

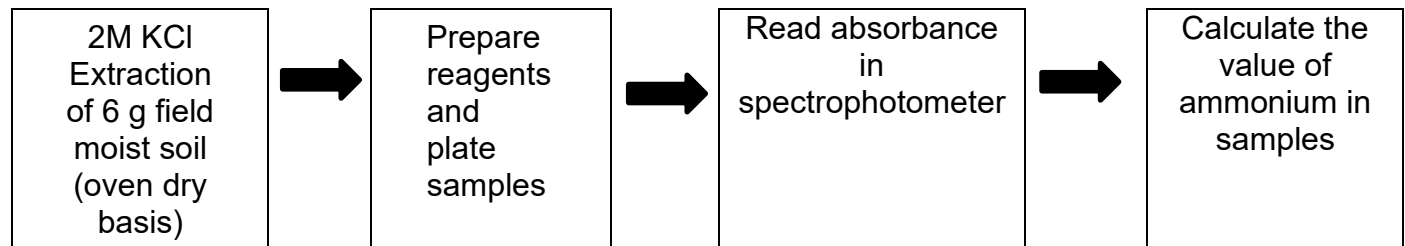
SOP: Determination of Soil Extractable Ammonium via the Salicylate Method

Overview:

The following standard operating procedure (SOP) for ammonium is accomplished via **the salicylate method**. The salicylate method was created as an alternative to the indophenol method, a toxic phenol also used in ammonium determination^{1,2}

Sodium salicylate is used as the **phenolic reagent** necessary for ammonium determination. Ammonium and salicylate react in the presence of a **nitroprusside catalyst**^{1,2}. Citrate and sodium potassium tartrate are both added as **complexing agents** preventing the precipitation of metals. This is necessary for soil extracts but is not needed for water samples¹.

The hypochlorite addition results in the **chlorination** of the phenolic compound, leading to color development necessary for the colorimetric analysis of ammonium².



Safety:

All standard safety protocols and online safety training via UIUC [Division of Research Safety \(DRS\)](#) are required.

Personal protection (PPE) for this procedure include:

Eye Protection: Safety goggles

Body Protection: Lab coat

Hand Protection: Gloves

Particularly hazardous substances: Sodium Nitroprusside (nitroferricyanide) is toxic if swallowed. It should never come in contact with acidic solutions because hydrogen cyanide can be produced. Seal with parafilm and store in desiccator immediately after use. Make sure to check Safety Data Sheet if unsure about how to handle this chemical. Specific details on these substances are incorporated in the **Detailed Procedure** below.

Instrumentation & Consumables:

Standards preparation

- 1.5 mL microcentrifuge tubes
- Ammonium standard

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- Location: Chemicals Only Fridge
- 2 M Potassium Chloride (KCl)
- 1000 μ L pipette and tips

Reagent preparation

- Reagent A
 - Analytical balance (three decimal places sensitivity)
 - 500 mL Erlenmeyer flask wrapped with tin foil
 - 250 mL Nalgene bottle wrapped with tin foil
 - Sodium Salicylate
 - Location: Organic reagents shelf
 - Sodium Citrate
 - Location: Organic reagents shelf
 - Potassium Sodium Tartrate (CAS 304-59-6 6381-59-5)
 - Location: Organic reagents shelf
 - Sodium Nitroprusside
 - Location: Reagent desiccator
- Reagent B
 - 250 mL Nalgene bottle
 - Sodium Hydroxide
 - Location: Inorganic reagents shelf
 - Bleach (5.25% - 6.5% Sodium Hypochlorite)
 - Location: Cabinet under the sink in S-27, fridge in S-23.
 - 2000 μ L pipette
 - Small beaker (100 mL)

Colorimetry

- 96 well microplates
- Microplate spectrophotometer
- Pipettes and tips (20 μ L – 200 μ L)

Detailed Procedure:

I. 2M KCl Preparation

1. In a 1L volumetric flask, dissolve 150 g KCl into 1L 18.2 M Ω water.
2. Calculate in advance how much 2M KCl reagent you will need for all samples. Each sample will require 30 mL of 2M KCl.
 - i. Using the proportion above in step 1, the amount of KCl needed for your desired volume can be calculated.

