Introduction: Nanotechnology involves the study of matter that is between 1-100 nm in size. In this experiment, you will synthesize silver nanoparticles (AgNPs) using herbal extracts (oregano, sage, and mint), explore the properties of this colloidal suspension, use UV-Visible Spectroscopy to measure absorbance, and calculate the concentration of the synthesized particles.

Background Reading:


Safety Information:
- Wear Goggles
- Wash hands, and wipe down workspace with wet wash cloth after working with nanoparticles (Silver stains hands and clothing).
- Store nanoparticle waste in scintillation vials, and do not dispose into the trash or down the drain.

Materials:
- 5.0 g each of sage, oregano, and mint leaves, washed and dried with a paper towel
- 24 scintillation vials
- 3 (250 mL) Erlenmeyer flasks
- 3 (250 mL) beakers
Day 1

Warm-Up
Names of group members: _______________________________________________________

Define assigned terms and give examples of each.

Demonstration Activity:  (Distinguish between a solution and a colloid.)
Turn off the lights in the classroom.
Shine a flashlight beam through the scintillation vials labelled "A" and "B," and record your observations.

Observations:
Light through vial "A": _________________________________________________________
Light through vial "B": _________________________________________________________

Questions:
Which vial contains a solution? Explain.

Which vial contains a colloid? Explain.

**Experimental Procedure A:**

- Label 3 scintillation vials with your name and the name of the herbal extract you are about to prepare.
- Mass 5.0g of the herb.
- Use a pair of scissors to shred the leaves into a 250 mL Erlenmeyer flask.
- Add 100 mL of distilled water using the 100 mL graduated cylinder.
- Heat the contents of the flask on the hot plate for 5 minutes.
- Decant the liquid into the 250 mL beaker by filtering through a funnel lined with a coffee filter.
- Pour the leaf extract into the three labelled scintillation vials.
- Store the vials in the refrigerator.
- Clean-up your work station.

**Homework:** Review the warm-up questions for tomorrow, and review the procedure for tomorrow.

**Day 2**

**Warm-Up and Homework Review:**

1. If you begin with a stock solution that has a concentration of 1 mM of AgNO₃ and remove 10 mL of the AgNO₃ stock and add 10 mL of water, what is the final dilution of the solution in % dilution by volume and concentration of AgNO₃ in mM?
2. If you begin with a stock solution that has a concentration of 1 mM of AgNO₃ and remove 18 mL of the AgNO₃ stock and add 2 mL of water, what is the final dilution of the solution in % dilution by volume and concentration of the AgNO₃ in mM?

**Experimental Procedure B:**

(Use your time wisely when following the procedure below)

- Label 5 scintillation vials (5% extract, 10% extract, 20% extract, 30% extract, and 50% extract).
- Add 1.0 mL of extract into the 5% vial, 2.0 mL of extract into the 10% vial, 4 mL of extract into the 20% vial, 6.0 mL of extract into the 30% vial, and 10.0 mL of extract into the 50% vial using the automatic pipette set for 1000 μ (1.000 mL).
- Add 19.0mL of 1 mM AgNO₃ into the 5% vial, 18.0 mL of 1 mM AgNO₃ into the 10% vial, 16mL of 1 mM AgNO₃ into the 20% vial, 14.0 mL of 1mM AgNO₃ into the 30% vial, and 10.0 mL of 1mM AgNO₃ into the 50% vial, and mix.
- Record your observations after 5 minutes and at 10 minutes after that.
- Label 5 scintillation vials (5% extract blank, 10% extract blank, 20% extract blank, 30% extract blank, and 50% extract blank).
- Add 1.0 mL of extract into the 5% vial, 2.0 mL of extract into the 10% vial, 4 mL of extract into the 20% vial, 6.0 mL of extract into the 30% vial, and 10.0 mL of extract into the 50% vial using the automatic pipette set for 1000 μL (1.000 mL).
- Add 19.0 mL of deionized water into the 5% vial, 18.0 mL of deionized water into the 10% vial, 16 mL of deionized water into the 20% vial, 14.0 mL of deionized water into the 30% vial, and 10.0 mL of deionized water into the 50% vial, and mix.

**Observations:**

**After 5 minutes:**

Orange wisps appear in the 10%, 20%, or 30% vials.

**After 15 minutes:**

The vials get darker and show a color change.
After 20 minutes, rank the vials by color with the darkest vial being first:

After 24 hours, rank the vials by color with the darkest vial being first:

**Table and Calculations: (Complete for homework)**

<table>
<thead>
<tr>
<th>Volume of AgNO₃ added to Vial (mL)</th>
<th>Volume of Extract added to Vial (mL)</th>
<th>Extract in Vial</th>
<th>Concentration of AgNO₃ in Scintillation Vials (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.0</td>
<td>1.0</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>18.0</td>
<td>2.0</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>16.0</td>
<td>4.0</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>14.0</td>
<td>6.0</td>
<td>30%</td>
<td></td>
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<tr>
<td>10.0</td>
<td>10.0</td>
<td>50%</td>
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</tbody>
</table>

*Sample Calculation for % of Extract:*

\[(1.0\text{mL}/20.0\text{mL}) \times 100 = 5\%\]

*Sample Calculation for Concentration of AgNO₃ in Scintillation Vials:*

\[(19.0\text{ mL}/20.0\text{mL})(1.00\text{mM}) = 0.95\text{mM}\]

**Closing:**

Identify the wisps: _____________________________________________

Clean–Up your work station by wiping it down with a damp cloth.

