Wet Chemistry

- ***(The How, Why and What next?)***
  - ABSTRACT:
  - This is a general discussion of Wet Chemistry techniques including TSS, BOD and Nitrogen and Phosphorus. The intent is to cover approved methodologies, procedures, QA, data interpretation and troubleshooting.

Outline:

- *I Methods*
- *II TSS*
- *III BOD*
- *IV Calibration*
- *V Nitrogen*
- *VI Phosphorus*

I. Methods

- MUR
  - **Possibly this spring**
  - **19, 20 or 21st ED Standard Methods**
  - **Standard Methods**
  - **40 CFR 136**

II. TSS

- ***A. Filters (Preweighed or not)***
  - 1. Preparing
- ***B. Yield (10-200mg)***
- ***C. Filter Time (10min.)/Volume***
- ***D. Drying and Drying Studies***
  - the lesser of .5mg or 4%
- ***E. Duplicates and RPD***
  - 5% from average

% Difference vs. Relative Percent Difference

- $\frac{\text{Sample - Duplicate}}{\text{Sample}} \times 100$ $\qquad$ $\frac{\text{Sample - Duplicate}}{\text{(Sample + Duplicate)/2}} \times 100$
- Example: Sample = 100mg/l, Duplicate = 110mg/l
- $\%\text{Diff.} = \frac{100-110}{100} \times 100$ $\qquad$ $\text{RPD} = \frac{100-110}{(100+110)/2} \times 100$
- %Diff. = 10% $\qquad$ RPD = 9.5%

- ***F. Balances, Desicators and Ovens***
- ***G. Procedure***
  - 1. Sample mixing
  - 2. Assembling filter and seating filter
  - 3. Pour sample and time
  - 4. Allow about 3 minutes to dry
  - 5. Place in oven (104+/-1 for 1 hour)
  - 6. Remove to cool
  - 7. Weigh and reweigh

- ***H.QA***
  - 1. Duplicates
  - 2. Drying Study
  - 3. Blanks
  - 4. Spikes
  - 5. Filter Time
  - 6. Filter Yield

- ***I. Troubleshooting***
  - 1. Drying temp
  - 2. Desiccant
  - 3. Oils or other volatile 'solids'
  - 4. Filter size
  - 5. Mixing to avoid inhomogeneity

III. BOD

- ***A. Blanks***
- ***B. Seed Curve***
- ***C. GGA***
  - 6.6 Glucose-Glutamic acid solution: Add 0.150 g glucose and 0.156 g glutamic acid to distilled water and dilute to 1 l. Both reagents should be dried for 1 hour at 103øC before use. Add 6ml for each GGA. Prepare fresh daily.

- ***D. Nutrient Water***
  - 6.1 Magnesium Sulfate solution: Dissolve 22.5 g $MgSO_4 \cdot 7 H_2O$ in distilled water and dilute to 1 l.
  - 6.2 Calcium Chloride solution: Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 l.
  - 6.3 Ferric Chloride solution: Dissolve 0.25 g $FeCl_3 \cdot 6 H_2O$ in distilled water and dilute to 1 l.
  - 6.4 Phosphate buffer solution: Dissolve 8.5 KH_2PO_4, 21.75g $K_2HPO_4 \cdot 7 H_2O$, 33.4g $Na_2HPO_4 \cdot 7H_2O$ and 1.7 g NH_4Cl in about 300 ml distilled and dilute to 1 l. The pH should be 7.2 without adjustment.

D. Dilutions

- *Color*
- *Odor*
- *Historical Knowledge*
- *COD*
- *Use additional dilutions to span a wider range*

E. Saturation and Calibration

- 1. Altitude
- 2. Temperature
- 3. Membrane
- 4. Calibration/Drift
- 5. Zero

F. Aeration, pH and Chlorine

- ***1. Initial DO 7-9***
  - Aeration
  - a. Fish pump
  - b. shake
  - c. pour from bottle to bottle
- ***2. pH 6.5-7.5***
- ***3. Remove chlorine***
  - Add 1:5 H_2SO_4, KI and starch indicator (1ml/100ml)
  - Titrate with Na_2SO_3 untill clear
  - (must remain clear for 15 min.)

- ***G. Toxicity***
  - 1. If change in DO decreases with increasing sample -
- ***H. Calculations***
  - Seed Curve
  - GGA
  - Samples
  - 7-2-1

IV. Calibration Curves

- *STANDARD CURVE: A plot of concentrations of known analyte standards versus the instrument response to the analyte. Calibration standards are prepared by successively diluting a standard solution to produce working standards which cover the working range of the instrument. Standards should be prepared at the frequency specified by the method. The calibration standards should be prepared in the same manner as samples following sample preparation.*
- *Curve evaluation:*
- *The ability to predict behavior by establishing known outcome.*

- ***Why Linear?***
  - Easier to predict.
  - Less complex.
  - Fewer variables.
  - Greater certainty between standards.