Example: If 3.5L 2M KCl is needed for all samples—
~525 g of KCl will be added into 3.5 L 18.2 M Ω water.

II. 2M KCl or 0.5 M K₂SO₄ Extraction

1. Weigh 6 g (+/- 0.05) of field-wet soil on an **oven-dried basis** into a 50 mL centrifuge tube. Record exact weight of soil and centrifuge tube.

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- i. To calculate the amount of wet soil needed, see Example 2 under “Example Calculations” and/or SOP: GWC Standard Operating Procedure (Soils Lab 2023).
2. To the measured field-wet soil, add 30 mL of the 2M KCl extracting solution. Include blank samples, where no soil is added.
3. Shake samples for 1 hour on a reciprocating shaker, on low setting.
4. Remove from reciprocating shaker, and then let rest for at least 30 min for the soil can settle to the bottom.
5. Prepare new 50 mL or 15 mL centrifuge tubes for each extracted sample. Fold and place Whatman #42 filter papers over each new tube.
6. When samples are settled, filter the extracted sample solution into the new centrifuge tubes. Filter at least 1 mL of sample extract.
7. Filtered sample extracts should be stored in the refrigerator if being analyzed immediately, or frozen to preserve samples if immediate analysis is not possible.

III. Standards Preparation

1. Pour a small amount of the ammonium standard into a 15 mL centrifuge tube. This is to avoid any type of contamination on the reagent container.
2. Pipette the required amount of standard reagent (Table 1) to dilute in 2M KCl from 1000 mg/L to 100 mg/L into a 1.5 mL microcentrifuge tube. Proceed to pipette the required amount of 2 M KCl and shake the mixture. Serial dilution then continues from the previous diluted standard.
3. Refer to Table 1 for examples of dilutions that can be used for creating a standard curve. The amounts will change depending on the required detection range.
4. Once the standards are ready, they should be treated as samples when it comes to plating them on the well microplate. Follow the exact colorimetry procedure for preparing samples for the standards. Make sure to remember their location on the plate, since the absorbances will be needed to create the curve to convert absorbance readings of the samples to concentrations.

Table 1. Standards dilutions

Standard concentration (mg/L)	Amount of 2M KCl (μL)	Serial dilution of standards
		Amount of NH ₄ ⁺ -N standard (μL)
100	900	100 of 1000 mg/L
20	800	200 of 100 mg/L
10	500	500 of 20 mg/L
5	500	500 of 10 mg/L
2.5	500	500 of 5 mg/L
1.25	500	500 of 2.5 mg/L
0	500	-

NOTE: Serial dilution consists of performing the same dilution step repeatedly the previous diluted solution as the input to the next dilution in each step. To create a serial dilution a concentration factor and a dilution factor are needed. These concepts are explained in the following formulas:

$$\text{Concentration factor} = \frac{\text{volume}_{\text{initial}}}{\text{volume}_{\text{final}}}$$
$$\text{Dilution factor} = \frac{1}{\text{concentration factor}}$$

The purpose of using serial dilutions is making the standards with known concentrations to create a standard curve, also known as calibration curve. This tool represents the relationship between two quantities. In this case, it assigns an estimated concentration to the absorbance value of the sample. Without the calibration curve, the absorbance values don't have a meaning. All the absorbance values of the samples must lie between the lowest and the highest absorbance values of the created standards. This means that the concentrations of the standards should be planned around the expected/estimated range of ammonium concentrations of the samples. However, a standard curve involving colorimetry is usually only linear until a certain concentration. Samples outside the range of the standard curve therefore need to be diluted with 2M KCl (e.g., 0.2 mL extract + 0.8 mL 2M KCl to yield 5x dilution) to bring the concentration down to the range of the standard curve, and the sample concentrations will need to be corrected for the corresponding dilution factor.

