Tips for preservation of ammonia and total phosphorus

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Ammonia and total phosphorus samples must be preserved by adding sulfuric acid to a pH of <2 and cooling (refrigerating) at ≤ 6ºC (but not be frozen!) until analysis. This is a simple process but one that can lead to sample contamination if not done with care.

When a laboratory performs total phosphorus using the Hach TNT method (Hach Method 8190) they must neutralize acidified samples prior to beginning analysis. The determinative step in the method is very pH dependent. The proper pH is only achieved when the reagents are added in the exact amounts specified in the method. However, this requires the sample to be neutralized before beginning the digestion step. Below is a simplified approach that may help reduce the labor and minimize potential phosphorus contamination.

Suggested supplies needed:

- Adjustable volume mechanical pipets capable of measuring to 0.1 mL and disposable pipet tips.
  - A 0.1 to 1 mL
  - A 1-5 or 1-10 mL mechanical pipet.
- 5 N sulfuric acid solution (phosphorus free) such as NCL # S-90APF.
- 5 N sodium hydroxide solution (phosphorus free) such as NCL# S-48PF
- Phenolphthalein indicator, 0.5% aqueous solution such as NCL# P-18W
- Small plastic dropper bottle such as NCL # B-760 for use in dispensing the phenolphthalein indicator described below. The Short range pH paper (pH 0-6) to confirm the sample pH is <2.
- Mid-range pH paper (pH 5.5 to 8). This may be used to confirm the sample is neutralized as an alternative to the phenolphthalein indicator.
- 2 small bottles (<100 mL) with caps, clean 50 mL beakers or disposable 50 mL beakers such as NCL# BE-50D. Disposable beakers are preferred because they help minimize the contamination risk.
- Sample bottles to be used to store the preserved ammonia and total phosphorus samples. Wide mouth 250 mL or 500 mL polyethylene or polypropylene bottles are recommended.
- Erlenmeyer flasks, 125 to 250 mL size. The flasks must be acid washed with dilute (10%) HCl prior to use.

Procedure:

1. Collect and split all samples in a normal manner.
2. Pour off representative portions of sample into each of two clean, acid washed wide-mouth 250 or 500 mL bottles. For this example, 2- 500 mL wide mouth bottles will be used. A single 500 mL sample bottle will provide enough volume to allow the laboratory to test ammonia and total phosphorus.
3. To preserve 2-500 mL bottles, pour about 15 mL of 5N sulfuric acid (H_2SO_4) into a disposable beaker (or small clean bottle or beaker).
4. Place a tip on the 1to 5 mL mechanical pipet. Adjust the dial to the 2 mL mark (use 1 mL if the 250 mL bottle is being used).
5. Add 2 mL of the 5N H_2SO_4 to each 500 mL sample bottle using the mechanical pipet. Draw the acid out of the disposable beaker and discard any remaining acid. **DO NOT** pour the remaining acid back into the stock acid bottle as this could contaminate it.
6. Cap the bottle and mix thoroughly.
7. Pour a few drops of preserved sample onto the 0-6 pH paper to confirm the pH is <2. In most cases, 2 mL of 5N H_2SO_4will be more than enough to reduce the pH to <2 of most domestic wastewater in Wisconsin. If not, add another mL of H_2SO_4 and recheck. Add the same amount of acid to each sample

and note the amount added on the sample collection or chain-of-custody records, and that the pH was confirmed to be <2.
8. Label samples appropriately and refrigerate at ≤ 6ºC (but not frozen) until analysis. Samples for ammonia and total phosphorus must be analyzed within 28 days of collection.

Neutralization of sample aliquots for total phosphorus for labs using the Hach method 8190 (TNT method)

1. The general concept here is to add the proportional amount of 5N sodium hydroxide (NaOH) needed to neutralize the pH of the sample. This is why the exact amount of acid must be added to the samples during the preservation process. It is also why mechanical pipets are needed to add the acid and base. This process requires accuracy and precision which cannot be achieved using serological pipets.
2. Pour off 100 mL of preserved sample into a clean 125 or 250 mL Erlenmeyer flask. Use the graduation marks on the side of the flask to determine the 100 mL.
3. Add 1 to 2 drops phenolphthalein indicator to the flask containing the sample.
4. Pour about 15 mL of 5N NaOH solution into a clean bottle or disposable beaker.
5. Since the normality of the acid and NaOH are the same, one only needs to be concerned with the volumes. If 2 mL of acid was added to 500 mL then 2 mL of NaOH must be added per 500 mL of sample to neutralize the pH. Here, we are only using 100 mL of sample so only 1/5 of the NaOH or 0.4 mL is needed.
6. Place a tip on the 0-1 mL mechanical pipet and adjust the volume to 0.4 mL. Add 0.4 mL of 5N NaOH to the 100 mL of sample and swirl to mix. If the solution shows a slight pink color, add one drop of 5N sulfuric acid to discharge the color. If the color is not pink, add 5N NaOH drop-wise until the solution turns a faint pink, then add a drop of 5N H_2SO_4 to discharge the color.
    a. Alternatively, mid-range (pH 5.5 to 8) pH paper may be used to confirm the pH has been neutralized to a pH of 6-8. Pour a few drops of sample onto the pH paper and check the color against the chart provided.
    b. **DO NOT use a pH meter to neutralize the pH** because of the risk of contamination. The buffers used to calibrate the pH meter contain very high amounts of phosphorus and pose a serious contamination risk. Use phenolphthalein indicator or pH paper only.

The above neutralization process is not 100% foolproof and some experimentation may be required to determine the exact amount of NaOH required. Again, some fine-tuning will likely be required. Make sure to note the amount of NaOH that was used to neutralize the sample on the total phosphorus bench records. Also note the reagent code of the acid and NaOH used on the bench records for traceability purposes.

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