

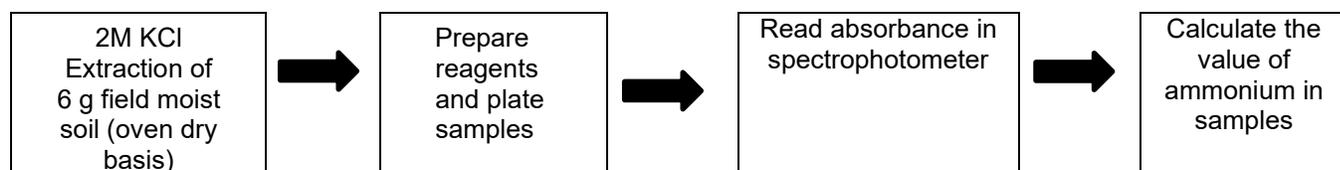
SOP: Determination of Soil Extractable Ammonium via the Salicylate Method

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 mL microcentrifuge tubes
- Ammonium standard
 - Location: Chemicals Only Fridge
- 2 M Potassium Chloride (KCl)

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- 1000 μ L pipette and tips

Reagent preparation

- Reagent A
 - Analytical balance (three decimal places sensitivity)
 - 500 mL Erlenmeyer flask wrapped with tin foil
 - Sodium Salicylate
 - Location: Organic reagents shelf
 - Sodium Citrate
 - Location: Organic reagents shelf
 - Sodium Tartrate
 - 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
 - 2000 μ L pipette
 - Small beaker (100 mL)

Colorimetry

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

Detailed Procedure:

Can use 0.5 M potassium sulfate, but values cannot be directly compared*

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 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.
 - i. To calculate the amount of wet soil needed, see *Calculations Example 2* (pg. 7).

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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 hr on a reciprocating shaker, on high 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 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 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)
100.00	900	100 of 1000 mg/L
20.00	800	200 of 100.0 mg/L
10.00	500	500 of 20.00 mg/L
5.00	500	500 of 10.00 mg/L
2.50	500	500 of 5.00 mg/L
1.25	500	500 of 2.50 mg/L
0	500	-

NOTE: Serial dilution consists of performing the same dilution step over and over using 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 lay between the lowest and the highest absorbance values of the created standards. This means that the concentrations of the standards should be planed around the expected/estimated rage of ammonium concentrations of the samples.

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 tartate
 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. Solution 1 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

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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 uL reagent A	200 uL reagent A*	200 uL reagent A	500 uL reagent A	500 uL reagent A
600 uL sample	800 uL sample	800 uL sample	80 uL sample	40 uL sample
150 uL reagent B	200 uL reagent B	200 uL reagent B	500 uL reagent B	500 uL reagent B
Microplates-----	Microplates-----	Microplates-----	Microplates-----	Microplates-----
50 uL reagent A	128 uL reagent A*	128 uL reagent A	139 uL reagent A	144 uL reagent A
200 uL sample	45 uL sample	45 uL sample	22 uL sample	12 uL sample
50 uL reagent B	128 uL reagent B	128 uL reagent B	139 uL reagent B	144 uL 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. Make sure all reagents are back in the shelves.
3. 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, see corresponding Excel calculation template (Example 1).

1. Generate a scatter plot graph for the standard curve. Add a trendline and check if the R² value is acceptable. In general, an R² > 0.97 is necessary.

Anything below 0.090 should be redone and checked for procedural errors. An example of the absorbance values and graph is provided below.

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

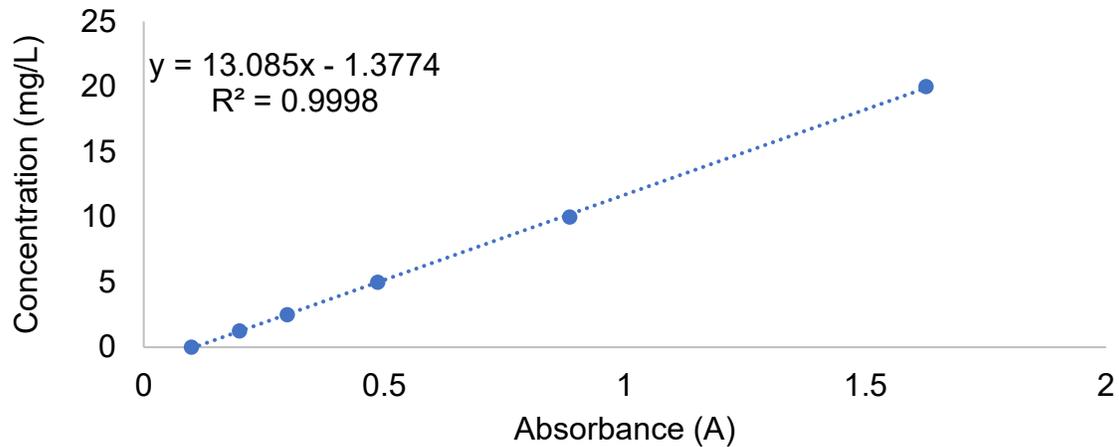


Figure 1. Example of ammonium standard curve, with absorbance values recorded from spectrophotometer and concentration of each standard created through serial dilutions.

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	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).			
10				
11				
12	=TREND(known_ys, known_xs,new_xs)			
13	=TREND(concentration_values,absorbance_values,sample_value)			
14	=TREND(B\$2:B\$7,A\$2:A\$7,C2)			
15	LOCK the "concentration_values" and "absorbance_values"			
16	i.e., add the dollar sign after each letter. DO NOT lock sample value.			
17				
18				
19				

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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