

A Laboratory Exercise on Semiquantitative Analysis of Ions in Nutrient Solutions

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ABSTRACT

A student laboratory exercise on the development of nutrient deficiency and toxicity symptoms was linked to analytical techniques and ion chemistry by having the students determine which ions in the nutrient solutions were missing or present in excess. Qualitative analytical methods for Cl, Ca, Mg, Na, K, Mn, S, ammonium, and nitrate were made semiquantitative by using standards. Quantitative analytical techniques for B, Fe, P, and bicarbonate were simplified. Most reagents and samples were in dropper bottles, allowing students to rapidly run the tests by adding the proper number of drops of sample and reagent(s) to a test tube. For most elements, a color change or precipitation denoted a positive test. Most reagents have stored well for 2.5 yr, minimizing reagent preparation after the 1st yr.

CLASS LABORATORY EXPERIMENTS in hydroponics, the soilless culture of plants, are popular for demonstrating plant nutrient deficiency symptoms (Epstein, 1986; Moore, 1974). Generally, nutrient deficiency symptom laboratories are mostly observational in nature with students focusing on symptom development. Tissue or nutrient solution analysis to confirm the nutrient deficiency is usually not done due to a lack of analytical equipment. However, visual nutrient deficiency symptoms have limited usefulness as a diagnostic procedure.

Introductory inorganic chemistry laboratories often perform qualitative analysis of ions in solution when students learn ion chemistry in a hands-on setting. A weakness of this classic qualitative analysis laboratory experience is that the ion solutions analyzed, particularly the unknowns, are artificial. In the real world, plant scientists know what they are analyzing be it irrigation water, fertilizer solution, plant tissue, or soil.

In order to extend the learning experience in a plant nutrient deficiency laboratory and link it more closely to analytical chemistry, qualitative analysis laboratory techniques were utilized to analyze nutrient solutions. The objective was to determine if an ion or element was present at deficient, adequate, or toxic levels.

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NUTRIENT SOLUTIONS

Nutrient deficiency solutions for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B, and Mo were prepared according to Hoagland and Arnon (1950) except that Fe was provided as Fe-EDTA at 90 μM Fe, and CaCl_2 was used instead of CaSO_4 for the minus-N solution. The complete solution was a Hoagland Solution Number 1.

Various element toxicities were induced by adding one of the following: 460 μM $\text{B}(\text{OH})_3$, 90 μM MnCl_2 , 20 mM NaCl, or 20 mM NaHCO_3 . The toxic levels of B and Mn were ten times the normal Hoagland Solution Number 1 level.

Ammonium toxicity was induced by growing plants in a modified Hoagland Solution Number 1. The solution for ammonium toxicity had CaCl_2 substituted for $\text{Ca}(\text{NO}_3)_2$, K_2SO_4 substituted for KNO_3 , and 15 mM ammonium as $(\text{NH}_4)_2\text{SO}_4$.

Extra volumes of all but the minus-Zn, -Cu, and -Mo solutions were prepared for student analysis. Analytical techniques for Zn, Cu, and Mo were not attempted because the low concentrations of these elements are either difficult to detect with simple techniques or difficult-to-handle reagents are needed. Zinc analysis requires organic solvent extraction with carbon tetrachloride (Epstein, 1986), Cu analysis requires toxic cyanides, and Mo analysis requires atomic absorption spectrophotometry (American Public Health Association, 1985). The all-nitrate-N, all-ammonium-N, nine deficient, and four toxic nutrient solutions were assigned numbers and placed in 30-mL-glass-dropper bottles.

