AGRICULTURAL MATERIALS

A 5-Day Method for Determination of Soluble Silicon Concentrations in Nonliquid Fertilizer Materials Using a Sodium Carbonate-Ammonium Nitrate Extractant Followed by Visible Spectroscopy with Heteropoly Blue Analysis: Single-Laboratory Validation

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A 5-day method for determining the soluble silicon (Si) concentrations in nonliquid fertilizer products was developed using a sodium carbonate (Na_2CO_3)–ammonium nitrate (NH_4NO_3) extractant followed by visible spectroscopy with heteropoly blue analysis at 660 nm. The 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method can be applied to quantify the plant-available Si in solid fertilizer products at levels ranging from 0.2 to 8.4% Si with an LOD of 0.06%, and LOQ of 0.20%. This Si extraction method for fertilizers correlates well with plant uptake of Si ($r^2 = 0.96$ for a range of solid fertilizers) and is applicable to solid Si fertilizer products including blended products and beneficial substances. Fertilizer materials can be processed as received using commercially available laboratory chemicals and materials at ambient laboratory temperatures. The single-laboratory validation of the 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method has been approved by The Association of American Plant Food Control Officials for testing nonliquid Si fertilizer products.

The recent elevation of silicon (Si) from fringe element to plant-beneficial substance by The Association of American Plant Food Control Officials (AAPFCO) at its midyear meeting February 19–23, 2012, in San Antonio, TX (1) has been long awaited. This validation process began with researchers and Si product manufacturers long before AAPFCO approval of the new category, termed "Beneficial Substances or Compounds" under the T-9.1 category "Secondary and Micro Plant Nutrients" (2). This new term was defined by AAPFCO (2) as "any substance or compound other than primary, secondary, and micro plant nutrients that can be demonstrated by scientific research to be beneficial to one or more species of plants." However, Si was only given tentative approval at that time pending development and validation of a standardized testing method for determining plant-available Si from fertilizer sources (3). The single-laboratory validation (SLV) of the 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method presented here received full approval for use at AAPFCO's February 2012 meeting elevating silicon to official status (4).

To date, no other official method in the United States has been approved for determining available Si from solid fertilizer sources. AAPFCO approval of this 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method for determining plant-available Si from nonliquid fertilizers (4) was anticipated, considering the first research into the use of Si as a fertilizer was reported in 1840 (5). Additionally, increased plant Si concentrations, associated with reduction in rice (*Oryza sativa* L.) blast disease (*Magnaporthe grisea* M.E. Barr), was recorded nearly a century ago in Japan (6). Since then, research has extended to other grasses and grains such as barley (*Hordeum vulgare* L.; 7), corn (*Zea mays* L.; 8), oats (*Avena sativa* L.; 9), pasture (10) and turf grasses (11); sugarcane (*Saccharum officinarum* L.; 12), and wheat (*Triticum aestivum* L.; 13), and to dicotyledonous crops such as cucumber (*Cucumis sativus* L.; 14), grapes (*Vitis vinifera* L.; 15), pepper (*Capsicum* L.; 16), pumpkin (*Cucurbita pepo* L.; 17), soybean (*Glycine max* (L.) Merr.; 18), and tomato (*Solanum lycopersicum* L.; 19). Beneficial effects from Si fertility have included increased stress tolerance (disease, insect, drought, salt, nutrient imbalance, low and high temperature) and yield increases with or without stress (20). However, this is not a complete list of crop plants and responses seen from Si fertility.

In 1999, the first International Conference on Silicon in Agriculture was held in Fort Lauderdale, FL. Since then, conferences have been held every three years; the most recent, The V International Conference on Silicon in Agriculture, was held in Beijing, China in September 2011.

Although Si is ubiquitous in nature, making up 25.7% of the earth's crust (21), not all forms of silicon found in soils or fertilizer products are soluble and plant-available (20, 22). The

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form of Si in soils that is soluble and available for plant uptake is monosilicic acid [$Si(OH)_4$], more commonly referred to as silicic acid or soluble silicon (23).

Worldwide Si removal from soils by crops has been estimated at 210–224 mega tons annually (24). Although the first U.S. patent on a solid Si fertilizer was issued in 1881 (25), prior to AAPFCO approval in 2012, U.S. fertilizer manufacturers could neither register Si products as beneficial substances nor include Si concentrations on fertilizer product labels. Purchasers of fertilizer products had no means of evaluating and comparing products based on their Si content or Si supplying capacity to meet plant uptake needs.

Scientists in Japan have been at the forefront in evaluating methods for determining the Si available from different slag sources. Various extractants including citric acid ($C_6H_8O_7$), ethanoic (acetic) acid (HOAc/CH_3COOH), and 4.0 pH ammonium acetate (CH_3COONH_4) buffer solution, and cation-exchange resins have been tested in attempts to find a valid method for determining available silicon (26, 27). The currently accepted method in Japan for evaluating plant-available Si from slag sources uses a hydrochloric acid (HCl) extractant method (28). However, these methods have been inefficient in determining plant-available Si from fertilizer sources, tending to either over- or underestimate plant-available Si (22).