- ***If the curve is bad- Now What?***
- ***Corrective action or Start over?***
  - If r<.995
  - Deleting standards
  - Not enough standards
  - Lack of reproducability (unpredictable)
  - Hi blanks
  - Non-linear
  - Drift

Basic linear regression by least squares

- *LINEAR Eq.: x=(y*b)/m or y=mx+b*
- *where m = slope or rise over run*
- *b = intercept (curve crosses the y axis)*
- *(Sxy - (Sx * Sy / n))*
- *Slope =----------*
- *(Sx^2 - ((Sx)^2 / n))*
- *intercept = (Sy/n) - (m *Sx / n)*

The Correlation Coefficient:

- *An estimate of the remainders derived from the regression.*
- $$r = \frac{(Sxy - (Sx * Sy / n))^2}{((Sx^2 - ((Sx)^2 / n)))*(Sy^2 - ((Sy)^2 / n))}$$
- *For our purpose it must be >0.995*
- *The correlation coefficient only indicates variance from the averages; therefore it is not the best indicator of curve quality.*

Other factors for Evaluation

- ***Slope vs. RF***
  - Response Factor is the ratio of Concentration/Response and represents the instantaneous slope for a given point.
- ***Variance of RF***
  - The average of the RFs should be close to the slope. The variance of the RFs is an excellent indication of curve quality. RSD <10%
- ***Intercept - Should not be greater than the lowest standard.***
- ***Hi or Low Slope***
- ***Linear Calibration Range Study - LCR***
  - LCR is simply plugging the response of each standard into the line function and determining the variance from predicted value (conc.). These should be <20% and ideally <10%
- ***Visual***
  - Equal distributaion of data points on either side

Method Limitations - Beer's Law and the Analytical Process

- *Noise is most often the function of the detector*
- *Quenching - At high concentration, Beer's law will not continue to behave in a strict linear fashion*
- *Limiting factors of method or reagents. Most methods are intended to operate at specific trace ranges. Dilution after reaction or extraction beyond trace levels may limit available reagents for reaction.*
- *Range - a defined range of standards bracketing expected results.*
- *Blanks should be matched for matrix and standards.*
- *Interference - turbidity or cloudiness may require filtering.*
- *Dilution and general pipetting technique - It is critical to use consistent and precise dilution techniques for standards and all spiking or QC functions. Use of varying means for dilutions can add significant variance for each standard.*

B. Corrective Actions

- *Perform Maintenance*
- *Clean glassware*
- *Check dilutions and be consistent*
- *Remake standard and reagents (INCLUDING STOCKS)*
- *Re-run standards*
- *Re-zero*

Avoid:

- *Deleting standards*
- *Running extra standards*
- *Excessive re-zeroing*
- *"Special" Behavior (i.e. using one cuvette for standards and another for samples)*

V. General QA for Calibration

- *A. Blanks*
- *B. Standards*
- *C. Second Source (Initial Instrument Calibration +/-10%)*
- *D. LCS (+/-15%)*
- *E. Continuing Calibration Verfication (+/-15%)*
- *F. Duplicates (1 in 10 +/-20%)*
- *G. Spikes (Matrix and Matrix Spike Duplicates 1 in 20 +/-20%)*

VI. Nitrogen

- ***A. Nitrite***
  - 1. Buffer-color reagent: To 250 mL of distilled water, add 85ml conc. Phosphoric Acid, 10.0 g sulfanilamide and 0.5g N-(1-naphthyl) ethylenediamine dihydrochloride. Stir until dissolved. Add 136g of Sodium Acetate ($CH_3COONa\ 3H_2O$) and stir until dissolved. Dilute to 500ml with DI Water.
  - 2. pH (Neutral to start)
  - 3. Spectrophotometer at 543nm
  - 4. Filtering
  - 5. Color development 15min at ph 1.5 to 2. Read within 2 hours

- ***B. Nitrate***
  - 1. Cadmium Reduction and analyze as Nitrite
    - **a. Column Efficiency**
    - **b. Flow**
    - **c. Regenerating Cadmium (HCl and CuSO4)**
  - 2. Ion Chromatography

- ***C. Ammonia (Ion Selective Electrode)***
  - 1. Log
  - 2. Slope Check
  - 2. Relative vs. Absolute mV
  - 4. Temperature
  - 3. Soaking Probes
  - 4. pH (>11) and Gas Sensing Electrodes

- ***D. TKN***
  - 1. Digestion
  - Reagents:
    - **6.2 Digestion Reagent: Dissolve 267g K_2SO_4 in 1300 mL of deionized water, add 400ml conc. sulfuric acid and dilute to 2L with deionized water.**
    - **6.3 Copper Sulfate Solution: Dissolve 25.115g $CuSO_4$ in deionized water and dilute to 1l.**