PLANTS

Leaf cuttings of *Tolmiea menziesii* (Pursh) Torr. & A. Gray were rooted in 2 mM CaCl_2 in solution culture (Hershey, 1989), placed in plastic jars in continuously aerated nutrient solutions, and set on a greenhouse bench for symptom development. The aeration manifold was like that of Hershey and Merritt (1986) as modified by Sadof and Hershey (1989). There were four replications per treatment, but not all jars were labeled for the students. The unknown jars were labeled for the instructors, and were used later to test the student's ability to discern

the identity of the deficient or toxic element via symptom identification and solution analysis. The seed-propagated crops more often used in nutrient deficiency laboratories, such as tomato (*Lycopersicon esculentum* Mill.) (Epstein, 1986) or sunflower (*Helianthus annuus* L.) (Moore, 1974) could also be used. The houseplant *Tolmiea*, known as piggyback plant, was chosen for a change of pace and because its rosette form requires no staking.

ANALYTICAL PROCEDURES

Qualitative analysis procedures for Cl, Ca, Mg, Na, K, Mn, ammonium, nitrate, and sulfate (Masterton and Slowinski, 1978) were used with modifications. Standard qualitative analysis procedures usually utilize samples with 20 mM concentrations of each ion. This presented sensitivity problems for certain ions since a Hoagland Solution Number 1 has ion concentrations of 15 mM or less (Hoagland and Arnon, 1950). To overcome the problem, solutions for the least sensitive tests, Mn and Mg, were evaporated to dryness in an oven and reconstituted to one-tenth of the original volume prior to class. With the exception of Cl, the other tests were sensitive enough to detect deficient or adequate ion levels. The Cl test was only used to detect a Cl excess.

Quantitative analytical procedures for B (John et al., 1975, bicarbonate (Chapman and Pratt, 1961), phosphate (Watanabe and Olsen, 1965), and Fe (Chaney et al., 1972) were simplified by minimizing the solution volumes utilized, measuring reagent volumes by drop-count instead of pipettes, and visually estimating solution color instead of using spectrophotometers.

For the majority of tests, 10 drops of sample are added to a 12- by 75-mm-glass-test tube and the appropriate number of drops of one or more reagents added. A positive test is indicated by a change in solution color or formation of a precipitate. Sections of 512 square, plastic plug flats, used for seed germination in bedding plant production, are utilized as inexpensive racks for the 12- by 75-mm-disposable-test tubes. Each plug cell is 14 mm wide and 21 mm deep. The flats are available from Maryland Plants and Supply, Baltimore, MD. Most reagents and nutrient solutions are in 30-mL-dropper bottles. Standard solutions of specified concentrations are available for each ion and allow the student to judge if the nutrient solution concentration is higher or lower than the standard. Deionized water is available in dropper bottles so students can determine what a negative test result looks like. Preparation of reagents for each test are summarized in Table 1. Details for each test are described below.

Chloride. To 10 drops of sample add two drops of 0.1 M AgNO₃. A cloudy white precipitate of AgCl indicates the presence of chloride. The dropper bottle of AgNO₃ solution is wrapped in aluminum foil to prevent photodegradation of AgNO₃. A 10-mM-NaCl solution is used as a standard. This test is not sensitive enough to detect the normal 18μM-chloride concentration in Hoagland Solution Number 1, so it is used to detect an excess of chloride.

Table 1. Directions for achieving desired molarity of reagents necessary for the nutrient solution analysis laboratory. The instructions for each test are given in the text.

