Chemical Speciation Analysis of Sports Drinks by Acid–Base Titrimetry and Ion Chromatography: A Challenging Beverage Formulation Project

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Chemical Speciation Analysis of Sports Drinks by Acid–Base Titrimetry and Ion Chromatography: A Challenging Beverage Formulation Project

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In this three-part laboratory project, students enrolled in quantitative analysis, introductory analytical chemistry, or instrumental analysis classes standardize a sodium hydroxide solution and then analyze commercially available sports drinks by titrimetric analysis of the triprotic citric acid [H_3Cit; $C_3H_4(OH)(COOH)_3$], dihydrogen phosphate ($H_2PO_4^-$), and dihydrogen citrate (H_2Cit^-) and by ion chromatography for chloride (Cl^-), total phosphate, and citrate. Though sodium and potassium concentrations are provided as part of the nutritional data, an extra session of the lab project could also analyze for the cations by ion chromatography. The sports drink formulation calculations combine the often loosely related theory and lab aspects of acid–base chemistry, chemical speciation, titrations, chromatography, linear regression, and error propagation through detailed spreadsheet analyses.

Chemical speciation, a central theme in many undergraduate analytical chemistry classes, has been described in numerous articles dealing with theory and calculations. Theoretical approaches for calculating speciation include numerous commentaries on distribution (alpha) diagrams *(1–3)* and spreadsheet applications for acid–base titrations *(4–8)*. However, there is a paucity of laboratory experiments to illustrate why speciation is important in real-world applications or how one determines the formulation of a commercial product in the laboratory.

Real-world samples and project-based approaches pique students' interest and enhance their learning much more than experiments that follow cookbook recipes or analyze stock standards *(9)*. Thus, to stimulate student interest, this project-based laboratory focuses on determining the formulation of popular commercially available sports drinks and powders. The examples used in this article include the analysis of Powerade and Gatorade liquid beverages. A survey of the chemical education literature indicates numerous laboratory experiments that focus on the analysis of beverages. In one creative endeavor, an extensive Web-based general chemistry curriculum focused exclusively on concepts related to the invention of Gatorade *(10)*. Other relevant examples that use food and beverages as a lab focus include the chromatographic determination of phosphate in cola *(11–12)*, titrimetric and colorimetric determination of phosphate in cola *(13)*, iodimetric analysis of vitamin C in sports drinks *(14)*, densitometric determination of sugars in commercial beverages *(15)*, enzymatic and spectrophotometric determination of glucose in fruit drinks *(16)* and sports drinks and sodas *(17)*, HPLC detection of taurine in sports drinks *(18)*, titrimetric analysis of total acids in cola *(19)*, spectrophotometric analysis of food colors in sports drinks *(20–21)*, capillary electrophoresis separation of caffeine and total phosphate and citrate in soda *(22)*, and the headspace GC–MS of volatile flavors in candy and gum *(23)*. Though all of these experiments are interesting examples of analyzing real-world food and beverage samples, none focuses on the complexity of determining multiple species to formulate a beverage product or on the speciation analysis of citrate and phosphate.

Project Description

The relevant ingredients in Gatorade include citric acid (H_3Cit), salt (NaCl), sodium citrate (Na_3Cit), and potassium dihydrogen phosphate (KH_2PO_4), listed in the relative order on the nutritional information label. The other components, water, sugars, flavors, ester gum, and food colors, are not analyzed in this experiment though the analysis of glucose *(17)* and food dyes *(20–21)* in sports drinks and volatile flavor components *(23)* have been described elsewhere. A similar list of ingredients in Powerade includes citric acid, salt, potassium citrate (KH_2Cit, in place of Na_3Cit), and potassium dihydrogen phosphate. The use of potassium (as opposed to sodium) salts for two ingredients in Powerade provides an interesting complement to the Gatorade analysis.

A series of challenging calculations from the lab data allow students to determine the concentration of the stated components required to formulate the sports drinks. Ion chromatographic data for chloride, total phosphate, and citrate along with the nutritional analysis of sodium and potassium serve to test the quality of the analysis, as well as provide an exercise in unit conversions and clues for which species of phosphate and citrate contribute significantly to the observed pH of the beverage. As part of the lab conclusion, a student challenge question might be: if you were a food chemist working in the sports drink sector, how much of each ingredient has your competitor added and how might you improve on the recipe without infringing on patents? Another possibility to make the project even more relevant is to have students formulate and taste test their own drinks from food-grade reagents.

