ADVANCED Lab Exam Review

April 21, 2015

Why are we here?

A brief history of lab exam results

How to best prep for an exam?

Why should we care?

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WI DNR Lab Certification

ADV 1.1.4 Define oxidizing chemicals.

Historical: 2 questions ~80% pass

An oxidizing chemical is one that oxidizes another chemical, the reducing chemical. In doing so, it becomes reduced. This type of reaction is called an oxidation-reduction, or redox, reaction. The oxidizing chemical GAINS an electron, while the reducing chemical LOSES an electron.

This issue can be confusing. You might have learned mnemonics such as "LEO the lion says GER", where LEO stands for Lose Electron=Oxidation and GER stands for Gain Electron = Reduction. Another familiar one is "OIL RIG", or Oxidation Is Losing (electrons); Reduction Is Gaining (electrons).

An oxidizing chemical, or oxidizer, actually becomes reduced during the reaction, because it gains electrons. In this case, "Gains Electrons = Reduction" refers to the process involved, not the compound. Oxidizers are reduced during redox reactions and reducers are oxidized.

ADV 1.1.4 Define oxidizing chemicals.

Historical: 2 questions ~80% pass

Common oxidizing agents that may be found in small labs include:

- Oxygen gas (O2), ozone (O3), halogens (fluorine, chlorine, bromine).
- Hypochlorites: such as household bleach, chlorination chemicals.
- Nitric acid (HNO3), Nitrate salts such as sodium or potassium nitrate (NaNO3, KNO3).
- Permanganates and persulfates: such as potassium permanganate (KMnO4).

- **For BOD,** oxidizers you might use include: bleach, hypochlorite
- **For Ammonia**, oxidizers you might use include: none
- **For Total Phosphorus**, oxidizers you might use include: ammonium persulfate
- **For Chlorine Residual**, oxidizers you might use include: hypochlorite, potassium permanganate

ADV 1.1.4 Define oxidizing chemicals.

Historical: 2 questions ~80% pass

Generally speaking, chemicals whose names end in "**–ate**" or "**ite**" are those that contain a significant amount of bound oxygen, and thus are frequently oxidizers.

There are two main categories of oxidizing agents:
(1) reagents that contain an oxygen-oxygen bond, and
(2) reagents that contain metal-oxygen bonds.

Examples of oxidizing agents containing an O—O bond include oxygen gas (O_2), ozone (O_3), and hydrogen peroxide (H_2O_2).
The most common oxidizing agents with metal-oxygen bonds contain either hexavalent chromium (Cr+6) or heptavalent manganese (Mn+7). Common Cr+6 reagents include chromate (CrO_3) and sodium or potassium dichromate ($Na_2Cr_2O_7$and $K_2Cr_2O_7$). The most common Mn+7 reagent is potassium permanganate ($KMnO_4$).

ADV 1.1.4 Define oxidizing chemicals.

Historical: 2 questions ~80% pass

NFPA 704 is a standard maintained by the National Fire Protection Association (NFPA) . It defines the commonly named, "fire diamond" used by emergency personnel to quickly and easily identify the risks posed by nearby hazardous materials. The four divisions of the “fire diamond” are typically color-coded, with blue indicating level of health hazard, red indicating flammability, yellow (chemical) reactivity, and white containing special codes for unique hazards. Each of health, flammability and reactivity is rated on a scale from 0 (no hazard; normal substance) to 4 (severe risk).

Oxidizers are designated in the white, “special” code area, using a code of “OX” or “OXY”

ADV 2.2.1 Explain the significance of the ≤ 6°C preservation requirement for samples.

Historical: 36% pass

The upper limit for sample temperature is 6°C. The only limiting criterion for the lower acceptable range for sample temperature is that samples must not be frozen, as freezing samples can change the physical or chemical nature of certain analytes. Consequently, when ch. NR 219, Wisconsin Administrative Code (which governs analysis of wastewater samples) was revised, this code specifies a temperature of "less than or equal to 6°C" with a footnote that specifies that samples also are not to be frozen.

The most critical thing to remember is that the overall goal of sample preservation has not changed. Therefore, labs should still consider the target sample preservation temperature to be 4°C. If autosamplers or refrigerators appear to be creeping upwards of 4°C, then corrective action should be initiated to provide more cooling to samples. This may include adjusting (and noting in maintenance logs) that the thermostat was adjusted to reduce cooling temperature.

ADV 3.2.4 Discuss proper pipetting techniques.

