-visible spectrophotometer. When students calculate the mean and standard deviation of their triplicate measurements, they see that absorbance data are typically good to three

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significant figures. Mean absorbance data are collected for each of the assigned solutions, the class data are pooled, and Beer's law plots are prepared at both wavelengths. What students observe is good adherence to Beer's Law at 394 nm where the maximum absorbance is about 1.5, but considerable downward curvature in the Beer's Law plot at 544 nm for absorbances above 2. This leads to an in-class discussion of the limitations to Beer's Law due to the presence of stray and scattered light, the outcome of which is a maximum absorbance guideline for the preparation of standards to be used in UV-Visible absorption analyses.

2. Determination of the Active Ingredients in Dristan By UV Absorption Spectroscopy
This laboratory requires three to four 3-hour laboratory periods.

The use of Beer's Law described above prepares students quite well for this experiment, which has been described by Williams et al. [4]. The absorption spectra of the active ingredients of Dristan, pheniramine (maleate salt, PAM) and L-phenylephrine (PEH), overlap completely, thereby requiring the use of simultaneous equations for the determination. Students must measure molar absorptivities for both ingredients at two wavelengths using Beer's Law plots and then demonstrate absorbance additivity by using *t* tests to compare measured and calculated absorbances of known mixtures of PAM and PEH.

3. Determination of Calcium in Over-the-Counter (OTC) Tablets by Atomic Absorption Spectrometry (AAS)
This experiment requires three 3-hour laboratory periods.

At this point students have a good feel for molecular absorption, so we now introduce a determination requiring atomic absorption. After briefly lecturing on the differences between molecular and atomic absorption, we have students perform an experiment based on one developed by Quigley, who used Bufferin as the OTC tablet [5]. We ask students to adapt this procedure to some other OTC tablet of their choice, which requires them to decide on stock solution concentrations and dilutions to give test solutions within the range of their standards (5–40 ppm Ca). Such adaptations are commonly required by practicing analysts, and asking students to become involved in experimental design is useful pedagogy. Students then repeat the measurements on new test solutions containing La^{3+} as a Ca^{2+} releasing agent [5]. Those with OTC

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tablets containing phosphate see a significant increase in the reported Ca level due to the releasing effect of the La^{3+}, which leads to an in-lab discussion of this effect.

4. Determination of Calcium in Over-the-Counter (OTC) Tablets by Ion Selective Electrode

This experiment requires two to three 3-hour laboratory periods.

In the next two determinations we introduce electrochemical methods (potentiometry and polarography) by a combination of in-lab lectures and handouts.

In the first of these, students repeat the analysis of their OTC tablet by using an ion-selective electrode to compare the mV reading for a solution containing their dissolved tablet with a standard curve (mV vs. log $[Ca^{2+}]$) and by standard addition. Both determinations are done in triplicate, and the mean values from both are compared in a subsequent written report with the % Ca value they obtained previously by AAS. From this comparison, students realize that different analytical methods often give different answers because interferences do not affect all methods to the same extent. For example, OTC tablets containing phosphate are likely to give better results by standard addition than by use of a standard curve. These comparisons are also a good source for in-lab discussions.

5. Determination of Lead in Wine Bottle Tops by Differential Pulse Polarography (DPP)

This experiment requires two 3-hour laboratory periods.

This laboratory is the result of the discovery of a bag of Pb-containing wine bottle tops at the home of one of the authors. We had students dissolve about 1 g of a bottle top by heating the sample for 20–30 minutes in 35 mL of 6M HNO_3 and then analyze the diluted sample for lead using DPP with standard addition. The tops were virtually 100% Pb, which led to a discussion of why these tops are used less frequently than in the past. Students then are asked to precipitate out the Pb^{2+} from their sample and standards by the addition of SO_4^{2-} for waste disposal.

6. Determination of the pK_a Values of Sulfonated Naphthols by UV Absorption Spectroscopy

One of the most important topics covered in Quantitative Analysis is acid-base equilibrium. Perhaps the key concept in acid-base chemistry, with regard to its

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importance in other areas of chemistry, is the pK_a. While many students can readily provide a definition of this parameter, most have only a vague appreciation of its significance and no idea about how it is measured. This experiment is designed to alleviate these deficiencies by having students measure the pK_a of sulfonated naphthols by absorption spectroscopy.

Sulfonated naphthols are good choices for this experiment because a variety of these derivatives having adequate water solubility are readily available. Moreover, their pK_a values can be determined easily by measuring the absorbance of the base form of the naphthol as a function of pH.