Note the use of NH₄-N standard which is considered an "N basis" in contrast to an ion basis as NH₄⁺. Ammonium-N on an N basis refers to only the N for each sample, while the ion basis includes the entire NH₄⁺ ion. For determining ammonium-N in soil samples, an N basis should be done using ammonium-N standards because of this distinction.

IV. Colorimetry Reagents

1. Reagent A (Wrap in Aluminum foil)
 - i. In a 500 mL Erlenmeyer flask, add:
 1. 100 mL water
 2. **6.5 g** sodium salicylate
 3. **5 g** sodium citrate
 4. **5 g** sodium tartrate
 5. **0.025 (+/- 0.005) g** sodium nitroprusside (nitroferricyanide)
 - ii. NOTE: Always wear gloves when handling nitroprusside. Wrap in parafilm, and store in desiccator immediately after use.
 - iii. Mix solution thoroughly, until completely dissolved. This may include the use of a magnetic stirrer.
 - iv. Reagent A can be stored in a Nalgene bottle. **Store Reagent A separately from Reagent B.**
2. Reagent B
 - i. In a 150 mL Erlenmeyer flask, add:
 1. 100 mL 18.2 MΩ water

2. **6 g NaOH**
3. **2 mL Bleach** (sodium hypochlorite)
- ii. Percent sodium hypochlorite varies by bottle, but within the range of 5-6.5% can be utilized.
- iii. Pour approximately ~3-5 mL bleach into a beaker, and pipette out 2 mL into Erlenmeyer flask.
- iv. Store Reagent B in a Nalgene bottle. Shelf life for Reagent B is indefinite but is recommended to be replaced every 2-4 months. Store Reagent A separately from Reagent B.

V. Colorimetric Analysis

1. Preparing samples with reagents (Table 2) into 96-well plates OR cuvettes.
 - i. Add Reagent A
 - ii. Add Sample
 - iii. Add Reagent B
2. Invert (if using cuvettes)
3. Let samples sit (1-4 hours)
4. Read at 650 nm

Table 2. Reagent and sample proportions

<u><1 mg/L (LL 0.02 mg/L)</u>	<u><1 mg/L (High Ca/Mg)</u>	<u>1-5 mg/L (LL 0.2 mg/L)</u>	<u>1-10 mg/L (LL 0.2 mg/L)</u>	<u>1-20 mg/L (LL 0.5 mg/L)</u>
Cuvettes-----	Cuvettes-----	Cuvettes-----	Cuvettes-----	Cuvettes-----
150 µL reagent A	200 µL reagent A*	400 µL reagent A	500 µL reagent A	500 µL reagent A
600 µL sample	800 µL sample	140 µL sample	80 µL sample	40 µL sample
150 µL reagent B	200 µL reagent B	400 µL reagent B	500 µL reagent B	500 µL reagent B
Microplates-----	Microplates-----	Microplates-----	Microplates-----	Microplates-----
50 µL reagent A	128 µL reagent A*	128 µL reagent A	139 µL reagent A	144 µL reagent A
200 µL sample	45 µL sample	45 µL sample	22 µL sample	12 µL sample
50 µL reagent B	128 µL reagent B	128 µL reagent B	139 µL reagent B	144 µL reagent B

* For samples with High Ca/Mg, reagent A should be concentrated per 100 mL 18.2 MΩ water. Solution 1 proportion becomes: 12 g sodium salicylate, 10 g sodium citrate, 10 g sodium potassium tartrate, and 0.05 g sodium nitroprusside.

VI. Clean Up

1. Dial back the pipettes to their corresponding volume.
2. Dispensette: clean IMMEDIATELY after use. Failure to do so will result in KCl crystallization in the dispensette, making it difficult to clean out.

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3. Make sure all reagents are back on the shelves.
4. Liquid waste from Ammonium reagents should be kept in a separate waste container. DO NOT combine or mix with Nitrate waste. Sodium Nitroprusside (nitroferricyanide) should not be combined with acidic solutions (see “Safety” section for more details).