Ongoing concerns

Make sure you know how to pipet properly. The most important rules to follow are:

- Pipet with a slow, smooth action.
- Hold the pipet vertically when drawing liquid in.
- Only immerse the tip slightly when drawing liquid in, otherwise you will coat the outside of the tip with liquid, which will be transferred along with the volume inside the pipet.
- When dispensing the liquid hold the pipet vertically, but keep the sidewall of the receiving vessel at 45 degrees. Pipet against the sidewall or into the liquid that's already there.

ADV 3.2.6

Historical: 52% pass

Explain how the accuracy of autopipettors is verified.

Calibration of autopipettors can be done either photometrically or gravimetrically. The photometric approach is rarely used due to the need for expensive instrumentation and reagents.

The gravimetric approach is what most labs use. This approach assumes that purified water (i.e., lab reagent water) weighs approximately 1.0 gram per milliliter, mL). The exact density of water is based on temperature and can be obtained from a reference table.
Pipets are tested by pipetting consecutive aliquots (specific milliliter volume) of reagent water and comparing the resulting mean and standard deviation of the weight of each aliquot to the nominal weight (based on 1 mL = 1 gm). The accuracy of all **mechanical** pipets must be verified quarterly by analyzing the weights resulting from at least four replicate pipettings.

Replicate analyses must meet acceptance criteria or use of the pipet should be discontinued until the problem has been corrected.

If you are checking an adjustable volume pipet, at least three different volumes should be tested: 10% of maximum volume, mid volume and maximum volume.

ADV 4.1.1 Define Conductivity.

Historical: 58% pass

- Conductivity or specific conductivity is a measure of a material's ability to conduct an electric current. The ability of water to conduct an electric current is driven by the number of ions dissolved in the water. The more dissolved ions, the greater the conductivity.
- These "ions" result from the ionization of salts and other chemicals when they become dissolved in water. For example, if you add a pinch of table salt (NaCl) to a liter of deionized water, the salt quickly dissolves. During this process, the NaCl gets broken down into two ionic parts: Na+ and Cl-. It is the presence of these ions (dissolved solids) in water that causes conductivity.
- Drinking water has a conductivity about 100 times greater than that of deionized water. Seawater has a conductivity about 1,000,000 times greater than that of deionized water.

ADV 4.1.1 Define Conductivity.

Historical: 58% pass

- Increasing temperature can make ions in the water move faster. Faster ionic movement leads to increased conductivity. Conductivity levels falsely increase approximately 2% per °C.
- Conductivity can be estimated by measuring the amount of total dissolved solids (TDS) in a sample. Because dissolved ions cause conductivity, conductivity has been shown to have a direct correlation to the amount of total dissolved solids (TDS) in a sample. The concentration (mg/L) of TDS in a water sample can be "approximated" by multiplying conductivity by 0.64.

ADV 4.1.2

Historical: 60% pass

Discuss how conductivity relates to laboratory reagent water quality.

In theory, lab reagent water should be "pure" and thus contain no dissolved solids or ions. Therefore one would expect the conductivity of lab reagent water to be zero.

Pure water is actually a poor conductor.

If water has even a tiny amount of such impurities, then it can conduct electricity much better, because impurities such as salts separate into free ions in aqueous solution by which an electric current can flow.

Conductivity gives us a measure of water quality. The ASTM has defined Type I reagent water as water having a maximum conductivity of 0.056 µS/cm at 25°C. ASTM "Type II" water has a maximum conductivity of 1.0 µS/cm at 25°C. Conductivity means ions are present and the presence of ions clearly means that the water is not "pure". Conductivity is useful as an indication that ion exchange resin is overloaded, that a reverse osmosis membrane has been breached, or simply that your reagent water may not be of sufficient quality for use in testing.

ADV 4.1.2

Historical: 60% pass

Discuss how conductivity relates to laboratory reagent water quality.

The drawbacks to using conductivity alone as a means of verifying water quality are:

1. Conductivity ONLY measures substances that ionize…i.e. form ions. You can dissolve 1000 ppb of sugar in pure water and still not exceed ASTM Type I water criteria for conductivity.
2. It is virtually impossible to measure conductivity accurately to Type I or Type II levels without a closed system and VERY sensitive conductivity equipment. The nominal levels of CO2 in the atmosphere will cause gaseous CO2 to enter pure water causing a chemical reaction which increases conductivity.

Conductivity & Reagent Water "takeaways"

- Water is electrically neutral.
- Water that is not 100% pure may contain some measure of dissolved solids
- Dissolved solids are often salts, which "ionize" into ⊕ and ⊖ "fragments".
- Conductivity essentially measures these dissolved substances and provides an indication of the water quality.
- Conductivity is not a perfect tool, because it cannot measure dissolved organic compounds which do not ionize.