The 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method presented here originated from the research of Pereira et al. (29), which showed resin Amberlite® (Rohm and Haas Co., Philadelphia, PA) and Na_2CO_3-NH_4NO_3 to be the best extractants of plant-available Si from solid Si sources. They determined that the time period for extraction that best correlated with plant uptake was between 5 and 9 days. Buck et al. (22) showed that liquid and solid Si fertilizer sources required different test methods, by testing for total Si using the extractants HCl and hydrofluoric acid (HF) for liquids, and testing for soluble silicon using the extractant Na_2CO_3-NH_4NO_3 for solid fertilizer sources.

Pereira et al. (30) continued work on a 5-day extraction procedure using 100 mL of a low M Na_2CO_3-NH_4NO_3 extractant added to 0.1 g of fertilizer product followed by shaking for 1 h at ambient temperature. This analytical method for soluble silicon using the heteropoly blue method (31) correlated well with plant uptake. However, a more rapid means (< 5 day) was pursued.

The heteropoly blue method is a colorimetric method for determining Si that uses ascorbic acid ($C_6H_8O_6$) to reduce the silico-molybdate complex formed under acid conditions to an intensely blue complex (31), which forms in direct proportion to the concentration of analyte (32) and is quantitated in fertilizer extracts by using visible spectroscopy at 660 nm. The heteropoly blue method requires that Si be in the form of silicic acid. This is referred to as molybdate-reactive silica and is generally considered to be less than three units SiO_2 polymerized (33).

Following the original trials of Pereira et al. (29) and further research by Buck et al. (22), an accelerated 2 h extraction method at elevated temperature was tested (30). The test portion was shaken in an 85°C water bath for 2 h, followed by 2 h of cooling. The Accelerated Method was used as a basis for optimization testing. Optimization tests showed major sensitivities to bath temperature and test portion weight, in addition to minor sensitivities in bath time, time to reading, and cooling time. Poor reproducibility of the Accelerated Method, including problems in maintaining water bath volume and constant temperature in the laboratory setting, increased worker burn risk, high HorRat values (34), and low predictive capability (r^2), resulted in a need to revisit the Original 5-Day Method (29). The HorRat is an accepted tool in analytical chemistry for determining the precision (repeatability and reproducibility) of an analytical method. The HorRat equation (listed under *Precision,* below) is a ratio of the observed RSD to predicted RSD (PRSD), and tends to conservatively overestimate variability at the extremes, both high and low (34, 35). Reported Si extraction detection limits are reported as LOD, the true net signal level predicted to result in detection, and LOQ, the signal level above which the measurement can be performed at a stated relative uncertainty level (36).

The 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method presented herein includes the following optimizations to the Original 5-Day Method: testing is conducted within a prescribed range of ambient laboratory temperatures; test portion weights and volumes of extractant are increased; talc, magnesium silicate-[$Mg_3Si_4O_{10}(OH)_2$], is used as the spiked matrix for recovery evaluation; and larger aliquots of extract are analyzed for fertilizer materials containing low concentrations of soluble Si.

SLV

Method Optimization

Optimization testing was conducted on the Accelerated Method to determine the effects of six variables: bath temperature, bath time, cooling time, read (time) delay, volume of each extractant, and sample weight on the % Si extracted from six fertilizer sources (wollastonite, slag, potassium silicate-liquid, Ca/Mg blend, silica gel, and silicic acid). Eight runs were conducted for each of two variants of each test factor. An optimization matrix was developed using seven variables in a standard Youden square design (YSD; 37). Factors and their variants included: bath temperature, 60 and 90°C; bath time, 1.75 and 2.25 h; cooling time, 1.75 and 2.25 h; read delay, 50 and 70 min; volume of Na_2CO_3, 45 and 55 mL; volume of NH_4NO_3, 45 and 55 mL; and test portion weight, 0.095 and 0.105 g. The difference (D) in % silicon extraction was determined using the following formula:

$$D_F = \bar{x}^{(+)} - \bar{x}^{(-)}$$

where F = factor tested, X = % Si from each variant run, and (+) and (–) are the highest and lowest variant levels, respectively. The differentials in % Si were then plotted versus the seven variables.

Interference Agent Testing

Interference testing was performed by adding 1 mL potential interfering reagent (all ACS reagent grade materials were used for interference testing and were obtained from Fisher Scientific Pittsburgh, PA) to 1 mL of the wollastonite extractant solution at the dilution step to obtain a representative 50/50 (v/v) mixture. Separate extractions were conducted for each potential interfering agent: monocalcium phosphate [$Ca(H_2PO_4)_2$]; iron (II) sulfate ($FeSO_4 \cdot 7H_2O$); potassium chloride (KCl); and ammonium nitrate (NH_4NO_3). Wollastonite was chosen as the Si fertilizer source for interference testing because of its known Si extraction value (2.54 %) when processed at 75°C with a 2 h sample shaking time. An initial 10% reduction in Si recovery

prompted further interference testing using a 50/50 (v/v) blend of KCl and wollastonite at ambient laboratory temperature (22–25°C).

Test Materials

The Si sources analyzed during this SLV included: Vansil® (wollastonite) obtained from R.T. Vanderbilt Co, Norwalk, CT; SILI-CAL™ (slag) obtained from Calcium Silicate Corp., Lake Harbor, FL; magnesium silicate (talc) obtained from Atlantic Equipment Engineers, Bergenfield, NJ; AgrowSil™ (Ca/Mg silicate blend) obtained from Harsco Metals & Minerals, Sarver, PA; and AG-SIL 25® (K_2SiO_3-liquid) obtained from PQ Corp., Valley Forge, PA.

