External Standards or Standard Additions? Selecting and Validating a Method of Standardization

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Several recent articles highlight an interest in including problem-based learning activities in the analytical chemistry curriculum (*1–4*). A report on two workshops on curricular developments in analytical chemistry, for example, includes a recommendation that problem-based learning be incorporated into the analytical curriculum (*5*). Numerous examples of innovative problem-based laboratory projects have been reported (*5–12*).

A common feature of many problem-based laboratories is a lengthy independent project involving the analysis of "real-world" samples (*1*). Students research the literature, adapting and developing a method suitable for their analyte, sample matrix, and problem scenario. Because these projects encompass the complete analytical process, students must consider issues such as obtaining a representative sample, selecting a method of analysis, developing a suitable standardization, validating results, and implementing appropriate quality assessment/quality control practices. With a few exceptions (*13, 14*), however, most textbooks and monographs suitable for an undergraduate course in analytical chemistry provide only limited coverage of these important topics. The need for short laboratory experiments emphasizing important facets of method development is evident.

Analytical textbooks do a good job of discussing the advantages and disadvantages of external standardizations and standard additions. Students readily appreciate that there is a significant savings in time and work when analyzing samples using an external standardization. Students also know, or at least recall, that matrix effects often necessitate a standard addition. What is less certain is whether students actually understand what a matrix effect is, or how to determine if a particular analysis is subject to serious matrix effects. Telling students that an unknown or complex matrix may require a standard addition does not provide them with the tools to determine if a standard addition is required.

The experiment reported here, which is suitable for an introductory course in analytical chemistry, illustrates the importance of matrix effects on selecting a method of standardization. Students also learn how to use a spike recovery to validate an analytical method, and obtain a practical experience in the difference between an external standardization and a standard addition.

The Problem Scenario

Although the purpose of this experiment is to have students think critically about methods of standardization, this fact is intentionally withheld from them. Instead, students are informed only that the goal of the experiment is to develop a quantitative spectrophotometric method for *p*-nitrophenol (PNP), an environmentally significant toxic contaminant listed as a priority pollutant by the EPA. PNP forms naturally during the biodegradation of the insecticide parathion and, in turn, biodegrades to 4-nitrocatechol (*15*). Excessive exposure to PNP may induce methemoglobinemia, in which an abnormally large percentage of the iron in hemoglobin is present as Fe^{3+} instead of Fe^{2+}, decreasing its capacity for transporting and releasing O_2 to cells.

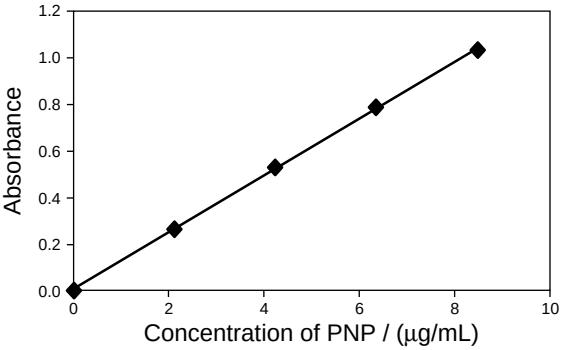

Figure 1. Typical external-standard calibration curve for PNP prepared using a stock solution of 26.5 µg/mL PNP in a matrix consisting of a pH 10.4 buffer.

Students complete the experiment in small groups, gathering data and answering questions posed by the instructor. As the lab progresses, individual groups come together to share ideas, to provide opportunity for a more detailed discussion by the class, and to discover the experiment's hidden lesson.

Background Information (What the Students Don't Know!)

PNP is a weak acid with a pK_a of approximately 7.2. The basic *p*-nitrophenolate ion is yellowish, with a maximum absorbance near 400 nm, and the weak acid form is colorless. The absorbance of a solution of PNP, therefore, exhibits a significant pH-dependent matrix effect, approaching an absorbance of zero for pH levels less than 5 and a maximum value for pH levels greater than 9.5. This information is withheld from students, who are told only that aqueous solutions of PNP are yellow. In the remainder of this paper, information in *italics* is not revealed to the students until later in the experiment.