ADV 5.1.2 Define pH.

Historical: 49% pass

pH is a range of numbers (0-14) expressing the relative acidity or basicity of a solution. pH values less than 7 are considered acidic, and those greater than 7 are considered basic. Mathematically, the pH value is the negative logarithm of the molar hydrogen-ion concentration in a solution.

- pH = -log [H+]
- Since the scale is logarithmic, the pH changes by one for every power of ten change in hydrogen-ion concentration. interpolated.

$$[H+] = 10^{0} \text{ to } 10^{-14}$$

ADV 5.2.1

Historical: 62% pass

Discuss the testing differences between BOD and cBOD.

The only difference between samples analyzed for BOD and those analyzed for cBOD cBOD is NOT the letter "c"; rather, it is the addition of a chemical inhibitor to all samples for which cBOD is determined.

ammonia (NH3) or

In the absence of nitrogenous demand and nitrifying organisms, BOD and cBOD values should be equivalent. This is because the inhibitor theoretically suppresses only Nitrosomonas sp., the microorganism which is responsible for the first reaction in the nitrification bio-chemical reactions. In a sample in which no nitrification is expected to occur, adding the inhibiting agent should not change the results, thus explaining why, in these cases, BOD and cBOD would be expected to be equivalent.

In practice, however, a low bias has been reported for cBOD results relative to BOD results, when nitrification would not be expected. This may be due to a toxic affect that the inhibitor agent has on microbial species other than Nitrosomonas.

ADV 5.2.8

Historical: 68%, 74% pass

Discuss factors that would result in excessive DO depletion in blanks.

(1) CALIBRATION

The single greatest cause for blank "failures" (DO depletion greater than 0.2 mg/L) stems from calibration problems.

Blank depletions due to calibration errors generally tend to be SMALL effects (depletion of 0.2 - 0.5 mg/L)

The effect can be either LOW or HIGH bias (blanks deplete > 0.2 mg/L or GAIN > 0.2 mg/L).

Since it violates laws of physics to gain oxygen, and if the final DO is greater than the initial DO, this is nearly always a sign of calibration errors.

The basic problem is that errors in calibration cause the initial DO reading to be biased high (or the final DO reading is biased low). The net result is that it appears to be a DO depletion.

(2) SUPERSATURATION

If the initial DO of the blank is above the saturation point, all of this DO will come out of the solution during incubation (sometimes seen as micro-bubbles just underneath the bottle stopper.) This appears to be depletion, but it is actually degassing.

ADV 5.2.8

Historical: 68%, 74% pass

Discuss factors that would result in excessive DO depletion in blanks.

(3) CONTAMINATION (organic matter + micro-organisms)
Contamination, when it occurs, tends to be LARGE effect (i.e. DO depletions of > 0.5 mg/L).

Contamination problems will ALWAYS result in excessive depletions. Note that contamination from organic material or micro-organisms alone will NOT cause an exceedance in blanks.

There must be contamination from BOTH organic matter and microorganisms. Without the presence of microorganisms, there is nothing to break down the waste material and thus no oxygen will be utilized. Even if there is microbial contamination, without the presence of waste material, there is nothing for the microorganisms to break down and thus no - or minimal - oxygen will be utilized. Be aware that over-engineered water purification systems can result in insufficient water utilization creating a stagnancy within the water system. This can become a breeding ground for microbes, and thus the use of water from a purification system may be the cause of failures.

ADV 5.2.13

Explain the potential reasons why GGA results could be unacceptably high.

HIGH BIAS IN GGA

Historical: 62% pass

(1) Nitrification

Seed source selection is critical; if the plant process includes recycling final effluent into primary clarifiers, you could be adding nitrifying organisms to the seed (if you use primary effluent as seed material). To determine if nitrification is occurring, try adding a nitrification inhibitor. Compare GGAs seeded with domestic wastewater vs. commercial (Polyseed, BOD seed). If nitrification is occurring, select another source (that does not receive final wastewater) or use a commercially obtained synthetic seed.

(2) Cold GGA

If you don't warm the GGA to room temperature (20 — 3°C) before use, results will be consistently high.

(3) Contamination – organic matter

The contamination is likely "dirty glassware", providing a food source. Your blanks may even meet depletion criteria because -despite availability of a food source (the "crud") - there is no source of bugs and therefore no oxygen can be used. GGAs will typically fail high due to the extra oxygen consumed by the bugs as they attack both the GGA and the "crud".

ADV 5.2.13

Historical: 62% pass

Explain the potential reasons why GGA results could be unacceptably high.

HIGH BIAS IN GGA

Contamination can also result from insufficient rinsing of the DO probe after measuring highly concentrated samples.