METHOD

Applicability Statement

This 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method is applicable to the detection of soluble Si in nonliquid fertilizer products, blended products, and beneficial substances at Si concentrations of 0.2–8.4%, with an LOD of 0.06% Si, and an LOQ of 0.20%.

Method Principle

Soluble Si from solid Si fertilizer sources is extracted at ambient room temperature using a dilute Na_2CO_3-NH_4NO_3 extractant. The extractant solution is analyzed by manual spectrophotometry at 660 nm using the heteropoly blue method. Soluble Si is reported as % Si.

Caution Statement

Safety glasses, gloves, and lab coats should be worn at all times. NH_4NO_3 is a strong oxidizing agent and should not be used near flames, heating or ignition sources, combustible materials, or reducing agents to avoid potential combustion or explosive hazards. NH_4NO_3 should be separated from all organic materials present within the laboratory. Check with government agencies for any applicable regulatory licensing requirements before obtaining or using NH_4NO_3. Flexible vinyl gloves should be worn when mixing Na_2CO_3 and NH_4NO_3, as the extraction reagent is caustic. Care should be taken to avoid skin or eye contact. Spills should be cleaned up immediately. If contact is made with skin or eyes, flush with tap water immediately and seek medical attention.

Gloves, goggles, face shields, and lab coats should always be worn when handling concentrated sulfuric acid. Sulfuric acid is extremely corrosive and dehydrating, causing severe burns when in contact with skin or eyes. Rinse immediately with fresh water if sulfuric acid comes in contact with skin or eyes and seek immediate medical attention. When diluting concentrated sulfuric acid, always add sulfuric acid to water and not water to sulfuric acid, as the latter will result in intense fuming and spattering, and should be avoided.

Apparatus

All glass, plastic, and labware including pipets and weighing papers/boats, etc. used were obtained from either VWR, (Radnor, PA), or Fisher Scientific (Pittsburgh, PA). The quantities of each needed will depend on the number of samples being analyzed. Because silica (SiO_2) is a constituent of glassware commonly used in laboratory settings, and detection limits are at 0.06% Si, it is important to adhere to these strict cleaning instructions.

(**a**) *Glassware*.—Glassware should be thoroughly acid-cleaned before use in reagent preparation by soaking in 4% HNO_3 solution for 30 min and rinsed three times with deionized water. Allow glassware to air dry prior to use. Glassware items needed include 100 mL to 1 L volumetric flasks, 1 L beakers, and assorted 5 to 50 mL calibrated pipets. All glassware (including flasks and pipets) must conform to Class A tolerances as recommended for routine laboratory applications.

(**b**) *Plasticware*.—Plastic storage bottles used for reagent storage and test sample extraction should be cleaned well with warm tap water, followed by triple rinsing with deionized water. Plasticware items needed include: 50 to 1 L graduated cylinders, 200 mL Nalgene volumetric flasks with screw closure; 250 mL Nalgene Erlenymer flasks with screw closure; 50 mL centrifuge or test tubes with screw closure; 250 mL to 1 L Nalgene or fluorinated ethylene propylene (FEP) narrow-mouth storage bottles with screw closure; and 20 L wide-mouth dispensing bottles with screw closure (also Nalgene or FEP).

(**c**) *pH meter*.—Accumet™ AB15 Basic pH/mV benchtop meter (Fisher Scientific or equivalent).

(**d**) *Touch agitator*.—Thermolyne Maxi Mix II 12v Vortex Mixer, Model M37615 (Barnstead Thermolyne Corp., Dubuque, IA), or equivalent.

(**e**) *Analytical balance*.—Mettler AG200, accuracy to 0.1 mg (Mettler Toledo, Columbus, OH), or equivalent.

(**f**) *Orbital platform shaker*.—LabLine Orbit (Melrose Park, IL) shaker, model 3590 equipped with timer and adjustable rpm control, or equivalent.

(**g**) *Wrist-action shaker*.—Burrell (Pittsburgh, PA), VWR, or equivalent.

(**h**) *Spectrophotometer*.—Jasco (Easton, MD) V-630, equipped with a 10 mm flow cell, peristaltic sipper & SpectraManager software package, or equivalent. The flow cell should be cleaned at least 15 min before the start of test sample readings, and upon completion of readings. Clean the flow cell by flushing with a 10% (v/v) solution of HCl. Allow the acid solution to remain in the cell for a minimum of 5 min. Dump and dispose of the acid solution and pass water through the cell (about 100 mL). Read the Á value. If necessary, rezero the instrument and continue flushing with water until the Á value is stable. For the manual cell, allow the quartz cell to sit in a dilute HCl solution (10%, v/v) for a minimum of 5 min. Rinse thoroughly with water three times. Blot dry with a soft tissue and visually examine for streaking or discoloration of the cell. Use a cotton-tipped swab to gently remove any stains, then rinse the cell thoroughly with water three times. Repeat this process as needed. Check the Á of water to confirm a stable reading before test sample analysis.