Reagent	Preparation
Chloride	
0.1 M AgNO ₃	17 g AgNO ₃ L ⁻¹
10 mM NaCl	584 mg NaCl L ⁻¹
Sulfate	
1 M BaCl ₂	244 g BaCl ₂ ·2H ₂ O L ⁻¹
1 mM H ₂ SO ₄	56 μL conc. H ₂ SO ₄ L ⁻¹
Phosphate	
Reagent A	12 g ammonium molybdate [(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O] in 250 mL H ₂ O and 0.3 g antimony potassium tartrate (C ₈ H ₄ K ₂ Sb ₂ O ₁₂ ·3H ₂ O) in 100 mL H ₂ O are added to 1 L of 2.5 M H ₂ SO ₄ (148 mL conc. H ₂ SO ₄ L ⁻¹), mixed well, and brought to 2 L
Reagent B	0.25 g ascorbic acid in 50 mL of Reagent A (make fresh)
1 mM KH ₂ PO ₄	136 mg KH ₂ PO ₄ L ⁻¹
Nitrate	
18 M H ₂ SO ₄	conc. H ₂ SO ₄
0.2 M FeSO ₄	2.8 g FeSO ₄ ·7H ₂ O in 50 mL (make fresh)
10 mM KNO ₃	1.01 g KNO ₃ L ⁻¹
Ca	
6 M NH ₄ OH	400 mL 30% NH ₄ OH L ⁻¹
1 M potassium oxalate	184 g K ₂ C ₂ O ₄ ·H ₂ O L ⁻¹
5 mM CaCl ₂	735 mg CaCl ₂ ·2H ₂ O L ⁻¹
Mg	
6 M HCl	500 mL conc. HCl L ⁻¹
Mg reagent	0.1 g <i>p</i> -nitrobenzene-azoresorcinol L ⁻¹ of 25 mM NaOH (1 g L ⁻¹)
6 M NaOH	240 g NaOH L ⁻¹
5 mM MgSO ₄	1.23 g MgSO ₄ ·7H ₂ O L ⁻¹
Ammonium	
6 M NaOH	240 g NaOH L ⁻¹
10 mM NH ₄ Cl	535 mg NH ₄ Cl L ⁻¹
K	
10 mM KCl	746 mg KCl L ⁻¹
Na	
10 mM NaCl	584 mg NaCl L ⁻¹
Mn	
6 M HNO ₃	400 mL conc. HNO ₃ L ⁻¹
Sodium bismuthate	Solid NaBiO ₃
100 μM MnCl ₂	20 mg MnCl ₂ ·4H ₂ O L ⁻¹
B	
Buffer masking reagent	125 g ammonium acetate (CH ₃ COONH ₄) and 7.5 g Na-EDTA in 200 mL H ₂ O; slowly add 62.5 mL glacial acetic acid
Azomethine-H reagent	0.45 g Azomethine-H and 1 g ascorbic acid in 50 mL H ₂ O (make fresh)
500 μM B(OH) ₃	31 mg B(OH) ₃ L ⁻¹
Fe	
2.5 mM TPTZ (2,4,6 tri-pyridyl-s-triazine)	780 mg TPTZ L ⁻¹
4 M sodium acetate	544 g CH ₃ COONa·3H ₂ O L ⁻¹
2 M HCl	165 mL conc. HCl L ⁻¹
Ascorbic acid	Solid
100 μM Fe-EDTA	36.7 mg Fe-EDTA L ⁻¹
Bicarbonate	
Phenolphthalein indicator	2.5 g phenolphthalein L ⁻¹ of 1:1 ethanol/water
Methyl orange indicator	1 g methyl orange L ⁻¹
25 mM H ₂ SO ₄	1.39 mL conc. H ₂ SO ₄ L ⁻¹
10 mM NaHCO ₃	840 mg NaHCO ₃ L ⁻¹

Sulfate. To 10 drops of sample add three drops of 1 M BaCl₂ and mix well. A white, finely divided precipitate of BaSO₄ indicates the presence of sulfate. A 1-mM-H₂SO₄ standard is used. Hoagland Solution Number 1 has 2 mM sulfate.

Phosphate. To one drop of sample add four drops of

Reagent B (Table 1) and 20 drops of deionized water. Mix and let stand for 10 min. A blue solution indicates the presence of phosphate. A 1-mM-KH₂PO₄ standard is used. Hoagland Solution Number 1 contains 1 mM phosphate.

Nitrate. Add 10 drops of sample to a test tube. Carefully add 20 drops of 18 M H₂SO₄. Add the acid slowly, mixing continuously. Be careful because the tube becomes hot. Cool the tube under a water tap. Holding the tube at a 45° angle, let five drops of 0.2 M FeSO₄ run down the side of the tube and form a layer over the acid. The FeSO₄ should be prepared fresh for each lab. Let the tube stand for a few minutes. A brown ring at the junction of the two layers indicates the presence of nitrate. Ten millimolar KNO₃ serves as a standard.