(4) Contamination – bugs

The contamination source may be from “bugs” in the lab reagent water, possibly from a bad filter in a DI system. As long as your glassware is clean, blanks will meet depletion criteria. If there is no “food source” (e.g., "crud" on the glassware) to keep bugs going and expending oxygen, GGAs will generally fail high due to the extra oxygen consumed by the bugs as they attack the GGA.

Contamination, when it occurs, tends to be a LARGE effect (i.e. DO depletions of > 0.5 mg/L).

NOTE: Contamination from either “bugs” or BOD material alone will cause high bias in GGA but is not likely to cause an exceedance in blanks.

There must be contamination from BOTH “bugs” AND waste material for contamination to result in blank exceedances. This explains a common statement from lab analysts that “my GGA is failing high, but my blanks are fine”.

ADV 5.2.15

Historical: 70%, 80% pass

How might you know if toxicity is occurring in the BOD test.

- In order to ensure detection of sample toxicity, one must have prepared a sufficient number of sample dilutions and the individual dilutions must consist of significant differences in volume of the original sample. One other consideration is the degree to which BOD concentrations in individual dilutions differ. There must be a distinct trend in the data for the sample to be designated "toxic" and reported as such on the DMR.
- The first symptom of
  BOD concentration a
  sample volume in th
  that we're looking fo
  than two data points
  with greater sample
  merely be a functior
  dilution which confi

Would you trust your $$$ with a financial manager who uses only two points to identify a trend?

ADV 5.2.15

Historical: 70%, 80% pass

How might you know if toxicity is occurring in the BOD test.

- Therefore, at least three (3) dilutions are necessary to effectively detect sample toxicity, but 5 to 7 or more dilutions is preferred. These results do not mean a toxic sample because the range of the data is well within the precision capability of the test itself.
- The next concern is that individual sample dilutions be sufficiently different to be able to detect a trend. For example, if dilutions of 100 mLs and 125 mLs are used, normal variability associated with the BOD test alone may make it difficult to discern any differences in BOD related solely to sample volume used.

100, 125, and 150 mLs are too close

- The final consideration is to carefully evaluate the magnitude of difference between individual results. For example, consider the following data:
  - a BOD of 6 for a 300 mL sample volume
  - a BOD of 7 for a 200 mL sample volume
  - a BOD of 8 for a 100 mL sample volume

ADV 5.2.15

Historical: 70%, 80% pass

How might you know if toxicity is occurring in the BOD test.

Certainly it is true that these results "slide" downward with increasing sample volume. Before jumping to the conclusion that this is a "toxic" sample, one has to remember that BOD is a bioassay rather than a test which adheres to the more strict laws of chemistry. As a bioassay, BOD is not an exact science. In fact, Standard Methods suggests GGA control limits that represent

Given the expected a
all three results to th
case that this is a tox
results all "slide" in t

45, 85, 180 is significant

6,7,8 is not!

On the other hand, if a lab obtained the following results:

- over-depletion for a 50 mL sample volume **(~45 BOD)**
- a BOD of 85 for a 25 mL sample volume
- a BOD of 180 for a 10 mL sample volume

These results strongly suggest a toxic sample. The results are well above the reporting limit and there is a clear trend. Even the over-depleted 50 mL dilution could represent a BOD result as low as 45 mg/L.

ADV 5.2.16

Historical: 49% pass

What factors may cause toxicity in the BOD test?

- Toxicity is the term used to define the conditions which would result in an apparent decrease in BOD concentration as the volume of sample increases. The phenomenon is frequently referred to as "sliding BOD" in reference to the typical observation that BOD "slides" down as sample volume used for analysis increases.
- Sample toxicity can be caused by virtually anything that would adversely affect the health of the sample microbial population (which is required to utilize oxygen during the process of waste decomposition). Some things that would cause a toxic effect include:
  - high concentrations of heavy metals (e.g., chromium),
  - sample pH extremes,
  - concentrations of various inorganic (e.g., cyanides) and organic (e.g., pesticides) parameters. **Toxins cause toxicity!**

ADV 5.2.18 Discuss the problems associated with over-dechlorinating a sample.

Historical: 32% / 55% / 74% pass

Sodium sulfite is used to dechlorinate for BOD, because sodium thiosulfate has a significant oxygen demand if any excess is present. Because it is important to add only as much sodium sulfite as you need for dechlorination and no more, the operator must first determine how much chlorine is present before dechlorination. The excess could deplete DO and interfere with the test.