(**i**) *Sample grinder*.—Capable of grinding to a fineness passing a 300 μm sieve, Micron Bantam Mill (Micron, Summit, NJ) or equivalent.

(**j**) *Refrigeration unit*.—0.31 m^3 capacity and capable of maintaining a temperature of 4°C (GE, Louisville, KY, or similar brand).

(**k**) *Drying oven*.—Blue-M, Stabil-Therm, or similar

convection oven capable of holding a constant temperature of 105 ± 2°C (VWR).

Reagents and Calibration Standards

Chemicals for reagent preparation and interfering agents were purchased from VWR and Fisher Scientific. All water used for reagent preparation was obtained from municipal tap water processed by softening, carbon filtration, reverse osmosis (0.45 micron) and deionization using a Feed Water Solutions (Tampa, FL) system to produce 18 mΩ water.

Silicon stock solution standard of 1,000 mg/L containing 0.5 M NaOH was obtained from Acros Organics, Part No. 19629100 (Fisher Scientific). If other Si stock standards are used, the Si must be in the chemical form of SiO_2 and traceable to a National Institute of Standards and Technology Standard Reference Materials listing. Prepare fresh Si calibration standard solutions weekly. Reagents and Si calibration standard solutions were prepared using a w/v method.

Reagents

Cap all reagents tightly after preparation to prevent evaporation and shake prior to removal of any aliquot(s). Once prepared, transfer reagents immediately from glass flasks to plastic Nalgene storage bottles and store at ambient laboratory temperature (22 to 25°C) or at 3–5°C if refrigeration is required (e.g., for ascorbic acid).

(**a**) *Sodium carbonate solution, 0.094 M.*—Fill a 19 L plastic dispensing bottle with 18 L water. Add 180 g anhydrous Na_2CO_3. Stir to dissolve. Cap container tightly. Prepare fresh solution monthly.

(**b**) *Ammonium nitrate solution*, *0.20 M.*—Fill a 19 L plastic dispensing bottle with 18 L water. Add 288 g NH_4NO_3. Stir to dissolve. Cap container tightly. Prepare fresh solution monthly.

(**c**) *Na_2CO_3-NH_4NO_3 extractant solution, 9.4 pH.*—After preparation of solutions (**a**) and (**b**) above, add 50 mL of each solution to a plastic beaker, stir, and verify a pH of 9.4 ±0.05 for the mixed solution using a pH meter and following manufacturer's instructions. *Note*: If solution pH is not within the required range, dispose of solution and make fresh items (**a**) and/or (**b**) and repeat (**c**) until the correct solution pH is achieved.

(**d**) *Ammonium molybdate solution, 0.42 M.*—Add 75 g ammonium molybdate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] to a 1 L beaker. Add 500 mL water. Dissolve. To avoid splattering, slowly add 100 mL concentrated (18.4 M) sulfuric acid (H_2SO_4). Cool. Transfer to a 1 L volumetric flask. Dilute to 1 L with water. Transfer to a plastic storage bottle. Cap tightly. Prepare fresh solution weekly.

(**e**) *Tartaric acid solution, 1.33 M.*—Add 200 g tartaric acid to 1 L beaker. Add 700 mL water. Stir. Transfer to 1 L volumetric flask. Dilute to 1 L with water. Transfer to a plastic storage bottle. Cap tightly. Prepare fresh solution if not used within 3 days.

(**f**) *Ascorbic acid solution, 0.017 M.*—Add 3 g ascorbic acid to 1 L volumetric flask. Dilute to 1 L with water. Stopper flask and mix by inverting 10 times. Transfer to a plastic storage bottle. Cap tightly and refrigerate. Once prepared, this reagent must be used within 3 days and allowed to come to room temperature before use.

(**g**) *Intermediate Si standard solution, 50 mg/L.*—Prepare a 50 mg/L intermediate standard by diluting 5 mL of the stock 1000 mg/L Si standard to 100 mL in a volumetric flask. Transfer immediately to a plastic storage bottle. Prepare fresh solution weekly.

(**h**) *Silicon spike solution, 500 mg/L.*—Pipette 50 mL of silicon stock solution standard into a 100 mL volumetric flask. Dilute to 100 mL with water. Transfer to a plastic storage bottle. Prepare fresh solution weekly.

Calibration Standards

Prepare fresh calibration standard solutions weekly.

(**a**) *Blank, 0 mg Si/L.*—Add 10 mL sodium carbonate–ammonium nitrate extraction solution to a 1 L volumetric flask. Dilute to 1 L with water. Stopper flask and mix by inverting 10 times. Transfer to a plastic storage bottle.

(**b**) *Standard 1, 0.25 mg Si/L.*—Add 10 mL sodium carbonate–ammonium nitrate extraction solution to a 1 L volumetric flask. Add 5 mL intermediate silicon standard solution by pipetting. Dilute to 1 L with water. Stopper flask and mix by inverting 10 times. Transfer solution to a plastic storage bottle. Cap tightly.

(**c**) *Standard 2, 0.50 mg Si/L.*—Add 10 mL sodium carbonate–ammonium nitrate extraction solution to a 1 L volumetric flask. Add 10 mL intermediate silicon standard solution by pipetting. Dilute to 1 L with water. Stopper flask and mix well by inverting 10 times. Transfer to a plastic storage bottle. Cap tightly.