Speciation analysis for Gatorade is described by the two mass balance expressions (eqs 1 and 2) that relate the analytical (formal) concentration of sodium (C_{Na}) and citrate (C_{Cit}) to the ingredients that are added to prepare the beverage.

$$C_{Na} = \left[Na^+\right]_{NaCl} + 3\left[Na^+\right]_{Na_3Cit} \quad (1)$$

$$C_{Cit} = \left[H_3Cit\right] + \left[Na_3Cit\right] \quad (2)$$

The speciation for Na^+ by compound added cannot be obtained explicitly since the total sodium and the total citrate come from three different compounds: NaCl, Na_3Cit, and H_3Cit. Thus, in eqs 1 and 2, the expressions for NaCl, Na_3Cit, and H_3Cit represent the concentration added by the manufacturer and not the equilibrium concentration. The more standard mass balance, given by eq 3, can be written and solved explicitly for total citrate and its equilibrium species.

$$C_{\mathrm{Cit}} = \left[\mathrm{H_3Cit}\right] + \left[\mathrm{H_2Cit^-}\right] + \left[\mathrm{HCit^{2-}}\right] + \left[\mathrm{Cit^{3-}}\right] \quad (3)$$

The speciation analysis of Powerade requires a similar logic and mass balance approach (eqs 4 and 5) where KH_2PO_4, KH_2Cit, and H_3Cit are the concentrations added by the manufacturer of the beverage and not the equilibrium concentrations.

$$C_{\mathrm{K^+}} = \left[\mathrm{K^+}\right]_{\mathrm{KH_2PO_4}} + \left[\mathrm{K^+}\right]_{\mathrm{KH_2Cit}} \quad (4)$$

$$C_{\mathrm{Cit}} = \left[\mathrm{H_3Cit}\right] + \left[\mathrm{KH_2Cit}\right] \quad (5)$$

The major twist for Powerade is that there are three sources of acidic protons: H_3Cit, KH_2PO_4, and KH_2Cit, which makes the calculation a bit different from the calculation for Gatorade. The detailed calculations for both beverages are provided in the Supplemental Material.[W]

Hazards

Standard precautions should be taken when handling acids and bases.

Procedure

The entire lab portion of the project can be completed in a single 4-hour lab period or as many as 3–4 lab periods depending on which parts the student is expected to perform versus which information the instructor will provide. The Supplemental Material[W] includes a full write-up and report form for the three parts of the project. We combine the first and second part of the experiment in a single, 4-hour lab. In part 1, students prepare a 0.1000 M NaOH solution and standardize it with potassium hydrogen phthalate. Part 2 is devoted to the potentiometric or colorimetric titration of the sports drink samples. The introduction of new colorless beverages (Gatorade Ice flavors and Powerade Arctic Blast flavor) allows students to easily perform colorimetric titrations. For beverages that have added food dyes, a potentiometric titration is sufficient. Alternatively, some food dyes may be removed from beverages by stirring with activated charcoal followed by gravity filtration or specific wavelengths can be monitored with a spectrometer.

Though the use of ion chromatography (IC) is not required for speciation of Gatorade if the chloride and either phosphate or potassium concentrations are provided (or are measured by alternative analytical methods), the addition of an IC component provides confirming data and stimulates greater interest for students in labs that have such instrumentation. Because detailed discussions of IC instrumental methods have appeared previously in this *Journal (24–26),* the details of the IC analysis are provided in the Supplemental Material.[W]

Results

Student results for all the species analyzed and the formulation recipe for the Powerade lemon–lime and the Gatorade watermelon ice liquid beverages are shown in Table 1 along with the concentrations determined directly or by simple calculation from the nutritional label (shaded portion of the table). To further relate the lab work to speciation theory, students are asked to prepare a spreadsheet for graphing the distribution functions of citrate and phosphate species as a function of pH. The data include the equilibrium concentrations: $[H_3Cit]$, $[H_2Cit^-]$, $[HCit^{2-}]$, $[Cit^{3-}]$, $[H_3PO_4]$, $[H_2PO_4^-]$, $[HPO_4^{2-}]$, $[PO_4^{3-}]$, $[H^+]$, and $[OH^-]$ and the analytical concentrations of phosphate, citrate, chloride, sodium, and potassium. For the analytical concentrations, students use their lab results.