In the Lab

Using a stock solution of nominally 25 µg/mL PNP *in a matrix consisting of a pH 10.4 buffer*, each group prepares a set of external standards with PNP concentrations of less than 10.0 µg/mL (diluting with distilled water, as they are unaware of the stock solution's matrix). After obtaining a visible spectrum and selecting a wavelength for further analysis, each group measures the absorbances for its external standards, using distilled water as a blank. A typical external standard calibration curve is shown in Figure 1.

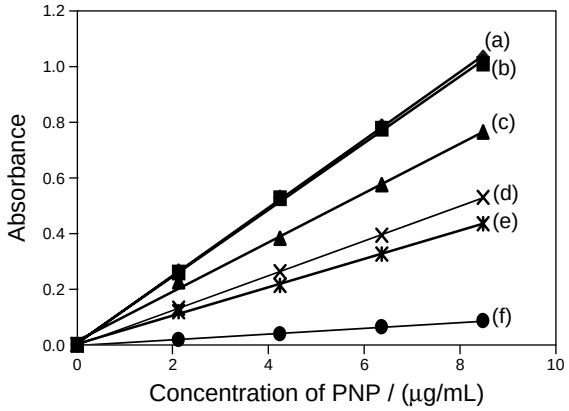

Figure 2. Typical external-standard calibration curves for PNP at pH (a) 10.4, (b) 9.18, (c) 7.41, (d) 7.00, (e) 6.86, and (f) 6.01.

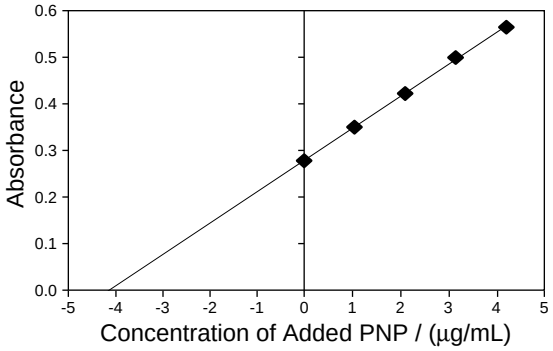

Figure 3. Typical standard-addition calibration curve for the analysis of a "real" sample that is 8.48 μg/mL PNP in a nitrogen-free mineral salts buffer.

To evaluate its calibration curve, each group is provided with a 10–12-μg/mL PNP calibration standard *prepared in a pH 10.4 buffer*. After diluting the calibration standard to bring its absorbance within their calibration curve's range, the group determines its concentration and compares it to the known value. Typical results show an error of less than ±1%, convincing students that their external standardization is valid.

Each group is then provided with a "real" sample *whose matrix is a nitrogen-free mineral salts broth mimicking that obtained from a kinetic study of the biodegradation of PNP* (*16*). *The true concentration of PNP in their sample is in the range of 8–10 μg/mL.* Students analyze the "real" sample using their external standard calibration curve *for a pH 10.4 matrix*, typically reporting concentrations of PNP between 4 and 5 μg/mL (*a determinate error of approximately 50%*). When asked to express confidence in their group's analysis of the "real" sample, most students indicate that they believe their work is accurate. This is not surprising, because they have no reason to anticipate a matrix effect.

At this point the instructor reminds the students of the possibility of matrix effects and introduces the idea of spiking a sample with a known concentration of PNP and determining a spike recovery, %*R*

$$\%R = \frac{C_{\text{spike}} - C_{\text{sample}}}{C_{\text{added}}} \times 100\ \%$$

where C_{sample} and C_{spike} are the experimentally determined concentrations of PNP in the unspiked sample and in the spiked sample, respectively, and C_{added} is the known concentration of PNP added during the spike. If an analytical method is accurate, then a spike recovery near 100% is expected. Groups determine a spike recovery on the "real" sample using their stock PNP solution *whose matrix is a pH 10.4 buffer*. Typical recoveries are greater than 150%, convincing students that the analysis is flawed.

In the ensuing discussion, students, with prompting from the instructor, recognize that PNP's acid–base chemistry might account for a pH-dependent matrix effect. Each group then prepares an additional external-standard calibration curve at a different buffered pH, using a nominally 25-μg/mL solution of PNP in distilled water. The results (Fig. 2) show that the matrix's pH does affect the calibration curve's slope. Groups then verify the difference between the matrices of their external standards and the "real" sample using pH test strips.