- Procedure:
  - **9.2.1 Place 50.0 mL of sample or an aliquot diluted to 50 mL in a 100 mL Kjeldahl flask and add 10 mL sulfuric acid-potassium sulfate solution (7.2), 2ml copper sulfate solution (6.3) and 2-3 boiling beads. Evaporate the mixture in the Kjeldahl apparatus until SO3 fumes are given off and the solution turns colorless or pale green. Then increase temperature and digest for an additional 30 minutes to pale green color. Cool the residue and add 44 mL deionized water.**
  - **9.2.2 Make the digestate alkaline by careful addition of 6 mL of sodium hydroxide solution (6.4) without mixing. Mix sample (blue should appear; then clear). If sample does not clear, add 5N H2SO4 dropwise until clear (no more than 5ml).**
- 2. Distillation
- 3. Interferences and Recovery

VII. Phosphorus

- ***Forms of Phosphorus***

  - 3.1 Total Phosphorus (P) - all of the phosphorus present in the sample, regardless of form, as measured by the persulfate digestion procedure. (00665)

    - **3.1.1 Total Orthophosphate (P, ortho) - inorganic phosphorus [(PO4)-3] in the sample as measured by the direct colorimetric analysis procedure. (70507)**

    - **3.1.2 Total Hydrolyzable Phosphorus (P, hydro) - phosphorus in the sample as measured by the sulfuric acid hydrolysis procedure, and minus predetermined orthophosphates. This hydrolyzable phosphorus includes polyphosphorus. [(P2O7)-4, (P3O10)-5, etc.] plus some organic phosphorus. (00669)**

    - **3.1.3 Total Organic Phosphorus (P, org) - phosphorus (inorganic plus oxidizable organic) in the sample measured by the persulfate digestion procedure, and minus hydrolyzable phosphorus and orthophosphate. (00670)**

- 3.2 Dissolved Phosphorus (P-D) all of the phosphorus present in the filtrate of a sample filtered through a phosphorus-free filter of 0.45 micron pore size and measured by the persulfate digestion procedure. (00666)
  - **3.2.1 Dissolved Orthophosphate (P-D, ortho) as measured by the direct colorimetric analysis procedure.(00671)**
  - **3.2.2 Dissolved Hydrolyzable Phosphorus (P-D, hydro) as measured by the sulfuric acid hydrolysis procedure and minus predetermined dissolved orthophosphates. (00672)**
  - **3.2.3 Dissolved Organic Phosphorus (P-D, org) as measured by the persulfate digestion procedure, and minus dissolved hydrolyzable phosphorus and orthophosphate. (00673)**

- 3.3 The following forms, when sufficient amounts of phosphorus are present in the sample to warrant such consideration, may be calculated:
  - **3.3.1 Insoluble Phosphorus (P-I) = (P) - (P-D). (00667)**
    - 3.3.1.1 Insoluble orthophosphate (P-I, ortho) = (P, ortho) - (P-D, ortho). (00674)
    - 3.3.1.2 Insoluble Hydrolyzable Phosphorus (P-I, hydro) = (P, hydro) - (P-D, hydro). (00675)
    - 3.3.1.3Insoluble Organic Phosphorus (P-I, org) = (P, org) - (P-D, org). (00676)
- 3.4 All phosphorus forms shall be reported as P, mg/L, to the third place.

- ***D. Procedure***
  - 9.1 Total Phosphorus
    - **9.1.1 Transfer 50 mL of sample or an aliquot diluted to 50 mL into a 125 mL Erlenmeyer flask and add 1 mL of 5 N sulfuric acid (6.2), 1 drop of phenolphthalein and one boiling chip.**
    - **9.1.2 Add 0.4 g ammonium persulfate (6.4), mix and boil gently for approximately 30-40 minutes or until a final volume of about 10 mL is reached. psi). Cool, add 40 mL DI Water.**
    - **9.1.3 Add 1 drop of phenolphthalein, add 5N NaOH dropwise until sample turns pink. Titrate dropwise with 5N H_2SO_4 to clear and check pH (pH should be 5-8).**
    - **9.1.4 Determine phosphate as outlined in (9.2) Orthophosphate.**

– 9.2 Orthophosphate

- 9.2.1 To develop color, treat as in 9.1.3 above. Add 8ml of color reagent.
- 9.2.2 After 20 minutes, measure the absorbance at 880 nm with a spectrophotometer and determine the phosphorus concentration from the standard curve. The color is stable for at least 30 minutes. For concentrations in the range of 0.01 to 0.3 mg P/L, a 5 cm cell should be used.

Questions?

Thank you for your time!

Greg Hudson

804.550.3971

GregHudson@envirocompliance.com