The internal consistency of the formulation data can be determined by calculating the theoretical pH from the measured analytical concentrations and then comparing the result with the measured pH of the beverage. We use the charge balance equation along with a pointer function *(27)* to cal-

Table 1. Comparison of Experimental and Reported Data for Analysis of Powerade and Gatorade

Analyte	Powerade Conc/(mg/L)		Gatorade Conc/(mg/L)	
	Exp	Rep	Exp	Rep
Total phosphate[a]	22.7±0.7[h]	—	320±2	304
Total citrate[b]	2364±6	—	2846±17	—
Citric acid[c]	2010±12	—	2320±17	—
Chloride[d]	348±2	348	425±2	380
Total sodium[e]	224±3	225	480±24	465
Total potassium[f]	127±13	127	132±6	125
pH[g]	2.85±0.11	2.82	2.96±0.13	3.26
Formulation (ingredient)	Quantity added/ (mg/L)		Quantity added/ (mg/L)	
Citric acid	2010	—	2320	—
KH_2PO_4	33	—	460	—
Na_3Cit	—	—	765	—
KH_2Cit	691	—	—	—
NaCl	574	—	700	—

[a]Analyzed by ion chromatography; may also be analyzed by colorimetry.
[b]Determined by IC; may also be analyzed by ISE or potentiometry.
[c]Determined by calculation from titrimetric data and calculation.
[d]Determined by IC; may also be analyzed by ISE or argentometric titration.
[e]Determined by calculation; may be analyzed by IC, ISE, or flame atomic emission spectroscopy.
[f]Determined by calculation; may be analyzed directly by IC, ISE, or flame atomic emission spectroscopy.
[g]Determined by pH meter measurement.
[h]The confidence intervals for titration data are calculated from the standard deviation of three colorimetric determinations. The confidence intervals for IC data are calculated from the standard deviation of five samples run at 5% dilution of the liquid beverages with Milli-Q water and normalized to full concentration. For sodium in Gatorade and potassium in Powerade, the errors represent the propagated standard deviations.

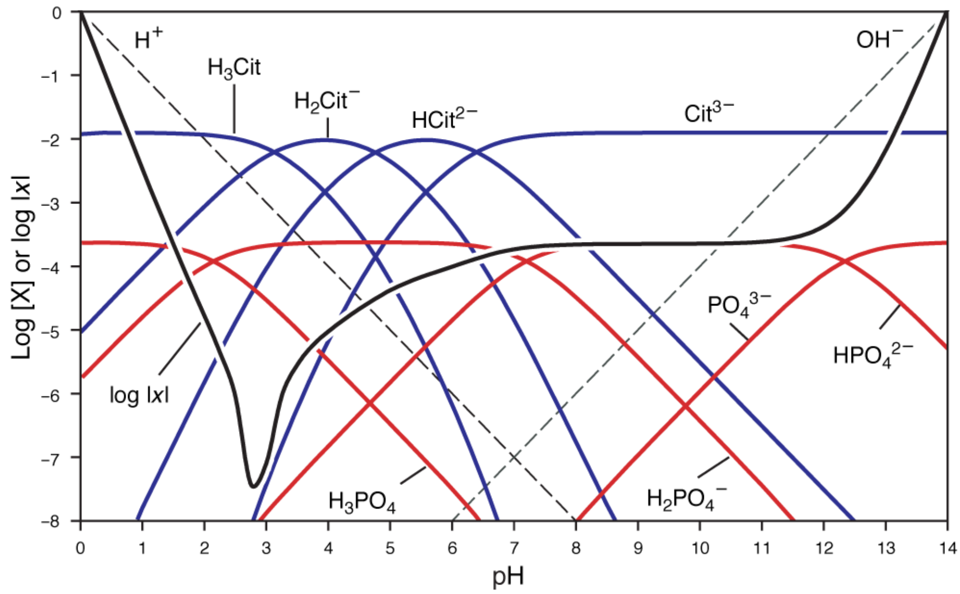

Figure 1. Speciation of Powerade with a spreadsheet distribution function analysis. The analytical concentrations used for the analysis are 12.5 mM citrate, 0.239 mM phosphate, 9.82 mM chloride, 9.82 mM sodium, and 3.2 mM potassium. The (scaled) pointer function indicates a predicted pH of 2.85.