The most common dechlorinating agent is sulfite. The following forms of the compound are commonly used and yield sulfite (SO_2) when dissolved in water. The greater the amount required to neutralize a standard concentration of chlorine, the greater the oxygen depletion affect.

ADV 5.2.18 Discuss the problems associated with over-dechlorinating a sample.

Historical: 32% / 55% / 74% pass

Theoretical mg/L Required

Dechlorination Chemical to Neutralize 1 mg/L Cl2

Sodium thiosulfate (solution)	0.56 mg/L
Sulfur dioxide (gas)	0.90
Sodium meta bisulfite (solution)	1.34
Sodium bisulfite (solution)	1.46
Sodium sulfite (tablet)	1.78

Theoretical values may be used for initial approximations, to size feed equipment with the consideration that under good mixing conditions 10% excess dechlorinating chemical is required above theoretical values. Excess sulfur dioxide may consume oxygen at a maximum of 1.0 mg dissolved oxygen for every 4 mg SO2.

NOTE: Standard Methods specifies that sodium sulfite be used for dechlorination of BOD samples.

ADV 5.3.1

Historical: 63%, 65%, 83%, 93%

Discuss the importance of TSS in wastewater analyses.

Total suspended solids (TSS) are those which are visible and in suspension in the water.

They are the solids which can be removed from wastewater by physical or mechanical means such as sedimentation, flocculation, or filtration. TSS will include the larger floating particles and consist of silt, grit, clay, fecal solids, paper, fibers, particles of food, garbage, and similar materials. Suspended solids are approximately 70% organic and 30% inorganic. TSS determinations may be used to assess wastewater strength, process efficiency, and loadings.

By reducing the TSS in your effluent discharge, you are going to get better disinfection, which will reduce your fecal coliform and/or E. coli counts, allowing you to maintain compliance.

ADV 5.3.1

Historical: 63%, 65%, 83%, 93%

Discuss the importance of TSS in wastewater analyses.

High TSS can block light from reaching aquatic vegetation. Photosynthesis is inhibited as the amount of light passing through the water is cut down. Without photosynthesis, aquatic plants produce less oxygen, which is a significant source of DO. If light is completely blocked from bottom dwelling plants, the plants will stop producing oxygen and will die. As the plants are decomposed, bacteria will consume what oxygen (DO) is present in the water. Low DO is a major contributor to fish kills.

High concentrations of TSS can also result in an increase in surface water temperature, because the suspended particles absorb heat from sunlight. Higher temperatures consequently result in a reduced ability of the water to hold DO.

Bottom line on TSS and wastewater

- High TSS interferes with disinfection efficiency
- High TSS reduces light for aquatic plants
  - Without light, plants die
  - Plants provide oxygen, so less plants = less DO
  - Dying plants suck up available DO (demand)
  - Low oxygen results in fish kills
- High TSS raises surface water temp. (particles absorb sunlight)…which also lowers DO

TSS

Organic 70%

Inorganic 30%

ADV 5.4.2 Explain the operating theory of an ammonia electrode.

Historical: 20%, 43%, 72%

A "gas-sensing" type electrode is used for ammonia analysis. The three key parts of the ammonia electrode are an internal pH electrode (complete with reference electrode), a hydrophobic (impermeable to water) gas permeable membrane, and an electrolyte solution that fills the minute gap between the membrane and the pH bulb.