The experimental procedure is simple. Students prepare a 250-mL solution of a given naphthol in its base form by dissolving an amount of it in 0.010 M Na_3PO_4 to give a maximum absorbance of 1 to 1.5 in the range from 280 to 450 nm. A 100-mL aliquot of this solution is placed in a beaker along with pH electrodes and a stir bar. A Hewlett-Packard 8452A UV-visible spectrophotometer is set up in the overlay mode, and spectra are obtained on aliquots of this solution as the pH is changed by the addition of 10 to 100 μL volumes of 2 M or 4 M HCl. By returning each aliquot to the original solution before a subsequent addition of HCl, students are able to change the pH without significantly affecting total solution volume. As a consequence nice isosbestic points are obtained. Students generally overlay 7–9 spectra extending over a pH range from that of the initial solution (about 11.7) down to about 7.

We have students determine the pK_a in two ways: from the inflection point of a plot of A (base form) as a function of pH and from the intercept of a plot of the Henderson-Hasselbalch equation (equation 1), where A_t is the absorbance of the base form at a wavelength where only it absorbs and at a pH where only it is present, and A is the absorbance at this same wavelength at pH values where both forms are present.

$$\mathrm{pH} = \mathrm{p}K_a + \log\,[A/(A_t - A)] \qquad (1)$$

We hold an in-lab discussion of these relations to ensure that students understand how pK_a can be extracted from these plots. The pK_a data for several naphthols obtained by a recent Chem 40 class are given in Table 1.

The data in Table 1 are used for class discussion. We first ask students to apply their knowledge of organic chemistry to explain why the pK_a values of the naphthols are so

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TABLE 1. The pK_a's of Sulfonated Naphthols.

Naphthol Derivative	pK_a (standard deviation)
1-OH, 2-SO_3^-	9.45 (0.02)
1-OH, 4-SO_3^-	8.19 (0.01)
2-OH, 6,8-SO_3^-	8.91 (0.01)
2-OH, 3,6-SO_3^-	9.46 (0.02)
2-OH, 7-SO_3^-	9.20 (0.02)

much lower than those of aliphatic alcohols. This leads to a discussion of the effect of conjugation on acid strength. Next, we ask students whether conjugative effects can also be used to rationalize the variation in the pK_a values in Table 1. Since the SO_3^- group can stabilize a negative charge at its position of substitution on the ring, the low pK_a of the 1-OH, 4-SO_3^- derivative in Table 1 can be understood by enhanced stability of the base form due to the electron-donating effect of the O^- group, which builds up negative charge at the position of SO_3^- substitution. A similar effect might be expected for the 1-OH, 2-SO_3^- derivative, but the higher pK_a for this derivative indicates relative stabilization of the acid form. A possible reason for this is the existence of intramolecular hydrogen bonding between the adjacent –OH and SO_3^- groups, which could lead to such a stabilization. It is worth noting that the highest pK_a among the 2-OH derivatives in Table 1 is seen for 2-OH, 3,6-SO_3^-, which also has these groups on adjacent positions. In any case, the discussion of the data from this experiment not only clarifies student understanding of pK_a but also provides an opportunity for students to see that what they learn in one course (organic chemistry) can have relevance in another (analytical chemistry).

7. Determination of Caffeine and Aspartame in Beverages by HPLC

In this experiment we have students determine the ppm of caffeine and aspartame in common beverages by comparing HPLC data on these beverages with those from a standard solution containing about 200 ppm of caffeine and 2000 ppm of aspartmame. Bidlingmeyer [6] has published an HPLC experiment dealing with the analysis of artificial sweeteners and additives in beverages, which employs a C_{18} column and a mobile phase of 20% CH_3OH / 80% 1 M acetic acid-acetate buffer (pH 3.0 to 4.5). We

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TABLE 2. The ppm levels of caffeine and aspartame in common beverages.

Beverage	ppm caffeine	ppm aspartame
Cola #1	113	
Diet Cola #1	107	690
Caffeine-free Cola	<5	770
High-caffeine Cola	203	
High caffeine non-Cola	160	
Commercial Caffeine water	224	
Coffee (brewed	469	
Bottled ice-tea	81	

replaced the CH_3OH with CH_3CN (to avoid formation of methyl acetate on standing [5]) and set the pH at 3.5. This mobile phase enables us to achieve base-line resolution of both components with retention times of 4.14 and 5.08 min for caffeine and aspartame, respectively, on a 25-cm C_{18} column containing 5-μm particles. A variable-wavelength detector set at 254 nm was used for detection. Student results from our most recent Chem 40 class are given in Table 2. The cola and diet cola values are in reasonable agreement with those reported by Bidlingmeyer [6], while the reported value for caffeine water is 200 ppm.

We have students calculate, in the laboratory, the following chromatographic parameters using their beverage data: capacity factors (k'), selectivities (α), resolution (R_s), and number of theoretical plates (N). An in-laboratory discussion of the meaning of these parameters then follows.