(**d**) *Standard 3, 1.0 mg Si/L.*—Add 10 mL sodium carbonate–ammonium nitrate extraction solution to a 1 L volumetric flask. Add 20 mL intermediate silicon standard solution by pipetting. Dilute to 1 L with water. Stopper flask and mix by inverting 10 times. Transfer to a plastic storage bottle. Cap tightly.

(**e**) *Standard 4, 2.0 mg Si/L.*—Add 10 mL sodium carbonate–ammonium nitrate extraction solution to a 1 L volumetric flask. Add 40 mL intermediate silicon standard solution by pipetting. Dilute to 1 L with water. Stopper flask and mix by inverting 10 times. Transfer to a plastic storage bottle. Cap tightly.

Extraction

Dry control test portions (e.g., wollastonite and talc) for 2 h at 105 ± 5°C. All other fertilizer materials need not be dried and should be processed on an as-is moisture basis.

(**a**) *Grind test sample*.—Grind fertilizer material to pass a 300 µm sieve (USA standard No. 50).

(**b**) *Weigh test portion*.—Weigh out a 0.2 g test portion with a variance not to exceed ± 0.005 g. Transfer to a 250 mL tared plastic flask, weigh again after transfer, and record test portion weight.

(**c**) *Addition of extraction solution*.—Add 100 mL each of sodium carbonate and ammonium nitrate solutions using a plastic graduated cylinder.

(**d**) *Shaking of extraction sample*.—Cap flask tightly and shake solution at 140 rpm (table unit) or 60 rpm (wrist action shaker) at ambient temperature (25°C ± 3°C) for 60 min ± 1 min.

(**e**) *Resting of extraction sample*.—Remove from shaker and let stand undisturbed for 5 days. *Note*: Begin 5 day timer at start of shaking.

(**f**) *Spiking procedure*.—Prepare a spiked talc sample by extracting talc using steps (**a**–**c**) above. Before step (**d**), add 3 mL 500 mg/L Si spike solution to the talc test sample. This

talc-spiked test sample is processed and used for the matrix spike recovery test to verify that soluble (spike) rather than insoluble Si (talc) is extracted and reported using this method.

(**g**) *Test sample duplication*.—Make a duplicate of at least one of the unknown test samples.

Heteropoly Blue Analysis

(**a**) *Test solution preparation*.—At the end of 5 days, transfer 2 mL (4 mL for materials expected to be < 3% Si) of resting extraction sample, step (**e**) above, by Eppendorf or similar plastic pipet (do not move or agitate test solution prior to pipetting) to a 200 mL polypropylene volumetric flask. Dilute to 200 mL with water. Stopper flask and mix by inverting 10 times. Pipette 20 mL of diluted test solution into a plastic test tube. *Note*: Either proceed directly to step (**b**) or aliquot may be allowed to sit overnight prior to proceeding.

(**b**) *Calibration standard analysis*.—Prepare Si calibration standards, blank, and standards 1–4, by pipetting 20 mL of each standard or blank solution into a plastic test tube.

(**c**) *Reagent additions*.—Add prepared reagents (*Reagent* section, above) as follows: Add 2 mL ammonium molybdate solution and mix well for 10 s using a touch agitator. Wait 10 min. Add 2 mL tartaric acid solution. Stopper test tube and mix well for 10 s using a touch agitator. Wait 5 min. Add 2 mL ascorbic acid solution. Stopper test tube and mix well for 10 s using a touch agitator.

(**d**) *Color development*.—Allow test sample, blank, and standards to stand for 60 ±1 min for color development. Color gradation from blue to purple should be seen with increasing Si concentration.

Manual Spectrophotometer Analysis

(**a**) *Spectrophotometer setting*.—Set the spectrophotometer wavelength to 660 nm and mode to *A*.

(**b**) *Flushing of flow cell*.—Flush the flow cell or cuvette three times with water before initiating blank and standard sample readings, after reading standard 4, and after every three test samples. Flushing is used to prevent coating of the flow cell.

(**c**) *Absorbance readings*.—Read and record *A* data for blank, standards, and test samples. Determine the linear correlation equations using concentration versus *A* data by graphing or linear regression using Microsoft Excel, MS Corp. (38) or similar program. Calculate % Si concentration in test samples using the equations below.

Calculations

Si concentration in test samples.—To determine the soluble (% Si) in the fertilizer product test sample, use the following equation:

$$\% \text{ Si} = [((K \times A) + B) \times Vi / W \times Vf / Va] / 10{,}000$$

where K = coefficient K1, or slope factor from standard curve, *A* = absorbance of test solution, B = intercept from the standard curve, Vi = initial volume in mL (200 mL for our test sample), Va = test portion aliquot volume taken for dilution in mL, (2 or 4 mL), Vf = final volume in mL (200 mL for our sample), W = test portion weight in mg (200 mg for our sample), and 10 000 = conversion from mg/L to %.

Spike recovery.—To determine the % Si spike recovery, use the following equation:

$$\% \text{ Si spike recovery} = (C_f - C_u) \times 100 / C_a$$

where C_f = observed fortified test sample concentration, C_u = observed unfortified test sample concentration, C_a = calculated concentration of spike using a test portion weight basis, rather than a solution basis (500 mg/L × 0.003 L/(0.2 g × 1000 mg/g) × 100 for our test sample).