Calcium. Put 10 drops of sample in a test tube, then add five drops of 6 M NH₄OH and mix. Add five drops of 1 M K₂C₂O₄ (potassium oxalate), mix and let stand for 1 min. A white precipitate of calcium oxalate is confirmation for the presence of Ca. Hoagland Solution Number 1 has 5 mM Ca, so 5 mM CaCl₂ is used as a standard.

Magnesium. Put 12 drops of sample in a test tube, add three drops of 6 M HCl, and three drops of magnesium reagent (Table 1). The solution will turn yellow. Stir the solution and add, drop by drop, 6 M NaOH until the solution turns purple or a blue precipitate forms. A medium-blue precipitate of magnesium hydroxide with adsorbed magnesium reagent indicates that Mg is present.

The Mg test is one of the least sensitive, so the 2 mM Mg in Hoagland Solution Number 1 is too low to give a positive test. To successfully use the test, the sample solutions are concentrated tenfold, e.g., 100 mL of sample is evaporated to dryness in an oven and dissolved in 8 mL deionized water and 2 mL of 6 M HCl. Fifteen drops of this concentrated solution are used in place of the 12 drops of sample and three drops of 6 M HCl.

Use 5 mM MgSO₄ as a standard since it is the minimum detectable concentration with this test.

Ammonium. Put 10 drops of sample into a 50 mL plastic beaker. Moisten a piece of red litmus paper with deionized water and stick it on the convex side of a small watch glass. Add 10 drops of 6 M NaOH to the beaker and gently swirl to stir. Cover the beaker with the watch glass, convex side down. The litmus paper gradually turns blue if ammonium is present due to formation of ammonia gas. Care must be taken that no solution splashes on the litmus paper and gives a false positive. The watch glass can be removed after the litmus paper turns blue, and you may be able to detect the odor of ammonia.

Hoagland Solution Number 1 has no ammonium. The all-ammonium solution has 15 mM ammonium. Ten millimolar NH₄Cl is used as a standard.

Potassium. Potassium and Na are determined by flame emission tests. A bunsen burner or inexpensive, portable propane torch is needed along with a platinum wire loop.

Dip the end of the platinum wire in 12 M HCl, and flame the tip of the wire until it imparts no color to the

flame. Dip the wire loop in the sample and place in the flame. A violet flame, lasting 0.5 s indicates the presence of K.

If the sample solution contains Na, the violet K flame will be obscured by the stronger yellow Na flame. If this is the case, view the flame through one or two layers of cobalt blue glass or blue plastic film, which filter out the yellow color due to Na and allow the violet flame to be observed.

The Hoagland Solution Number 1 has 6 mM K. Ten millimolar KCl is the standard.

Sodium. Run the Na flame test as for K. A strong yellow flame, persisting for 1 to 2 s confirms the presence of Na. Ten millimolar NaCl is used as a standard.

Manganese. Put 10 drops of sample solution in a test tube, and add 10 drops of 6 M HNO₃. With a stainless steel spatula, add about 0.1 g of solid NaBiO₃ (sodium bismuthate) and mix. There should be a little solid bismuthate in excess. Let the mixture stand for a few minutes. A purple solution confirms the presence of Mn as permanganate. Sodium bismuthate oxidizes Mn to permanganate.

This test is not sensitive enough to detect the 9 μM Mn in Hoagland Solution Number 1. Therefore, samples are concentrated tenfold, e.g., 100 mL of sample is evaporated to dryness and dissolved in 5 mL of deionized water and 5 mL of 6 M HNO₃. Twenty drops of this concentrated sample is used in place of the 10 drops of sample and 10 drops of HNO₃. A 100 μM MnCl₂ solution is used as a standard.

Boron. Put 10 drops of sample in a plastic, disposable, 1-mL cuvette. Borosilicate glass test tubes should not be used because they may produce a false positive reaction. Add 8 drops of buffer masking reagent and 2 drops of azomethine-H reagent (Table 1). Mix thoroughly, and allow the color to develop at room temperature for at least 30 min. A yellow solution indicates the presence of B.