In the second part of this experiment, we change columns and mobile phases to demonstrate how these changes affect the separation between caffeine and aspartame. Some examples include.

1. The use of 20% CH_3CN / 80% water (no buffer) leads to longer retention times for both analytes, presumably due to interactions with ionized silanols on the column.

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2. The use of 30% CH_3CN / 70% water (no buffer) gives incomplete resolution of the peaks and a 50:50 mobile phase (no buffer) gives complete overlap.

3. The use of 30% CH_3CN / 70% water (no buffer) and a 25-cm C-phenyl column (5-μm particles) yield base-line resolution, but the order of retention is now reversed from that with the C_{18} phase (retention times = 3.69 min for aspartame and 4.99 min for caffeine).

4. The replacement of CH_3CN by CH_3OH leads to increased retention times for both components, showing that the latter solvent is a weaker eluant.

5. The coffee sample listed in Table 2 showed a large peak with the retention time of aspartame using the original mobile phase and column, despite the fact that no sweetener was present. The use of 30% CH_3CN / 70% water (no buffer) and a 25-cm C-phenyl column showed no peak for aspartame, which confirmed that the peak seen with the original mobile phase and column was an interferent with a similar retention time to that of aspartame.

These series of demonstrations brings home quite nicely the versatility of reverse-phase HPLC separations.

Pedagogical Outcomes from the Current Chem 40 Syllabus

The current Chem 40 syllabus, with its almost exclusive emphasis on instrumental methods, bears little resemblance to a classical Quantitative Analysis Laboratory syllabus, which features gravimetric and titrimetric procedures. Omission of the former is justified by the vanishingly small chance that any of our students will encounter this procedure in the future. Titration procedures are discussed in the lecture portion of the course, and all students at Union do a titration in Introductory Chemistry. Moreover, our past experience convinces us that even freshman-level students have little difficulty becoming quite proficient with the simple titrations previously done in Chem 40. As a consequence, we are confident that students can, on their own, readily develop competency in a titration procedure if required to do so in the future.

The use of “real” samples for quantitative work has several advantages, besides the obvious one of being more appealing to students than commercial unknowns. This appeal can be enhanced by allowing students to choose their samples, as in the OTC

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and beverage analyses. Moreover, the presence of interferents is not “fixed” as it is in commercial samples, but is dependent on student choice (i.e., phosphate in OTC samples, apparent aspartame peak in coffee), which makes for more enlightening for in-lab discussions. Waste disposal issues are much reduced with the samples used in the current syllabus. Only the lead and HPLC analyses create waste that must be disposed of in a special way. We have students participate in this process by precipitating the lead as $PbSO_4$, which helps sensitize them to the need to consider safe waste disposal in an analysis.

With exception of the glassware calibration portion of the first laboratory, students perform all experiments in groups of two or three. Laboratory group membership is changed for each experiment, so that students have the opportunity to work with partners having a variety of talents and personalities. Students often learn best from each other in these situations, and potential employers want employees who have the ability to work with others. Once formed, laboratory groups are allowed to divide work as they see fit. Because our laboratory sections are small (12 students maximum), it is not difficult for the instructor to monitor the contributions of all students in a given group. In addition, the use of laboratory groups reduces chemical use and waste disposal problems.

Each student submits a formal, written laboratory report (Introduction, Experimental, Results, Discussion) for three of these experiments. The report grade depends on the quality of both the results and the writing. For the other experiments, students prepare abbreviated reports containing results, relevant calculations, and discussion, as directed in the experiment. On occasion, we allow students to rewrite portions of their reports. As a consequence, students now have ample opportunity to work on their scientific-writing skills. The downside of this is that the workload has increased substantially for both students and instructors.

Brief quizzes on the laboratory experiments are given to ensure that students are familiar with the experiments while they are being performed; each quiz covers the chemistry and procedures underlying the experiment. These quizzes also help to ensure that all of the students can become active rather than passive participants in the group work. The average of the quiz marks counts the same as one laboratory report in determining the overall laboratory grade.

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The overall laboratory grade, which counts as 40% of the overall course grade, is then determined by the combination of laboratory report grades, quiz grades, and our evaluation of individual student laboratory notebooks. We feel that this grading system is far superior in evaluating overall student performance than the "results-only"-based system used in Chem 40 up through the mid-1980s.

The feedback we get from student comments and end-of-course evaluations is highly positive regarding the current Chem 40 laboratory syllabus. Although some students dislike the repetitive aspect of the first experiment, others appreciate the opportunity to develop their pipetting skills. All of the other experiments receive overwhelmingly positive endorsements from the students. It is worth noting that we have yet to receive any negative feedback concerning the work load distribution in any laboratory group.

More information about Chem 40 is available on the Web at http://chandler.union.edu /chem40/Chem40Home.html.

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