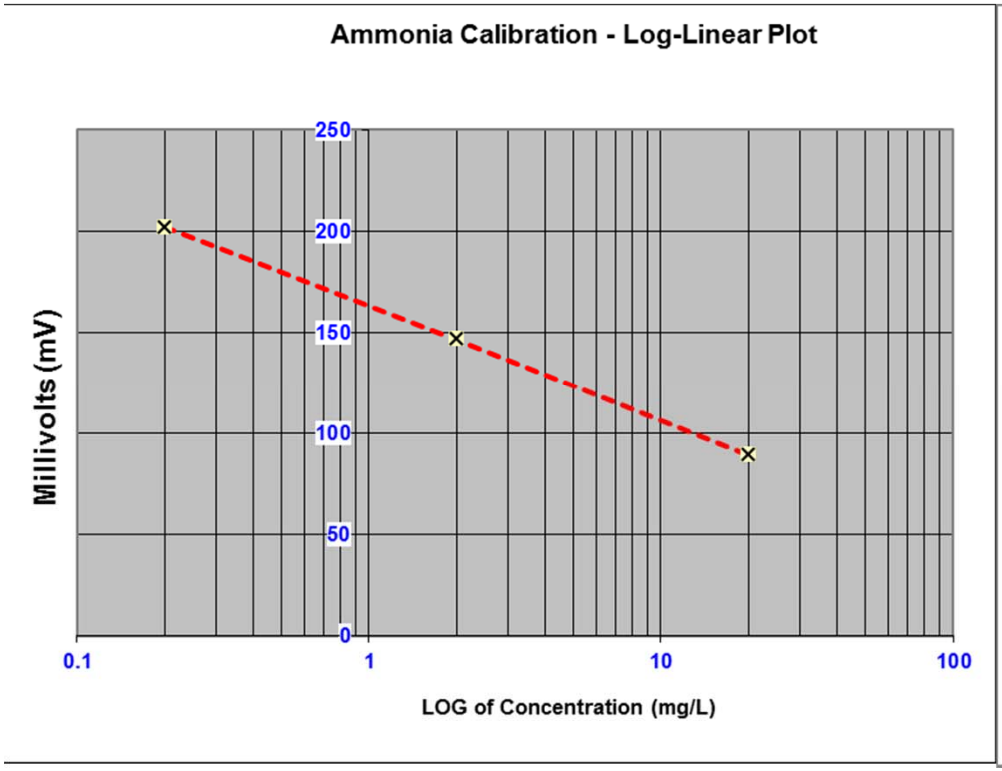
As ammonia, in gaseous form, diffuses across the gas-permeable membrane, a reaction takes place between ammonia and the water in which the electrolytes are dissolved. This reaction causes a change in the pH of the internal electrolyte solution which is in turn sensed by the pH electrode. The change in potential (measured as millivolts) is proportional to the concentration of ammonia in the sample.

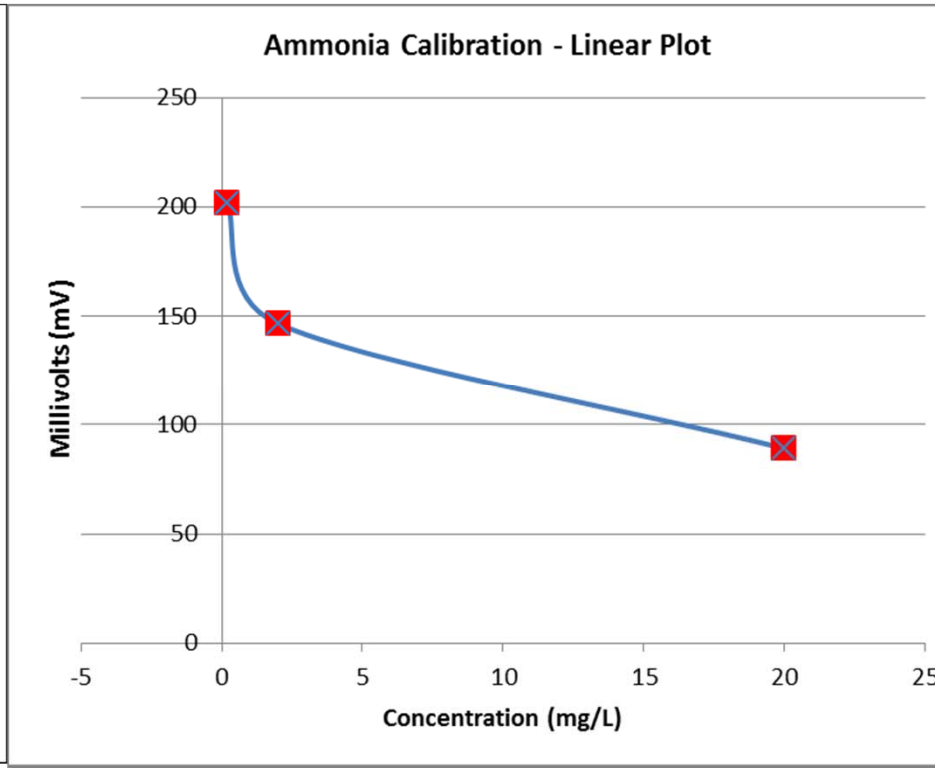
Whereas most calibration relationships between analyte and instrument response are linear, it is important to note that the relationship between ammonia concentration and millivolts (electrode potential) is logarithmic. This explains why plots of ammonia calibrations use semi-logarithmic paper. In order to obtain a linear calibration, the log of ammonia concentration must be plotted against millivolt response.

ADV 5.4.2

Historical: 20%, 43%, 72%

Explain the operating theory of an ammonia electrode.





A linear plot simply does not work!

It MUST be log-linear (logarithmic)

ADV 5.4.3

Historical: 11% , 81% pass

Explain why temperature is the only variable that affects electrode response and why each ten-fold change in concentration should result in a slope (net difference in millivolt response) of -54 to -60 millivolts.