Precision

To obtain precision, method repeatability SD and repeatability RSD values were calculated for each test sample. Calculated values were compared to the PRSD using the HorRat equation (34):

$$PRSD = 2C^{-0.1505}$$

where C = sample concentration expressed as a mass fraction (or decimal part/hundred).

Statistical Analysis

A target range for HorRat (34) values (RSDr/PRSD) was set at 0.3 to 1.3. Statistical analysis was performed using the statistical database in Microsoft Excel (38).

Quality Control

When processing unknowns, adhere to these quality control procedures:

(**a**) *Control*.—Include one or more control test samples of known Si concentration, preferably within the predicted range of Si in test samples, for added accuracy. No certified check samples are currently available, so the known should be prepared from materials different from those used as calibration standards.

(**b**) *Duplication*.—Duplication of at least one of the unknown materials being analyzed is needed to ensure that the process produces similar results.

(**c**) *Blank*.—A blank is used to ensure that contamination of reagents and glassware has not occurred.

(**d**) *Spiking*.—Spike a test portion of talc to check extraction recovery efficiency, referred to as matrix spike recovery. The talc spiked test sample is used to verify that the Si extracted and reported using this method is soluble Si (spike) rather than insoluble Si (talc).

Comments

Because of the chemical reactions of various Si compounds, such as potassium silicate (K_2SiO_3), magnesium silicate ($MgSiO_3$), and calcium silicate ($CaSiO_3$) to form soluble Si prior to plant uptake, fertilizer, soil, and plant tissue, i.e., soluble silicon, is reported as elemental Si in %, mg/kg, or g/kg rather than as monosilicic acid [$Si(OH)_4$].

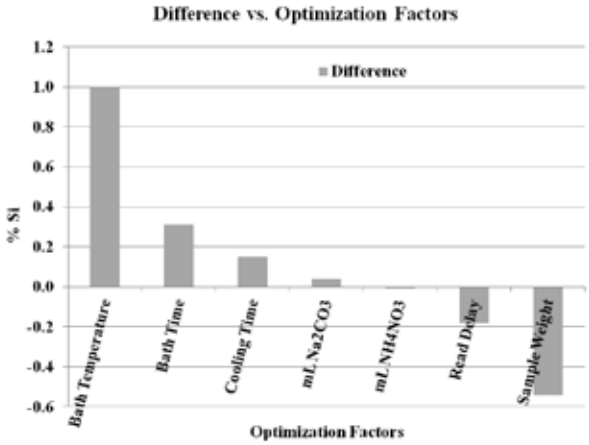

Figure 1. Differences in % Si extracted from six Si fertilizer sources due to changes in seven method variables (optimization factors).

Results and Discussion

Method Optimization

The original 5-Day Extraction Method of Pereira et al. (29) was further developed by Buck et al. (22), who compared the efficacy of various extractants. Optimization of the extraction method was achieved using 0.094 M Na_2CO_3 and 0.2 M NH_4NO_3 as extractants with a 1 h sample shaking time (initiation of the 5-day extraction time period) at ambient temperature. In an attempt to expedite sample processing (Accelerated Method), Pereira et al. (30) tested the effects of altered water bath temperature (85°C), bath time (2 h), and cooling time (2 h). Sources of variability in the Accelerated Method were evaluated using ruggedness test methodology (37).

Factors selected for optimization testing were considered major variables in the extraction procedure. These seven factors were tested and evaluated using a standard YSD (37). During testing, bath temperature span was increased to determine the feasibility of using 65°C as the water bath temperature, a potential optimization step, as fertilizer laboratories routinely have a bath set at 65°C for testing phosphorus availability from phosphate fertilizer sources (39). The differentials in extracted Si were calculated for each variable by source (major factor minus minor factor), averaged by variable, then ranked according to the magnitude of % Si differential (Figure 1).

Optimization tests revealed high sensitivity in Si extraction levels based on bath temperature and test portion weight with smaller differences seen for bath time, read delay, and cooling time. With the exception of bath temperature, factors were equally distributed around the nominal test parameters as originally stated by the Accelerated Method developers (30). Although acceptable factor differences are a function of concentration, at the Si concentration levels needed for validation of the Original 5-Day Method, differences above 0.1 were insufficient for analysis of plant-available Si in fertilizer sources, thus warranting further consideration.

Tabulation of optimization factors provided confirmation of sensitivity issues (Table 1); although reasonable variations can be expected to occur during routine laboratory analysis, the high temperature (Accelerated Method) also required frequent analyst attention to maintain proper water bath volume due to high evaporation rates. Optimization testing resulted in lowering of the bath temperature to 75°C to alleviate these issues, and an initial study of potential interferences, accuracy, and repeatability testing was conducted using 11 Si fertilizer sources (Table 2).

Table 1. Tabulation of optimization test factors[a]

No.	Optimization factor	Major value	Method value	Minor value
1	Sample weight (g)	A = 0.105	0.1	a = 0.095
2	Na_2CO_3 solution volume (mL)	B = 55	50	b = 45
3	NH_4NO_3 solution volume (mL)	C = 55	50	c = 45
4	Water bath temperature (°C)	D = 90°C	85	d = 60°C
5	Bath time (h)	E = 1.75.	2	e = 2.25
6	Cooling time (h)	F = 1.75	2	f = 2.25
7	Read delay (min)	G = 50	60	g = 70

[a] Major (upper case) and minor (lower case) values represent upper and lower predicted extremes in variance from method values.