VII. Calculations

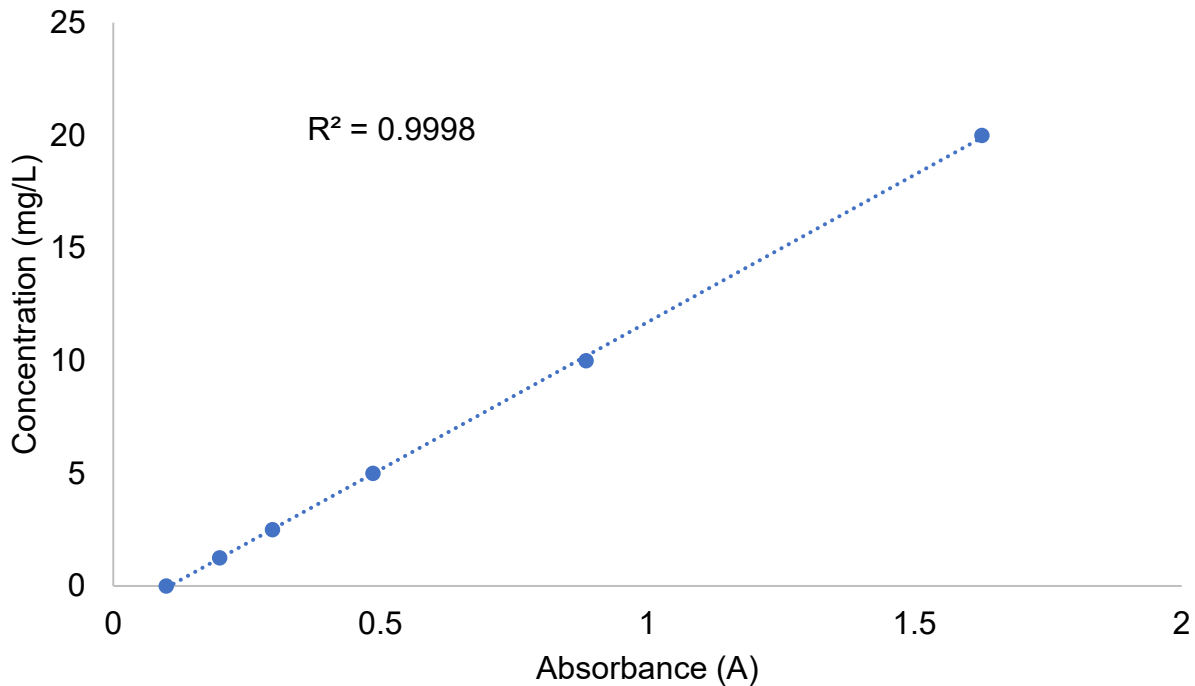
Measurement of ammonium is usually expressed in units of mg/L. To convert absorbance into concentration, Example 1 under “Example Calculations”.

1. Generate a scatter plot graph for the standard curve (Table 1). Add a trendline and check if the R^2 value is acceptable. In general, an $R^2 > 0.99$ is necessary. Anything R^2 below 0.99 should be redone and checked for procedural errors. An example of the absorbance values and graph is provided below (Table 3, Fig. 1).
2. Conversions can also be done from mg/L to mg kg^{-1} soil (see Example 3 under “Example Calculations”.

Table 3. Example of recorded absorbance values from standard samples analyzed through the spectrophotometer.

Absorbance values (A)	0.099	0.199	0.298	0.486	0.885	1.626
Concentration of standards (mg/L)	0	1.25	2.5	5	10	20

Figure 1. Example of ammonium standard curve, with absorbance values recorded from spectrophotometer and concentration of each standard created through serial dilutions.



Example calculations:

Example 1. Sample conversion from absorbance to mg/L (i.e., “ppm”).