Prepare azomethine-H reagent just before lab and store in a refrigerator.

Hoagland Solution Number 1 contains 46 μM B as undissociated boric acid, B(OH)₃. A 500 μM B(OH)₃ standard is used to demonstrate toxic concentrations. For an optimal level standard, use one drop of 500 μM B(OH)₃ and 9 drops of deionized water in place of the 10 drops of sample.

Iron. Add 20 drops of sample to a test tube followed by one drop of 2.5 mM TPTZ (2,4,6-tripyridyl-*s*-triazine), two drops of 4 M sodium acetate, and two drops of 2 M HCl. Add a pinch of ascorbic acid using a stainless steel spatula and mix. A blue solution indicates the presence of Fe. The ascorbic acid reduces the ferric Fe in Fe-EDTA to ferrous Fe which forms a chelate with TPTZ.

The Hoagland Solution Number 1 was modified to contain 90 μM Fe as Fe-EDTA. The standard is 100 μM Fe-EDTA.

Bicarbonate. This test is usually used for water analysis where carbonate and bicarbonate are the major ions

which buffer the solution pH. For this lab, the nutrient solutions contain appreciable orthophosphates, which behave like bicarbonate in the titration. Thus, this test does not confirm the presence of bicarbonate. However, high readings from this test are likely to be due to bicarbonate, rather than phosphate.

Using a graduated cylinder, put 25 mL of sample into a 125-mL-Erlenmeyer flask. Add two drops of phenolphthalein indicator (Table 1).

If the solution turns pink, titrate with 25 mM H_2SO_4 until colorless. The titration is done with a burette supported by a ringstand or by counting the drops of acid added using a dropper. Record the milliliters of acid used and designate as y . To convert the number of drops to milliliters, divide by the appropriate number determined by adding drops to a 10-mL-graduated cylinder.

Next add two drops of methyl orange indicator (Table 1). Titrate to the orange endpoint, again recording the milliliters of acid added, and designating it as z .

Approximate the millimoles of carbonate as y and the millimoles of bicarbonate as $2z$. Normally, y will be zero. A 10 mM NaHCO_3 standard is used.

CLASS APPLICATION

The analysis lab was conducted the week after the solution culture study was started. Each student was given a data sheet listing the 13 tests and indicating which solutions were to be tested. Deionized water, an appropriate standard for the ion or element, Hoagland Solution Number 1, and the solutions deficient and toxic for that ion or element were tested. Therefore, there were four or five solutions for each test. Equipment and samples for each test were at a single location, so one or two students could complete the test for one element and then move on to the next. After the instructor demonstrated to the class how each test was performed, a class of 15 students took about 1 h to complete the tests. Laboratory safety was stressed since concentrated acids or bases are used in many of the tests (Nagel, 1989).

After deficiency/toxicity symptoms on solution culture plants were well developed, students were asked to identify what was wrong with the unknown plants and then confirm their diagnosis by analyzing the nutrient solution. A few trick plants were included to test students' knowledge of the limitations of nutrient deficiency symptoms. For example, new shoots of Sunburst honeylocust

(*Gleditsia triacanthos* var. *inermis* Willd. 'Sunburst') were placed in a Hoagland Solution Number 1. Students typically guess that the solution is deficient in Fe because the youngest leaves are bright yellow, but solution analysis does not confirm the diagnosis. Sunburst honeylocust was selected by horticulturists for the unusual appearance of the new leaves (Lancaster, 1974). The yellow leaves are normal, not an Fe deficiency. There are thousands of cultivars of variegated plants which could be used instead (Yokoi and Hirose, 1978). Other potential trick plants, whose symptoms appear to be caused by nutrient-deficient solutions, but are not, include plants grown with low root temperatures to induce phosphorus deficiency, plants overdosed by herbicides which suffer from chlorosis, or insect-damaged plants.

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