Response of any electrode is governed by physics. The slope of electrode response between concentrations of standards that are exactly ten-fold different from one another is governed by the Nernst factor.

At 25 °C the Nernst equation calculates to 59.16. This represents the **theoretical** electrode slope (millivolts per ten-fold increase in concentration) at 25 degrees C, the base temp. for electrode analysis. A Nernst equation value (slope) of -54 millivolts is associated with a temperature of 0°C, and a slope of -60 mV translates to 30 degrees C. Therefore, one would only expect a slope of 60 or more if the temperature of the calibration solution was 30°C. At 20°C, the **theoretical** slope is 58.15 mV.

Many ion meters display the slope as a percentage of the theoretical. For example, a 98.5% slope equals a slope of -58.27 mV (at ~~20°~~ 25 C).

ADV 5.4.3

Explain why temperature is the only variable that affects electrode response and why each ten-fold change in concentration should result in a slope (net difference in millivolt response) of -54 to -60 millivolts.

- 0° C -54.2 mV
- 5° C -55.2 mV
- 10° C -56.2 mV
- 15° C -57.2 mV
- 20° C -58.2 mV
- 25° C -59.2 mV
- 30° C -60.2 mV

Nuts to Nernst!

$$\text{Nernst factor} = 2.3 \times \frac{RT}{nF}$$

$$= \frac{2.3 \times [\text{constant}] \times \textbf{temperature}}{[1] \times [\text{constant}]}$$

$$= \frac{[\text{constant}] \times [\text{constant}] \times \textbf{temperature}}{[\text{constant}] \times [\text{constant}]}$$

so… temperature is the only variable!

…and that's why temperature is critical for electrode determinations

ADV 5.4.6

Discuss why, although an approved technique, the Nessler method is not a good choice for ammonia determinations.

The Nessler method has concentration range limitations; this technique is only appropriate for levels of 0.05 to 1.0 ppm. More important, however, is the concern over the toxicity of the Nessler reagent itself, both to the environment and the health risks it poses for analysts.

Nessler reagent contains 100 grams of mercuric iodide (HgI_2) per liter. The Nessler technique calls for the addition of 2 mL Nessler reagent to 50 mL of sample in a Nessler tube. Based on the percent composition of mercury in the Nessler reagent, each 2 mL of Nessler reagent contains 88 mg of pure mercury.

Disposal becomes an issue because the contents of just ONE Nessler tube would have to be diluted with about 130 gallons of reagent water to make it safe to drink. Consequently, dumping samples down the sink after analysis is not an option, as the mercury will just end up in the sludge, making landspreading more problematic. All Nessler samples should be treated as hazardous waste and disposed of accordingly.

ADV 5.5.1

Historical: 16% pass

Discuss the calibration blank required for phosphorus testing.

Calibration blank

A calibration blank is a standard containing no added analyte, but all the other reagents that are in other calibration standards, such as color reagent, and digestion reagent (if standards are digested). In many cases, the calibration blank may be virtually identical to a method blank, but they serve different purposes.

Calibration blanks are not used to zero the instrument; their purpose is to represent the response of a zero concentration standard.

ADV 5.5.1

Historical: 27.1% pass

Discuss the calibration blank required for phosphorus testing.

Generally, **Calibration blanks**

- Consist of the solvent used plus all of the same reagents used to prepare the calibration standards.
  - If the standards are digested - the same as the samples are, then the calibration blank consists of reagent water plus all other reagents including the combined color reagent.
  - If the standards are not digested, therefore handled differently than the samples, then the calibration blank consists of reagent water plus combined color reagent, but not the digestion reagents.
- Indicate the absorbance response of a zero concentration standard (0.0 mg/L).
- This blank is not used to zero the instrument.

The absorbance of this blank is measured and used in the calibration curve as (x = concentration = zero; y = response = measured absorbance). It is possible for the measured absorbance of this blank to be zero, but it is not expected to be zero.

ADV 5.5.6

Historical: 66% pass

Explain what might be the cause of slow color development in samples or standards.

When potassium antimonyl tartrate is absent, old, or weak, the color reaction proceeds slowly.

SLOW color development?

Create a mnemonic

P roceeds

A t

T urtle speed

ADV 5.6.1

Historical: 44% pass

Explain how the chlorine electrode works.

- The electrode is based on iodometric measurement of chlorine.
- Ic
- Ic
- Tl
- Tl
refe
- Tl
rela

1. Add **iodide** (reagent)
2. **Iodide** reacts with **chlorine** to produce **iodine**
3. **Iodine** level = **chlorine** level

- The iodine-sensing element develops a potential that depends on the iodide level in solution.
- The meter measures the difference between these potentials (which therefore provides the iodine concentration).
- Iodine concentration = total residual chlorine concentration.