Table 2. Silicon fertilizer source materials processed using optimization test procedures for estimated total and soluble Si at three different processing temperatures

Si source	Total Si, %	Soluble Si, %	Comment
Wollastonite	24.2	3.6	85/75°C/ambient
Slag	20.3	0.5	85/75°C only
Talc	28.5	0.1	85/75°C/ambient
Ca/Mg silicate blend	12.0	2.2	85/75°C/ambient
Silica gel	46.7	5.8	85/75°C/ambient
K_2SiO_3-liquid	9.70	7.6	85/75°C/ambient
Silicic acid	36.0	6.4	85/75°C only
Monocal, Si blend	12.1	1.8	85/75°C only
KCl-Si blend	12.1	1.8	85/75°C only
$FeSO_4$- Si blend	12.1	1.8	85/75°C only
NH_4NO_3- Si blend	12.1	1.8	85/75°C only

Each of the 11 Si fertilizer source materials and mixtures was subjected to further optimization testing. Total Si was determined gravimetrically using AOAC *Official Method* **963.02** (39). Soluble Si concentrations were determined at stable temperatures, but due to a lack of variability in test results for materials of similar composition, test runs at ambient temperature for all materials were not considered necessary by AAPFCO's review committee. Extractable Si varied from 0.35 to 78.35% of total Si depending on fertilizer source, confirming the need for a method to differentiate soluble Si from total Si in fertilizer materials (Table 2).

Interference Agent Testing

Known potential interferences with colorimetric Si analysis occur with phosphate, chloride, iron, and ammonium nitrate, with the main effects of interfering compounds occurring during color formation (40). The unadulterated wollastonite product used for interference testing analyzed at 2.54% soluble Si, with

Table 3. Effects of four known interference compounds on Si extraction using wollastonite as the selectivity control[a]

Sample	Rep 1% Si	Rep 2% Si	Mean	Recovery, %
Wollastonite plus $Ca(H_2PO_4)_2$	1.21	1.20	1.21	95.04
Wollastonite plus $FeSO_4$	1.42	1.20	1.31	103.11
Wollastonite plus NH_4NO_3	1.32	1.29	1.30	102.36
Wollastonite plus KCl	1.19	1.12	1.16	90.94

[a] Expected Si extraction from wollastonite is 1.27%.

Table 4. Interference testing of a 50/50 blend of KCl and wollastonite on Si extraction at ambient laboratory temperature (22 to 25°C)[a]

Sample	Rep 1% Si	Rep 2% Si	Rep 3% Si	Mean	Recovery, %
Wollastonite	2.47	2.69	2.55	2.57	100.00
Wollastonite plus KCl	1.29	1.31	1.27	1.29	100.00

[a] Expected Si extraction was 2.50% for wollastonite and 1.29% for the KCl-wollastonite blend.

Table 5. Comparison of HorRat values for two Si extraction methods, the 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction (SLV 5-Day) and Accelerated methods, using five Si sources

Si source	HorRat, SLV 5-Day	HorRat, Accelerated
Wollastonite	0.79	6.58
Ca/Mg silicate blend	0.98	1.92
Talc	13.12	5.76
Silica gel	1.6	1.73
K_2SiO_3-liquid	1.54	1.37

an expected extraction level of 1.27% Si under noninterfering conditions for a 50/50 blend of wollastonite and potential interfering agent at 75°C. Testing of wollastonite samples for potential interference revealed that KCl, more specifically the chloride ion (Cl^-), reduced Si recovery by nearly 10% during the analytical process (Table 3). Other potential interfering agents tended to be within ±5% of expected extraction levels. Therefore, the AAPFCO review committee recommended that when analyzing chloride-mixed Si fertilizer sources, the fertilizer samples be prewashed on a fine filter before processing to lessen the tendency for chloride-induced Si recovery reduction. However, analysis of prewashed fertilizer samples showed soluble Si to be removed in addition to chloride. Additional testing of a 50/50 (v/v) blend of wollastonite and KCl showed no chloride interference when samples were processed at ambient laboratory temperatures of 20–22°C (Table 4). A possible explanation is that interference testing at 75°C resulted in a saturated extract and the addition of KCl had a salting-out effect. Another possible reason could be precipitation or agglomeration to non-molybdate-reactive silica during cooling. Regardless of the reason for these reductions in soluble Si at

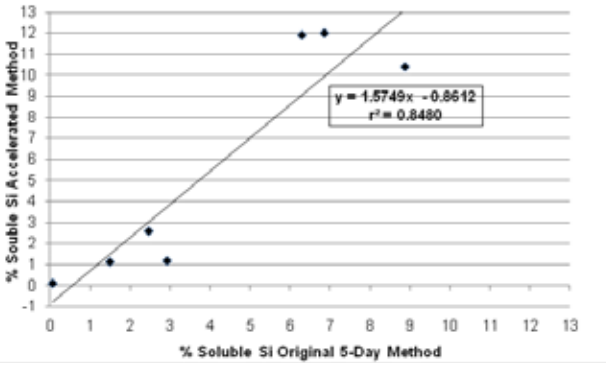

Figure 2. Linear correlation between Si extracted from seven fertilizer sources using either the Accelerated (2 h, 75°C) or Original 5-Day Method.