Drop the disposable pipette tips in the trash can.

Store all vials in the refrigerator, and wash your hands.
**Homework:** Complete the calculations for Day 2 and read the procedure for Day 3.

**Day 3**

**Warm-Up:** (~10 minutes)

Questions on the Whiteboard:

1. Silver atoms pack together to form a unit cell that is face-centered-cubic (fcc). These unit cells repeat in a silver crystal. Study the arrangement of atoms on your paper, and assume that one atom is spherical. How many silver atoms are in the fcc unit cell for silver?

2. If the diameter of the AgNPs made is 30 nm, what is the volume of the AgNP (assume that one AgNP has a spherical shape)?

3. The volume of the unit cell for silver is $0.4073 \, \text{nm}^3$. Knowing the volume of the unit cell, the number of atoms in the unit cell, and the volume of an AgNP, calculate the number of atoms in the silver nanoparticles you synthesized. Report your answer to two significant digits.

**Experimental Procedure C:**

- Optional: Add a drop of dish detergent to the vials to stop the aggregates of silver from getting larger and falling out of solution.
- Use a micropipette to perform a 1/3 dilution of AgNP by adding 1 mL or 1000 μL of colloid to a cuvette with 2 mL or 2000 μL of nano-pure water (deionized water). Perform the same dilution for the corresponding % of extract dissolved in water; this will constitute the blank.
- Use a micropipette to perform a 1/6 dilution by adding 1 mL or 1000 μL of colloid from the 1/3 dilution to a cuvette with 1mL or 1000 μL of nano-pure water (deionized water).
- Record absorbance values for all the different concentrations of colloids at a wavelength of 406 nm.
- Absorbance values have to be less than 1.000 and above 0.100 to be valid.
- Perform subsequent dilutions, if necessary, to obtain absorbance values in this range.

**Data Table:**

<table>
<thead>
<tr>
<th>Wavelength = 406 nm</th>
<th>Molar-Absorptivity Constant (ε) = 145x10^8 M^-1cm^-1</th>
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</thead>
<tbody>
<tr>
<td>Name of Extract: Sage</td>
<td>Name of Extract: Oregano Name of Extract: Mint</td>
</tr>
<tr>
<td>Dilution of AgNP: 1/3rd</td>
<td>Dilution of AgNP: 1/6th Dilution of AgNP: 1/6th</td>
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</tbody>
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<table>
<thead>
<tr>
<th>% of Extract</th>
<th>Absorbance</th>
<th>( c = \frac{A}{\epsilon l} )</th>
<th>% of Extract</th>
<th>Absorbance</th>
<th>( c = \frac{A}{\epsilon l} )</th>
<th>% of Extract</th>
<th>Absorbance</th>
<th>( c = \frac{A}{\epsilon l} )</th>
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Calculate concentration “c” in molarity using the molar–absorptivity constant given above.

**Graph:** Draw a graph of Absorbance (y-axis) vs. % Extract (x-axis) on a sheet of graph paper.

**Calculations:** Calculate the concentration of nanoparticles for each % of extract and for each herbal sample using the following sample calculations.

*For 5% Oregano Extract*

- Concentration of AgNO3 in scintillation vial = 0.95 mM
- Concentration from absorbance (\( c = \frac{A}{\epsilon l} \)) = \( 4.17 \times 10^{-11} \) M
- Convert to mM = \( 4.17 \times 10^{-8} \) M (1000) = \( 4.17 \times 10^{-8} \) mM
- Account for dilution factor (Multiply by 6 or 3 depending on the extract) = \( 2.50 \times 10^{-7} \) mM

- Volume of AgNP with 30 nm diameter = \( 1.4 \times 10^4 \) nm³
- Volume of face-centered cubic unit cell of Ag atoms = \( 0.04073 \) nm³
- Number of atoms in a unit cell of silver = 4 atoms

- Estimated concentration of silver in AgNP vial:

\[ \frac{(2.50 \times 10^{-7}) \times (1.4 \times 10^4) \times (4)}{(0.04073)} = 0.210 \text{ mM of Ag in colloidal suspension of AgNP} \]
% of Ag that became AgNP from original AgNO₃ concentration:
\[(0.210/0.95) 100 = 22\%\]

Closing:
- Clean up work station using a damp cloth.
- Discard pipette tips into the trash.
- Leave all scintillation vials with the colloids at your station.
- Empty cuvettes into a special container marked “solid metal waste.”

Questions:
1. Compare the concentrations from the visual analysis you performed 24 hours after the synthesis and the concentrations of AgNP you obtained from the calculations. Account for discrepancies between the visual analysis and the calculated concentrations of nanoparticles.

2. Which of the herbs produced the highest concentration of silver nanoparticles, and what was the concentration of the leaf extract that produced this high concentration?

3. Which herbal extract seemed to be the least effective in producing silver nanoparticles? What was the lowest concentration of particles produced?
4. Why might the results from similar experiments with the same herbs show some variation in concentration of nanoparticles?

5. What controls the size of nanoparticles formed? How can the size of nanoparticles be changed in this experiment?

6. What might happen if the aggregates of nanoparticles become very large? Why might this happen?