ADV 5.7.11

Historical: 25%, 39% pass

Explain how Nitrate Plus Nitrite Nitrogen are involved in process control:

Nitrate + Nitrite

___NO2 = Nitrite NO3 = Nitrate

The levels of nitrate and nitrite become important due to their involvement in de-nitrification and disinfection.

Nitrite levels should be very low throughout the entire treatment process. High levels of nitrite (NO2) in the system indicate there may be a problem with the nitrification cycle.

Nitrosomonas bacteria are harder to kill than Nitrobacter bacteria. If the Nitrobacter bacteria are killed off, the Nitrosomonas bacteria will continue working on the ammonia (NH3) and you will have a jammed cycle with high levels of nitrite (NO2). An effluent with high nitrite (NO2) concentrations will be difficult to disinfect because of the tremendous chlorine demand it poses.

ADV 5.7.11

Historical: 25%, 39% pass

Explain how Nitrate Plus Nitrite Nitrogen are involved in process control:

Denitrification is an anaerobic process (meaning without oxygen) in which the oxygen bound in nitrate (NO3-) becomes the primary oxygen source for microorganisms. When bacteria break apart nitrate (NO3-) to gain the oxygen (O2), the nitrate is reduced to nitrous oxide (N2O), and nitrogen gas (N2). Since nitrogen gas has low water solubility, it tends to escape as gas bubbles. These gas bubbles can become bound in the settled sludge in clarifiers and cause the sludge to rise to the surface.

An advantage of denitrification is the production of alkalinity (which will help buffer against pH changes) and an increase of pH. Approximately 3.0 to 3.6 mg of alkalinity (as CaCO3) is produced per milligram of nitrate reduced to nitrogen gas. Optimum pH values for denitrification are between 7.0 to 8.5.

ADV 6.1.3

Historical: 33%, 42% pass

Define Linear Regression.

Linear regression is a statistical tool for determining the relationship that exists between a dependent variable (instrument response, such as absorbance) and an independent variable (concentration), for a given set of data (calibration standards). As with other statistical tests, the more data provided, the more accurate the relationship will be defined. For instance, a linear regression based on seven (7) calibration standards spanning a concentration range of 0.1 to 1 ppm will be far more accurate than one based on only three (3) standards over the same concentration range.

As the name suggests, linear regression results in an equation for the straight line which describes the relationship. The important parameters that result from a regression are the slope and intercept of the resultant line. The correlation coefficient can also be calculated to provide an estimate of the strength or validity of the relationship between concentration and response.

ADV 6.2.7

Historical: 33%, 56% pass

Discuss how to identify if results exceed the calibration range of an instrument and action to be taken.

Samples having RESPONSES (not concentration) greater than that of the most concentrated standard of an initial calibration, established using at least 3 different standard concentrations, must be diluted and reanalyzed.

When samples cannot be diluted and reanalyzed (i.e., beyond holding time, or insufficient sample remains), sample results shall be reported with appropriate qualifiers or narrative warnings.

It is critical to note that a calibration is established based upon absolute response as a function of concentration. Subsequently, the determination of whether or not a sample exceeds the calibration range is based on its absolute response rather than concentration.

ADV 6.2.7

Historical: 33%, 56% pass

Discuss how to identify if results exceed the calibration range of an instrument and action to be taken.

What is the proper way of determining whether a sample requires dilution?

Given that:

SR = Sample Response (absorbance, etc.)

SC = Sample Concentration

UCSR= Upper Calibration Standard Response (absorbance)

UCSC= Upper Calibration Standard Concentration

The following sample would require dilution and reanalysis

SR= 0.915 | SC= 0.98 mg/L | UCSR=0.900 | UCSC= 1.00 mg/L

Because….even though the concentration is within the calibration range, the RESPONSE is not (0.915 > 0.900)

ADV 6.2.7

Historical: 33%, 56% pass

Discuss how to identify if results exceed the calibration range of an instrument and action to be taken.

Even though the concentration determined by linear regression is less than that of the highest calibration standard, the sample must be diluted because we are really calibrating response. Response is the KNOWN (independent variable). Sample concentration is the UNKNOWN.

The following sample would NOT require dilution and reanalysis:
SR= 0.875 | SC= 1.10 mg/L | UCSR=0.900 | UCSC= 1.00 mg/L

…because even though the sample concentration exceeds the calibration range (the concentration of the uppermost calibration standard), the sample response is well below that of the uppermost calibration standard.

Focus on response not concentration