	A	B	C	D
1	Absorbance (A)	Concentration (mg/L)	Samples (A)	Samples (mg/L)
2	0.074	0	1.329923274	113.5300274
3	0.082	1.25	1.150895141	97.39359339
4	0.097	2.5	1.355498721	115.8352322
5	0.124	5	1.636828645	141.1924856
6	0.183	10	1.150895141	97.39359339
7	0.292	20	1.023017903	85.86756912
8				
9	<p>Green (left two) columns are from standard curve. Orange (right two) columns are from sample readings. The standard curve is used to convert the sample absorbance (A) into concentration (mg/L).</p>			
10				
11				
12	<p>=TREND(known_ys, known_xs,new_xs)</p>			
13	<p>=TREND(concentration_values,absorbance_values,sample_value)</p>			
14	<p>=TREND(B\$2:B\$7,A\$2:A\$7,C2)</p>			
15	<p>LOCK the "concentration_values" and "absorbance_values"</p>			
16	<p>i.e., add the dollar sign after each letter. DO NOT lock sample value.</p>			
17				
18				
19				

Example 2. Calculation to determine fresh weight needed on an oven dried basis.

<u>Sample ID</u>	1	Sample ID: sample name/number
<u>Soil before drying (g)</u>	9.97	Soil before drying (g): weigh 10 g (+/- 0.5) of wet soil and record exact weight to 0.00 decimal place.
<u>Tin Mass</u>	1.76	Tin mass: record weight of tin that soil sample is placed in.
<u>Soil + Tin after drying (g)</u>	9.85	<i>Soil samples should be oven dried @ 105°C for at least 24 h.</i>
<u>Soil after drying (g)</u>	8.09	
<u>GWC (%)</u>	23.23856613	Soil and Tin after drying: record weight of soil and tin after samples has been oven dried.
<u>GWC in 6 g soil</u>	1.394313968	Gravimetric Water content (GWC): = ([soil before drying - soil after drying]) / [soil after drying] * 100 = (9.97-8.09)/(8.09)*100 = 23%
<u>Fresh Weight needed</u>	7.394313968	
<u>Actual Weight (g)</u>	7.4	
<u>Weight (g) oven dried basis</u>	6.004613842	GWC in 6 g soil: =6*(Water content %) = 6*23% = 1.39 g
		Fresh weight needed: =6+(WC in 6 g soil) = 6+1.39 = 7.39 g fresh weight needed
		Actual weight: Actual fresh weight recorded, +/- 0.05 of the fresh weight calculated above. = 7.40 g
		Oven dried basis weight: =(Actual Weight) / (1+[Water Content %] /100) = (7.4) / (1+ 23/100) = 6.005 g oven dried basis used

Example 2. Conversion from extract concentration to soil basis (mg/kg soil).

L	M	N	O	P
Samples (mg/L)	Extraction Vol (L)	Actual Weight (g)	Soil Mass (kg)	mg/ kg soil
113.5300274	0.03	7.53	0.00753	452.310866
97.39359339	0.03	7.63	0.00763	382.9368023
115.8352322	0.03	7.73	0.00773	449.5545882
141.1924856	0.03	7.44	0.00744	569.3245388
97.39359339	0.03	7.35	0.00735	397.524871
85.86756912	0.03	7.21	0.00721	357.2853084

Orange (left most column) is from sample readings.

Extraction volume: each soil sample was extracted in 30 mL of 2M KCl.

Actual weight (g): recorded wet mass used for soil extraction.

Soil mass (kg): =(actual weight) / 1000

mg/kg soil: =(concentration * extraction vol) / (soil mass kg)

References:

Nelson, D. W. (1983). Determination of ammonium in KCl extracts of soils by the salicylate method. *Communications in Soil Science and Plant Analysis*, 14(11), 1051–1062. <https://doi.org/10.1080/00103628309367431>

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Suggested reading:

Nelson, D. W. (1983). Determination of ammonium in KCl extracts of soils by the salicylate method. *Communications in Soil Science and Plant Analysis*, 14(11), 1051–1062. <https://doi.org/10.1080/00103628309367431>

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