After the class is given a stock solution of nominally 100 μg/mL PNP in distilled water, each group reanalyzes its "real" sample using the method of standard addition. A typical standard addition calibration curve is shown in Figure 3. Errors of less than ±3% are usually obtained.

After completing the experiment, students are asked to consider how they can adapt this analysis so that an external standardization is possible. Most students recognize that the simplest approach is to add NaOH to each sample and external standard, ensuring that the pH of each solution is sufficiently basic so that essentially all the PNP is in the form of *p*-nitrophenolate. Maintaining a pH of approximately 13 meets this requirement and is the approach used in the literature (*16, 17*).

Hazards

In solid form, *p*-nitrophenol poses a moderate health hazard (*18*). Acute inhalation or ingestion of PNP may lead to headaches, drowsiness, nausea, and cyanosis (blue color in lips, ears, and fingernails). Contact with skin and eyes may cause irritation; absorption through the skin is also possible. To avoid the risks associated with handling solid PNP, students are given only dilute solutions of PNP. At the solution concentrations used in this experiment, the hazards associated with PNP are minimal, although students should use appropriate caution when handling any chemical. Safety glasses are mandatory and safety gloves are recommended.

Summary

Students derive three specific benefits from completing this experiment. First, discovering for themselves that a sample's matrix may significantly affect the results of an analysis reinforces their understanding of the importance of considering matrix effects when developing analytical methods. Second, showing students how a recovery is used to evaluate accuracy provides them with a tool that can be used in later laboratory work. Finally, the practical experience of completing an analysis using both an external standardization and a standard addition reemphasizes the difference between these two methods of standardization.

[w]Supplemental Material

Copies of laboratory handouts, notes for instructors, and sample data sets are available in this issue of *JCE Online*.

Literature Cited

1. Wenzel, T. J. *Anal. Chem.* **1995,** *67*, 470A–475A.
2. Wenzel, T. J. *Anal. Chem.* **1998,** *70*, 790A–795A.
3. Wenzel, T. J. *Anal. Chem.* **1999,** *71*, 693A–695A.
4. Wenzel, T. J. *Anal. Chem.* **2000,** *72*, 293A–296A.
5. Curricular Developments in the Analytical Sciences; *http://www.chem.ku.edu/TKuwana/CURRICULARDEVEL--FINAL%20HTML/*. (accessed Mar 2002)
6. Hughes, K. D. *Anal. Chem.* **1993,** *63*, 883A–888A.
7. Fitch, A.; Wang, Y.; Mellican, S.; Macha, S. *Anal. Chem.* **1996,** *68*, 727A–731A.
8. Wilson, G. S.; Anderson, M. R.; Lunte, C. E. *Anal. Chem.* **1999,** *71*, 677A–681A.
9. Hope, W. W.; Johnson, L. P. *Anal. Chem.* **2000,** *72*, 460A–467A.
10. Werner, T. C.; Tobissen, P.; Lou, K. *Anal. Chem.* **2001,** *73*, 84A–87A.
11. Henderleiter, J.; Pringle, D. L. *J. Chem. Educ.* **1999,** *76*, 100–106.
12. Dorey, R. C.; Draves, J. A.; Stanitski, C. L. *J. Chem. Educ.* **1999,** *76*, 752.
13. Harvey, D. T. *Modern Analytical Chemistry*, McGraw-Hill: Dubuque, IA, 2000.
14. Kenkle, J. *A Primer on Quality in the Analytical Laboratory*; Lewis: Boca Raton, FL, 2000.
15. The University of Minnesota Biocatalysis/Biodegradation Database; *http://umbbd.ahc.umn.edu*. (accessed Mar 2002)
16. Duong, M. H.; Penrod, S. L.; Grant, S. B. *J. Chem. Educ.* **1997**, *74*, 1451–1454.
17. Merkx, M.; Averill, B. A. *Biochemistry* **1998**, *37*, 8490–8497.
18. MSDS at *http://www.jtbaker.com/msds/n6040.htm*. (accessed Mar 2002)