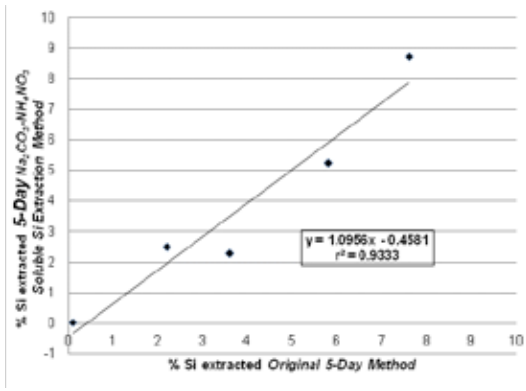

Figure 3. Linear correlation between Si extracted from five fertilizer sources using either the 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction or the Original 5-Day Method.

75°C, Cl is not expected to interfere with soluble Si analytical results for Si-blended fertilizers containing Cl at blended rates ≤50/50 (v/v) when samples are processed at ambient laboratory temperatures 20–22°C with no prewashing of samples.

Precision

The Accelerated Method at 75°C did not meet accuracy and repeatability requirements. High HorRat (34) values above AOAC-recommended upper limits (about 1.3; 41) were observed for all five Si sources tested (Table 5). A low correlation in Si extraction between the Accelerated and Original 5-Day Methods (r^2 =0.848) was also shown (Figure 2; 29, 30). Si recovery levels were also reduced by 20 to 50% for $CaSiO_3$ compounds (common solid Si fertilizer sources) when extraction was performed using the Accelerated Method (30) compared with the Original 5-Day Method (data not shown; 29). After reviewing the results from the Accelerated Method (30) were reviewed, it was determined that a need to revisit the Original 5-Day Method (29) was warranted. Optimizations to improve accuracy and repeatability of the Original 5-Day Method included: raising sample weight from 0.1 to 0.2 g; increasing extractant volume to 200 mL; analysis of 4 mL extract for low level (< 3% soluble Si) fertilizer sources; and analysis of 2 mL extract for fertilizer samples containing ≥ 3% soluble Si.

Results from this optimization work were compared with the results of both the Original 5-Day Method (29) and the

Table 6. Silicon extraction detection limits using a 0.2 g sample and the 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method

Mean % Si	SD	LOD[a] % Si	LOQ[b] % Si
0	0.02	0.06	0.20

[a] Detection limit LOD refers to the true net signal level predicted to result in detection.

[b] Determination limit LOQ refers to the signal level above which the measurement can be performed at a stated relative uncertainty level.

Table 7. Comparison of Original 5-Day and 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Methods (SLV 5-Day) in extraction of soluble Si from five Si sources

Si source	Original 5-Day Method, % Si extracted	SLV 5-Day Method, % Si extracted
Wollastonite	3.60	2.31
Ca/Mg silicate blend	2.20	2.50
Talc	0.10	0.03
Silica gel	5.80	5.27
K_2SiO_3-liquid	7.60	8.74

Accelerated Method (30; Table 5 and Figure 3). In the SLV of the 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method, talc acted like a true blank, with Si extraction levels below detection limits (Table 5). A strong correlation was exhibited between Si extraction values resulting from the 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction and Original 5-Day methods (r^2=0.933; 29; Figure 3). Raising sample weight improved LOD and LOQ limits (Table 6). Significant improvement in extraction from low-level samples over the Accelerated Method (30) resulted from analysis of an increased extract volume (4 mL versus 2 mL) using the SLV 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method (data not shown). Increased recovery from liquid Si fertilizer sources was also obtained using the 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction when compared with the Original 5-Day methods (Table 7; 29). Repeatability using the 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method was also improved for most samples when compared with the Accelerated Method (Table 5; 30).

Conclusions

The 5-Day Na_2CO_3-NH_4NO_3 Soluble Si Extraction Method described here is for the extraction and analysis of soluble Si in solid Si fertilizer sources. Silicon is extracted at ambient room temperature using a dilute Na_2CO_3-NH_4NO_3 extractant solution, and measured manually using visible spectroscopy at 660 nm with heteropoly blue; soluble Si is reported as % Si in the fertilizer source. The method is applicable to the detection of soluble Si in nonliquid fertilizer products, blended products, and beneficial substances at Si concentrations of 0.2 to 8.4%, with an LOD of 0.06% Si and an LOQ of 0.20%. Although liquids were included to demonstrate agreement with the Original 5-Day Method (29), a low bias of Si recovery (90.1%) and low correlation with plant uptake (r^2=0.75) was observed during analysis of liquid-soluble Si fertilizer sources (22). This is in agreement with our findings for K_2SiO_3's total Si versus soluble Si (Tables 2 and 6). It is suggested that when analyzing liquid Si fertilizer products for plant-available Si, either direct analysis after mixing or a total Si method using a stronger solvent, such as HF (CAS No. 7664-39-3) be used (22).

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