culate the pH. The pointer function, which plots the log$|x|$, where x is given by

$$
\begin{aligned}
x = {} & \left[H_2Cit^-\right] + 2\left[HCit^{2-}\right] + 3\left[Cit^{3-}\right] \\
& + \left[H_2PO_4^-\right] + 2\left[HPO_4^{2-}\right] + 3\left[PO_4^{3-}\right] \\
& + \left[OH^-\right] + \left[Cl^-\right] - \left[H^+\right] - \left[Na^+\right] - \left[K^+\right]
\end{aligned} \tag{7}
$$

has its minimum value at the solution pH. The pointer results by spreadsheet analysis (see the Supplemental Material) are provided for Powerade in Figure 1. The theoretical pH calculated from the pointer function for Powerade is within the experimentally determined confidence limits for the measured pH. The theoretical pH of Gatorade is 0.15 units higher than the confidence limits predicted for the beverage from lab data. The accuracy of the pH results is indicative of the overall accuracy of the data listed in Table 1.

Conclusion

Since potassium (and thus phosphate) is known exactly in Gatorade while sodium (and thus chloride) is known exactly for Powerade, it is reasonable to expect differences in accuracy. Though the recipes for both sports drinks remain unconfirmed owing to their proprietary nature, the internal agreement between the calculated and measured pH and nutritional values listed on the label indicate that the formulation is accurate for both beverages. Both the similarities and differences between the formulations of the two brands of sports drinks should be useful for provoking discussion among students. Some topics for discussion may include whether differences are due to avoiding patent infringements *(10)* or better effectiveness for rehydration *(28)*. While calculations needed to obtain the final recipe are challenging, students are exposed to a scenario not unlike what they may find in a commercial beverage formulation lab.

This lab has been used in a variety of formats for the past ten years.[1] We have had a number of students successfully analyze sports drinks and other beverages like Kool-Ade and Tang for potassium, sodium, sugars, food dyes, and volatile flavors as well as the analytes described in this project. Some alternative procedures we have used for determining analytes are included in the Table 1 notes. The lab has always been met with great student interest and excitement owing to the familiarity and relevance of the samples, especially to student athletes.

To assess the effectiveness of the 2004 version of this experiment in promoting student learning, we used the Student Assessment of Learning Gains (SALG) Web-based assessment instrument *(29)*. This instrument was designed to assess factors that promote student learning rather than those parts of class that students enjoy most. Compared with nine other aspects of the class, the titrimetric and IC parts of this lab received two of the three highest scores (4.4 and 4.6, respectively, on a 5-point scale) for what helped the students best learn the course material. Though a statistical analysis among the 11 different components was not appropriate owing to the small sample size and the large heterogeneity of variance, the high scores are nonetheless indicative that the labs are of pedagogical importance to the students.

We also use an ACS standardized analytical chemistry exam to compare the students' theoretical understanding each year. The 2004 version of the sports drink extended project and its related calculations consumed almost one-third of the total lab time by including sessions on spreadsheet analysis of linear regression, error propagation, acid–base speciation, and titration modeling in addition to the lab work on standardization, titrimetric analysis, and ion chromatography. With this extensive emphasis on a single project, the standardized exam scores were statistically the same as in prior years. Thus, the first complete test of this project as described in this article did not decrease students' abilities to learn the theories of analytical chemistry.

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[W]Supplemental Material

Student instructions and report forms for the three parts, pointer spreadsheet calculations, and notes for the instructor are available in this issue of *JCE Online*.

Notes

1. Presented in part as an oral presentation and workshop at a Project Kaleidoscope National Workshop, Colorado Springs, CO, October 24–26, 1997.

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